Hidden diversity in the spotlight: Integrative taxonomy of European cicada groups (Cicadidae) with emphasis on acoustics

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Part I:

General introduction

European cicadas (Cicadidae): Charismatic, popular and poorly known

Cicadas (Cicadidae) are among the most well-liked insect groups. This is especially true for the Mediterranean Basin. Cicadas are mentioned in Greek mythology by Aristotle, Plato and Socrates and can be purchased as southern French souvenirs made of wood, ceramic or metal. Their popularity is mainly owing to the calling songs, produced by males with a pair of timbals situated on the dorsal side of the abdomen and boosted by almost hollow abdomens. Some species like *Cicada orni* Linnaeus, 1758 or *Lyristes plebejus* (Scopoli, 1763) are persistent singers forming choruses, and their loud songs dominate the sensation of typical Mediterranean summer days. Large African, Australian and American species reach noise intensities of almost 110 dB, close to the pain threshold of the human ear (Petti, 1997).

However, cicadas are scientifically relatively badly known. Among the approximately 70 species currently described for Europe nearly 20 were published newly this century (Gogala, 2015; www.cicadasongs.eu). Taxonomy is still a major part of studying biodiversity and the crucial foundation for conservation. Since the taxonomy of cicadas is poorly investigated, it is not surprising that cicadas have played a minor role for conservation strategies in Europe up to now. The unique Red Lists were published for Germany with very few species listed (Achtziger, 1992; Remane *et al.*, 1998); the United Kingdom is the only state to establish a biodiversity action plan for their sole cicada species, *Cicadetta montana*, which symptomatically has most probably been extinct before (Pinchen & Ward, 2002; Joint Nature Conversation Committee, 2007).

We assume three facts to be crucial for the deficits in cicada research and taxonomy. 1) Many cicada species are very difficult to observe visually or to catch. 1.1) They are well camouflaged on trunks and branches of woods. 1.2) They are extremely wary, stop singing when a potential danger approaches and move to the reverse side of branches or fly away deftly. 1.3) Some species preferentially sing high up in the canopy of trees. 2) Based on a morphological approach only, the cicada diversity is essentially underestimated. Songs often evolve more rapidly than the morphology (e.g. Alexander & Moore, 1962; Marshall *et al.*, 2011). These songs are an important component of specific-mate recognition systems (SMRS; Paterson, 1985; Den Hollander, 1995; Villet, 1995) and, therefore, excellent markers of the early stages of reproductive isolation among populations. Songs must be inherited and not learnt since generations are temporally separated (Marshall *et al.*, 2011). Acoustic characters have been used extensively to reveal hidden taxonomic diversity when morphological traits are missing or weak (e.g. Dugdale & Fleming, 1978; Gogala & Trilar, 2004; Quartau & Simões, 2006; Puissant & Sueur, 2010;

Front page: *Cicada orni* (top left), *Lyristes plebejus* (top right), *Tibicina haematodes* (bottom left), *Cicadetta montana* s. str. (bottom right).

Marshall *et al.*, 2011). Therefore, cicada taxonomy must be studied mainly in the field where songs can be recorded. 3) Smaller cicadas sing faintly and often in a high carrier frequency range (e.g. Gogala & Trilar, 2004, Puissant & Sueur, 2010). These songs are hardly audible for elderly people. Their discovery used to be limited to younger human beings or, nowadays, to the availability of technical devices such as high-quality portable recorders and ultrasonic detectors (see **Fig IN.1**).

Fig IN.1. Field work with an ultrasonic 'Pettersson' microphone. The microphone is mounted into a parabolic reflector of a car and is connected to a bat detector.

Cicadas form the superfamily Cicadoidea within the order Hemiptera and the suborder Auchenorrhyncha and contain more than 1300 known species worldwide (e.g. Moulds, 2005; Sanborn, 2014). Although they are taxonomically close to other Auchenorrhyncha, their airborne emitted songs, their large body sizes and their lack of ability to jump make them unique. This is reflected in a practical aspect: The methods to explore cicadas are completely different to the work with other Auchenorrhyncha. Therefore, interestingly, many cicadologists do not work on other Auchenorrhyncha and vice versa.

Higher classification of the cicadas is under debate and all concepts are based only on their morphology (but see Marshall *et al.*, 2016). Moulds (2005) suggested one European family (Cicadidae) and three subfamilies (Cicadinae, Cicadettinae and Tettigadinae). Currently this is the most popular concept (Puissant, 2006; Sanborn, 2014). Other authors prefer categorising into the two subfamilies Cicadinae and Tibicininae (Gogala, 2015; www.cicadasongs.eu) or the analogue families Cicadidae and Tibicinidae (Boulard & Mondon, 1995). The two main concepts differ for Europe in the placement of the genus *Tibicina*; once being part of the Tibicininae respectively Tibicinidae together with the majority of small species and once seen as a single European genus in the Tettigadinae. Moulds' study (2005) implies that the genus *Tibicina* derived from one of the most ancient cicada lineages worldwide.

Cicadas have a long-lasting, subterranean larval development (compare **Fig IN.2**). Not much is published or known about the lifespans of European species to this day: A period of 3 to 5 years was normally observed in raising experiments for *Cicadatra atra* (Olivier, 1790) and 3 or 4 years for *Cicada orni* (Boulard, 1992; Puissant, pers. comm.). Kudryasheva (1979) calculated a mean larval life of 5.9 years for *Cicadetta montana* s. l. in central Russia and Pinchen & Ward (2002) observed 6 to 10 years for England, while Schedl (1973; 2000) estimated probably 2 years for Central Europe. American species of the genus *Magicicada* are famous. Millions of individuals hatch within a few days after a subterranean larval life of exactly 13 or 17 years, depending on the species (e.g. Alexander & Moore, 1962). Metapopulations form so-called 'broods' which are spatially and temporally isolated. Generally, long-lasting larval lives contrast with brief adult stages of a few weeks. Cicadas are normally not known to fly long distances (Simões & Quartau, 2007), and their life cycles imply low population vagility. These features result in high genetic and acoustic phylogeographic structures (Buckley *et al.*, 2001; Hill *et al.*, 2009; Marshall *et al.*, 2011) and make cicadas potential model organisms for studying evolutionary processes such as speciation. Many species also appear stenoecious and are therefore good indicators for habitat choice.

Methods to investigate cicadas acoustically for taxonomic purposes have been developed within a few decades to a high standard. Nevertheless, Sueur (2001) estimated that songs of only 12% of the known cicada species have been published worldwide and many descriptions of new species are still based solely on morphology, especially in tropical regions. Unfortunately, studies investigating phylogeny with molecular data continue to be scarce (e.g. Buckley *et al.*, 2006; Marshall *et al.*, 2011; Popple, 2013; Nunes *et al.*, 2014) and limited mainly to mitochondrial DNA (*COI* and *COII*). The few nuclear markers examined so far in cicadas were hardly informative when answering recent cladogenic events. Next-generation sequencing is necessary for taxonomists to target the fast evolving protein-coding genes of cicadas (Owen *et al.*, 2015). This current disadvantage of the group can be compensated partly by in-depth acoustic research. Hereby, song patterns serve as nuclear encoded predictors of the most parsimonious evolution as well as indicators of recent hybridisation exposed by intermediate singing specimens (Drosopoulos *et al.*, 2006; Marshall *et al.*, 2011).

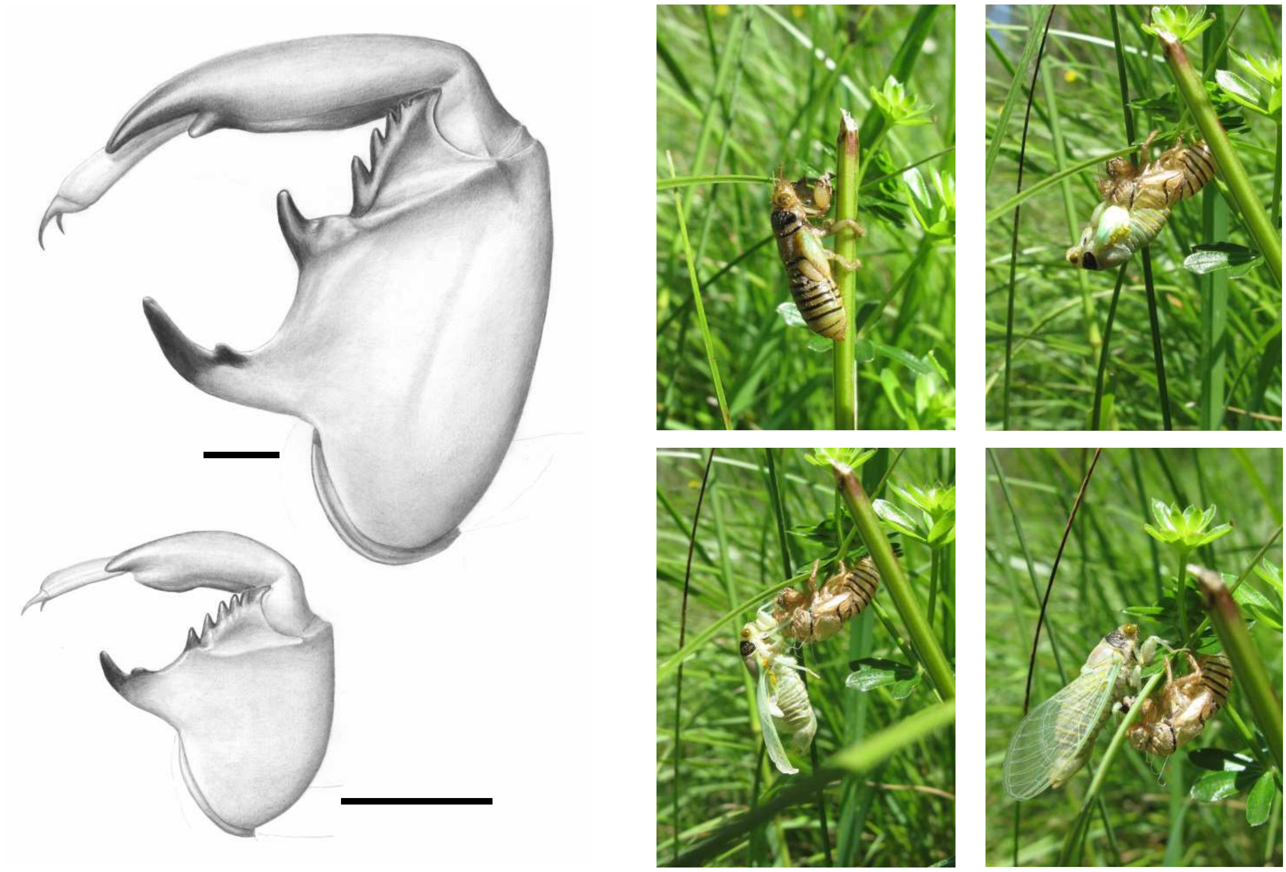

Fig IN.2. Cicada life cycle. Subterranean living larvae have fore legs (tibiae and femora) which develop to digging shovels [top left: *Lyristes plebejus*, bottom left: *Tettigettula pygmea* (Olivier, 1790), bars = 1 mm. Drawings of the exuviae by T. Hertach]. After several years the subterranean life is completed: A *Cicadetta montana* s. str. female eclosing from the exuviae and drying (top and bottom right).

From the precedent paragraphs the following major questions for the current European cicada taxonomic research can be inferred: Can undetected species or even species complexes be discovered with the use of bioacoustics (in regard to taxa which might have remained undiscovered until now owing to speciation under morphological stasis)? Are such distinct lineages supported by molecular approaches? Can combined data sets explain evolutionary processes and biogeographical aspects? Does in-depth field work alter the faunistics of European countries in a significant way? Are species endangered and do we need particular strategies for the maintenance of diversity?

The author of this thesis dealt with several of these general questions a few years ago: Hertach (2004; taxonomy), Hertach (2007; taxonomy, ecology, conservation), Hertach (2008a; faunistics), Trilar & Hertach (2008; taxonomy, faunistics). Some aspects could be delved into in more detail in this thesis. The *Cicadetta montana* species complex served as a model group since it was badly known, widespread and supposedly rich in taxa. We focussed on the cicadas of Italy and Switzerland.

Cicadetta montana species complex: A model group at the brink of human acoustic perception?

Cicadetta montana s. l. are rather small cicadas with wing-spans around 40 to 50 mm and body lengths of 15 to 23 mm, a generally black habitus, but conspicuous orange to reddish caudal margins of tergites. *Cicadetta montana* (Scopoli, 1772) was the fourth cicada species described for Europe after the large species *Cicada orni* Linnaeus, 1758, *Tibicina haematodes* (Scopoli, 1763) and *Lyristes plebejus* (Scopoli, 1763). Scopoli's description is short and rather unspecific. He does however refer to the Carniola region in Slovenia around Idrija ("In montanis Carnioliae circa Idriam"; Scopoli, 1772). Many subsequent authors recognised different morphologic phenotypes and regarded the variability in colouration and wing shape as new species (Goeze, 1778; Fabricius, 1803; Samouelle, 1819; Hartwig, 1857; Fieber, 1876), variations or forms (Fieber, 1876; Schumacher, 1924). None of these early classifications were convincing, and thirty years ago there was a widely accepted consensus that three closely related species exist only (Duffels & Van der Laan, 1985): *Cicadetta montana* (Scopoli, 1772), *Cicadetta fangoana* Boulard, 1976, a Corsican endemite with remarkable spots on the thorax, and *Cicadetta concinna* (Germar, 1821) [= *C. podolica* (Eichwald, 1830)] with infuscations at the apical cells 1 to 3.

Only since 1999 *C. montana* has been shown to be a complex of cryptic species (Gogala & Trilar, 1999; Puissant & Boulard, 2000; Gogala & Trilar, 2004; Hertach, 2004). The different songs, some of them with complex patterns, provided first evidence of this hidden diversity. It is surprising that widespread species remained undiscovered, even ones present in countries investigated far above-average like Switzerland and Germany. It appears this diversity was beyond the human perception. In fact, the calling songs of the *Cicadetta montana* group species are in high carrier frequency ranges (10-20 kHz). They can be heard in a distance of 40 to 50 metres by a young accurate ear and are the most effective way to prove the presence of this complex of species. Interestingly, a very old reference was found during this project, indicating that two scientists were aware of different song patterns in *Cicadetta montana* s. l. already in the middle of the 19th century and correctly interpreted them as proper species (Bremi, 1849). However, they did not publish accurate descriptions of these species and their knowledge was forgotten. Species belonging to the *Cicadetta montana* complex are among the most difficult European cicadas to locate and catch. Furthermore, they are sensitive to even slightly unfavourable weather conditions with clouds or wind and then stop their acoustic activity.

Gogala & Trilar (2004) demonstrated, with recordings taken in the localities around Idrija, that *Cicadetta montana* s. str. is identical to the simplest, long-lasting song pattern. At the beginning of this thesis, three species had been documented with acoustic methods for very few locations in northern Italy (Trilar &

Hertach, 2008): *Cicadetta montana* s. str., *Cicadetta brevipennis* Fieber, 1876 (sensu Gogala & Trilar, 2004) and *Cicadetta cerdaniensis* Puissant & Boulard, 2000. The same diversity was found for Switzerland (Hertach, 2007). Some species differ significantly in ecological niches with respect to vegetation classes, altitudinal ranges and seasonal patterns (Hertach, 2007). Molecular or in-depth morphological studies on the newly recognised taxa were lacking.

We analysed different data sets dealing with the acoustics, morphology, genetics, ecology and chorology of cicadas and combined the results towards integrative decisions. In the last years such an 'integrative taxonomy' has become a standard procedure when working with taxonomically difficult groups and is suitable to elevate the evidence of species delimitation (e.g. Schlick-Steiner *et al.*, 2010; Yeates *et al.*, 2011; Carstens *et al.*, 2013). In fact, some *C. montana* s. l. taxa proved to be intricate.

Species delimitation is a practical application of the theoretical species concepts using operational criteria (De Queiroz, 2007). Up to 20 different concepts exist to define the basic unit of life (Hey, 2006), namely the species, some of them being conflicting with one another. This in return implies that species delimitation and classification can differ owing to the chosen species concept. The 'morphological' and the 'biological species concepts' have the longest traditions, but are inflexible towards alternative or integrative approaches. Many concepts have fundamental difficulties incorporating or explaining hybridisation. This is a major problem. Hybridisation of species is commonplace in plants, but is often seen as unnatural and unusual in animals. The role and importance of hybridisation of animals was clearly underrated in older studies and is now estimated to affect more than 10% of animal species in wildlife (Mallet, 2005), with some groups showing more than one third of the species hybridizing (Mallet, 2008).

This situation with multiple disagreeing concepts is unsatisfactory and led to endless controversial discussions in the scientific community. De Queiroz (2007) postulated a single criterion as the property of a species in his 'Unified Species Concept', namely the "separately evolving metapopulation lineage". Properties formerly accounting for species concepts like monophyly or reproductive isolation are herein only relevant to the extent that they contribute to the evidence of lineage separation. We approve of the 'Unified Species Concept' theory and have adapted it to this thesis. However, the term 'separately evolving' can be misleading with respect to sporadic gene flow. The concept is helpful when combining data sets, even though results of different methods are incongruent.

The author's territory: Italy and Switzerland or from the Mediterranean Basin to the Alps

Italy and Switzerland were mainly chosen as investigation areas for this thesis because of obvious deficits in the knowledge of cicada faunistics in those countries and their proximity to the university and author's domicile. Italy is assumed to form one of the European centres of cicada diversity. It has a long north-south extension, many parts are typically Mediterranean and mountainous habitats can be found all over the country, the latter supposedly decisive for *Cicadetta montana* s. l. species. These factors contrast with the poor knowledge of cicadas in general (summarised by Servadei, 1967) and on *C. montana* s. l. in particular (Trilar & Hertach, 2008).

Several long trips (in total 27 weeks, 20,000 km on a scooter) were made for data collection in all Italian mountainous regions between 2008 and 2013 (**Fig IN.3**). This extensive data collection enables us to give a complete overview on the *Cicadetta montana* s. l. species diversity. Potential contact zones of closely related taxa were detected and forced us to in-depth investigations for subsequent taxonomic considerations. Song patterns were recorded from many local populations, specimens caught whenever possible and ecological parameters noted. Some specimens were prepared for molecular analyses. First molecular work was conducted by collaborators of the University of Connecticut (Beth Wade, Chris Simon; Chapters 2, 3) and later in the Biogeography laboratory of the University of Basel (Chapter 4). The Italian trips also served to ameliorate substantially the knowledge of other cicada species not closely related to *C. montana*. However, those species are only in the focus of this thesis for Switzerland, not for Italy.

Switzerland, situated more northern, was expected to have less cicada species with more restricted distribution areas including the xerothermic Valais and sub-mediterranean southern Ticino. Very old references indicate the presence of cicadas for Switzerland (Von Haller, 1765; Fuesslin, 1775). It was Pillet (1993) who eventually wrote a more detailed study. He focussed on the Valais and presents five species with precise distribution maps: *Cicada orni*, *Lyristes plebejus*, *Cicadetta montana* s. l., *Tibicina haematodes* and *Tibicina quadrisignata* (Hagen, 1855). He also concluded that these five species were the only ones occurring in Switzerland. With the discovery of *Cicadetta montana* forming a species complex, his statement needed a revision (see Hertach, 2004; 2007).

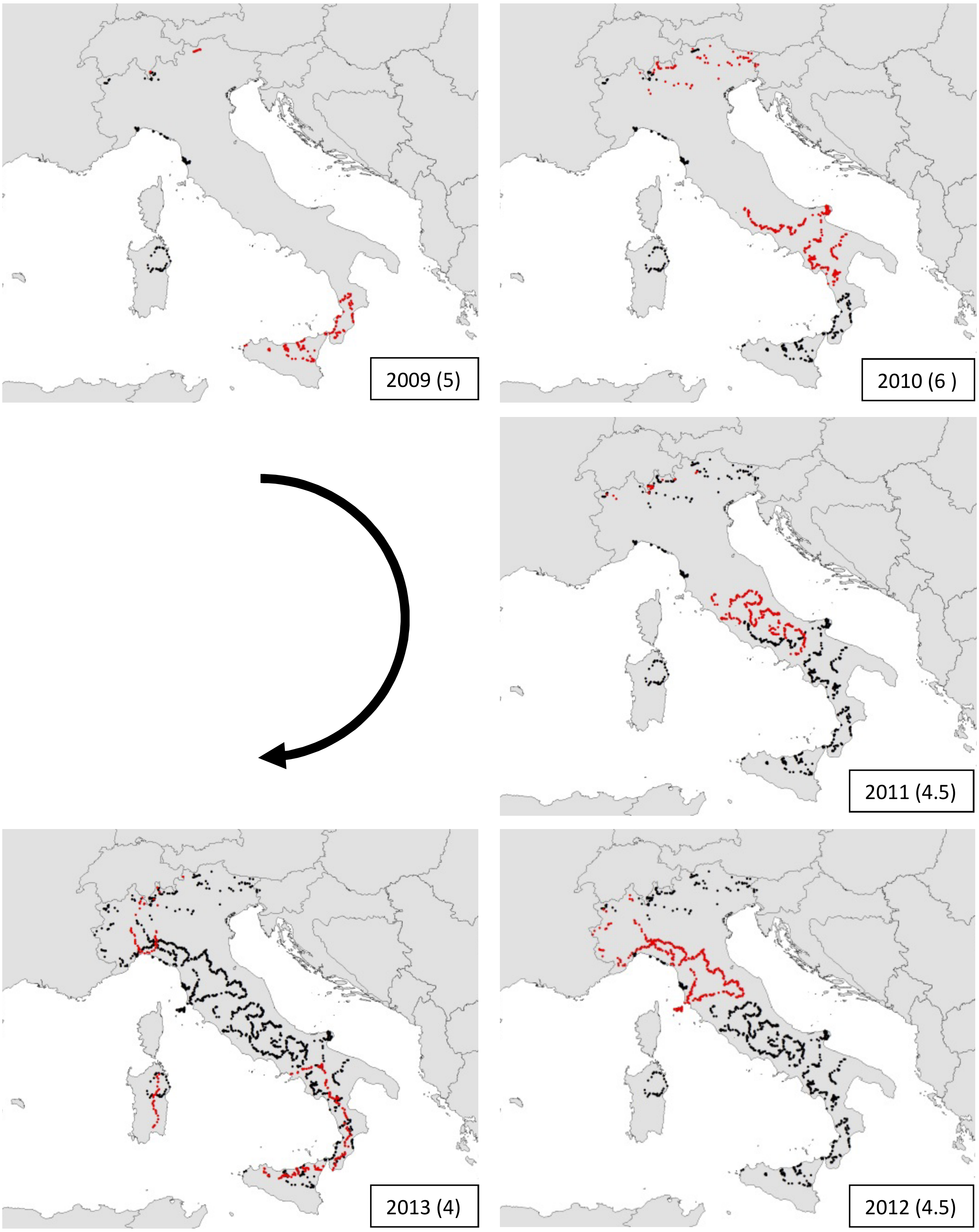

Fig IN.3. Personal data collection in Italy from 2009 to 2013. Dots are records of cicada species (black: found in previous years, red: new finds). Arrow = time order, number in brackets = duration of travels in weeks.

Additional deficits towards a complete Swiss cicada faunistics, which are not linked to the *Cicadetta montana* species complex, remained unresolved: 1) There was no sampling across the whole country using modern methods. 2) A comprehensive survey of the collections and literature was lacking. 3) Different references listed species for Switzerland which have not been observed in recent field work (e.g. *Cicadatra atra*; Haupt, 1935; Nast, 1972; 1987; Schedl, 2000). 4) Determination problems seemed obvious in the genus *Tibicina*, which contains species of remarkable size with wing-spans up to 8 cm (Pillet, 1993; Vernier, 1996; Sueur *et al.*, 2003). Difficulties are opposite to the *Cicadetta montana* complex: The species can easily be distinguished morphologically, exhibiting different colouration patterns, but the continuous powerful songs are very similar. At the beginning of the thesis we expected eight to eleven mostly rare autochthonous species. Thus, a doubled number in comparison to Pillet (1993) could result in the end.

Five regional projects were established to investigate the Swiss cicada fauna in more detail: The Cantons of Basel-Land, Basel-Stadt, Aargau and Solothurn (Hertach, 2008b), the Canton of Ticino (Hertach, 2010; Hertach & Pollini Paltrinieri, 2012), the Canton of Graubünden (Grisons) (Hertach, 2013a), the Leman Basin (Hertach, 2013b) and later a random sample of the remaining Swiss regions (Hertach, 2015) were explored. These projects were financed usually by regional authorities and not published (**Fig IN.4**). They focussed on gathering records in the field; the subsequent analyses of ecology and threat resulted in local conservation proposals.

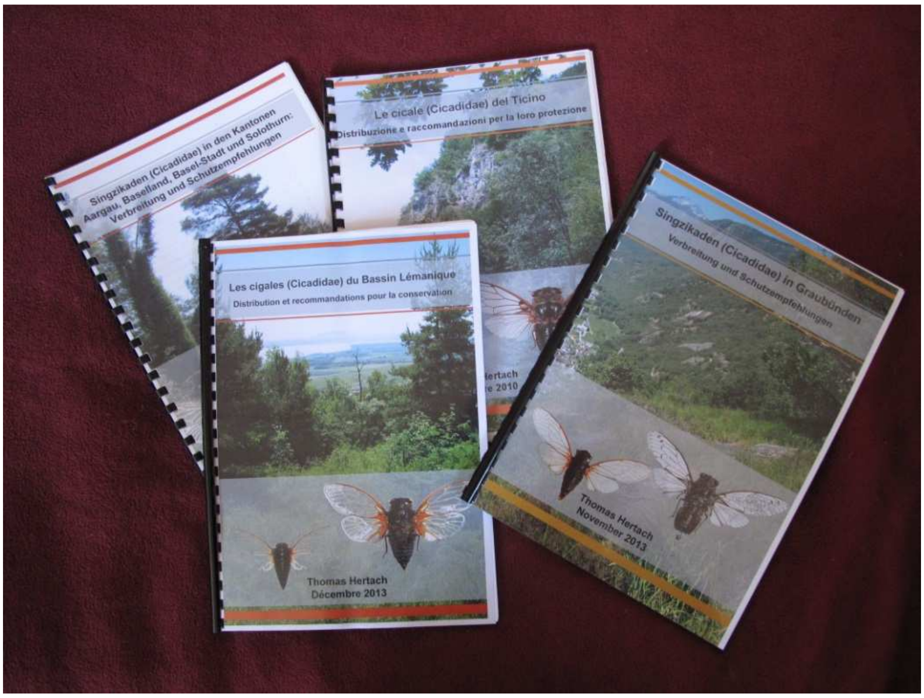

Fig IN.4. Reports of regional cicada inventories completed between 2008 and 2013.

Some taxonomic problems concerning the Italian and Swiss cicada species could only be resolved when data from additional countries was added. Slovenian (Matija Gogala, Tomi Trilar) and French (Stéphane Puissant) colleagues kindly provided their field data. Furthermore, we visited the Alsace in 2009, the French Pyrenees in 2011 and Thuringia in Germany in 2014.

Questions and chapters

Following questions were elaborated within this thesis: **1) Which different species-specific song patterns can be detected in Italy and Switzerland as operational taxonomic units (especially in the model group *Cicadetta montana* s. l.)? 2) Which cicada species are historically and currently part of the Swiss fauna? 3) Is it possible to consolidate species delimitation with the help of integrative taxonomy (acoustics, genetics, morphology, ecology and chorology)? 4) Can evolutionary processes, based on the combined data sets, be assumed? 5) Which biogeographical aspects are helpful to explain the diversity? 6) Can habitat preferences be derived from our observations? 7) Does the cicada diversity have an impact on conservation strategies?**

Part II of the thesis is concerned with the *Cicadetta montana* species complex in Italy and Switzerland. Chapters (1 to 5) are presented in chronological order. In Chapter 1 we describe a species with a distinct song pattern first found in Sicily, which is new to science. Closely related song patterns have been recognised step by step as complex groups of different taxa. Chapter 2 elucidates the so-called '*Cicadetta cerdaniensis* song group', Chapter 4 the '*Cicadetta brevipennis* song group', both of them with a vast European distribution. Chapter 5 is dedicated to the Italian *Cicadetta montana* s. l. species diversity not treated in the previous chapters. Chapter 3 demonstrates the results of molecular analyses and species delimitation models conducted by our American colleagues with material sampled from all over Europe. At the beginning of this study, species belonging to the *Cicadetta montana* complex were considered morphologically indistinguishable (with the exception of *C. fangoana* and *C. concinna,* see above). First morphological traits are presented in Chapters 1, 2 and 4 and a comprehensive study is in preparation (Hertach & Baur, compare Part IV).

Part III of this thesis portrays a first national synthesis of the current and historical knowledge of Swiss cicadas (Chapter 6). This synthesis serves as a basis for a book in the series Fauna Helvetica 'Cicadas (Cicadidae) of Switzerland', planned for the following months, and for the Red List assessment, according to the IUCN criteria, in collaboration with the 'Bundesamt für Umwelt' (Bafu) and the 'Swiss Central Fauna

Data Bank' (CSCF) (IUCN, 2012; Cordillot & Klaus, 2011). Finally, all chapters are discussed in Part IV (General discussion).

REFERENCES PART I

Achtziger R. 1992. Rote Liste gefährdeter Singzikaden (Homoptera, Auchenorrhyncha, Cicadidae) Bayerns. *Schriftenreihe Bayerisches Landesamt für Umweltschutz*, München 111: 99.

Alexander RD, Moore TE. 1962. The evolutionary relationships of 17-year and 13-year cicadas, and three new species (Homoptera, Cicadidae, *Magicicada*). *Miscellaneous Publications of the Museum of Zoology, University of Michigan* 121: 1-59.

Boulard M. 1992. Identité et bio-écologie de *Cicadatra atra* (Olivier, 1790), la Cicale noire, in Gallia Primordia Patria (Homoptera, Cicadoidea, Cicadidae). *Ecole pratique des hautes Etudes, Biologie et Evolution des Insectes* 5: 55-86.

Boulard M, Mondon B. 1995. *Vie et Mémoires de Cigales. Provence, Languedoc, Méditerranée*. Barbentane: Édition de l'Équinoxe, 159 pp. + CD.

Bremi H. 1849. Uebersicht der schweizerischen Rhynchoten. *Mittheilungen der Naturforschenden Gesellschaft in Zürich* 34: 325-339.

Buckley TR, Cordeiro M, Marshall DC, Simon C. 2006. Differentiating between hypotheses of lineage sorting and introgression in New Zealand alpine cicadas (*Maoricicada* Dugdale). *Systematic Biology* 55(3): 411-425.

Buckley TR, Simon C, Chambers GK. 2001. Phylogeography of the New Zealand cicada *Maoricicada campbelli* based on mitochondrial DNA sequences: Ancient clades associated with Cenozoic environmental change. *Evolution* 55(7): 1395-1407.

Carstens BC, Pelletier TA, Reid NM, Satler JD. 2013. How to fail at species delimitation. *Molecular Ecology* 22: 4369-4383.

Cordillot F, Klaus G. 2011. *Gefährdete Arten in der Schweiz. Synthese Rote Listen, Stand 2010.* Bundesamt für Umwelt, Bern, Umwelt-Zustand Nr. 1120, 111 pp.

De Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* 56(6): 879-886.

Den Hollander J. 1995. Acoustic signals as specific mate recognition signals in leafhoppers (Cicadellidae) and planthoppers (Delphacidae) (Homoptera: Auchenorrhyncha). In: Lambert DM, Spencer HG, eds. *Speciation and the Recognition Concept, Theory and Application*. Baltimore: Johns Hopkins University Press, 440-463.

Drosopolos S, Eliopoulos E, Tsakalou P. 2006. Is migration responsible for the peculiar geographical distribution and speciation based on acoustic divergence of two cicadas in the Aegean archipelago? In: Drosopoulos S, Claridge M, eds. *Insect sounds and communication: Physiology, behaviour, ecology and evolution.* Boca Raton: CRC Press Taylor & Francis Group, 219-226.

Duffels JP, Van der Laan PA. 1985. *Catalogue of the Cicadoidea (Homoptera, Auchenorrhyncha) 1956–1980.* Series entomologica. Dordrecht, Boston, Lancaster: Dr. W. Junk Publishers, 414 pp.

Dugdale JS, Fleming CA. 1978. New Zealand cicadas of the genus *Maoricicada* (Homoptera: Tibicinidae). *New Zealand Journal of Zoology* 5(2): 295-340.

Fabricius JC. 1803. *Systema Rhyngotorum secundum ordines, genera, species, adiectis synonymis, locis, observationibus, descriptionibus – Ryngota*. Braunschweig: C. Reichard, 1-101.

Fieber FX. 1876. Les Cicadines d'Europe d'après les originaux et les publications les plus récentes. Deuxième partie: Descriptions des espèces. *Revue et Magazin de Zoologie* 4: 11-268.

Fuesslin JC. 1775. *Verzeichnis der ihm bekannten Schweitzerischen Inseckten.* Zürich/Winterthur: Bey dem Verfasser und in Commission bey Heinrich Steiner und Compagnie, xii + 62 pp.

Goetze JAE. 1778. *Entomologische Beyträge zu des Ritter Linné zwölften Ausgabe des Natursystems – 223. Geschlecht. Cicada, Zikade, Cigale, Baumheimiche, Baumgrylle*. Leipzig: Weidmanns Erben und Reich, 124-164.

Gogala M. 2015, ed. *Songs of European singing cicadas.* Available from: www.cicadasong.eu (accessed 25 January 2016).

Gogala M, Trilar T. 1999. The song structure of *Cicadetta montana macedonica* Schedl with remarks on songs of related singing cicadas (Hemiptera: Auchenorrhyncha: Cicadomorpha: Tibicinidae). *Reichenbachia* 33: 91-97.

Gogala M, Trilar T. 2004. Bioacoustic investigations and taxonomic considerations on the *Cicadetta montana* species complex (Homoptera: Cicadoidea: Tibicinidae). *Anais da Academia Brasileira de Ciências* 76(2): 316-324.

Haller Von A. 1765. *Kurzer Auszug einer Beschreibung der Salzwerke in dem Amte Aelen*. Bern: Dan. Brunner und Albr. Haller, 170 pp.

Hartwig P. 1857. Cicada Saxonica mihi. *Allgemeine deutsche naturhistorische Zeitung, Dresden* 3: 313-314.

Haupt H. 1935. 2. Unterordnung: Gleichfülger, Homoptera. In: Brohmer P, Ehrmann P, Ulmer G, eds. *Die Tierwelt Mitteleuropas*, Band IV, Insekten I. Teil. Leipzig: Quelle & Meyer, 115-262.

Hertach T. 2004. Beitrag zur Klärung des Artkomplexes *Cicadetta montana* – Bergzikade (Hemiptera, Cicadidae): Entdeckung einer Singzikadenart mit ungewissem taxonomischem Status in der Nordschweiz. *Mitteilungen der Entomologischen Gesellschaft Basel* 54(2): 58-66.

Hertach T. 2007. Three species instead of only one: Distribution and ecology of the *Cicadetta montana* species complex (Hemiptera: Cicadoidea) in Switzerland. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 80: 37-61.

Hertach T. 2008a. A new cicada species for Switzerland: *Tettigetta argentata* (Olivier, 1790) (Hemiptera: Cicadoidea). *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 81: 209-214.

Hertach T. 2008b. *Singzikaden (Cicadidae) in den Kantonen Aargau, Baselland, Basel-Stadt und Solothurn: Verbreitung und Schutzempfehlungen.* Otelfingen, unpublished, 27 pp.

Hertach T. 2010. *Le cicale (Cicadidae) del Ticino: Distribuzione e raccomandazioni per la loro protezione.* Otelfingen, unpublished, 33 pp.

Hertach T. 2013a. *Singzikaden (Cicadidae) in Graubünden: Verbreitung und Schutzempfehlungen.* Hedingen, unpublished, 30 pp.

Hertach T. 2013b. *Les cigales (Cicadidae) du Bassin Lémanique: Distribution et recommandations pour la conservation.* Hedingen, unpublished, 46 pp.

Hertach T. 2015. *Singzikaden (Cicadidae) in der Schweiz – Ergänzende Stichprobe als Basis einer Roten Liste.* Hedingen, unpublished, 16 pp.

Hertach T, Pollini Paltrinieri L. 2012. Le cicale (Hemiptera: Cicadidae) del Cantone Ticino (Svizzera). *Bollettino della Società ticinese di scienze naturali* 100: 43-51.

Hey J. 2006. On the failure of modern species concepts. *Trends in Ecology & Evolution* 21(8): 447-450.

Hill KBR, Marshall DC, Simon C, Chambers GK. 2009. Surviving glacial ages within the Biotic Gap: phylogeography of the New Zealand cicada *Maoricicada campbelli*. *Journal of Biogeography* 36(4): 675-692.

IUCN. 2012. *IUCN Red List categories and criteria version 3.1.* Second edition. Gland, Switzerland and Cambridge, UK: IUCN. iv + 32pp.

Joint Nature Conversation Committee. 2007. *UK action biodiversity plan – action plan for Cicadetta montana.* Available from: http://www.ukbap.org.uk/ukplans.aspx?ID=216 (accessed 5 March 2010).

Kudryasheva JV. 1979. *Larvae of singing cicadas (Homoptera, Cicadidae) of the Fauna of SSSR.* Moskva: Nauka, 159 pp. (in Russian)

Mallet J. 2005. Hybridization as an invasion of the genome. *Trends in Ecology & Evolution* 20(5): 229-237.

Mallet J. 2008. Hybridization, ecological races and the nature of species: empirical evidence for the ease of speciation. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 363: 2971-2986.

Marshall DC, Hill KBR, Cooley JR, Simon C. 2011. Hybridization, mitochondrial DNA phylogeography, and prediction of the early stages of reproductive isolation: Lessons from New Zealand cicadas (Genus *Kikihia*). *Systematic Biology* 60: 482-502.

Marshall DC, Hill KBR, Moulds MS, Vanderpool D, Cooley JR, Mohagan AB, Simon C. 2016. Inflation of molecular clock rates and dates: Molecular phylogenetics, biogeography, and diversification of a global cicada radiation from Australasia (Hemiptera: Cicadidae: Cicadettini). *Systematic Biology* 65(1): 16-34.

Moulds MS. 2005. An appraisal of the higher classification of cicadas (Hemiptera: Cicadoidea) with special reference to the Australian fauna. *Records of the Australian Museum* 57: 375-446.

Nast J. 1972. *Plaearctic Auchenorrhyncha (Homoptera). An annotated check list.* Warszawa: Polish scientific publishers, 550 pp.

Nast J. 1987. The Auchenorrhyncha (Homoptera) of Europe. *Annales Zoologici Warszawa* 40(15): 355-661.

Nunes VL, Mendes R, Marabuto E, Novais BM, Hertach T, Quartau JA, Seabra SG, Paulo OS, Simões PC. 2014. Conflicting patterns of DNA barcoding and taxonomy in the cicada genus *Tettigettalna* from southern Europe (Hemiptera: Cicadidae). *Molecular Ecology Resources* 14(1): 27-38.

Owen CL, Marshall DC, Hill KBR, Simon C. 2015. The phylogenetic utility of acetyltransferase (ARD1) and glutimany1 tRNA synthetase (QtRNA) for reconstructing Cenozoic relationships as exemplified by the large Australian cicada *Pauropsalta* generic complex. *Molecular Phylogenetics and Evolution* 83: 258-277.

Paterson HEH. 1985. The recognition concept of species. In: Vrba ES, ed. *Species and Speciation.* Pretoria: Transvaal Museum, Monograph No. 4, 21-29.

Petti JM. 1997. Chapter 24 Loudest. In: Walker TJ, ed. *The University of Florida Book of Insect Records.* Gainesville, FL: Department of Entomology & Nematology, University of Florida, 58-60.

Pillet JM. 1993. Les Cigales du Valais. *Bulletin de la Murithienne* 111: 95-113.

Pinchen BJ, Ward LK. 2002. The history, ecology and conservation of the New Forest Cicada. *British Wildlife* 13: 258-266.

Popple LW. 2013. A revision of the *Pauropsalta annulata* Goding & Froggatt species group (Hemiptera: Cicadidae) based on morphology, calling songs and ecology, with investigations into calling song structure, molecular phylogenetic relationships and a case of hybridisation between two subspecies. *Zootaxa* 3730(1): 1-102.

Puissant S. 2006. *Contribution à la connaissance des cigales de France: Géonémie et écologie des populations (Hemiptera, Cicadidae).* Bédeilhac/Aynat: Association pour la Caractérisation et l'Etude des Entomocénoses, 193 pp.

Puissant S, Boulard M. 2000. *Cicadetta cerdaniensis,* espèce jumelle de *Cicadetta montana* décryptée par l'acoustique (Auchenorrhyncha, Cicadidae, Tibicinidae). *Ecole pratique des hautes Etudes, Biologie et Evolution des Insectes* 13: 111-117.

Puissant S, Sueur J. 2010. A hotspot for Mediterranean cicadas (Insecta: Hemiptera: Cicadidae): new genera, species and songs from southern Spain. *Systematics and Biodiversity* 8(4): 555-574.

Quartau JA, Simões PC. 2006. Acoustic evolutionary divergence in cicadas: the species of *Cicada* L. in Southern Europe. In: Drosopoulos S, Claridge M, eds. *Insect sounds and communication: Physiology, behaviour, ecology and evolution.* Boca Raton: CRC Press Taylor & Francis Group, 227-237.

Remane R, Achtziger R, Fröhlich W, Nickel H, Witsack W. 1998. Rote Liste der Zikaden (Homoptera, Auchenorrhyncha). In: Bundesamt für Naturschutz, ed. *Rote Liste gefährdeter Tiere Deutschlands.* Bonn: Schriftenreihe für Landschaftspflege und Naturschutz 55: 243-249.

Samouelle G. 1819. *The entomologist's useful compendium; or, an introduction to the knowledge of British insects.* London: T. Boys, 496 pp.

Sanborn AF. 2014. *Catalogue of the Cicadoidea (Hemiptera: Auchenorrhyncha).* London: Academic Press/ Elsevier, 1002 pp.

Schedl W. 1973. Zur Verbreitung, Bionomie und Ökologie der Singzikaden (Homoptera: Auchenorrhyncha, Cicadidae) der Ostalpen und ihrer benachbarten Gebiete. *Berichte des naturwissenschaftlich-medizinischen Vereins Innsbruck* 60: 79-94.

Schedl W. 2000. Taxonomie, Biologie und Verbreitung der Singzikaden Mitteleuropas (Insecta: Homoptera: Cicadidae et Tibicinidae). *Berichte des naturwissenschaftlich-medizinischen Vereins Innsbruck* 87: 257-271.

Schlick-Steiner B, Steiner FM, Seifert B, Stauffer C, Christian E, Corzier RH. 2010. Integrative taxonomy: A multisource approach to exploring biodiversity. *Annual Review of Entomology* 55: 421-438.

Schumacher F. 1924. Auftreten der Bergzikaden in der Mark Brandenburg und Übersicht über die deutschen Formen (Hem. Homopt.). *Berliner Entomologische Zeitschrift* 4: 329-334.

Scopoli A. 1772. Observationes zoologicae. In: *Annus V, Historico Naturalis*, Lipsiae, 109-110.

Servadei A. 1967. *Fauna d'Italia. Rhynchota (Heteroptera, Homoptera, Auchenorrhyncha). Catalogo topografico e sinonimico.* Bologna: Edizione Calderini, 851 pp.

Simões PC, Quartau JA. 2007. On the dispersal of males of *Cicada orni* in Portugal (Hemiptera: Cicadidae). *Entomologia Generalis* 30(3): 245-252.

Sueur J. 2001. Audiospectrographical analysis of cicada sound production: a catalogue (Hemiptera, Cicadidae). *Mitteilungen aus dem Museum für Naturkunde Berlin, Deutsche Entomologische Zeitschrift* 48(1): 33-51.

Sueur J, Puissant S, Pillet JM. 2003. An Eastern mediterranean cicada in the West: first record of *Tibicina steveni* (Krynicki, 1837) in Switzerland and France (Hemiptera, Cicadidae). *Revue française d'Entomologie* 25: 105-111.

Trilar T, Hertach T. 2008. Three species of Mountain Cicadas *Cicadetta montana* (sensu lato) found in northern Italy. *Bulletin of Insectology* 61(1): 185-186.

Vernier R. 1996. Un chanteur isolé de *Lyristes plebejus* (Scop.) (Hemiptera, Cicadidae) à l'Allondon. *Bulletin romand d'entomologie* 14: 147-152.

Villet M. 1995. Intraspecific variability in SMRS signal: some causes and implications in acoustic signalling systems. In: Lambert DM, Spencer HG, eds. *Speciation and the Recognition Concept, Theory and Application*. Baltimore: Johns Hopkins University Press, 422-439.

Yeates DK, Seago A, Nelson L, Cameron SL, Joseph L, Trueman JWH. 2011. Integrative taxonomy, or iterative taxonomy? *Systematic entomology* 36(2): 209-217.

Part II:

Taxonomy and biogeography of the *Cicadetta montana* complex in Italy, Switzerland and adjoining regions

Chapter 1: Spectacular song pattern from the Sicilian Mountains: The new cicada species *Cicadetta anapaistica* Hertach, 2011* (Hemiptera: Cicadidae)

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ABSTRACT

Acoustic investigations of *Cicadetta montana* s. l. have revealed the presence of morphologically cryptic species in the last few years. This work describes the new cicada *Cicadetta anapaistica* Hertach, 2011 which was detected in the Madonie and Nebrodi Mountains (Italy, Sicily). The characteristic and sophisticated song is composed of three phrases, modulated on four typical power levels and three carrier frequency ranges. The song pattern is compared with those of the closely related *Cicadetta cerdaniensis* and *Cicadetta cantilatrix*. Quantitative and even qualitative intraspecific differences of the song structure among individuals exist which appear to allow individual-specific recognition in many cases. As in other species of the complex, reliable morphological differences between the new species and others in the complex have not been found. The species is currently only known to be endemic to forest and ecotone habitats in a small mountain range. Because of this limited distribution the species is likely to be vulnerable to habitat and climate changes.

*In the originally published text the species name is followed by the compulsory abbreviation ‘sp. n.’.

Front page: Italian *Cicadetta montana* complex habitats: Valley of Orba river, Piedmont (top left), near Pescasseroli, the Abruzzi (top right), near Pontinvrea, Liguria (bottom left), Monte Barro near Lecco, Lombardy (bottom right).

INTRODUCTION

Taxonomists have focussed on morphology when describing cicadas (Cicadidae, sensu Moulds, 2005) until the last few decades. In the 18th and 19th centuries many species and subspecies similar to *Cicadetta montana* (Scopoli, 1772) were described using morphological characters. Most authors later assigned them to two or three single taxa, since the characters showed large and overlapping variability (e.g. Metcalf, 1963; Duffels & Van der Laan, 1985; Nast, 1987; Schedl, 2000). With the introduction of bioacoustic methods, the taxonomy of cicadas was revised in some genera. Song activity is a very important mechanism used by male cicadas to attract females and is therefore species-specific (e.g. Cooley & Marshall, 2001). Acoustic research has revealed cryptic cicada species even in relatively well investigated regions, such as Europe (Quartau & Simões, 2006; Gogala & Trilar, 2004). In the last few years, *Cicadetta montana* s. l. has been shown to be a species complex according to different calling song patterns. Currently, ten European and one Korean species have been described within the complex: *Cicadetta montana* (Scopoli, 1772), *Cicadetta concinna* Germar, 1821, *Cicadetta fangoana* Boulard, 1976, *Cicadetta macedonica* Schedl, 1999, *Cicadetta cerdaniensis* Puissant & Boulard, 2000, *Cicadetta brevipennis* Fieber, 1876 (in Gogala & Trilar 2004), *Cicadetta cantilatrix* Sueur & Puissant, 2007, *Cicadetta hannekeae* Gogala, Drosopoulos & Trilar, 2008, *Cicadetta olympica* Gogala, Drosopoulos & Trilar, 2009, *Cicadetta kissavi* Gogala, Drosopoulos & Trilar, 2009 and *Cicadetta abscondita* Lee, 2008. Extended morphological and genetic analyses on the species complex are currently in progress.

Based on acoustic and morphological characters published so far, Trilar & Hertach (2008) reported three species, *Cicadetta montana* s. str., *C. brevipennis* and *C. cerdaniensis,* of the complex from northern Italy. Acoustically confirmed observations from southern Italy were missing up to now. In summer 2009, the author detected a completely new song pattern belonging to a species of the complex in Sicily. Since no old synonyms refer to taxa found in Italy or northern Africa, the species is described herein as new.

MATERIALS AND METHODS

This work was part of an ongoing project "*Cicadetta montana* species complex in Italy". The author conducted field work in the mountainous ecosystems in Calabria and Sicily between 22 June and 22 July 2009. Detection of calling songs in cicadas is the most effective way to prove the presence of different species. Mountain Cicadas sing in a high carrier frequency domain (10-20 kHz). The use of an ultrasonic detector (Pettersson D 200) with its microphone fitted into a small parabola (diameter 16 cm, adapted from Popov *et al.*, 1997) is very effective for revealing calls. Recordings were made with a Marantz PMD 660 (sampling frequency at 48 kHz) during the hottest hours with temperatures between 24 and 28 °C, when the sun was shining. The recordings are preserved in the Sound Archive of the Slovenian Museum of Natural History in Ljubljana and in the private archive of the author.

Songs were analysed using Raven Pro 1.3 (Cornell Lab of Ornithology) and Seewave (Sueur *et al.*, 2008) on the R software platform. Terminology of the variables used, follows as far as possible Sueur & Puissant (2007) to simplify the comparisons to the closely related species *Cicadetta cantilatrix* and *Cicadetta cerdaniensis*. Time variables were measured on the levels of phrase, echeme and inter-echeme durations. Long recordings were preferred. Frequency and power were registered over perfectly recorded sequences with duration of 30-50 seconds. The carrier frequency spectra were measured using a Hamming window (resolution 1024 points, 87.5% overlap). Maximal power per echeme was standardised for each recording to the average of the lowest echeme type. Afterwards delta power values were compared in the different recordings. Song patterns were compared with closely related songs of *Cicadetta cerdaniensis* (later in Chapter 3 et cetera recognised as an undescribed species) from the Insubrian Region (Italy/Switzerland) and the Ligurian cost (Italy) and *Cicadetta cantilatrix* from northern Switzerland and the Valais (Switzerland). The latter taxon has been discussed critically (Hertach, 2007), but subsequently turned out to represent a valid species. Statistical tests on song patterns were conducted first on the level of individuals [software for all analyses: Statistica 9.0 (StatSoft GmbH)]. Mean values and confidence intervals of variables revealed distinct differences among individuals within the species. Therefore, repeated measures ANOVAs were used to test various variables for significant differences between echeme types. Linear regression or Spearman rank correlations were used to investigate relationships between selected variables of interest.

Voucher specimens were collected with an entomological net. Morphological description of dry prepared specimens follows the terminology of Moulds (2005). The number of singing individuals was estimated for each observation point, put together for 5 x 5 km^2 cells and assigned to two categories of abundance (see Hertach, 2007). Pooled data is visualised in a distribution map according to the UTM coordinate system. Vegetation type and altitude were registered for all observation points.

Acronyms

(see Evenhuis, 2014)

ETHZ	Eidgenössische Technische Hochschule Zürich, Switzerland
NHMB	Naturhistorisches Museum Basel, Switzerland

RESULTS

Cicadetta anapaistica Hertach, 2011

Type material

The type series consists of twelve specimens all collected by the author from Italy in the Sicilian Nebrodi (4 males) and Madonie Mountains (4 males and 4 females) between 3 July 2009 and 12 July 2009. The male holotype is deposited in the NHMB, Switzerland. The paratypes are kept in the entomological collection of the ETHZ, Switzerland and in the collection of the author (**Table 1.1**).

Table 1.1. Type series in *Cicadetta anapaistica*.

Specimen	Sex	Location, Region	Altitude [m]	Longitude	Latitude	Date	Depos-itory
Holotype	M	Vallone Madonna degli Angeli, Madonie	1580	14°01.38'	37°50.38'	10.7.2009	NHMB
Paratype 1	M	Portella Creta, Nebrodi	1500	14°30.72'	37°54.35'	3.7.2009	Hertach
Paratype 2	M	Portella Creta, Nebrodi	1500	14°30.72'	37°54.35'	3.7.2009	Hertach
Paratype 3	M	Serra di Castagna, Nebrodi	980	14°34.78'	37°49.83'	4.7.2009	ETHZ
Paratype 4	M	Portella Calacudéra, Nebrodi	1570	14°39.99'	37°55.49'	4.7.2009	ETHZ
Paratype 5	M	Portella la Colla, Madonie	1410	14°00.41'	37°52.10'	9.7.2009	Hertach
Paratype 6	M	Vallone Madonna degli Angeli, Madonie	1580	14°01.38'	37°50.38'	10.7.2009	ETHZ
Paratype 7	M	Hotel Baita del Faggio, Madonie	1380	14°00.42'	37°52.45'	11.7.2009	ETHZ
Paratype 8	F	Pizzo Stefano, Madonie	1580	14°03.38'	37°53.67'	8.7.2009	Hertach
Paratype 9	F	Pizzo Stefano, Madonie	1560	14°03.50'	37°53.67'	8.7.2009	Hertach
Paratype 10	F	Piano di Farina, Madonie	1330	14°04.13'	37°51.49'	12.7.2009	ETHZ
Paratype 11	F	Piano di Farina, Madonie	1330	14°04.13'	37°51.49'	12.7.2009	ETHZ

Morphology

Diagnosis

The morphological distinction between the species of the Mountain Cicada complex is not clear at the moment. Males of *Cicadetta anapaistica* may be distinguished morphologically at least from two out of three other Italian species: from *Cicadetta montana* by the absence of black pigment on the radius and subcostal veins and the basal part of median vein, stockier habitus and completely blunt upper lobes of the pygofer; from *Cicadetta cerdaniensis* by the apical part of the meracanthus slightly directed laterally. Females of *C. anapaistica* may be separated only from *Cicadetta montana* by the same venation characters and the stockier fore wing ratio.

Description

Male holotype with variation of male paratypes: *Cicadetta anapaistica* looks very similar to the other species of the *Cicadetta montana* complex (**Fig 1.1**). It has a medium body length (17.4 mm in holotype, 17.4 ± 0.6 mm in paratypes, mean ± SD). Males are slightly stockier on average than other species of the *Cicadetta montana* complex, with a low body ratio (length to width, measured at frontal margin of mesonotum: 3.22 in holotype, 3.30 ± 0.09 in paratypes) and a low fore wing ratio (length to width: 2.38 in holotype, 2.36 ± 0.08 in paratypes).

Head: Black with brownish patch on epicranial suture. Postclypeus with longitudinal narrow groove, lateral yellowish, central black with an undulating boundary line. Rostrum reaching mid trochanter, labrum and mentum yellowish (paratypes sometimes darker), labium black. Compound eyes brownish, ocelli honey brown. Antennae dark brown to black with yellowish apical margins of scapes and pedicels.

Thorax: Pronotum and pronotal collar generally black (paratypes rarely with narrow yellowish band at frontal or posterior margins). Lateral angles of pronotal collar pronounced and with yellow to brown margin (sometimes missing in paratypes). Frontal to the lateral angles, pronotal collar with broad, lateral turned up margin, which is convex in shape (in paratypes sometimes extremely recessed, **Fig 1.1E**). Scutum, cruciform elevation and metanotum black, latter with margins getting more and more yellowish to the dorsal centre. Ventral side generally black, except membranes at bases of legs orange to brown, and yellowish spot on episternum II (sometimes missing in paratypes). Operculae not overlapping, kidney-shaped with black to dark brown base and yellowish distal part. Meracanthus relatively long with broad, rounded spike slightly curved and directed laterally (**Fig 1.1D**).

Abdomen: Abdomen triangular in cross section. Tergites I and II black, first with orange marked ribs anterad to timbals. Tergites III through VIII black with caudal margins orange (paratypes sometimes brown-red, rarely creamy), orange bands broad but strongly narrowed to dorsal ridge. Sternites III through VIII orange (paratypes sometimes brown-red, rarely creamy), sternites III through VI (paratypes sometimes VII) with basal brown to black spot reduced more and more towards end of abdomen. Sternite VIII 2.0 times longer than sternite VII (1.8 ± 0.1 in paratypes). Epipleurites orange, each with black spot. Timbals with two long

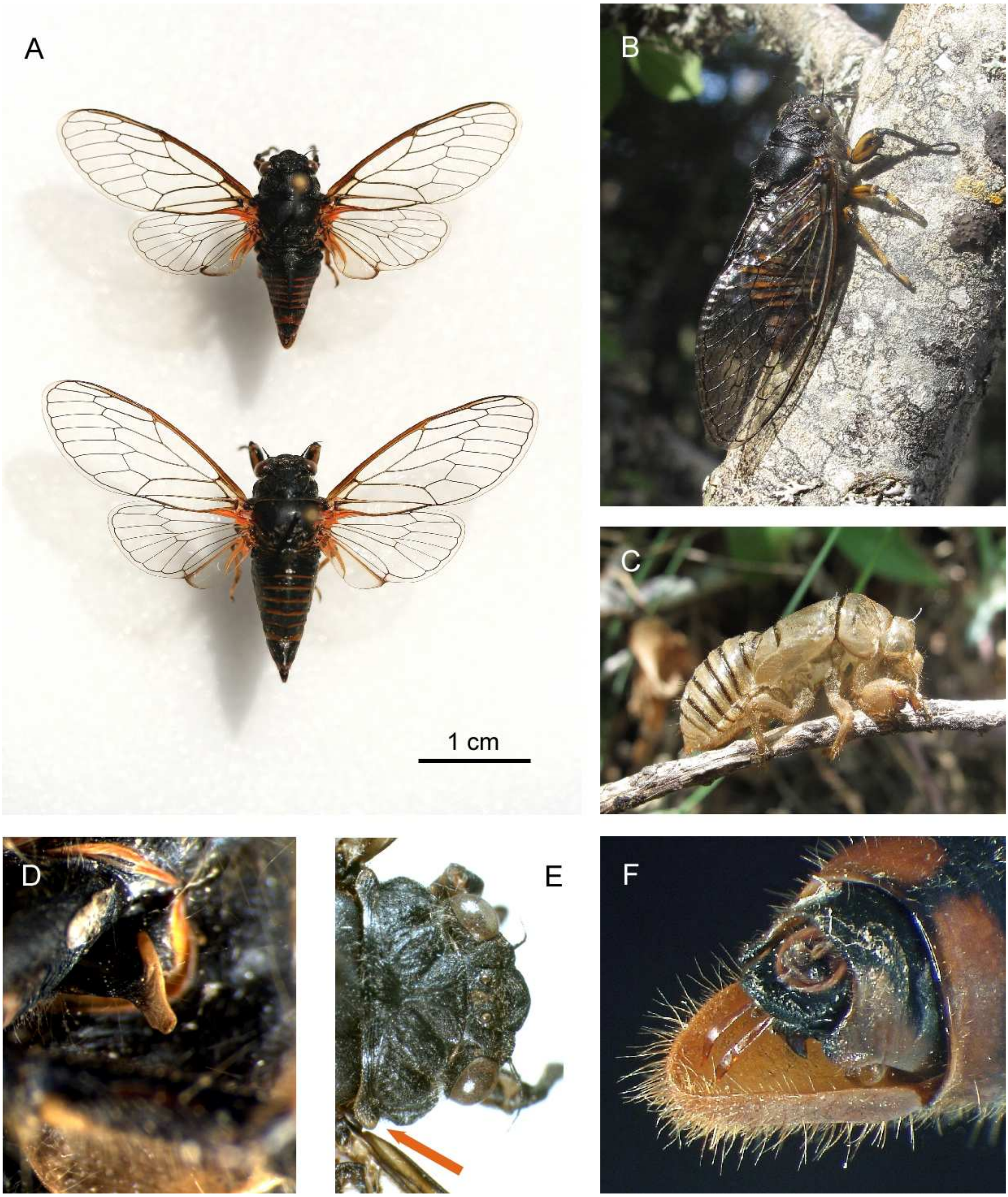

Fig 1.1. *Cicadetta anapaistica* morphology. (A) Male holotype (top) and female paratype 9 (below). (B) Male paratype 5 and (C) nymphal skin in natural surrounding in the Madonie Mountains. Detailed views of (D) meracanthus, (E) recessed shape of lateral pronotal collar (arrow) and (F) genitalia (all male paratype 7).

ribs and one of medium length (the latter optionally interrupted in paratypes). Ribs golden-brown (in paratypes also fainter).

Legs: All legs with yellow to orange and black fasciae and dots. Front femora with three spines, first one isolated, long and with various thickness, second and third shorter but robust.

Wings: Fore wing hyaline except for slightly yellowish basal cell and base part of cubital cell as well as for reddish pterostigma. Basal membrane orange to red. Median and cubitus anterior vein originating in one point at basal cell (one paratype with very short arculus in between). Colouration of veins rather constant: costal vein with dark brown exterior and light brown inner rim; radius and subcostal vein ochre; median and anterior cubitus vein ochre to dark brown up to nodal line (in paratypes rarely up to crossveins); cubitus posterior vein and first anal vein dark brown, basal lighter, apical darker. Distal veins with eight apical cells, dark or even black. Hind wing transparent except for reddish base of costal cell, orange margins of jugum and plaga, and dark brown spotted apical vannus margin. Veins dark, especially in distal part with six apical cells.

Genitalia (**Fig 1.1F**): Pygofer with rounded dorsal beak and rounded completely blunt upper lobes. Median lobe of uncus dark brown and curved upwards, rounded to slightly triangular, but broad. Claspers hooked and dark brown, hooks relatively small and pointed. Pseudoparameres flattened, especially in the central part, and with sharp end. Basal lobe of pygofer orange (in paratypes also yellow), touching main capsula. Anal tube and anal style orange.

Female paratypes: Females are slightly longer than males (19.7 ± 1.0 mm). The fore wing ratio is less than the mean of other species (2.36 ± 0.04), but not the body ratio. The colouration of some body and wing parts is normally slightly lighter than in males: On the head, the supra-antennal plates mostly have brownish margins. On the thorax, the pronotal collar often has the posterior margin yellowish and the parapsidal sutures are dark brown. On fore wings, the basal cell is normally not dark bordered, the median and cubitus anterior veins are mostly ochre to the crossveins and the cubitus posterior vein and first anal vein are brownish. Dark spots on the sternites are sometimes missing except for a symmetric pair on sternite VII. Sternite VII is caudally triangularly notched without clearly arched margins. Ratio of body length and ovipositor length (including sheath) 2.9 ± 0.2.

Acoustic behaviour

Diagnosis

The calling song of *Cicadetta anapaistica* shows affinities in general song composition to both *Cicadetta cerdaniensis* and *Cicadetta cantilatrix*. However, it can be distinguished very easily by the presence of a third phrase with a series of fast triple echemes. All other species of the complex sing in completely different patterns.

General composition of calling song

The decisive character for the distinction of the new species *Cicadetta anapaistica* is the calling song. Recordings of 33 to 40 individuals of *Cicadetta anapaistica* and a total duration of 414 minutes were assessed qualitatively. 19 to 24 singers (237 min) were measured in durations of phrases, 14 to 16 singers (86 min) in detail with holotype and paratype 5 included. The complicated song structure is composed of three phrases which alternate in a typical scheme (**Fig 1.2A**).

The song starts with 1 to 18 (often 3 to 6) short and low intensity echemes (E_1), emitted in irregular rhythm with pauses of normally one to three seconds (phrase 1, PH_1, **Fig 1.2A-B**). This first part seems to exhibit an activating and introductive character more than a high value for female attraction, but it is always present, even when full singing of the previous song was interrupted for a few seconds only. At very low stimulation level specimens emit these chirps for a longer time and with a more lazy rhythm and can fall into complete song pauses. In phrase 2 (PH_2) echemes (E_2) become clearly louder and more regular in rhythm (**Fig 1.2A and C**). They consist of a longer low intensity part (first part = FP_2) and a completely connected loud short part (second part = SP_2), which is similar to the phrase 2 in *Cicadetta cantilatrix* and *C. cerdaniensis*. The second phrase can be composed of a series of shorter (type phrase 2A, 16.4% of all observations) or longer echemes (type phrase 2B, 21.5%) or the echeme duration is intermediate or completely mixed with longer and shorter echemes (no type, 62.1%). Phrase 3 (PH_3) is shorter than phrase 2. This third phrase is a progression of very fast triple echemes, completely unknown in all other taxa of the *Cicadetta montana* species complex (**Fig 1.2A and D**). Two semi-loud and very short echemes (E_{3_1} and E_{3_2}) are followed by a faint longer echeme (E_{3_3}). A galloping impression is created by the typical inter-echeme durations. Basic rhythm is given by ED_{3_1} (echeme duration), $IED_{3\_1/2}$ (inter-echeme duration), ED_{3_2} and $IED_{3\_2/1}$. E_{3_3} is normally only introduced after some isolated E_{3_1} - E_{3_2} combinations and also stopped before emitting the last echeme groups.

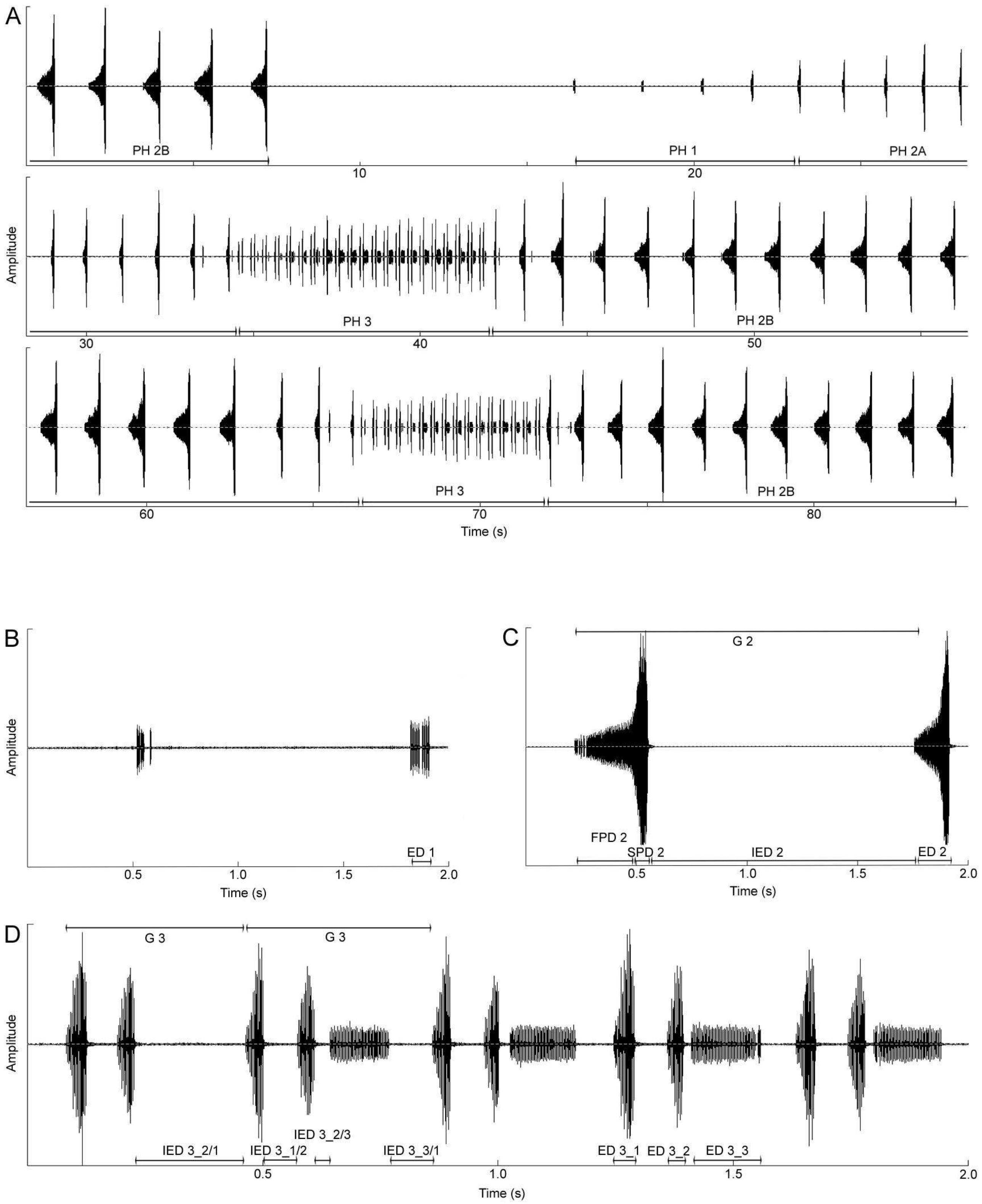

Fig 1.2. Calling song in *Cicadetta anapaistica*. (A) Typical composition with end of a former song and restart including phrases 1, 2 (types A and B) and 3 (85 s, Madonie Mountains). (B-D) Detailed view of the typical song elements with the measured duration terms (2 s): (B) Phrase 1, (C) phrase 2, (D) phrase 3. PH = phrase duration, ED = echeme duration, IED = inter-echeme duration, G = echeme/inter-echeme group duration.

After finishing phrase 3, *C. anapaistica* repeats another phrase 2. Phrases are typically arranged in the following way: 1–2–3–2–3–2–3–2. Phrases 2 and 3 can alternate up to 21 times before a break (mean = 5, n = 57). Phrase 1 is always at the beginning, sometimes also at the end of the song (34% of all phrases 1). First phrase 2 normally does not belong to the type 2B (94%, n = 31). Acoustical activity never ends with phrase 3. At lower stimulation level the song is shortened to 1–2(–1).

Time, power and frequency statistics

Measurements of the calling song characters in *C. anapaistica* are shown in **Table 1.2** (time) and **Table 1.3** (power and frequency). The coefficients of variation (CV) in **Table 1.2** give an impression of which time variables may be more (low value) or less (high value) important for species-specific information. The four lowest values all belong to echemes and inter-echemes of phrase 3, the highest values belong to phrase durations. The values of some time variables (PH_2, ED_2, FPD_2) are not distributed normally. Interestingly many time variables proceed in a strict scheme within the phrases. In phrase 2, first echemes (ED_2) are shorter than the last and especially the central while IED_2 and G_2 slightly increase from the beginning to the end. The progression in phrase 3 is complicated, but many differences between the first, central and last echeme/inter-echeme groups are distinctive. ED_{3_1} and ED_{3_2} increase as G_3. $IED_{3\_1/2}$ is constant. ED_{3_3} first clearly become longer and then slightly decrease. In $IED_{3\_2/3}$ and $IED_{3\_3/1}$ the patterns are opposite.

Calling males typically modulate echeme power (EP) in a strict pattern on four (rarely three or five) significant levels (repeated measures ANOVA). Interestingly, these differences between the levels are almost constant: 5 to 7 dB from EP_{3_3} to FPP_2, from FPP_2 to EP_{3_1}/EP_{3_2} and from EP_{3_1}/EP_{3_2} to SPP_2. The chronological steps are more complicated (**Fig 1.3**).

The carrier frequency domain of echemes (EF) is broad and high as in other Mountain Cicadas (**Fig 1.4**). The -20 dB range covers frequencies from 11.1 ± 0.5 up to 19.5 ± 1.3 kHz (mean ± SD). The centre frequency (median of energy) is around 14 kHz, the 1. quartile frequency around 13 kHz and the 3. quartile frequency around 15 kHz. The dominant frequency range is clearly shifted from specimen to specimen. The frequency is modified between the different echeme types within the song. Each specimen sings on three typical frequency ranges (rarely four or five) which are significant (repeated measures ANOVA). EF_{3_3} is 0.9–1.0 kHz lower in average than SPF_2 in centre, 1. and 3. quartile frequencies. Power and frequency are highly correlated in all measured individuals (Spearman rank correlation: $0.443 < R < 0.924$, $p < 10^{-7}$).

Table 1.2. Time variables of calling song in *Cicadetta anapaistica*. n_{ind} = number of singers, n_{obs} = number of measured/observed values, SD = standard deviation, CV = coefficient of variation, 1. QT = first quartile, 3. QT = third quartile, ED = echeme durations, IED = inter-echeme durations, N_2 and N_3 = number of echeme/inter-echeme groups (G_2 and G_3) per phrase, $IED_{2–3}$ and $IED_{3–2}$ = duration of transitions from phrase 2 to 3 and 3 to 2.

Variable	n_{ind}	n_{obs}	Mean [s]	SD [s]	CV	Median [s]	1. QT [s]	3. QT [s]	Min [s]	Max [s]
Phrases										
PH_1	11	73	19.8	20.0	100.6	11.3	6.7	27.4	0.5	88.6
PH_2 (all types)	19–24	311	28.3	20.6	73.1	23.5	15.4	33.9	2.0	159.2
PH_{2A}	19–24	51	25.9	13.6	52.5	25.0	16.2	33.6	9.8	75.5
PH_{2B}	19–24	67	30.2	22.2	73.6	24.3	18.7	33.5	8.8	159.2
PH_3	19–24	269	7.3	3.5	48.7	6.9	5.2	8.4	0.7	28.8
Echemes										
ED_1	6	128	0.064	0.021	33.2	0.067	0.050	0.079	0.014	0.156
ED_2	16	3758	0.189	0.103	54.4	0.150	0.113	0.244	0.033	0.581
FPD_2	16	3712	0.144	0.093	64.4	0.113	0.081	0.181	0.019	0.506
SPD_2	16	3712	0.046	0.016	34.0	0.050	0.038	0.050	0.013	0.100
ED_{3_1}	14	1859	0.041	0.012	28.2	0.038	0.033	0.049	0.010	0.085
ED_{3_2}	14	1859	0.035	0.007	20.5	0.034	0.029	0.041	0.008	0.059
ED_{3_3}	14	1660	0.088	0.025	29.0	0.090	0.077	0.103	0.004	0.162
Intervals										
IED_2	16	3579	0.995	0.256	25.7	0.938	0.825	1.106	0.067	3.356
$IED_{3\_1/2}$	14	1859	0.063	0.010	15.9	0.062	0.056	0.069	0.037	0.103
$IED_{3\_2/1}$	14	146	0.207	0.029	14.2	0.203	0.189	0.221	0.152	0.364
$IED_{3\_2/3}$	14	1660	0.024	0.015	61.7	0.023	0.013	0.032	0.000	0.093
$IED_{3\_3/1}$	14	1624	0.097	0.023	23.6	0.093	0.083	0.107	0.051	0.456
$IED_{2–3}$	9	84	0.487	0.482	99.0	0.253	0.230	0.411	0.165	2.060
$IED_{3–2}$	9	90	0.290	0.128	44.1	0.271	0.224	0.323	0.108	0.998
Groups (last two rows dimensionless)										
G_2	16	3579	1.193	0.303	25.5	1.125	0.975	1.350	0.167	3.469
G_3	14	1770	0.347	0.036	10.4	0.345	0.320	0.369	0.254	0.783
N_2	12	165	22.3	11.3	76.6	21	14	28	4	71
N_3	14	93	20.7	9.3	44.8	19	16	24	7	75

Table 1.3. Carrier frequency and power variables of calling song in *Cicadetta anapaistica*. n_{ind} = number of singers, n_{obs} = number of measured/observed values, SD = standard deviation, EF = echeme frequency (kHz), EP = delta maximal echeme power (dB), standardised to the average of EP_{3_3} for each calling male.

Variable	n_{ind}	n_{obs}	Mean	SD	Median	Min	Max
Centre Frequency (kHz)							
EF_1	6	123	14.0	0.9	13.8	12.4	15.6
FPF_2	14	376	14.0	0.9	14.0	11.8	15.8
SPF_2	14	376	14.6	0.9	14.4	12.3	16.5
EF_{3_1}	14	365	14.1	0.9	14.0	12.0	16.2
EF_{3_2}	14	364	14.0	1.0	14.0	11.8	16.1
EF_{3_3}	14	337	13.6	1.0	13.8	11.8	15.8
Delta Maximal Power (dB)							
FPP_2	14	376	6.6	2.6	6.4	0.7	14.7
SPP_2	14	376	17.3	2.9	17.2	11.0	25.0
EP_{3_1}	14	369	11.8	2.5	11.7	0.0	18.4
EP_{3_2}	14	369	11.4	3.1	11.6	-8.5	18.1
EP_{3_3}	14	340	0.0	1.8	0.1	-9.9	5.8

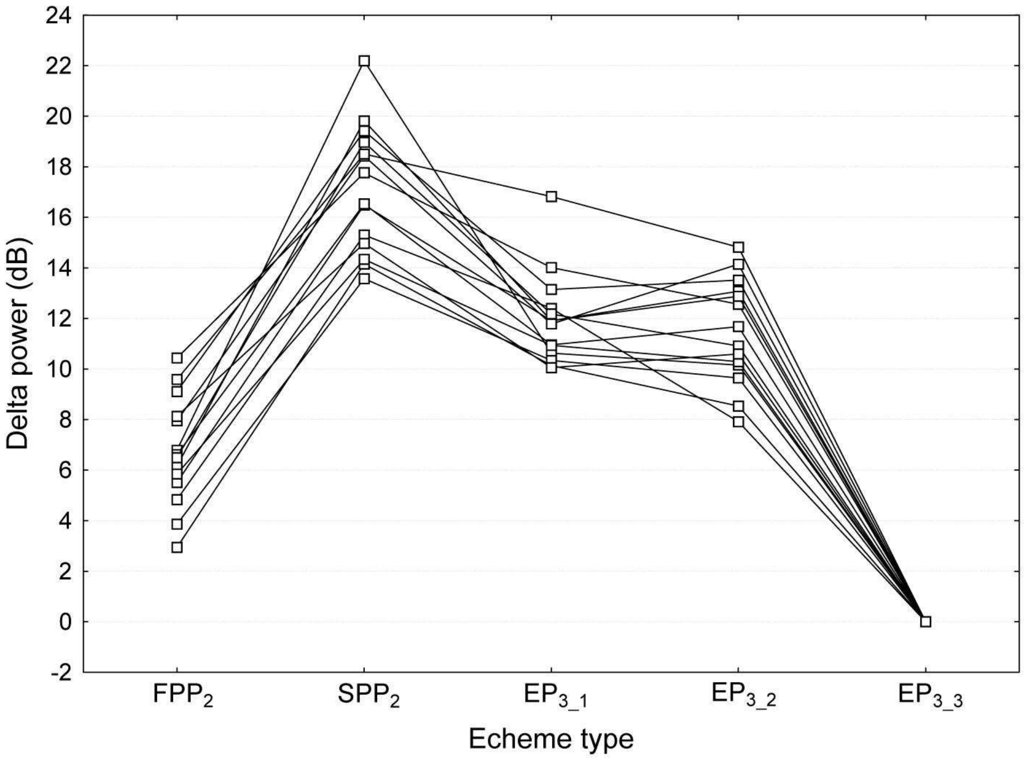

Fig 1.3. Power modulation between different echeme types in *Cicadetta anapaistica* demonstrated in chronological order. Mean values of 14 singers. Delta maximal echeme power (P) standardised to the average of EP_{3_3} for each male.

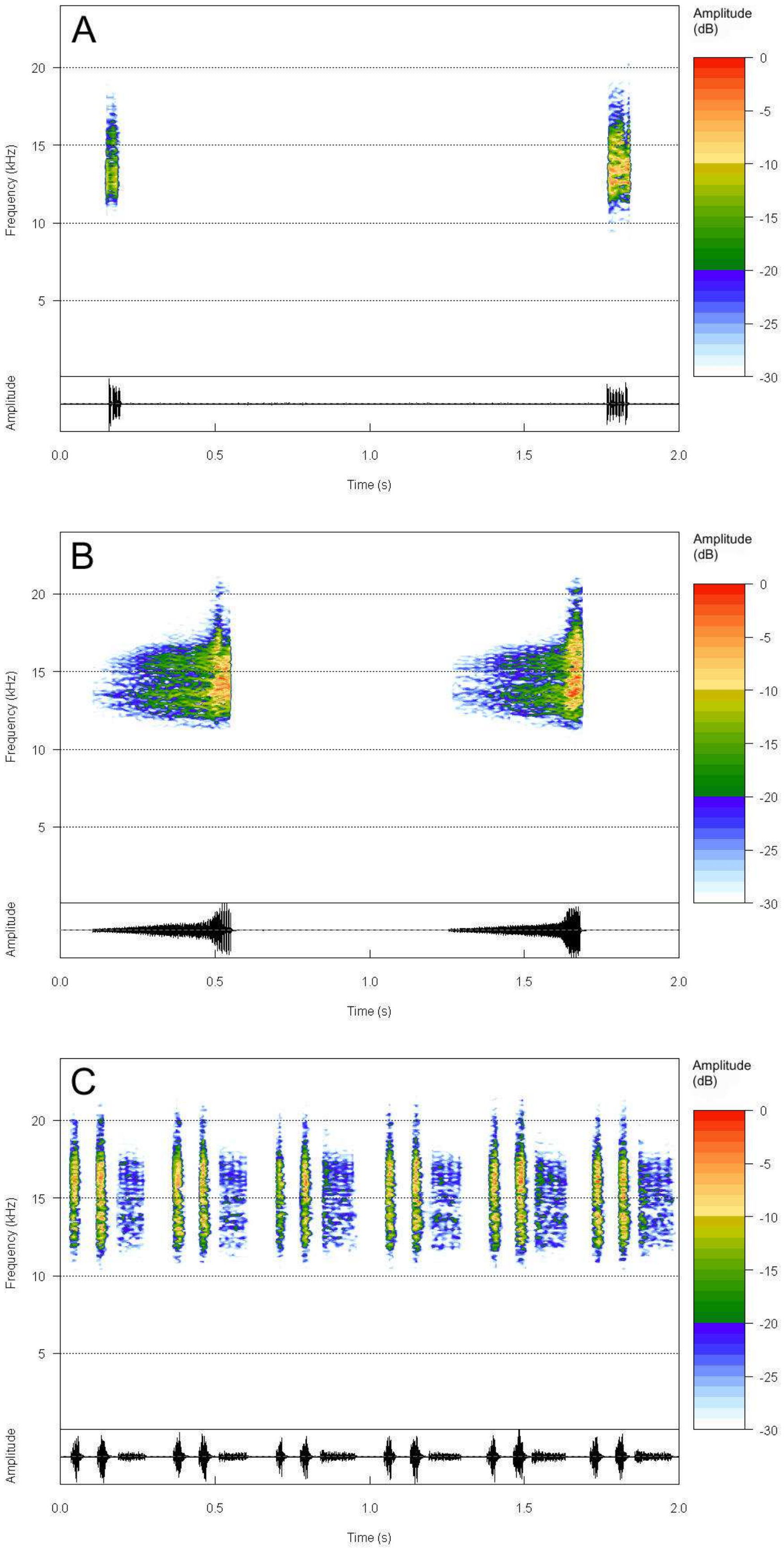

Fig 1.4. Carrier frequency domain of the typical song elements in *Cicadetta anapaistica*. (A) Phrase 1, (B) phrase 2, (C) phrase 3.

Intraspecific song variability and aberrations

As already indicated for power and carrier frequency, *C. anapaistica* songs vary at the individual level, visible in comparison of long recordings with many repeated phrases of the same specimen. General conditions like temperature or wind cannot account for this observed variation. Phrase 3 and the transitions from phrase 2 to phrase 3 are composed variably not only quantitatively but mainly qualitatively (**Fig 1.5A-D**). There are characteristic differences in position of first and last implemented E_{3_3} echemes (**Fig 1.5A ↔ 1.5C**), in the relative accent of E_{3_1} and E_{3_2} (**Fig 1.5B ↔ 1.5D**), in the manner to merge completely E_{3_2} and E_{3_3} echemes (**Fig 1.5D**, $IED_{3\_2/3} = 0$; 5.9% of all observations) or to interrupt inter-echemes with single (**Fig 1.5D**) or double beats (**Fig 1.5A**).

Moreover, song structure is obviously so complicated that aberrations to the normal song pattern could be recorded, which may have negative consequences in mating success. In phrase 3 individuals sometimes omit E_{3_3} echemes completely, probably when singing in lower stimulation. However, there are some individuals which simply seem not able to emit E_{3_3} echemes at all (**Fig 1.5E**). At least two specimens produced three instead of two short echemes followed by a longer one in phrase 3 (**Fig 1.5F**).

Comparison to the calling songs of *Cicadetta cerdaniensis* and *Cicadetta cantilatrix*

The song of the new species is compared with five sequences of *Cicadetta cerdaniensis* and five of *Cicadetta cantilatrix*. Duration of these sequences is from 2 to 5 minutes.

General song compositions of the three species are quite different (**Fig 1.6**). *Cicadetta cerdaniensis* and *C. cantilatrix* do not emit a few faint chirps regularly when they start to sing (phrase 1). Their phrase 1 is nearer to phrase 2 type A in *C. anapaistica*. Phrase 2 of all three species is comparable but with small differences (**Table 1.4**). In *C. cantilatrix* ED_2 and FPD_2 are clearly longer, but SPD_2 and IED_2 shorter in average than in the new species. In *C. cerdaniensis* ED_2 and FPD_2 have approximately the same duration in average compared to *C. anapaistica*, SPD_2 is shorter and IED_2 longer. Linear regressions between ED_2 and IED_2 reveal interesting divergence: The two variables are positively correlated in *C. cerdaniensis* ($R^2 = 0.33$), negatively correlated in *C. cantilatrix* ($R^2 = 0.38$) and not correlated in *C. anapaistica* ($R^2 = 0.06$, **Fig 1.7**). Phrase 3 in *C. anapaistica* is composed of characteristic distinctive triple echemes. *Cicadetta cerdaniensis* produces single echemes at an increased repetition rate. In *C. cantilatrix* phrase 3 normally does not exist. In very rare cases, this species emits a phrase very similar to phrase 3 of *C. cerdaniensis*, observed in Poland and northern Switzerland, which is probably a courtship song (compare Trilar *et al.*, 2006; Hertach, 2007).

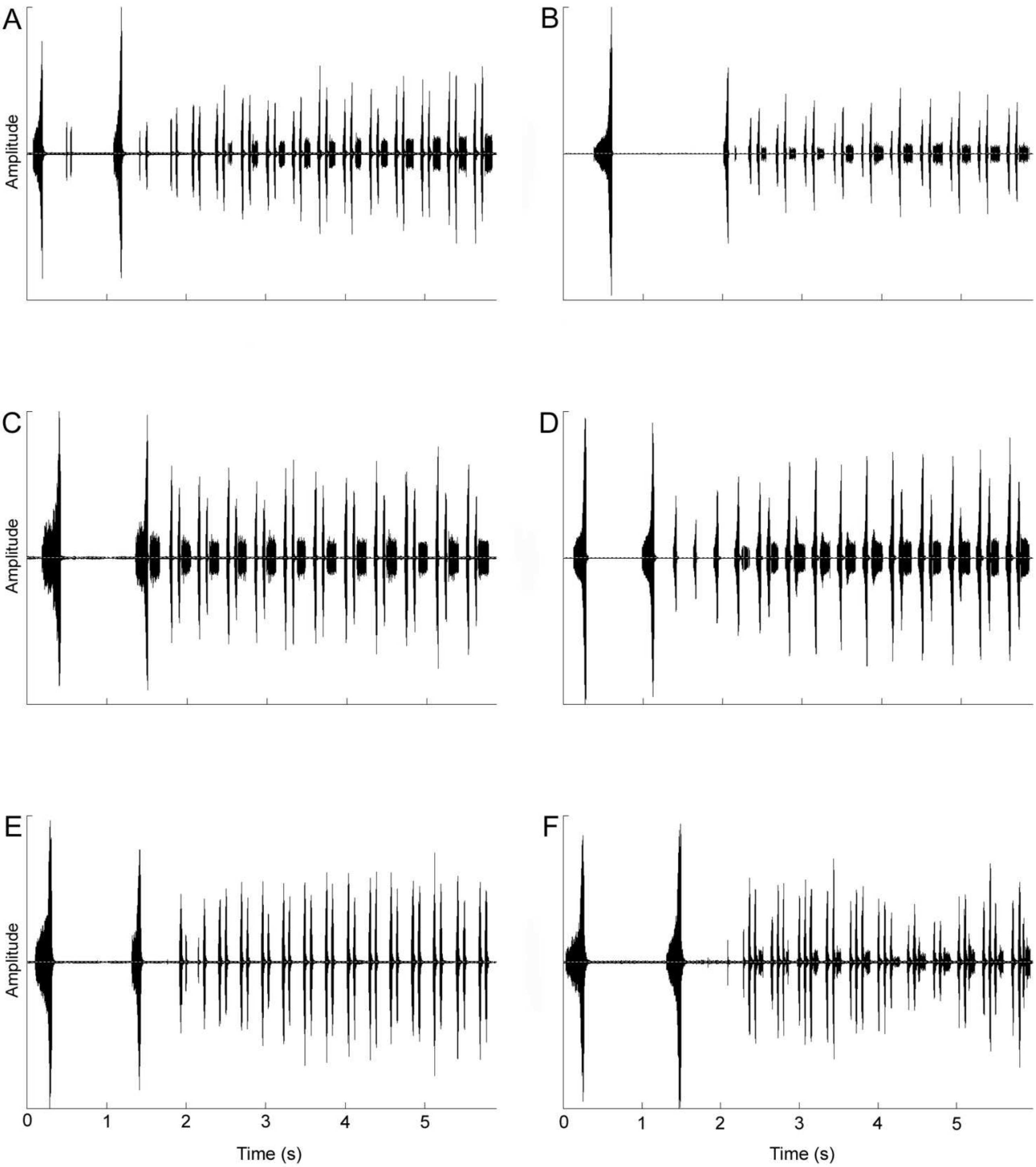

Fig 1.5. Intraspecific song variability in *Cicadetta anapaistica*. Sections showing qualitative intraspecific variability (A-D) and aberrations (E-F) from the end of phrase 2 to central part of phrase 3 (6 s).

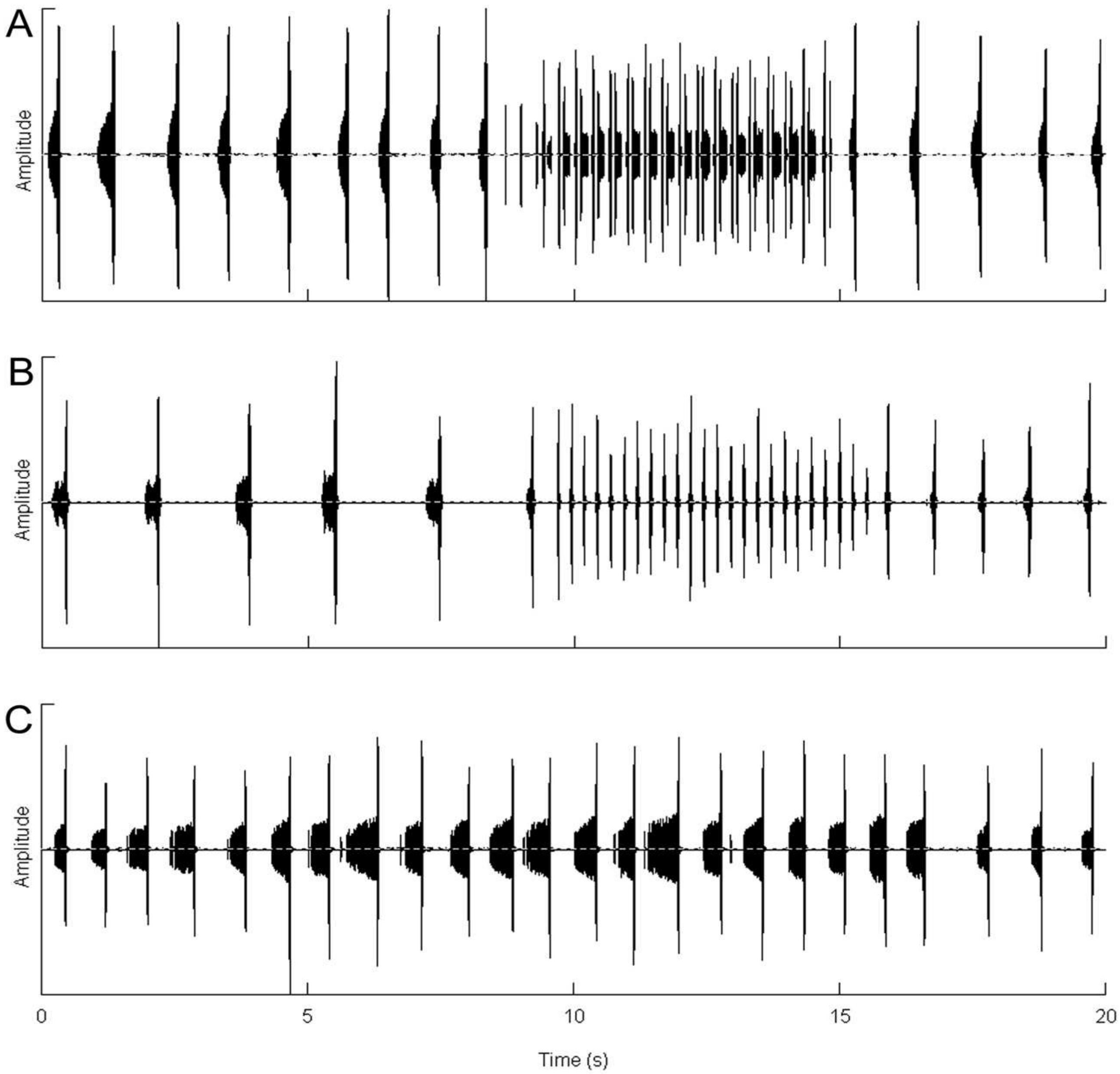

Fig 1.6. Comparison to closely related calling song patterns. (A) *Cicadetta anapaistica* (phrases 2–3–2, Nebrodi Mountains), (B) *Cicadetta cerdaniensis* (phrases 2–3–2, Lombardy, Italy), (C) *Cicadetta cantilatrix* (phrase 2, Basel-Land, Switzerland). 20 s sequences.

Table 1.4. Time variables in phrase 2 of the calling songs in the three species *Cicadetta anapaistica*, *Cicadetta cerdaniensis* and *Cicadetta cantilatrix*. n_{obs} = number of measured/observed values, mean value ± SD.

Species	**n_{obs} max**	**ED_2 [ms]**	**FPD_2 [ms]**	**SPD_2 [ms]**	**IED_2 [ms]**	**G_2 [ms]**	**FPD_2/SPD_2**
C. anapaistica	3758	189 ± 103	144 ± 93	46 ± 16	995 ± 256	1193 ± 303	3.1 ± 1.7
C. cerdaniensis	686	192 ± 82	154 ± 76	39 ± 11	1114 ± 305	1307 ± 358	4.0 ± 1.8
C. cantilatrix	808	514 ± 211	482 ± 209	32 ± 9	473 ± 289	992 ± 230	16.4 ± 9.7

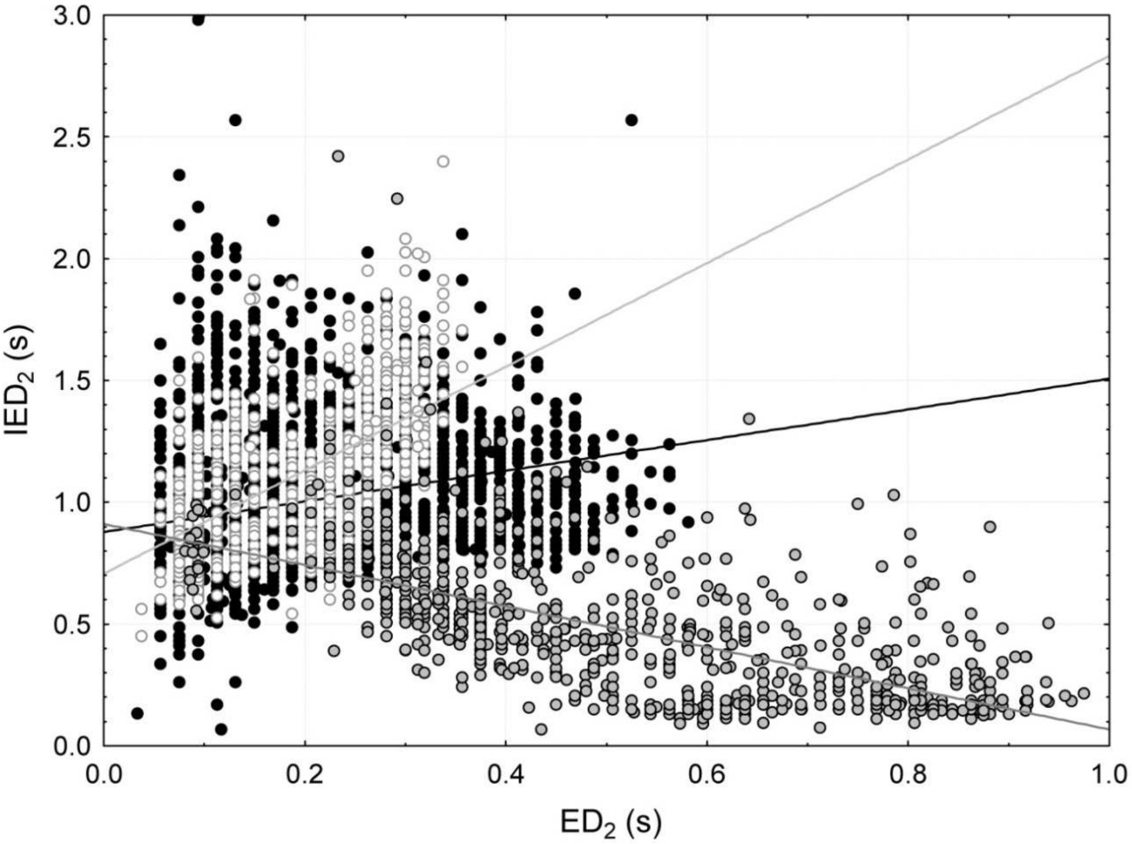

Fig 1.7. Echeme duration (ED_2) versus inter-echeme duration (IED_2) of phrase 2. *Cicadetta anapaistica* (black), *Cicadetta cerdaniensis* (white) and *Cicadetta cantilatrix* (grey) with linear regressions.

Etymology

‘*Anapaistica*’ is derived from the Greek word ‘anapaistos’ (lat. *anapaestus*). The most distinctive third song phrase reminds of the metrical foot ‘anapaest’. In song lyrics this metrical foot was originally defined as a completely quantitative rhythm (short–short–long syllables) by the ancient Greeks and Romans (Leonardt & West, 2000). The name therefore also links to two cultures that have strongly influenced Sicily for several centuries.

Distribution and ecology

Cicadetta anapaistica was found in 44 locations from the Sicilian Nebrodi and Madonie Mountains as the single representative of the *Cicadetta montana* complex. However, it is not the only species of the group in Sicily. *Cicadetta montana* s. str. was identified by acoustics at Mount Etna as well as at sites in

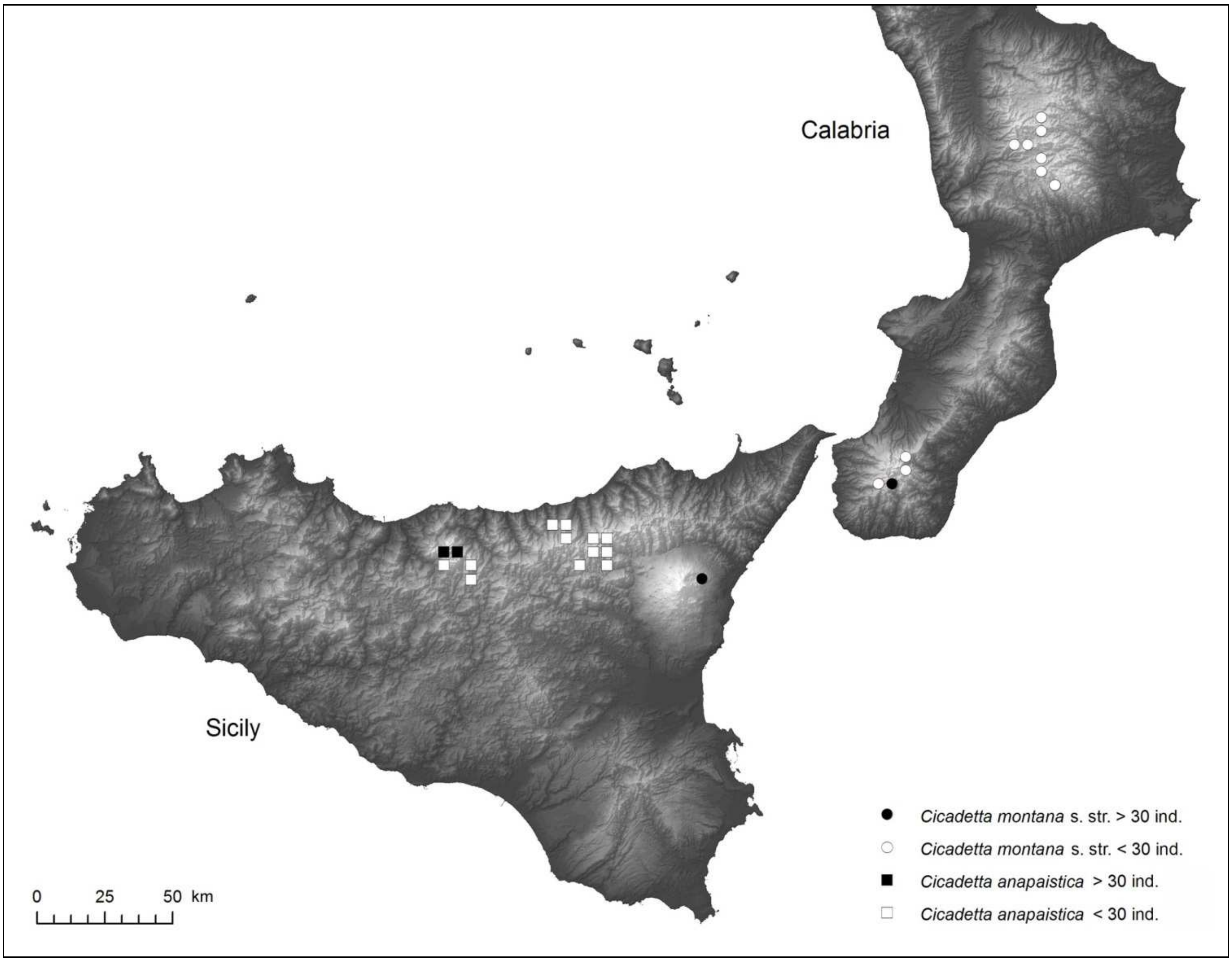

Fig 1.8. Distribution map of *Cicadetta montana* complex species in southern Italy. 5 x 5 km^2 cells with abundance during June and July 2009.

neighbouring Calabria by the author during the same stay (Aspromonte and Sila National Parks, **Fig 1.8**). Many locations in Calabria and Sicily did not yield any species of the *Cicadetta montana* complex. In particular, *C. anapaistica* could not be found in the mountainous Sicilian habitats Bosco della Ficuzza southern of Palermo, Erice near Trapani and in the northern part of the Madonie Mountains. Well-wooded mountains with more or less native vegetation have become scarce in Sicily except for the mentioned areas. The known distribution range of the species is very small and covers a territory of 70 x 20 km^2 only, including a gap of 30 km between the Nebrodi and Madonie Mountains.

The detected habitats of *C. anapaistica* range from 920 to 1750 m asl with a maximum abundance between 1300 to 1600 m (**Fig 1.9**). In central part of the Madonie Mountains the density of singing animals is clearly higher than in the Nebrodi Mountains. The majority of the Nebrodi Mountains is covered by a dense forest (*Fagus sylvatica* and different deciduous oaks). Well-exposed micro-habitats are scarce and the herb layer is often absent. Therefore, most of the singers have been observed along the inner artificial forest edges near roads (**Fig 1.10**, left). The Madonie Mountains are structured more diversely due to human extensive land use. Ecotone habitats from different woods to dry pastures are common (**Fig 1.10**, right). However, *C. anapaistica* is not selective with respect to the plant community (**Fig 1.9**). All listed vegetation types are occupied within the same dimensions, even artificially planted reforestations. Only the Holm Oak forests (*Quercus ilex*) are strictly avoided. Individuals sing in a broad vertical range from a few centimetres up to 10 m above ground sitting on small branches of bushes and normally moving to a new perch every few minutes. In the Nebrodi Mountains *C. anapaistica* was several times observed singing in very low blackberry shrubs (*Rubus* sp.).

The distribution of *C. anapaistica* is sympatric with only a few other cicada species. Many habitats are shared with *Tettigettula pygmea* (Olivier, 1790), and sometimes scattered *Cicada orni* Linné 1758 are present. The local abundance of *C. anapaistica* and *T. pygmea* appears negatively correlated which might indicate interspecific acoustic competition. The new species was first recorded on 3 July 2009 and last on 12 July 2009. The populations showed full singing activity during this period.

DISCUSSION

Cicadetta anapaistica Hertach, 2011 can be easily distinguished from all other known cicadas and especially representatives of the Mountain Cicada species complex by its characteristic third song phrase. The importance of bioacoustic investigations in cicadas and other groups can again be underlined. *Cicadetta anapaistica* is the eleventh species described in the *Cicadetta montana* complex for Europe. The species is characterised by a spectacular song pattern with three alternating phrases, modulated at three typical carrier frequency ranges and four power levels. Furthermore, the emission of regular triple echemes within the European cicadas is only known in *Cicadetta flaveola* (Gogala & Drosopoulos, 2006). Since the territory is not shared with closely related species and the number of characteristic song elements remains very high, individual-specific variations even in qualitative aspects occur. Individual recognition seems possible in many cases. Because of this variation *C. anapaistica* would be a good system to investigate individuality in acoustic female attraction. Song studies on insects and especially on cicadas are scarce at the individual level of conspecific populations (Seabra *et al.*, 2008), but well known in birds and frogs (e.g. Friedl, 2006). The CV values give an impression that variables are more static and more dynamic following the definitions of Gerhardt (1991). The static variables are important to bring conspecific sexes together. The dynamic variables perhaps influence the attractiveness of a singing male for a receptive female.

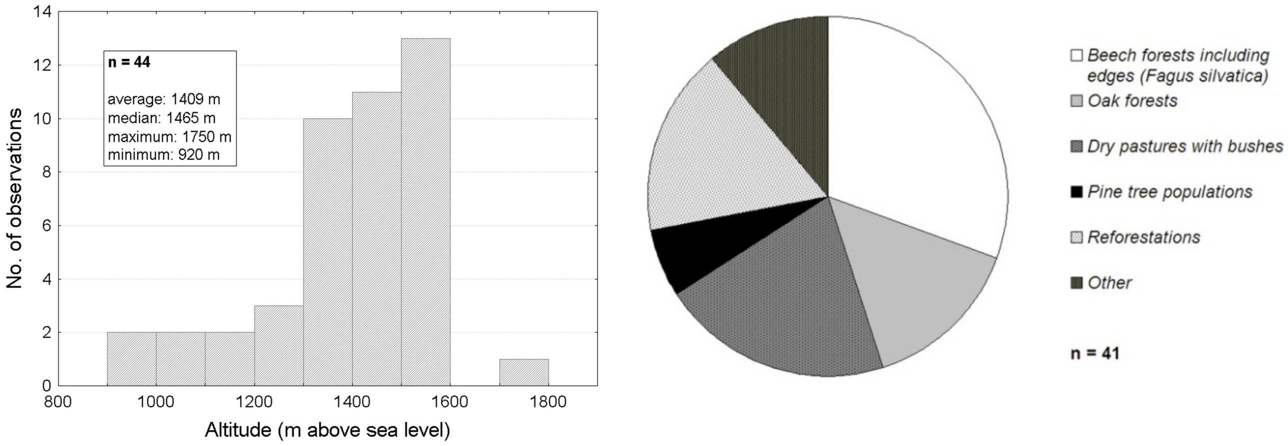

Fig 1.9. Altitudes above sea level (right) and vegetation types (left) regarding all observations points of *Cicadetta anapaistica*.

Fig 1.10. Typical habitats of *Cicadetta anapaistica*. Along an artificial forest edge with European Beeches (*Fagus sylvatica*) in the Nebrodi Mountains (left, breeding place), typical ecotone habitat in the Madonie Mountains (right).

Based on calling song, *Cicadetta cerdaniensis*, *C. cantilatrix* and *C. anapaistica* form a subgroup in the *Cicadetta montana* species complex. This was already suggested for the former two species by Sueur & Puissant (2007) and Gogala *et al.* (2008). This grouping must have an ancestor in common before splitting into three species with allopatric distribution. Phylogeny reconstructions will be interesting and helpful to test this hypothesis.

The distribution of *C. anapaistica* may be very small and split into two parts, each of them protected as a Regional Nature Park. The Nebrodi and Madonie Mountains form a biogeographic unit with high endemism (e.g. Adamović, 1933). It is not supposed that the new taxon occurs clearly beyond the observed range except possibly for the Peloritani Mountains, east of the current known distribution.

As in other representatives of the *Cicadetta montana* species complex, the structure of the vegetation is the decisive factor in habitat selection. Optimal habitats are composed of large ecotone systems between extensively grazed pastures and different sparse woods. A canopy covering of woody plants between 30 and 80% of the surface seems to be optimal for *C. anapaistica*. The habitat situation in the Madonie Mountains appears appropriate in many places, but with a tendency for too much open land. In contrast, in the Nebrodi Mountains only a small percentage of the protected area can be used as habitat due to dense forests. Interestingly, the Nebrodi Mountains are actually closer to the native vegetation. Before heavy deforestation impacted the Sicilian mountains, *C. anapaistica* possibly had a larger distribution area, focussing on mountainous natural ecotone habitats such as tree line in altitude, aridity border for woody plants, dynamic slide slopes and post-fire succession.

Changing habitat structure would cause a decline of the populations in the Madonie Mountains, if extensive grazing will be completely abandoned for decades. However, Mountain Cicadas larvae also suffer by intense or early grazing in spring (Hertach, 2007; Joint Nature Conversation Committee, 2007). Reforestation programmes should focus to re-establish an interconnection between the Madonie and Nebrodi Mountains.

Mediterranean mountainous ecosystems are among the most vulnerable areas in the world to climate change (Regato & Salman, 2008). Increasing temperatures and dryness could affect the populations by vegetation shift and changing species interactions. We do not know whether the observed co-distribution between *C. anapaistica* and *Tettigettula pygmea* is already a result of climate change or the species originally occurred sympatrically. In fact, the climate has become clearly warmer between 1960 and 1990 with negative consequences on the amphibian populations in northern Sicily (D'Amen & Bombi, 2009). The crucial factor is whether *C. anapaistica* will find an ecological niche when further cicada species could enter the habitats. However, *C. anapaistica* is supposed to be a vulnerable species because of its highly restricted distribution range. Nature protection programmes and land use management should focus on this attractive species in the future.

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REFERENCES CHAPTER 1

Adamović L. 1933. *Die pflanzengeographische Stellung und Gliederung Italiens.* Jena: Verlag Gustav Fischer, 259 pp.

Cooley JR, Marshall DC. 2001. Sexual signalling in periodical cicadas, *Magicicada* spp. (Hemiptera: Cicadidae). *Behaviour* 138: 827-855.

D'Amen M, Bombi P. 2009. Global warming and biodiversity: Evidence of climate-linked amphibian declines in Italy. *Biological Conservation* 142: 3060-3067.

Duffels JP, Van der Laan PA. 1985. *Catalogue of the Cicadoidea (Homoptera, Auchenorrhyncha) 1956–1980.* Series entomologica. Dordrecht, Boston, Lancaster: Dr. W. Junk Publishers, 414 pp.

Evenhuis NL. 2014. *Abbreviations for insect and spider collections of the world.* Available at: http://hbs.bishopmuseum.org/codens/codens-inst.html (accessed 20 May 2015).

Friedl TWP. 2006. Individual male calling pattern and male mating success in the European treefrog (*Hyla arborea*): Is there evidence for directional or stabilizing selection on male calling behaviour? *Ethology* 112: 116-126.

Gerhardt HC. 1991. Female choice in treefrogs: static and dynamic acoustic criteria. *Animal Behaviour* 42: 615-635.

Gogala M, Drosopoulos S. 2006. Song of *Cicadetta flaveola* Brullé (Auchenorrhyncha: Cicadoidea: Tibicinidae) from Greece. *Russian Entomological Journal* 15(3): 275-278.

Gogala M, Drosopoulos S, Trilar T. 2008. Present status of mountain cicadas *Cicadetta montana* (sensu lato) in Europe. *Bulletin of Insectology* 61(1): 123-124.

Gogala M, Trilar T. 2004. Bioacoustic investigations and taxonomic considerations on the *Cicadetta montana* species complex (Homoptera: Cicadoidea: Tibicinidae). *Anais da Academia Brasileira de Ciências* 76(2): 316-324.

Hertach T. 2007. Three species instead of only one: Distribution and ecology of the *Cicadetta montana* species complex (Hemiptera: Cicadoidea) in Switzerland. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 80: 37-61.

Joint Nature Conversation Committee. 2007. *UK action biodiversity plan – action plan for Cicadetta montana.* Available from: http://www.ukbap.org.uk/ukplans.aspx?ID=216 (accessed 5 March 2010).

Leonardt J, West ML. 2000. Metrik. In: Cancik H, Schneider H, eds. *Der Neue Pauly. Enzyklopädie der Antike*, *Band 8*. Stuttgart: Metzler Verlag, 109-129.

Metcalf ZP. 1963. *General Catalogue of the Homoptera, Fascicle VIII, Cicadoidea. Part 2: Tibicinidae.* Raleigh: University of North Carolina State College, 492 pp.

Moulds MS. 2005. An appraisal of the higher classification of cicadas (Hemiptera: Cicadoidea) with special reference to the Australian fauna. *Records of the Australian Museum* 57: 375-446.

Nast J. 1987. The Auchenorrhyncha (Homoptera) of Europe. *Annales Zoologici Warszawa* 40(15): 355-661.

Popov A, Beganovic A, Gogala M. 1997. Bioacoustics of singing cicadas on the Western Palaearctic: *Tettigetta brullei* (Fieber 1876) (Cicadoidea: Tibicinidae). *Acta entomologica slovenica* 5: 89-101.

Puissant S, Boulard M. 2000. *Cicadetta cerdaniensis,* espèce jumelle de *Cicadetta montana* décryptée par l'acoustique (Auchenorrhyncha, Cicadidae, Tibicinidae). *Ecole pratique des hautes Etudes, Biologie et Evolution des Insectes* 13: 111-117.

Quartau JA, Simões PC. 2006. Acoustic evolutionary divergence in cicadas: the species of *Cicada* L. in Southern Europe. In: Drosopoulos S, Claridge M, eds. *Insect sounds and communication: Physiology, behaviour, ecology and evolution.* Boca Raton: CRC Press Taylor & Francis Group, 227-237.

Regato P, Salman R. 2008. *Mediterranean Mountains in a Changing World: Guidelines for developing action plans*. Malaga: IUCN Centre for Mediterranean Cooperation, xii + 88 pp.

Schedl W. 2000. Taxonomie, Biologie und Verbreitung der Singzikaden Mitteleuropas (Insecta: Homoptera: Cicadidae et Tibicinidae). *Berichte des naturwissenschaftlich-medizinischen Vereins Innsbruck* 87: 257-271.

Seabra SG, Genage A, Quartau JA. 2008. Variation in the acoustic properties of the calling song of *Cicada barbara* and *C. orni* (Hemiptera: Cicadidae) at the individual and population levels. *Zoological Studies* 47(1): 1-10.

Sueur J, Aubin T, Simonis-Sueur C. 2008. Seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics* 18(2): 213-226.

Sueur J, Puissant S. 2007. Similar look but different song: a new *Cicadetta* species in the *montana* complex (Insecta, Hemiptera, Cicadidae). *Zootaxa* 1442: 55-68.

Trilar T, Gogala M, Szwedo J. 2006. Pyrenean Mountain Cicada *Cicadetta cerdaniensis* Puissant & Boulard (Hemiptera: Cicadomorpha: Cicadidae) found in Poland. *Polskie Pismo Entomologiczne* 75(2): 313-320.

Trilar T, Hertach T. 2008. Three species of Mountain Cicadas *Cicadetta montana* (sensu lato) found in northern Italy. *Bulletin of Insectology* 61(1): 185-186.

Chapter 2: Songs, genetics and morphology: Revealing the taxonomic units in the European *Cicadetta cerdaniensis* cicada group, with a description of new taxa (Hemiptera: Cicadidae)

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ABSTRACT

Recent acoustic studies have revealed that *Cicadetta montana* (Scopoli, 1772), which was once thought to be a single widespread Palaearctic cicada species, is actually a complex of many taxa. Although some song patterns are very distinct, others comprise groups of closely related species, as in the case of *Cicadetta cerdaniensis* Puissant & Boulard, 2000, *Cicadetta cantilatrix* Sueur & Puissant, 2007 and *Cicadetta anapaistica* Hertach, 2011. Seven spatially or behaviourally isolated metapopulations belonging to this song group from Italy and Switzerland were detected and investigated using acoustic, molecular and morphological methods. Taxonomic decisions in this group are challenging because of a lack of truly diagnostic morphological characters, variously coloured morphs, qualitatively intermediate song patterns in contact zones and strong temperature dependence of song duration characters. Molecular genetic studies suggest rapid speciation resulting in introgression or incomplete lineage sorting. It is only by using multiple sources of data that species can be delimited. The new species *Cicadetta sibillae* Hertach & Trilar, 2015** and the new subspecies *Cicadetta anapaistica lucana* Hertach, 2015*** were described using the microstructure of the male calling songs. *Cicadetta sibillae* occurs from southern Switzerland to central Italy, and is the most abundant cicada in the northern Apennine. *Cicadetta anapaistica lucana* is endemic to a small southern Italian distribution range, and seems to be threatened by habitat loss and fragmentation. There is strong evidence that current distribution patterns and phylogenetic relationships of the *Cicadetta cerdaniensis* group are linked to speciation events in Pleistocene glacial refugia in the Apenninian, Iberian and Balkan peninsulas.

**In the originally published text the species name is followed by the compulsory abbreviation ‘sp. n.’.
***In the originally published text the species name is followed by the compulsory abbreviation ‘ssp. n.’.

INTRODUCTION

Cicadas are well known for their songs, which males produce by the movement of paired timbals. These songs act as species-specific pre-mating barriers, and have been used extensively to reveal hidden taxonomic diversity (e.g. Dugdale & Fleming, 1978; Gogala & Trilar, 2004; Quartau & Simões, 2006; Puissant & Sueur, 2010; Marshall *et al.*, 2011). Songs appear to evolve more rapidly than morphological characters and provide a perfect tool to investigate the early stages of reproductive isolation (Marshall *et al.*, 2011). The description of such cryptic species has been enhanced within the last few decades by sensitive field equipment, analysis software and the addition of molecular data (e.g. Marshall *et al.*, 2008; 2011).

Cicada life histories imply low population vagility, with their multi-year nymphal and very brief adult stages. Cicadas are not known to fly long distances (Simões & Quartau, 2007) and are rarely translocated by humans (see Chapter 6 = Hertach & Nagel, 2013), resulting in high genetic and/or song phylogeographic structure (Buckley *et al.*, 2001; Hill *et al.*, 2009; Marshall *et al.*, 2011).

The Palaearctic *Cicadetta montana* complex (Mountain Cicadas) is an excellent example of a taxon with multiple, only recently discovered, cryptic species. Calling songs provided the first evidence of this hidden diversity. The existence of several song phenotypes in *Cicadetta montana* s. l. was described by Bremi (1849). He suggested that these song phenotypes might indicate the presence of two or three species in Switzerland, but he did not formally describe them. Perhaps because the songs of the *C. montana* complex species are in the upper frequency range of human hearing, his findings were not commented upon by subsequent authors for more than 150 years (Chapter 6 = Hertach & Nagel, 2013). In the last few years, *C. montana* s. l. has been shown to be a highly diverse species complex based on calling song patterns (e.g. Gogala & Trilar, 2004). Currently, twelve European and one Korean species of these small cicadas have been described (Gogala *et al.*, 2011; Chapter 1 = Hertach, 2011). Song patterns vary from very simple, as in *Cicadetta montana* s. str. (Scopoli, 1772) or *Cicadetta olympica* Gogala, Drosopoulos & Trilar, 2009, to very complex, as in *Cicadetta anapaistica* Hertach, 2011 and *Cicadetta hannekeae* Gogala, Drosopoulos & Trilar, 2008. Within the species complex some song patterns are closely related and form groups of species, although each species is still easily distinguishable according to qualitative song characters (Gogala, 2013). The widely distributed *C. cerdaniensis* song group contains three species (Chapter 1 = Hertach, 2011): *Cicadetta cerdaniensis* Puissant & Boulard, 2000, *Cicadetta cantilatrix* Sueur & Puissant, 2007 and *C. anapaistica*. All of these species emit a main song phrase based on the repetition of echemes with an important modulation in amplitude: initially of low intensity and with a final loud part.

Detailed fieldwork and analyses combining acoustic, molecular, spatial and morphological data suggest that this group is more complex in Italy and southern Switzerland than previously recognised. Seven metapopulations have been detected with uncertain taxonomic statuses. Here, we investigate species delimitation of these metapopulations in detail and provide distributional and ecological data. We also discuss their evolutionary history, biogeography and potential threats.

MATERIALS AND METHODS

Fieldwork

Fieldwork in the Italian mountainous ecosystems was conducted in June and July of 2007-2013. Swiss locations were investigated since 2004. Additionally, the Pyrenees Mountains were visited in June 2010 for comparison with *Cicadetta cerdaniensis* s. str. The detection of calling songs in cicadas is the most effective way to check for the presence of different species. This is especially true in the *C. montana* complex where individuals are wary, and are often very difficult to localise and catch.

Calling song recordings were made with a Marantz PMD 660 (sampling frequency at 48 kHz) in high temperature ranges (16-33 °C). The use of an ultrasonic detector (Pettersson D 200) with its microphone fitted into a small parabola (diameter 16 cm; see Gogala, 2013) was very effective for localizing the calls in their high carrier frequency domain (10-20 kHz). The equipment was able to cover a frequency range from at least 100 to 24,000 Hz. The temperature of the perch surface, where the singing individual was supposed to sit on trees, shrubs, or rarely in the herb layer, was measured with a TFA ScanTemp 410 infrared thermometer from the year 2010 onwards. Several measurements of the temperature were taken in close proximity to the singing individual with comparable exposures. The mean values were used for analyses to reduce measurement error. This temperature is not consequently identical with the body temperature of the individual (Sanborn *et al.*, 2011), but was a practical approach for efficient fieldwork.

Voucher specimens were collected with a net. One or two legs were removed from fresh specimens and preserved in ethanol for the collection of molecular data later. All specimens were pinned and dried.

Song analyses

Songs were analysed using Raven Pro 1.3 and Raven Pro 1.4 (Cornell Lab of Ornithology), and illustrations of oscillograms were produced with Seewave (Sueur *et al.*, 2008) on the R software platform (R Development Core Team, 2008). Our terminology for the variables used follows, as far as possible, Sueur & Puissant (2007) and Hertach, 2011 (= Chapter 1) in order to simplify comparisons with closely related species. Songs are hierarchically composed of pulses (basic signal related to the timbal movements), syllables (one group of in-and-out bucklings of both timbals), echemes (main unit characterised by the duration of timbal activity) and phrases (series of echemes in a strict scheme). This study differs in qualitative and not quantitative aspects between the so-called phrase 1 and phrase 2 defined by Puissant & Boulard (2000), Sueur & Puissant (2007) and Hertach, 2011 (= Chapter 1), as smooth transitions occur in all taxa. Analyses were conducted on two levels of work intensity. Best and longer recordings (2-5 min) within a perch temperature range of 23-28 °C were chosen in order to analyse all acoustic characters, predominantly the time and carrier frequency domains. As temporal variables turned out to be highly dependent on the temperatures, we used this relatively small range for the description of specific, comparable characters. Shorter sequences were used over the maximal temperature range of recordings to visualise the temperature dependency of duration characters, to count the timbal movements per echeme and to compare the power within parts of echemes and between echemes (Raven function: Max Power). Additional comments on the selection of the investigated variables and on the measurements in the song analyses are provided in **Appendix S2.1**.

Males produce their sound by activating the two timbals. We investigated the relationship between timbal activity and the microstructure of the acoustic signals for two individuals of *C. cantilatrix* kept indoors in a cage. Individuals were spontaneously singing with the aid of a warming lamp. First, recordings were made with intact sound production systems, then one timbal was inactivated and the individual recorded again (e.g. Fonseca, 1991; Sueur & Aubin, 2003; **Appendix S2.1**). The two timbals produced two pulses of similar quality, one when buckling inwards and a second when returning to the relaxed position, as in other small cicadas (e.g. Fonseca & Revez, 2002). Consequently, a complete syllable (group of in-and-out signals of both timbals) consists of four pulses in an oscillogram when contractions and relaxations of both timbals alternate (**Fig 2.1A**); however, some individuals produce only two signals visible in the oscillograms, when timbals are moved virtually synchronously (**Fig 2.1B**), or they produce three pulses with a typically alternating amplitude, for example when the buckling of timbal 2 is quasi synchronised with the relaxing of timbal 1 (half-synchronous; **Fig 2.1C**). Our outdoor studies, upon which the following data set is based, demonstrated that all three movement types could occur within one local population. Often the movement types are quite obvious and syllables are recognised as groups, but sometimes they were not. In these instances it was difficult to decide whether an individual had been moving the timbals synchronously or alternately (e.g. **Fig 2.1B**), and the duration of a syllable, varying between 5.5 and 8.5 ms for a large temperature range, helped to discover the movement type.

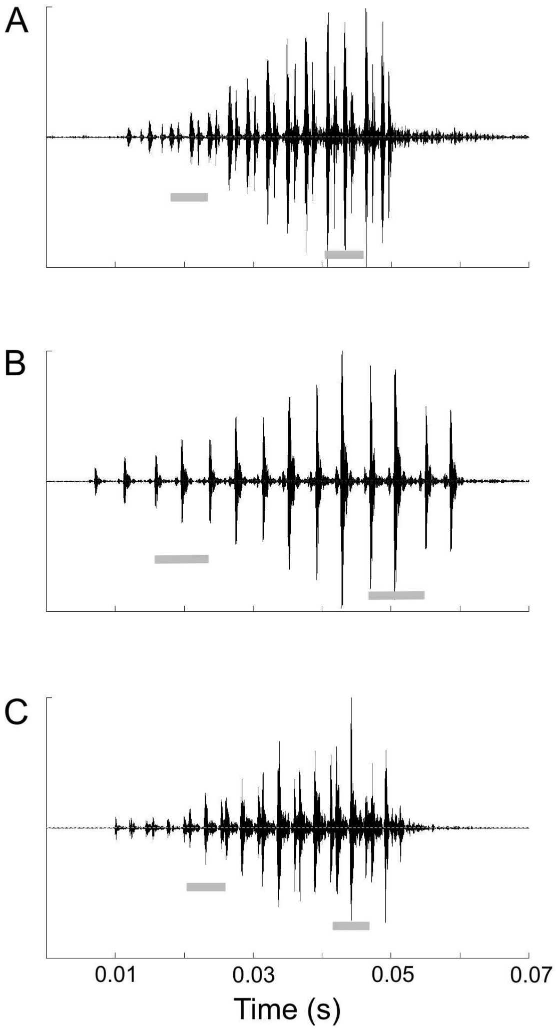

Fig 2.1. Timbal movement types in the *Cicadetta cerdaniensis* song group demonstrated in oscillograms (time versus amplitude). (A) alternating type with four signals per syllable, (B) synchronous with two signals per syllable visible (or one timbal not active/destroyed), (C) half-synchronous with three signals of variable amplitude visible. Grey bars = duration of one syllable (complete group of buckling inwards and restoring both timbals).

Song patterns recorded in the field from many different populations (**Fig 2.2**) were compared with closely related songs of *C. cerdaniensis* s. str. from the Pyrenees (France) and *C. anapaistica* from Sicily (Italy), including the type localities. Statistical tests on song variables were conducted in R between mean values of individuals from different taxa (R Development Core Team, 2008). A linear discriminant analysis (LDA) was performed with song variables for two taxa that were qualitatively indistinguishable. For the LDA, the function lda(…) was used, with priors set to equal values. Temperature-dependent variables were tested for significance between taxa with general linear models (analysis of covariance, ANCOVA). Measured values and log-transformed values exhibited relationships of similar dimensions in linear regressions. ANCOVA models were consequently tested for both, but the significance levels were always the same. Hence, we provide here data based on the untransformed values. First the ANCOVA model [R command: aov(variable~temperature*species)] checked for an interaction between the covariate ‘temperature’ and the categorical factor ‘species’. An existing interaction was characterised by significantly differing slopes and, therefore, rates of changes between taxa with temperature shifts. If slopes were not significantly different a second model was tested for the intercepts without any interaction [R command: aov(variable~temperature+ species)]. Afterwards an analysis of variance (ANOVA) between model 1 and model 2 was conducted to check whether removing the interaction significantly affected the fit of the model. Model 2 was more appropriate when not specified in the results. Spatial trends of some song variables were visualised as functions of the geographical latitude, as the north-south axis along the Apenninian peninsula explained most of the variability. Graphs were generated with the package ggplot2 (Wickham, 2009). The song recordings are preserved in the Slovenian Wildlife Sound Archive of the Slovenian Museum of Natural History (PMSL) in Ljubljana and in the private archive of the first author.

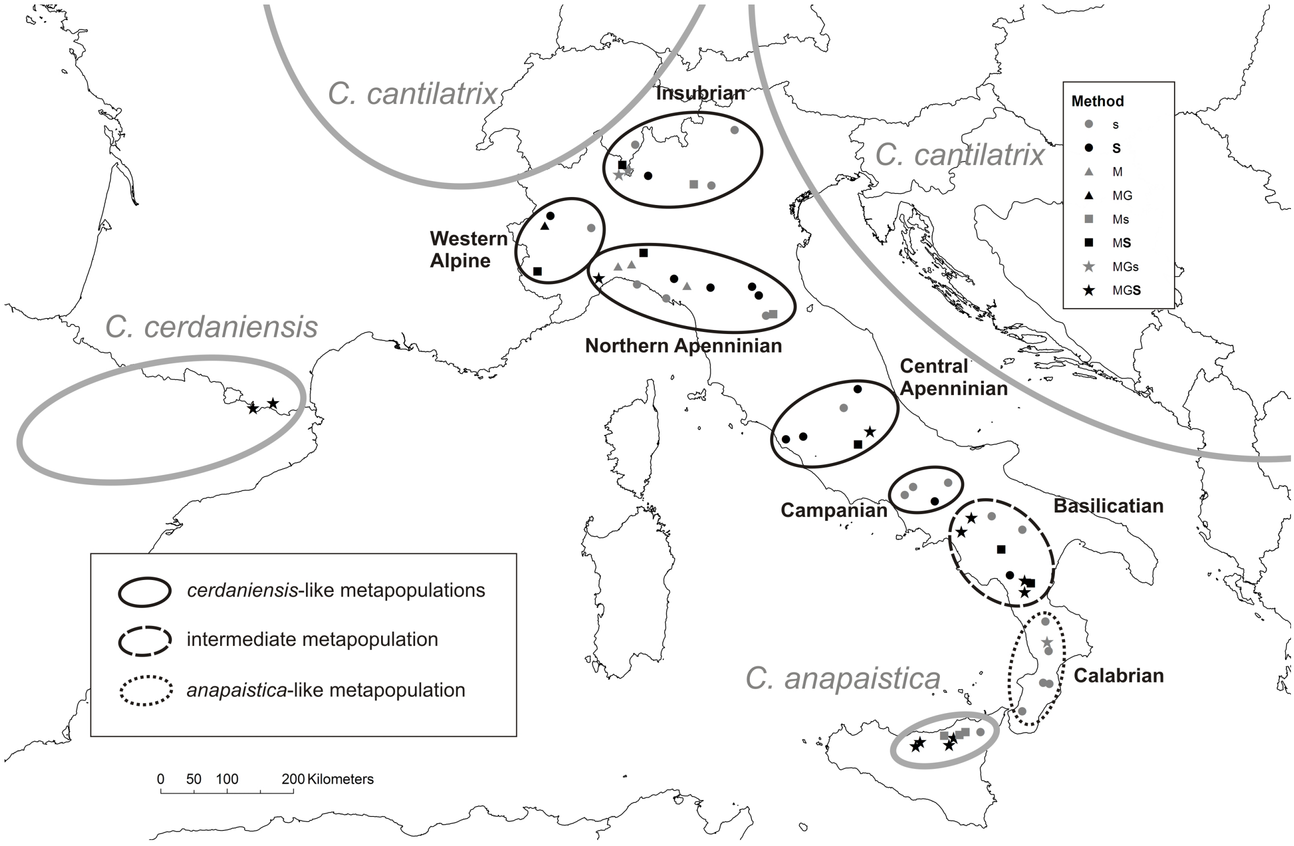

Fig 2.2. Chorological pattern of seven undetermined metapopulations in the *Cicadetta cerdaniensis* song group and distribution areas of described species in Southern Europe. Preliminary classifications with operational names according to qualitative song patterns. Map with locations of analysed data: s = some key variables of the song analysed, **S** = detailed song analyses (recordings between 23 °C and 28 °C perch temperature only), M = morphology, G = genetics.

Molecular phylogenetic analyses

Analyses of the mitochondrial gene segments from the cytochrome *c* oxidase subunits I and II (*COI* and *COII*) were conducted in this study. A total of 28 specimens were sequenced from the *C. cerdaniensis* song group and *Cicadetta fangoana* Boulard, 1976 was used as an out-group. For each cicada, genomic DNA was extracted from one leg with either the Qiagen DNeasy Tissue Kit (Qiagen) or the NucleoSpin Tissue Kit (Macherey-Nagel), following the manufacturers' instructions, except that the DNA digestion was conducted over 12-24 h. Polymerase chain reaction (PCR) was performed using the Takara Ex Taq kit and the following primers: C1J-2195 and TL2-N-3014 (*COI*) and TL2-J-3034 and TK-N-3786 (*COII*; Simon *et al.*, 1994). Amplified products were cleaned with ExoSAP-IT (Affymetrix) and cycle sequenced with the BigDye Terminator 1.1 or 3.1 (Applied Biosystems) cycle sequencing kit at one eighth the recommended concentration. The product was cleaned by Sephadex (GE Healthcare) filtration and sequenced with an ABI 3130 capillary sequencer. The resulting sequences were edited, aligned and checked by eye with Geneious (Biomatters Limited). Alignment was unambiguous and there was no length variation. *COI* and *COII* gene trees did not conflict as expected because of their common mitochondrial inheritance, and were concatenated for further analyses. PartitionFinder 1.0.1 (Lanfear *et al.*, 2012) was used to group predefined partitions and find the best-fitting model for each according to the Bayesian information criterion (BIC). The programme suggested a total of six partitions (two genes, with three codon positions each), which were taken as input for a maximum likelihood (ML) analysis (GARLI 2.0; Zwickl, 2006). A total of 100 bootstrap pseudoreplicates were performed, with the models of each partition unlinked and summarised on the ML tree with SumTrees (DendroPy 3.12.0; Sukumaran & Holder, 2010). Genetic distances between and within taxa were calculated with MEGA 5.1 (Tamura *et al.*, 2011). Both pairwise and ML K2P model-corrected distances were calculated for comparison with other studies. We recommend using best-fitting models to correct genetic distances, but these taxa are so closely related that the best-fitting model distances are nearly identical. We also present corrected genetic distance estimates in the form of ML branch lengths from our GARLI analysis using the best-fitting models for each of the data partitions, which is a much more statistically appropriate procedure for visualizing genetic distances among taxa.

Morphological analyses

Only song-identified males or females from single-species populations were used in the morphological analyses. Specimens of the seven metapopulations were compared with 20 *C. cerdaniensis* s. str. (19 males, one female, some of them kept in the MNHN and some in the private collection of Puissant) and 19 *C. anapaistica* (14 males, five females, including the type series), as well as other taxa. The morphological descriptions of dry prepared specimens follow the terminology of Moulds (2005). Some principal distances were photographed with a Leica DFC425 camera on a Leica M205 C stereomicroscope (fore wing) or a Keyence VHX 2000 digital photomicroscope (body) at different magnifications. They were afterwards measured from the photographs using ImageJ 1.47 (Schneider *et al.*, 2012). None of the characters investigated was strictly diagnostic. The best characters were tested for significance: categorical characters with χ^2 contingency tests and quantitative characters with Wilcoxon-Mann-Whitney rank sum tests (U-test).

Distribution pattern

Maps were produced with ArcGIS (map source: http://www.worldclim.org and http://www.diva-gis.org). The distribution map observation points were put together for 15 × 15 km^2 grid cells and visualised with the UTM coordinate system. Absence records were reported only if weather conditions were suitable for cicada activity, and only for Switzerland and Italy. The location of each specimen analysed in this study is given in **Fig 2.2 and Appendix S2.2**, and all records are listed in **Appendix S2.3**. We distinguish between local populations and metapopulations in the sense of Hanski & Gilpin (1991): Metapopulations are a set of conspecific local populations that presumably interact via individuals moving among populations.

Abbreviations and acronyms

Song variables

E_{x_y}	echeme type ‘y’ belonging to the phrase ‘x’ (all indexes analogue)
E_{a+b}	echemes of the phases ‘a’ and ‘b’
FP	first part of echeme
SP	second part of echeme
ED	echeme duration
$IED_{x\_y/z}$	inter-echeme duration between echeme types ‘y’ and ‘z’ belonging to the phrase ‘x’
G	echeme/inter-echeme group duration
PH	phrase and phrase duration
F	echeme carrier frequency
P	maximal echeme power

Museums (see Evenhuis, 2014)

ETHZ	Eidgenössische Technische Hochschule Zürich, Switzerland
MCSN	Museo Cantonale di Storia Naturale Lugano, Switzerland
MNHN	Muséum National d’Histoire Naturelle Paris, France
NHMB	Naturhistorisches Museum Basel, Switzerland
NMBE	Naturhistorisches Museum der Burgergemeinde Bern, Switzerland
PMSL	Prirodoslovni muzej Slovenije, Ljubljana, Slovenia

RESULTS

Song patterns

Our fieldwork in Italy and Switzerland revealed four qualitatively different song patterns within the *C. cerdaniensis* song group. The first pattern can be assigned to *C. cantilatrix* and is present in Switzerland north of the main ridge of the Alps (Chapter 6 = Hertach & Nagel, 2013) and in the Italian south-eastern Alps. This study focuses on the remaining three patterns, showing a complicated example of recent evolution. The most abundant pattern is very similar to that of *C. cerdaniensis* s. str. described from the Pyrenees (Puissant & Boulard, 2000), and was detected for Switzerland in 2004 and for Italy in 2007. The southernmost pattern was recently described as the new species *C. anapaistica* from Sicily (Chapter 1 = Hertach, 2011). In 2010, another song pattern was discovered that was qualitatively 'intermediate' to the '*cerdaniensis*'-like and the *C. anapaistica* pattern, and rather variable.

Distribution

Detailed fieldwork in the whole range of these three song types showed allopatric to parapatric distribution areas, often restricted to mountainous regions (**Fig 2.2**). The '*cerdaniensis*'-like song type occurs in the northern and central parts of Italy and in southern Switzerland, and is split into at least five disjunct areas, which are separated by gaps of up to 160 km. The '*anapaistica*'-like song type is not restricted to the Mountains of Sicily, as stated before (Chapter 1 = Hertach, 2011). A few additional smaller local populations were found on the mainland in Calabria during recent fieldwork. The 'intermediate' song type is endemic to the Basilicata and adjoining locations. Therefore, it is not only intermediate in song structure but also geographically. Most southern '*cerdaniensis*'-like and 'intermediate' metapopulations are split by a gap of a maximum of 58 km, whereas the gap between 'intermediate' and '*anapaistica*'-like metapopulations is at most 47 km. These gaps are characterised by low mountains and plains, which are mainly deforested. The relatively small distances between metapopulations lead to overlaps in song pattern qualities at both contact zones in the north and to the south of the 'intermediate' song type (see below).

Molecular phylogenetic analyses

The *C. cerdaniensis* song group forms three well-supported and geographically coherent clades for the ML mitochondrial phylogeny (**Fig 2.3**): *C. cantilatrix*, *C. cerdaniensis* s. str. from the Pyrenees and an Italian or Apenninian clade that contains taxa exhibiting the three song patterns treated here in detail, *C. anapaistica*, '*cerdaniensis*'-like and 'intermediate'. Within the Apenninian clade all *C. anapaistica* specimens from Sicily and the '*anapaistica*'-like specimen from Calabria form a monophyletic clade paraphyletic with the '*cerdaniensis*'-like and 'intermediate' specimens. Surprisingly, *Cicadetta brevipennis* Fieber, 1876, which has a very different song (see Gogala & Trilar, 2004; Hertach, 2007 and **Fig 2.6F**), form a polytomy with both *C. cerdaniensis* s. str. and the Apenninian clade (see Chapters 3 and 4). Eastern and central *C. brevipennis* cluster within the Apenninian clade and many western *C. brevipennis* cluster with *C. cerdaniensis* s. str. *Cicadetta cantilatrix* is monophyletic in the mitochondrial gene phylogeny.

Average pairwise genetic distances (ML corrected distances) among any song-defined taxa in the *C. montana* complex are at most 0.04 substitutions per site for the mitochondrial genes (see Chapter 3). Distances among *C. cantilatrix*, *C. cerdaniensis* s. str. and the Apenninian clade are approximately 0.025 substitutions per site (**Table 2.1**). Between *C. anapaistica* and the candidate taxa within the Apenninian clade, the distance is at most 0.01.

Morphology

Species of the *C. montana* species complex generally exhibit few interspecific morphological traits but remarkably high intraspecific variability. Only a very few taxa within the entire *C. montana* species complex are truly morphologically distinguishable with traditional comparative methods [e.g. *Cicadetta concinna* (Germar, 1821)]. Therefore, it is striking that 95.0% of the *C. cerdaniensis* s. str. specimens (n = 20) from the Pyrenees exhibit a predominant light (yellowish) basal junction of anal veins, whereas 95.5% (n = 66) of the specimens from the Apenninian genetic clade (*C. anapaistica*, '*cerdaniensis*'-like and 'intermediate') are dark (**Fig 2.4C-F**). Some specimens of the 'intermediate' metapopulation exhibit lighter colouration of the body than is normally found in any of the species of this complex.

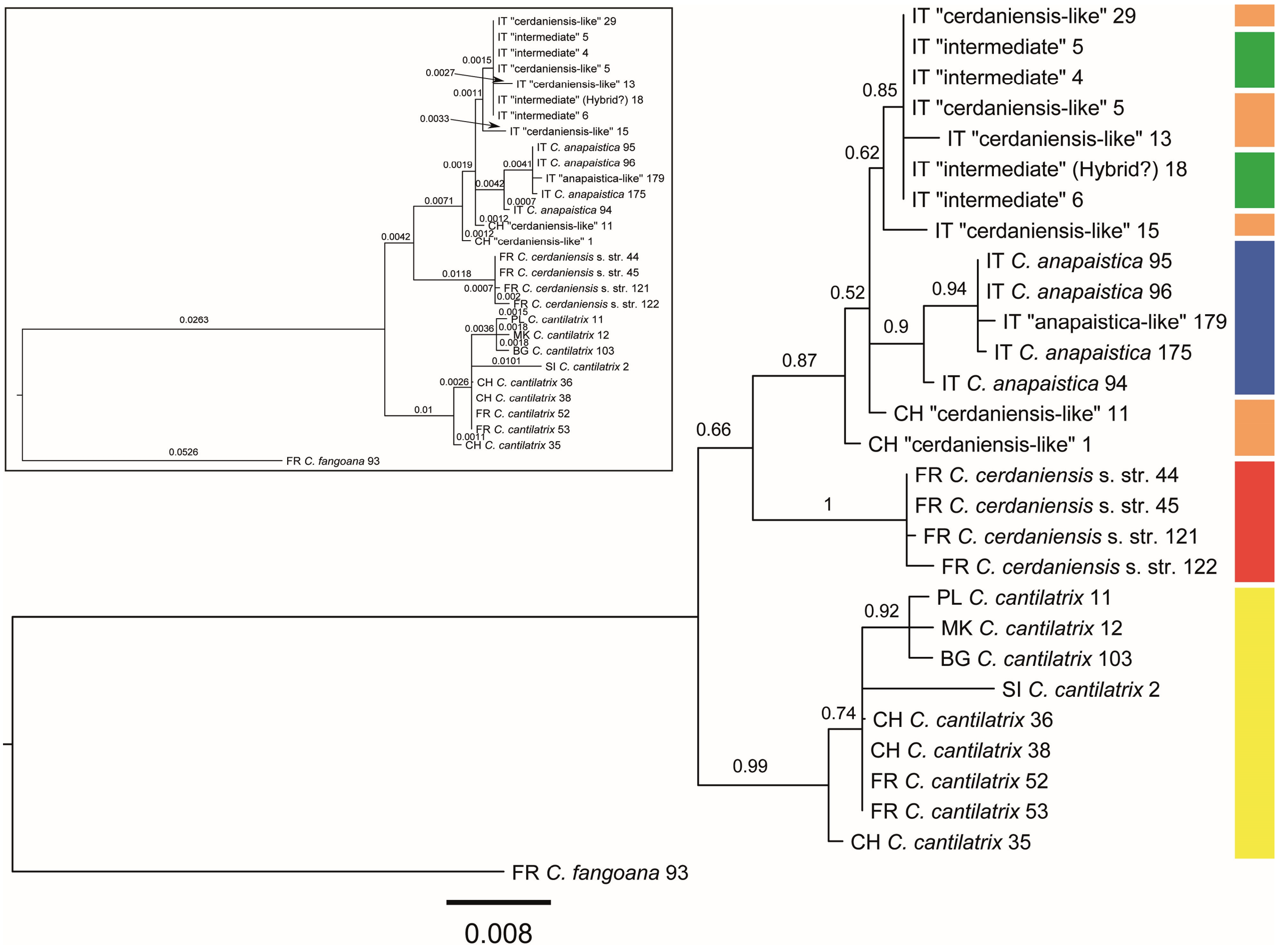

Fig 2.3. Cytochrome *c* oxidase subunits I and II (*COI, COII)* mitochondrial DNA concatenated phylogeny of the *Cicadetta cerdaniensis* song group. Maximum likelihood (ML, GARLI) phylogenies are shown with ML branch lengths (box on the left) and ML bootstrap support. Nodes with less than 50% bootstrap support have been collapsed. *Cicadetta fangoana* was selected as the out-group taxon. Specimen names include two-letter country codes followed by species or operational names (later on in this study: '*cerdaniensis*'-like = *Cicadetta sibillae*, 'intermediate' = *Cicadetta anapaistica lucana*). Specimen identification numbers are congruent with the type series for undescribed taxa. *Cicadetta cantilatrix* specimens originate from Switzerland, France, Poland, Slovenia, Macedonia and Bulgaria. Type localities of *C. cantilatrix, C. cerdaniensis* and *C. anapaistica* are included.

Integrative species delimitation

The 'Unified Species Concept' of De Queiroz (2007) views species as "separately evolving metapopulation lineages". A combination of song patterns, our mitochondrial gene phylogeny, distinctive morphological traits and a detailed knowledge of species ranges (including the spatial variability) can be used to delimit species under this concept.

The clearest result was the discovery that the Calabrian metapopulation unquestionably belongs to *C. anapaistica*, and therefore this species is not endemic to Sicily as previously thought. Obviously, *C. anapaistica*, ‘*cerdaniensis*’-like and ‘intermediate’ metapopulations are closely related and differ from the Pyrenean *C. cerdaniensis* s. str. based on mitochondrial DNA, colouration and a 450-km geographical separation. Additionally, ‘*cerdaniensis*’-like song patterns are distinguishable from *C. cerdaniensis* s. str. by the quantitative characters described below. Combining these facts, we consider the genetic distances between the ‘*cerdaniensis*’-like and *C. cerdaniensis* s. str. populations to be taxonomically relevant, rather than an artefact caused by the introgression of mitochondrial DNA from *C. brevipennis*. The qualitative song differences between ‘*cerdaniensis*’-like, ‘intermediate’ and *C. anapaistica* are important. Unresolved phylogenetic relationships and contact zones with variable song structures indicate that these taxa are young and not completely isolated. The mitochondrial phylogeny suggests that the ‘intermediate’ taxon is more closely related to the ‘*cerdaniensis*’-like taxon than to *C. anapaistica*; however, we assigned the most weight to the results of the detailed song analyses (see below). We decided to tolerate a small contact and putative hybrid zone with overlapping song patterns for ‘*cerdaniensis*’-like towards ‘intermediate’ metapopulations, but not a wider mixture zone without clear isolating mechanisms for ‘intermediate’ to *C. anapaistica*. We conclude that ‘*cerdaniensis*’-like metapopulations are a new species, whereas the ‘intermediate’ Basilicatian metapopulation is a new subspecies of *C. anapaistica*. The latter occurs in two colour morphs. The nominate form is subsequently called *Cicadetta anapaistica anapaistica*, whereas *C. anapaistica* refers to both subspecies.

Table 2.1. Genetic distances between three geographical clades in the *Cicadetta cerdaniensis* group based on cytochrome *c* oxidase subunits I and II (*COI*, *COII*) mitochondrial gene data. Average pairwise distances between taxa with uncorrected p-distances below the diagonal and maximum likelihood corrected distances above (K2P model, substitutions/site), within taxa divergence in the grey diagonal (p-distance/ML distance). Continuous line for monophyletic taxa, dotted line for taxa polyphyletic with ‘*C. brevipennis*-A’, broken line for taxa polyphyletic with ‘*C. brevipennis*-B’ (see Chapters 3 and 4). A putative hybrid specimen is excluded.

	North-eastern clade	**Western clade**	**Apenninian clade**		
	C. cantilatrix	*C. cerdaniensis* s. str.	*C. anapaistica* (nominate form)	‘*cerdaniensis*’-like	‘intermediate’
C. cantilatrix	0.006/0.007	0.027	0.029	0.027	0.027
C. cerdaniensis s. str.	0.026	0.001/0.001	0.024	0.021	0.022
C. anapaistica (nominate form)	0.027	0.023	0.002/0.002	0.010	0.009
‘*cerdaniensis*’-like	0.025	0.023	0.010	0.005/0.005	0.003
‘intermediate’	0.026	0.021	0.010	0.003	0.000/0.000

Cicadetta sibillae Hertach & Trilar, 2015

Cicadetta cerdaniensis s. l. (Hertach, 2007)
Cicadetta cerdaniensis (Trilar & Hertach, 2008; Chapter 1 = Hertach, 2011; Hertach & Pollini Paltrinieri, 2012)

Type material

The type series consists of 25 males and three females representing the central Apenninian (3), northern Apenninian (9), western Alpine (2) and Insubrian metapopulations (14). It is kept in the NHMB (holotype), MCSN, NMBE, PMSL and two private collections.

Holotype male: Verbatim label information: "nördl. Campigna, EMIL, I/43.8854°/11.7567°, 950 m asl/3.7.2012, leg. T. Hertach/Collection Code No. 5.020" (label rectangular, white, printed) and "HOLOTYPUS ♂/*Cicadetta sibillae* sp. n./Hertach & Trilar 2014" (label rectangular, light red with dark red margin, printed; NHMB).

Paratypes: All paratypes with labels "PARATYPUS XX Y, *Cicadetta sibillae* sp. n. Hertach & Trilar 2014" (label rectangular, white with red margin, printed) at which 'XX' is the number of the paratype and 'Y' the sex of the specimen. Numbers '20' and '24' do not exist. **Paratype males:** Monte San Giorgio, TI, CH, 45.9123°/8.9478°, 1040 m asl, 6.6.2005, leg. T. Hertach (paratype 1, coll. MCSN); Monte San Giorgio, TI, CH, 45.9123°/8.9478°, 1040 m asl, 27.6.2007, leg. T. Hertach (paratype 2, coll. Hertach); Monte San Giorgio, TI, CH, 45.9100°/8.9516°, 1000 m asl, 27.6.2007, leg. T. Hertach (paratype 3, coll. MCSN); Monte San Giorgio, TI, CH, 45.9100°/8.9516°, 1000 m asl, 27.6.2007, leg. T. Hertach (paratype 4, coll. Hertach); Pontinvrea, LIGU, I, 44.4267°/8.4250°, 550 m asl, 1.8.2008, leg. T. Hertach (paratype 5, coll. Hertach); Monte Bré, Sassa, TI, CH, 46.0084°/8.9851°, 880 m asl, 6.7.2009, leg. A. Rey (paratype 7, coll. Hertach); Lodrino, LOMB, I, 45.7043°/10.2808°, 930 m asl, 18.6.2010, leg. T. Hertach (paratypes 8 and 9, coll. Hertach); Monte Caslano, TI, CH, 45.9611°/8.8799°, 460 m asl, 24.6.2010, leg. T. Hertach (paratype 10, coll. MCSN); Monte San Giorgio, TI, CH, 45.9100°/8.9503°, 970 m asl, 11.7.2004, leg. T. Hertach (paratype 11, coll. MCSN); Rocca di Cambio/Rocca di Mezzo, ABRU, I, 42.2140°/13.4812°, 1390 m asl, 7.7.2011, leg. T. Hertach (paratypes 12 and 13, coll. Hertach); Verrecchie, ABRU, I, 42.0384°/13.2459°, 1010 m asl, 8.7.2011, leg. T. Hertach (paratype 14, coll. Hertach); E Celle, Susa, PIEM, I, 45.1304°/7.3548°, 870 m asl, 16.6.2012, leg. T. Hertach (paratype 15, coll. Hertach); Lottulo, Maira, PIEM, I, 44.4944°/7.2351°, 780 m asl, 18.6.2012, leg. T. Hertach (paratype 16, coll. NMBE); Mte. Tugello/Mte. Tobbio, LIGU, I, 44.5775°/8.7869°, 540 m asl, 21.6.2012, leg. T. Hertach (paratype 17, coll. Hertach); NE Vobbia, LIGU, I, 44.6074°/9.0495°, 520 m asl, 21.6.2012, leg. T. Hertach (paratype 18, coll. NMBE); Castello di Comano, TOSC, I, 44.3039°/10.1152°, 770 m asl, 24.6.2012, leg. T. Hertach (paratype 19, coll. Hertach); N Pregola, LOMB, I, 44.7548°/9.2915°, 1120 m asl, 18.7.2012, leg. T. Hertach (paratype 22, coll. Hertach); Monte Caslano, TI, CH, 45.9612°/8.8801°, 470 m asl, 22.6.2013, leg. T. Hertach (paratype 23, coll. Hertach); Monte d'Alpe, LOMB, I, 44.8022°/9.3149°, 1050 m asl, 3.7.2013, leg. T. Hertach (paratype 26, coll. Hertach); SW Brallo di Pregola, LOMB, I, 44.7341°/9.2759°, 970 m asl, 4.7.2013, leg. T. Hertach (paratype 27, coll. Hertach); Monte Bré, Sassa, TI, CH, 46.0085°/8.9853°, 880 m asl, 20.6.2009, leg. A. Rey (paratype 28, coll. Rey); Rasa, Monte Chiusarella, LOMB, I, 45.8660°/8.8133°, 700 m asl, 14.6.2007, leg. T. Trilar, T. Hertach (paratype 29, coll. PMSL). **Paratype females:** Monte Bré, Sassa, TI, CH, 46.0085°/8.9853°, 880 m asl, 20.6.2009, leg. A. Rey (paratype 6, coll. Hertach); N Campigna, EMIL, I, 43.8854°/11.7567°, 950 m asl, 3.7.2012, leg. T. Hertach (paratype 21, coll. NMBE); Monte Caslano, TI, CH, 45.9612°/8.8801°, 470 m asl, 22.6.2013, leg. T. Hertach (paratype 25, coll. Hertach).

Morphology

Diagnosis

The morphological distinction between the species of the *C. montana* complex is very difficult, and is not possible for all specimens. *Cicadetta sibillae* specimens are separated from the very closely related *C. cerdaniensis* s. str. with high probability by the predominantly dark basal junction of anal veins (92.3% versus 5.0%). From *C. montana* s. str., many specimens are distinguished by the outer rim of costa darker than the inner rim and the radius/subcostal veins in the fore wing (87.5% versus 9.5%, chi-square contingency test: $\chi^2 = 42.3$, $p < 0.001$) or by the cubitus anterior vein lighter than the median vein in the hind wing (85.7% versus 8.3%, chi-square contingency test: $\chi^2 = 42.2$, $p < 0.001$). *Cicadetta sibillae* has on average slender fore wings and a significantly lower wing ratio (length to width) than *C. a. anapaistica* (**Fig 2.5A**, only males considered, Wilcoxon rank sum test: $W = 79$, $p = 0.004$).

Description

Measurements: Body length: 18.0 mm in holotype specimen, 17.2 ± 0.7 mm (mean ± SD) in male paratypes, 18.5 ± 1.3 mm in female paratypes. Body width (abdomen, tergite II): 5.8 mm in holotype specimen, 5.6 ± 0.2 mm in male paratypes, 5.8 ± 0.5 mm in female paratypes. Fore wing length: 19.7 mm in holotype specimen, 18.9 ± 1.1 mm in male paratypes, 20.6 ± 1.0 mm in female paratypes. Fore wing width: 8.2 mm in holotype specimen, 7.7 ± 0.5 mm in male paratypes, 8.3 ± 0.4 mm in female paratypes.

Male holotype with remarks on the variability in the paratype series (**Fig 2.4A**): *Head:* Black with ochre patch on epicranial suture. Postclypeus with longitudinal narrow groove, black with lateral margins yellowish (paratypes sometimes additionally with a yellowish triangular patch at postclypeus towards the frontoclypeal suture), anteclypeus black. Rostrum reaching mid trochanter, labrum and mentum predominantly dark brown (paratypes often lighter), labium dark brown to black. Compound eyes and ocelli brownish. Antennae dark brown to black with lighter margins of scapes.

Thorax: Pronotum generally black with posterior margin of pronotal collar and its lateral angles yellowish (paratypes sometimes completely black, sometimes additionally with central suture partly marked yellowish). Lateral angles of pronotal collar pronounced but margin frontal to the lateral angles scarcely turned up and steadily narrowed towards the head in dorsal view (paratypes often turned up more strongly and convex in shape, compare **Fig 2.5B**). Scutum, cruciform elevation and metanotum black, the latter with

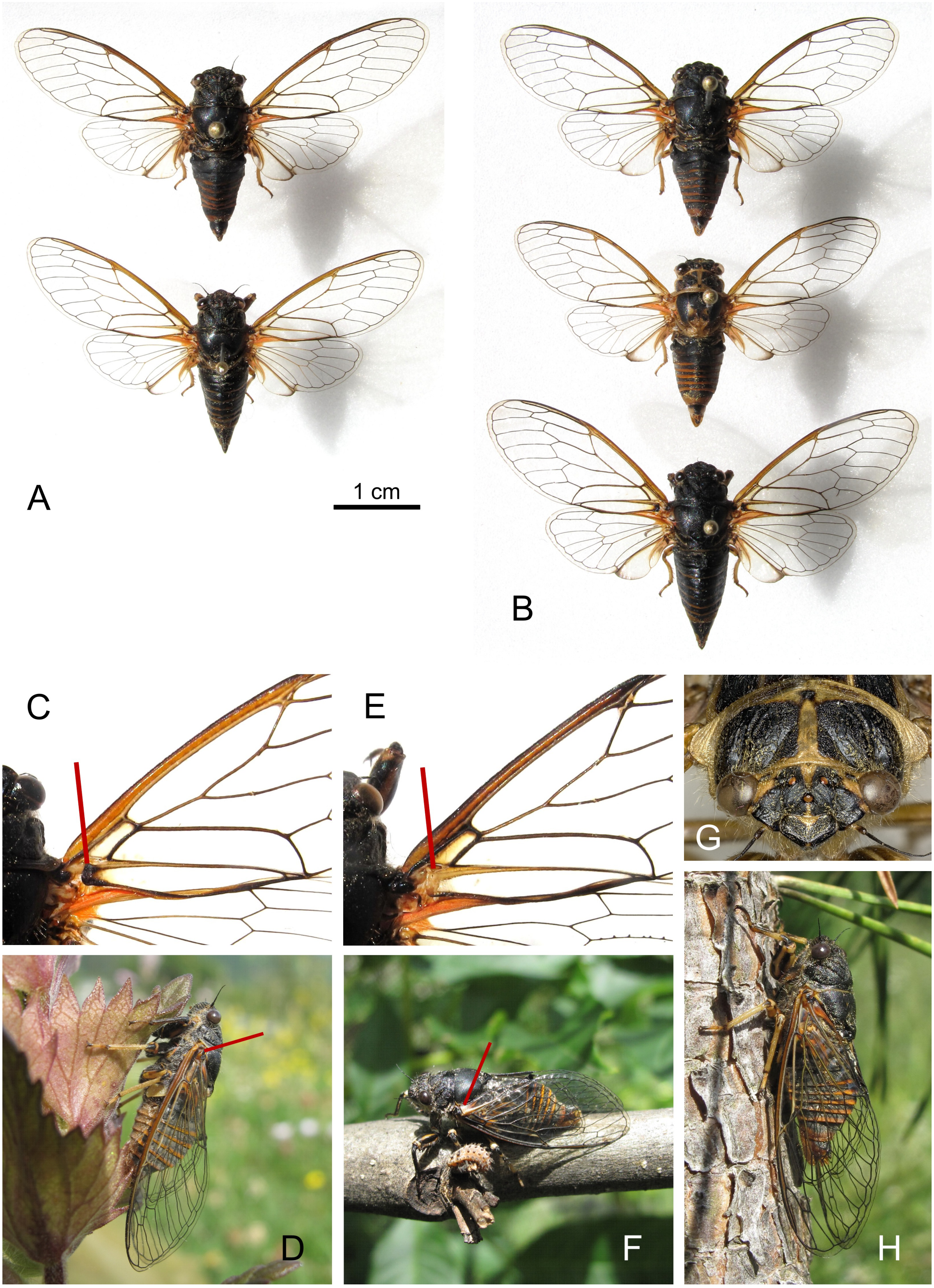

Fig 2.4. *Cicadetta cerdaniensis* group morphology. (A) *Cicadetta sibillae* type series with holotype specimen (top) and female paratype (below). (B) *Cicadetta anapaistica lucana* type series with holotype specimen (top), light morph paratype (centre) and female paratype (below). Basal junction of anal veins (red line) dark in *C. sibillae* (C-D) and light in *C. cerdaniensis* s. str. (E-F). Males of (D) *C. sibillae*, (F) *C. cerdaniensis* s. str. and (H) *C. a. lucana* in natural conditions. (G) Dorsofrontal view of head and pronotum in (dried) light morph paratype of *C. a. lucana*.

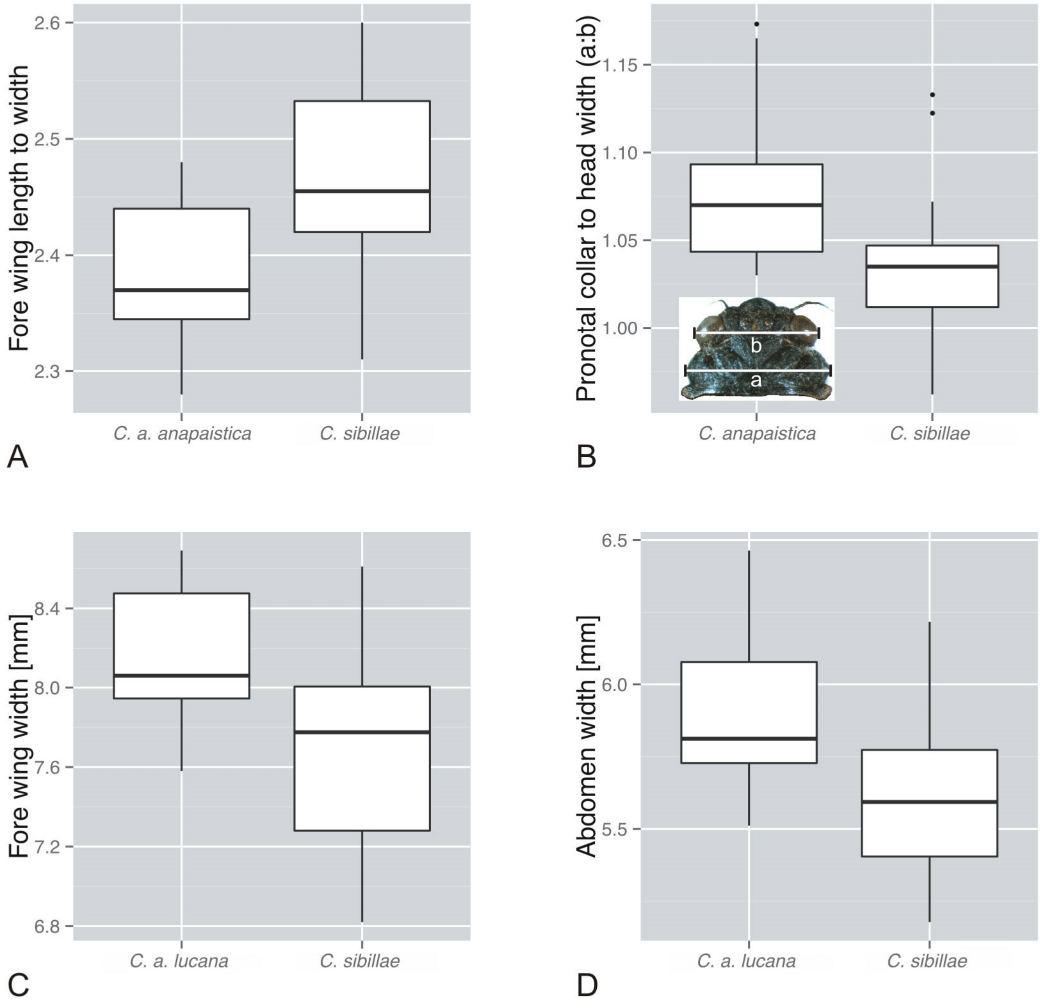

Fig 2.5. Morphometry of *Cicadetta sibillae* and *Cicadetta anapaistica*. Box plots of some significant but not diagnostic differences in sizes and ratios.

yellowish margins (paratypes sometimes with paired lighter spots on the cruciform elevation, rarely also on the scutum). Ventral side generally black, except membranes at bases of legs orange to brown and broad yellowish margin of episternum II (rarely missing in paratypes). Opercula not overlapping, kidney-shaped with black base and yellowish to brownish distal part. Meracanthus with spike of medium size, directed mainly caudally (paratypes very variable in shape and size).

Abdomen: Abdomen triangular in cross section. Tergites I and II black, first with orange to red brown marked ribs anterad to timbals. Tergites III-VIII frontal black and caudal red brown (some paratypes and fresh specimens orange). Sternites III-VII red brown with darker sectors frontal in the centre (paratypes sometimes orange or ochre instead of red brown, and dimensions and colour intensity of dark sectors highly variable). Epipleurites caudally orange brown, frontally darker. Timbals with three main ribs, two long and one shortened (the latter optionally interrupted in paratypes), and timbal plate.

Legs: All legs with yellow to orange and black fasciae and dots. Front femora with one longer and two shorter spines.

Wings: Fore wing hyaline except for slightly yellowish basal cell and for brownish to reddish pterostigma. Basal membrane orange to red. Median and cubitus anterior vein originating in one point at basal cell (five paratypes with median and cubitus anterior vein fused, on one or both sides, for approximately 1 mm). Colouration of basal veins ranging from ochre to dark brown (paratypes rarely and partly even black). Costal vein with darker exterior and lighter inner rim, radial/subcostal veins and median vein lightest (paratypes rarely other colour combinations). Basal junction of anal veins predominantly dark brown to black (**Fig 2.4C-D**, one paratype yellowish). Distal veins with eight apical cells (paratypes also seven or nine), dark or even black. Hind wing transparent, except for orange base of costal cell, orange margins of jugum and plaga and dark brown spotted apical vannus margin. Veins dark, especially in distal part with six apical cells. Radius and cubitus anterior vein lighter than median vein (paratypes sometimes same colour, especially in radius).

Genitalia: Pygofer very dark (paratypes lateral often with important orange brown portions) with rounded dorsal beak and rounded blunt upper lobes. Median lobe of uncus dark brown and curved upwards, rounded and broad. Claspers hooked and dark brown, hooks pointed. Pseudoparameres flattened, especially in the central part, and with sharp end. Basal lobe of pygofer dark brown (in paratypes often lighter ochre or red brown), touching main capsula. Anal tube and anal style reddish.

Female paratypes (**Fig 2.4A**): Female body and wing colouration is close to the lightest paratype males. On the thorax, all three females with paired lighter spots on the cruciform elevation and its lateral depressions and fine drawings along the parapsidal sutures. On the abdomen, dark spots on the sternites highly variable in dimensions and intensity. On fore wings, the basal veins less variable in colouration, from ochre to brown and never black, one specimen even with basal junction of anal veins yellowish instead of dark. Ratio of body length to ovipositor length (including sheath) 3.2 ± 0.2.

Acoustic behaviour

Diagnosis

The calling song of *C. sibillae* is very similar to *C. cerdaniensis* s. str. in qualitative aspects (**Fig 2.6B-C**); however, it is best distinguished by the speed of the fast phrase (phrase 3), which is slower in the new species and by the number of timbal movements of the ungrouped, short echemes forming this phrase (> 6.5 syllables, valid for 93% of investigated individuals in *C. sibillae*, 0% in *C. cerdaniensis* s. str.). Other species of the *C. cerdaniensis* species group differ qualitatively in the fast phrase 3: in *C. cantilatrix* the fast phrase is missing in the calling song and in *C. anapaistica* echemes of the fast phrase are grouped.

Composition of calling song

Recordings of 22 individuals of *C. sibillae* were analysed in detail in a perch temperature range of 23-28 °C (T_{mean} = 25.4 °C). They were compared with eight recordings of *C. cerdaniensis* s. str. in the same temperature range (T_{mean} = 25.0 °C). *Cicadetta sibillae* recordings originate from all five metapopulations (with a minimum of two and a maximum of six recordings), and the *C. cerdaniensis* s. str. recordings come from the most important local populations known in Osseja and Py (Puissant, 2006; eastern Pyrenees, **Fig 2.2**). Another 62 sound samples of *C. sibillae* and 16 of *C. cerdaniensis* s. str. were analysed for at least one variable in a broad temperature range.

The song structure is composed of two clearly distinct phrases (PH_2 and PH_3). A typical phrase 1 (PH_1) as in *C. cerdaniensis* s. str. (Puissant & Boulard, 2000) and as in *C. a. anapaistica* (Chapter 1 = Hertach, 2011) is very rare. Slow phrase 2 (PH_2) consists of a longer series of echemes composed of a low intensity part (FP_2) and a completely connected loud short part (SP_2), which is comparable with the main phrases in *C. cantilatrix, C. cerdaniensis* s. str. and *C. anapaistica*. Typical echemes slightly differ in qualitative or quantitative aspects between some taxa (**Fig 2.6**, right). Phrase 3 (PH_3) is a faster repetition of evenly distributed short single echemes, which is similar to the pattern in *C. cerdaniensis* s. str. Phrase 2 and phrase 3 are alternating, but the song never starts or ends with phrase 3 (**Fig 2.6C**). Interestingly, phrase 3 is almost equal in mean duration for all investigated taxa of this species group (PH_3; **Table 2.2**).

Measurements of the calling song characters within the defined temperature range in *C. sibillae* are reported in comparison with *C. cerdaniensis* s. str. in **Table 2.2**. The box plots of important time variables measured and the linear discriminant analysis show significant differences between the two taxa (**Fig 2.7**; **Table 2.3**).

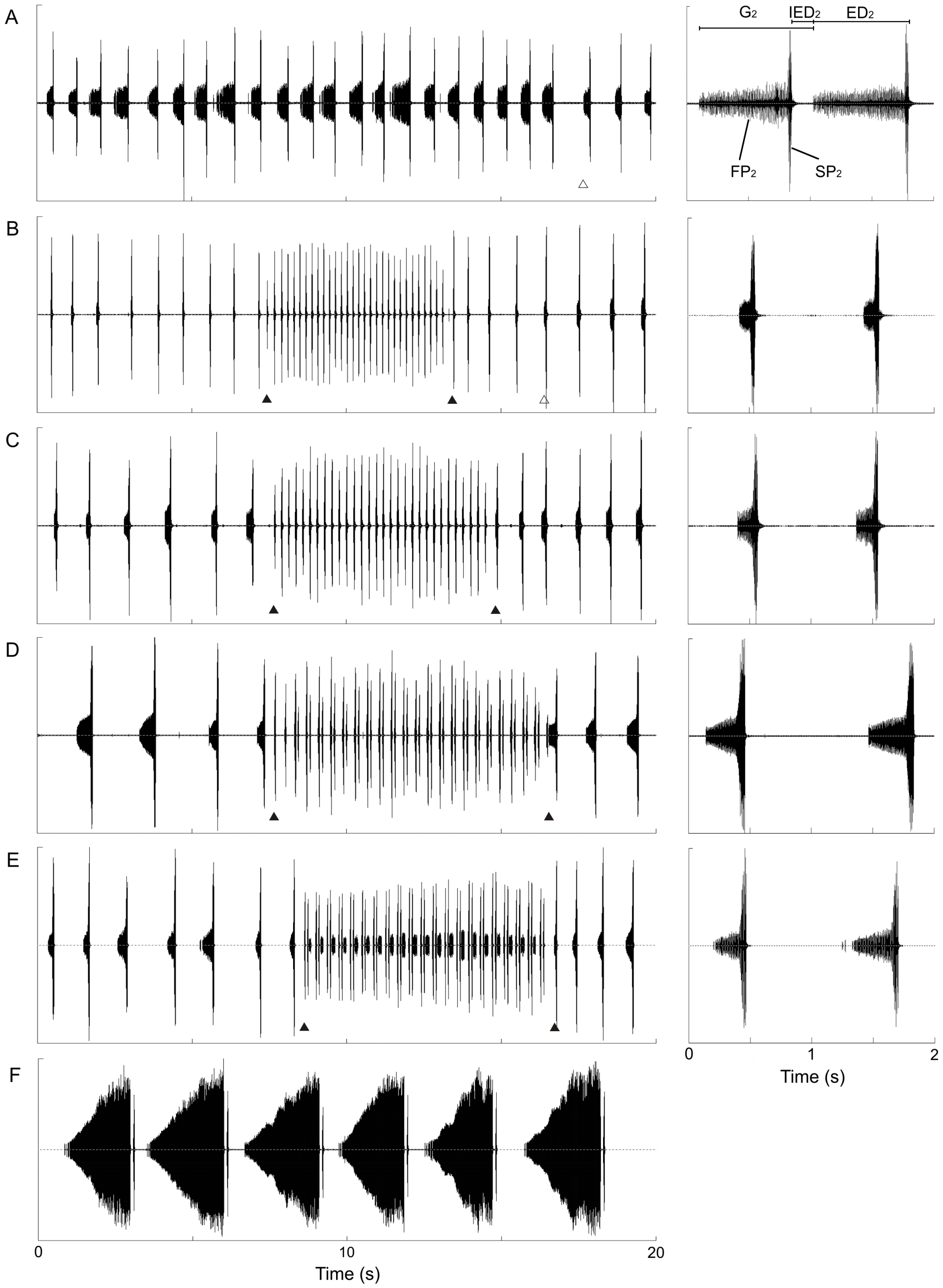

Fig 2.6. Oscillograms (time versus amplitude) of typical calling songs in the *Cicadetta cerdaniensis* group. (A) *Cicadetta cantilatrix* (phrases 2–1, Basel-Land, Switzerland), (B) *Cicadetta cerdaniensis* s. str. (phrases 1–3–1–2, eastern Pyrenees, France), (C) *Cicadetta sibillae* (phrases 2–3–2, Tuscany, Italy), (D) *Cicadetta anapaistica lucana* (phrases 2–3–2, Basilicata, Italy), (E) *Cicadetta anapaistica anapaistica* (phrases 2–3–2, Calabria, Italy). (F) *Cicadetta brevipennis* song pattern for comparison. 20 s sections on the left, 2 s sections of phrase 2 on the right with terms of variables. Triangles indicating the beginning of the phrases, black for typically clear transitions with phrase 3 involved, white for smooth transitions between phrases 1 and 2. For more details of phrases 3 see **Figs 2.8C-F and 2.11**.

Table 2.2. Measured variables of calling songs in the *Cicadetta cerdaniensis* group for a 23-28 °C perch temperature range. Mean values between individuals ± SD. N_{ind} = number of individuals, N_{obs} = number of measured/observed values. Notes: N_{obs} valid for subsequent variables, including power and carrier frequency domains of the same phrase, exceptions specified. PH_x values measured for more individuals and over a larger temperature range. Only the normal patterns are included in variable *Cicadetta anapaistica* songs ('luca_norm' and 'anap_norm').

	Variable	***C. cerdaniensis* s. str. (n_{ind} = 8)**	***C. sibillae* (n_{ind} = 22)**	***C. a. lucana* (n_{ind} = 8)**	***C. a. anapaistica* (n_{ind} = 10)**
Phrase 2(+1) Durations [s]	ED$_{1+2}$	0.097 ± 0.038 (n_{obs} ≈ 1350)	0.213 ± 0.083 (n_{obs} ≈ 2700)	0.284 ± 0.094 (n_{obs} ≈ 1000)	0.213 ± 0.069 (n_{obs} ≈ 2100)
	FPD$_2$	0.065 ± 0.031	0.169 ± 0.075	0.225 ± 0.087	0.161 ± 0.059
	SPD$_2$	0.036 ± 0.007	0.046 ± 0.008	0.059 ± 0.008	0.053 ± 0.011
	IED$_{1+2}$	0.967 ± 0.204	1.131 ± 0.218	1.138 ± 0.253	0.965 ± 0.135
	G$_{1+2}$	1.064 ± 0.238	1.344 ± 0.261	1.423 ± 0.275	1.258 ± 0.284
	PH$_2$	33.9 ± 11.5 (n_{obs} = 118)	23.1 ± 7.1 (n_{obs} = 195)	35.4 ± 23.1 (n_{obs} = 130)	33.6 ± 35.1 (n_{obs} = 313)
Phrase 3 Durations [s]	ED$_{3\_1}$	0.034 ± 0.004 (n_{obs} ≈ 1000)	0.050 ± 0.009 (n_{obs} ≈ 3400)	0.039 ± 0.010 (n_{obs} ≈ 650)	0.039 ± 0.007 (n_{obs} ≈ 1200)
	IED$_3$	0.208 ± 0.023	0.236 ± 0.035		
	IED$_{3\_1/2}$			0.076 ± 0.012	0.058 ± 0.005
	ED$_{3\_2}$			0.029 ± 0.005	0.034 ± 0.005
	IED$_{3\_2/1}$			0.223 ± 0.018	
	IED$_{3\_2/3}$				0.024 ± 0.011
	ED$_{3\_3}$				0.081 ± 0.009
	IED$_{3\_3/1}$				0.096 ± 0.013
	G$_3$	0.242 ± 0.027	0.286 ± 0.043	0.368 ± 0.023	0.332 ± 0.019
	PH$_3$	7.4 ± 1.9 (n_{obs} = 102)	7.1 ± 2.5 (n_{obs} = 185)	7.2 ± 1.7 (n_{obs} = 130)	8.0 ± 3.8 (n_{obs} = 269)
Power [dB]	SPP$_2$ - FPP$_2$	13.5 ± 2.1	11.8 ± 2.2	9.9 ± 2.2	10.8 ± 2.5 (n_{obs} ≈ 250)
Centre Frequency [kHz]	FPF$_2$	13.0 ± 0.7	13.3 ± 0.8	13.5 ± 0.5	14.3 ± 1.0 (n_{obs} ≈ 250)
	SPF$_2$	14.0 ± 1.2	14.2 ± 1.0	14.2 ± 0.7	14.9 ± 1.0
	EF$_{3\_1}$	13.8 ± 1.2	14.0 ± 1.1	13.9 ± 0.6	14.4 ± 1.0
	EF$_{3\_2}$			13.8 ± 0.7	14.4 ± 1.0
	EF$_{3\_3}$				14.0 ± 1.0
1. Quartile Frequency [kHz]	FPF$_2$	12.3 ± 0.7	12.5 ± 0.6	12.7 ± 0.3	13.3 ± 0.9
	SPF$_2$	13.1 ± 1.1	13.3 ± 0.8	13.3 ± 0.5	13.8 ± 1.0
	EF$_{3\_1}$	13.0 ± 1.0	13.1 ± 0.8	13.1 ± 0.4	13.5 ± 1.0
	EF$_{3\_2}$			13.0 ± 0.5	13.5 ± 1.1
	EF$_{3\_3}$				13.0 ± 1.0
3. Quartile Frequency [kHz]	FPF$_2$	14.0 ± 1.0	14.4 ± 1.0	14.5 ± 0.6	15.3 ± 1.0
	SPF$_2$	15.1 ± 1.4	15.2 ± 1.1	15.1 ± 0.8	16.0 ± 0.8
	EF$_{3\_1}$	14.8 ± 1.3	15.0 ± 1.2	14.8 ± 0.8	15.6 ± 0.9
	EF$_{3\_2}$			14.7 ± 0.8	15.5 ± 0.9
	EF$_{3\_3}$				15.2 ± 1.0

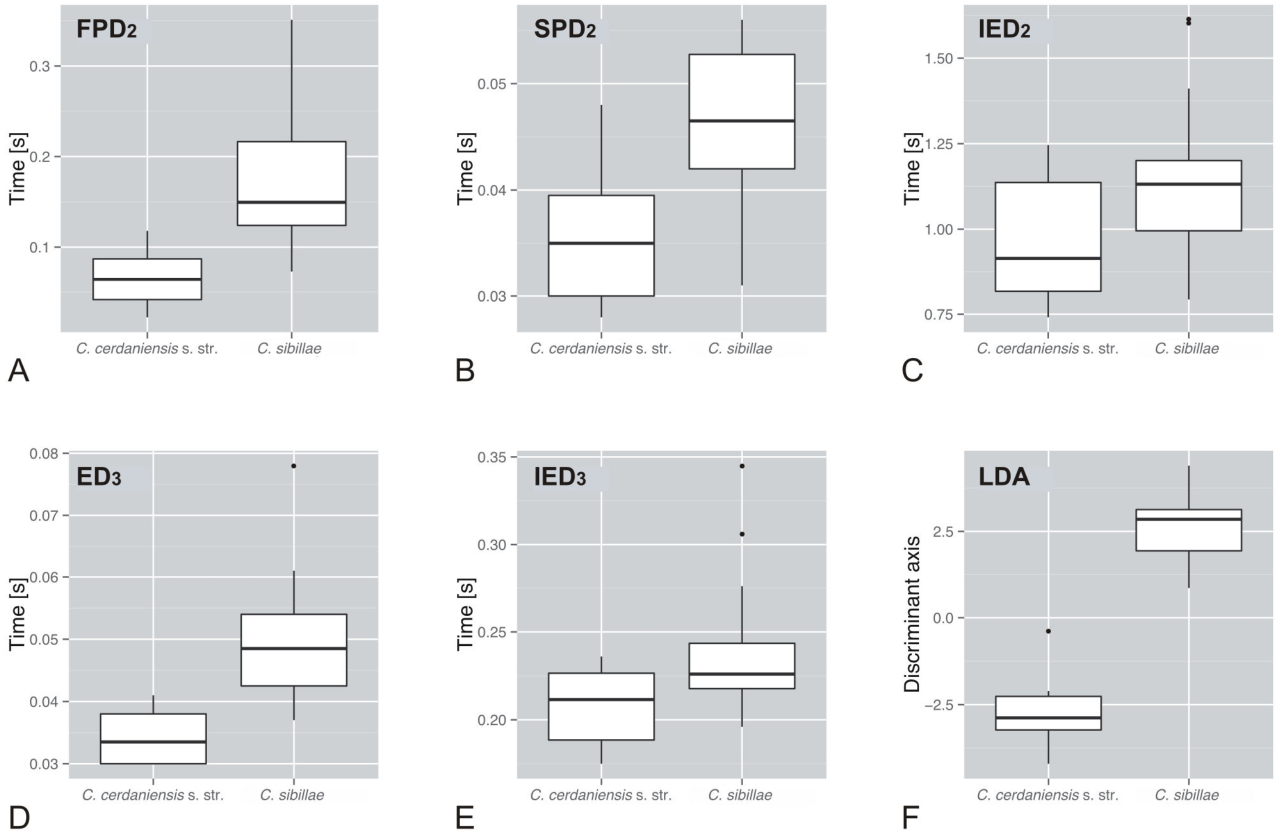

Fig 2.7. Song statistics of *Cicadetta cerdaniensis* s. str. (eight individuals) and *Cicadetta sibillae* (22 individuals) for a 23-28 °C perch temperature range. Box plots of important song duration variables and of the linear discriminant analysis (LDA) between 18 song variables. ED = echeme durations, IED = inter-echeme durations, FP = first echeme part, SP = second echeme part.

We tested in detail the assumptions from the field work that phrases 3 are emitted with different rates in *C. cerdaniensis* s. str. and *C. sibillae* (**Fig 2.8**). In fact, rates and durations are specific and correlate to the perch temperature. G_3 but also ED_3 are highly significant between species [**Fig 2.8A**, ANCOVA: F_{temp} (1, 85) = 38.8, $p_{temp} < 0.001$, $F_{species}$ (1, 85) = 66.8, $p_{species} < 0.001$ for G_3 and F_{temp} (1, 85) = 14.0, $p_{temp} < 0.001$, $F_{species}$ (1, 85) = 103.9, $p_{species} < 0.001$ for ED_3]. *Cicadetta sibillae* echemes have approximately the same duration as *C. cerdaniensis* s. str. when the temperature is shifted by +10 °C. Looking for a less temperature-dependent character, we counted the timbal movements of 20 short echemes for each individual, which turned out to be very constant within species and different between them: 5.40 ± 0.59 syllables in *C. cerdaniensis* s. str. and 7.64 ± 0.89 in *C. sibillae* [**Fig 2.8B**, ANCOVA: F_{temp} (1, 73) = 2.8, $p_{temp} = 0.097$, $F_{species}$ (1, 73) = 113.6, $p_{species} < 0.001$]. The mean values of the five metapopulations in *C. sibillae* vary in a very small range from 7.5 syllables (Campanian, Insubrian and northern Apenninian) to 7.8 (western Alpine) and 7.9 (central Apenninian), and are not correlated with latitude (**Table 2.4**). Out of 79 individuals investigated, only four of each species fall into the category with mean values between 6.0 and 6.4 and are impossible to separate.

Song variables are constant and without disruption between the different metapopulations in *C. sibillae* with one clear exception (**Table 2.4**). A spatial gradient is recognizable for the echeme durations in phrases 1 and 2 (ED_{1+2}) from north to south, with echemes of double durations on average in the Campanian and central Apenninian metapopulations, compared with the northern echemes. The high correlation with latitude indicates a cline. ED_{1+2} and IED_{1+2} are well and positively correlated in most individuals (linear regressions: $R^2_{median} = 0.54$, $n_{ind} = 22$), which is stronger than in any other taxa of the group (compare Chapter 1 = Hertach, 2011).

Echeme power (EP) is slightly reduced from that in phrases 1 and 2 (E_{1+2}) to that in phrase 3 (E_3) in both species being compared [1.3 ± 1.3 dB for *C. sibillae* (mean values of 39 individuals) and 1.1 ± 1.2 dB for *C. cerdaniensis* s. str. (mean values of 14 individuals)]. The carrier frequency domain (EF) is comparable with others from the *C. montana* complex, with a broad intraspecific variability, and is not suitable for species delimitation within the investigated group (**Tables 2.2 and 2.3**).

The song pattern of *C. sibillae* is very stable. No qualitative song variability and aberrations have been detected.

Table 2.3. Coefficients of the linear discriminant analysis (LDA) between 18 song variables in *Cicadetta cerdaniensis* s. str. and *Cicadetta sibillae.*

Variable	LD1
ED_{1+2}	-19.522
FPD_2	19.664
SPD_2	13.447
IED_{1+2}	2.716
FPF_2 (centre)	0.002
FPF_2 (1. quartile)	-0.003
FPF_2 (3. quartile)	0.002
SPF_2 (centre)	-0.005
SPF_2 (1. quartile)	0.006
SPF_2 (3. quartile)	0.003
SPP_2 FPP_2	0.011
ED_3	-43.834
IED_3	-1.534
EF_3 (centre)	0.004
EF_3 (1. quartile)	-0.005
EF_3 (3. quartile)	-0.003
EP_{1+2} EP_3	0.285
Syllables/E_3	1.913

Table 2.4. Spatial variation of selected song variables in *Cicadetta sibillae* as functions of the geographical latitude with linear regressions (23-28 °C). Song variables were normalised and standardised for better comparability. Example: ED_{1+2} values clearly decrease with increasing latitude and form a well-correlated cline, whereas ED_3 are independent of latitude and very constant between locations and metapopulations.

Variable	Slope	R^2
ED_{1+2}	-0.457	0.527
IED_{1+2}	-0.286	0.207
ED_3	0.028	0.002
IED_3	0.122	0.038
FPF_2 (centre)	0.207	0.108
Syllables/E_3	-0.055	0.007

Etymology

The species name '*sibillae*' is genitive of '*sibilla*', and is derived from the locality name 'Monte Sibilla' in the Monti Sibillini mountain group (Marche Region). The geographically closest local population is situated at the northern edge of the central Apenninian metapopulation. In addition, the name is indicative to the wife of the first author, with her first name being 'Sibille'. Finally, we hope that subsequent researchers judge our taxonomic conclusions in accordance with the origin of the word, which designates a wise or a truth-speaking woman.

Distribution and ecology

Cicadetta sibillae is one of the most widely distributed cicadas in Italy and reaches the southernmost parts of Switzerland and probably also France in the Maritime Alps, close to the Italian border (S. Puissant, pers. comm.). Our database consists of 267 locations, with observations of more than one thousand individuals (**Appendix S2.3**). The distribution range is well known and is split into at least five disjunct areas (**Figs 2.2 and 2.9**). The Insubrian metapopulation in the north reaches from Lake Garda and Bolzano to Lake Maggiore, and contains all Swiss populations in southern Ticino and the Mesocco Valley. Populations are scattered over relatively small suitable habitats but some local populations are very rich in individuals, as is the case at Monte San Giorgio (Meride, Ticino; **Fig 2.10B**), Monte Chiusarella (Varese), or Lodrino (Trompia Valley). The western Alpine metapopulation is a relictual fragment in very few locations around Lanzo, the Susa Valley, the Monferrato Hills and the Maira Valley. The most important occurrences are found in the northern Apennine, where the new species is generally the most frequent cicada. This metapopulation reaches at least from the Ormea region (Piedmont) in the west to the Monte Falterona and Campigna forest (Tuscany and Emilia-Romagna) in the east. Southwards, *Cicadetta sibillae* is abruptly replaced by *C. brevipennis*. The new species itself competitively excludes *C. brevipennis* in a central Apenninian

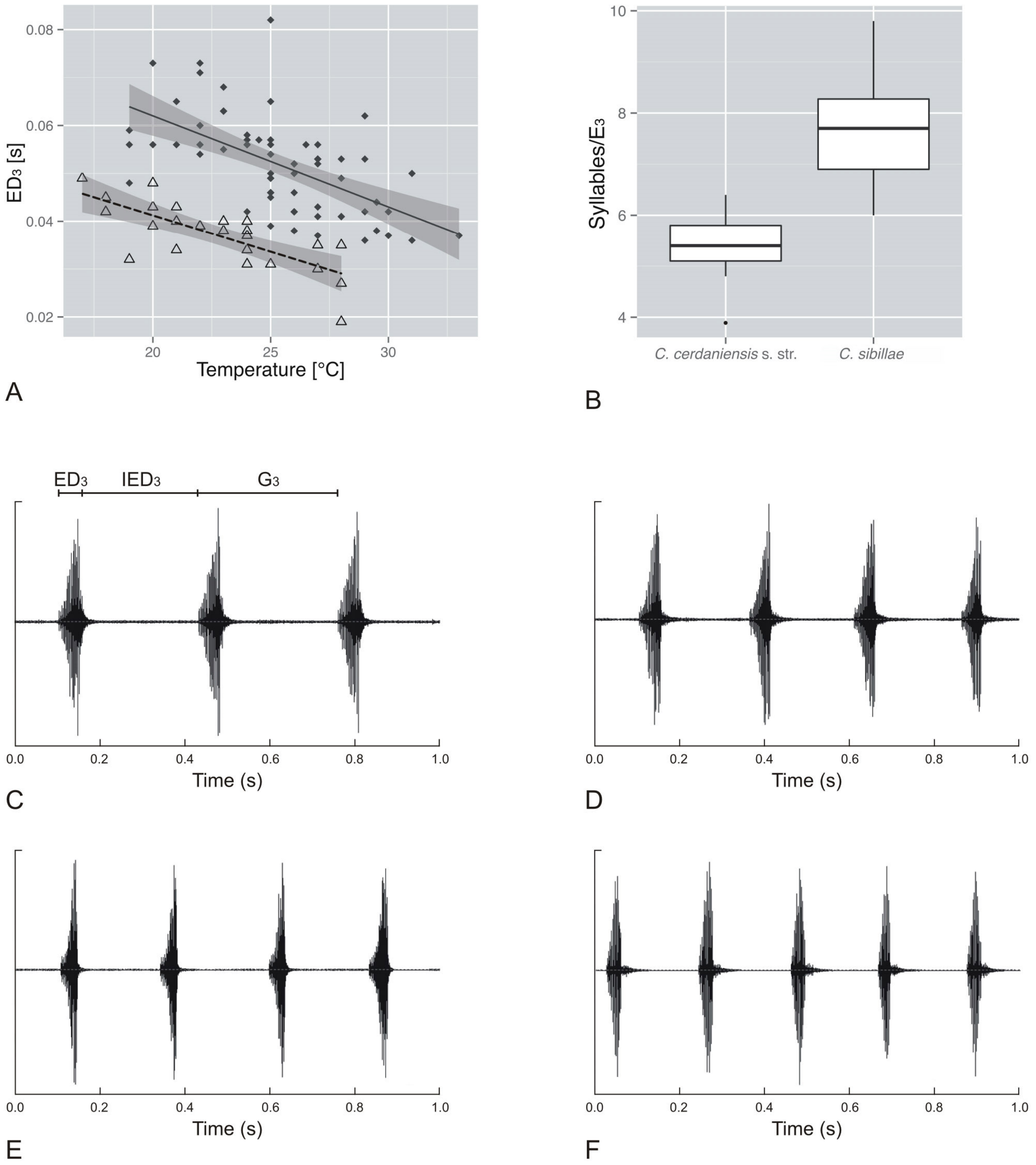

Fig 2.8. Echemes of phrase 3 in *Cicadetta cerdaniensis* s. str. (transparent triangles) and *Cicadetta sibillae* (dark diamonds). (A) Duration of echemes as a function of perch temperature, with linear regression trend lines and standard errors (grey areas). (B) Box plot of the number of syllables per echeme given as mean values of 20 echemes for each individual. (C-F) Oscillograms (time versus amplitude) close to the linear regression trend lines of (A): (C) *C. sibillae* at 23 °C perch temperature (with measured duration terms, northern Apenninian metapopulation), (D) *C. sibillae* at 28 °C (western Alpine metapopulation), (E) *C. cerdaniensis* s. str. at 23 °C (eastern Pyrenees), (F) *C. cerdaniensis* s. str. at 28 °C (eastern Pyrenees).

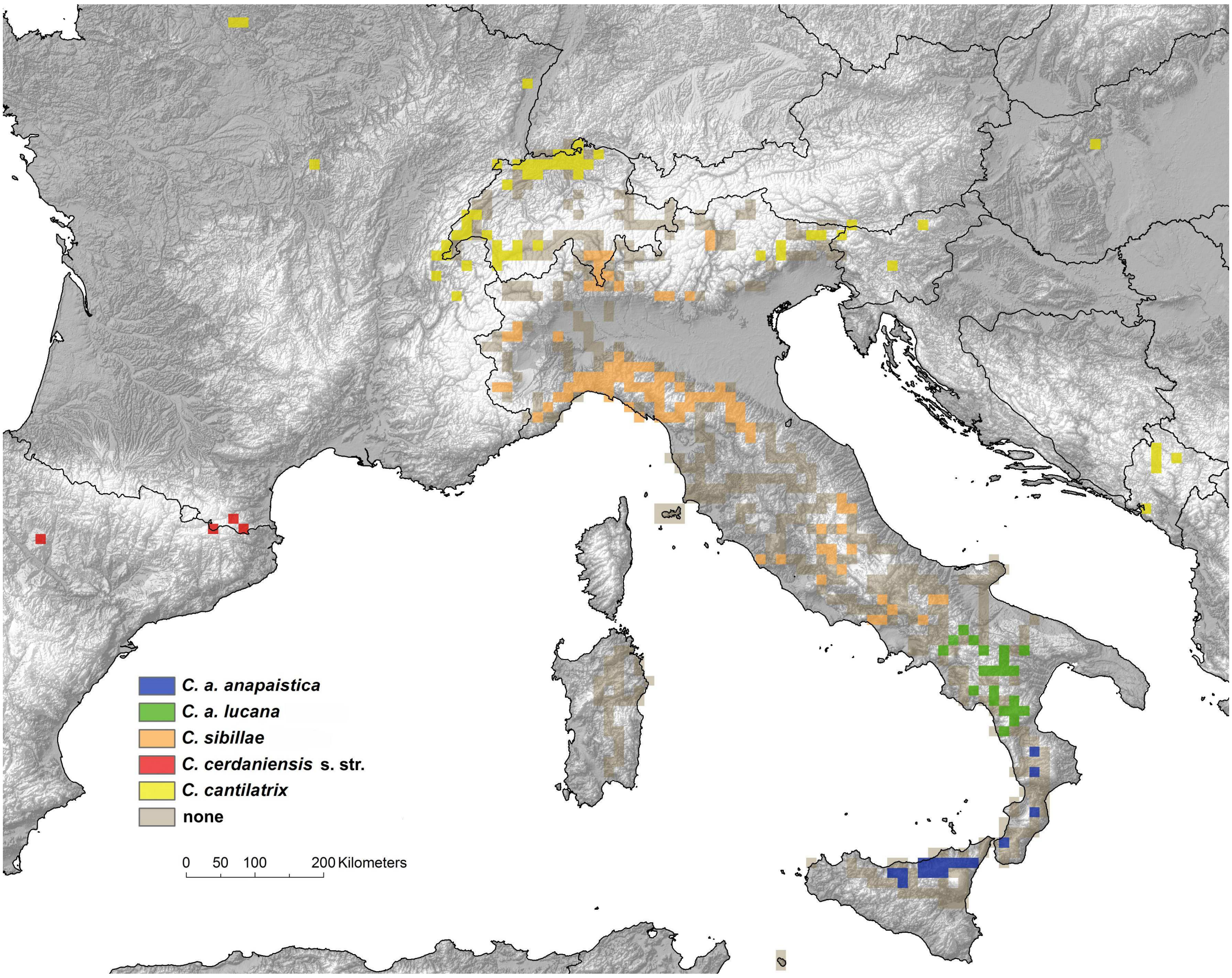

Fig 2.9. Distribution map of the species of the *Cicadetta cerdaniensis* song group (15 x 15 km^2 grid cells). Notes: Taxa colours correspond to **Fig 2.3**. Cells recently checked in the framework of the Swiss and Italian projects of the first author without detection of a taxon of this group are brownish. The *Cicadetta cantilatrix* distribution range is not completely visible. Published records of *Cicadetta cantilatrix* by Sueur & Puissant (2007), Trilar & Holzinger (2004), Hertach (2007), Brua & Hugel (2008), Trilar & Gogala (2012) and Hertach & Nagel, 2013 (= Chapter 6).

metapopulation from the Sibillini Mountains south- and westwards to the Simbruini Mountains. The southernmost metapopulation in Campania contains only a few scattered observations.

The altitudinal range of *C. sibillae* is from 20 m asl (Terme di Suio, Lazio) to 1430 m asl (Denti della Vecchia, Lombardy/Ticino), with a maximum abundance between 400 and 900 m asl. In peripheral metapopulations, the species is a habitat specialist. The Insubrian metapopulation prefers sparsely or semi-open woodland characterised by Downy Oak (*Quercus pubescens*), Turkey Oak (*Quercus cerris*), Hop Hornbeam (*Ostrya carpinifolia*) and Manna Ash (*Fraxinus ornus*) (**Fig 2.10B**). Western alpine cicadas are often found in sparse woods of the Black Pine (*Pinus nigra*) and the Scots Pine (*Pinus sylvestris*). The central Apenninian and

Campanian metapopulations are normally restricted to habitats with mesophilous, mountainous conditions, often on slopes or floors of smaller valleys. Conversely, the species is more of a generalist in the northern Apenninian metapopulation (compare **Fig 2.10A**). It has been found in Mediterranean shrub land influenced by fire near the coast as well as in vigorous European Beech forests (*Fagus sylvatica*). Nevertheless, the majority of populations are found in ecotone habitats with sparse oak woods and a well-developed grass layer. Individuals sing in a broad vertical range from a few centimetres up to 10 m above ground, but with a clear maximum between 2 and 5 m.

Cicadetta sibillae was observed syntopically with up to seven other cicada species in sparse oak forests (*Quercus* spp.) in the Apennine (Santerno Valley, Tuscany). Conversely, in the Alps and mesophilous, temperate habitats of the Apennine Mountains, it is sometimes the single cicada species. The new species was phenologically first recorded on 27 May (2005 and 2006, Ticino) and last on 1 August (2008, Liguria).

Cicadetta anapaistica lucana Hertach, 2015

Type material

The type series consists of 16 males and two females representing southern, central and northern populations. It is kept in the NHMB (holotype), ETHZ, NMBE and in one private collection.

Holotype male: Verbatim label information: “nördl. Mezzana Torre, BASI, I/40.0054°/16.1681°, 1030 m asl/18.7.2013, leg. T. Hertach/Collection Code No. 19.012” (label rectangular, white, printed) and “HOLOTYPUS ♂/*Cicadetta anapaistica lucana* ssp. n./Hertach 2014” (label rectangular, light red with dark red margin, printed; NHMB).

Paratypes: All paratypes with labels “PARATYPUS XX Y, *Cicadetta anapaistica lucana* ssp. n. Hertach 2014” (label rectangular, white with red margin, printed) at which ‘XX’ is the number of the paratype and ‘Y’ the sex of the specimen. Number ‘12’ does not exist. **Paratypes males, dark morph**: Timpa Falascoso, Viggianello, BASI, I, 40.0184°/16.1107°, 950 m asl, 7.7.2010, leg. T. Hertach (paratype 2, coll. Hertach); Serra Alberigo, Viggianello, BASI, I, 40.0014°/16.1171°, 1020 m asl, 7.7.2010, leg. T. Hertach (paratype 4, coll. Hertach); Timpone Rotondella, Morano Calabro, CALA, I, 39.8606°/16.0953°, 980 m asl, 17.7.2013, leg. T. Hertach (paratype 6, coll. Hertach); Serra Alberigo, Viggianello, BASI, I, 40.0012°/16.1170°, 1040 m asl, 17.7.2013, leg. T. Hertach (paratype 7, coll. Hertach); Serra Alberigo, Viggianello, BASI, I, 39.9980°/16.1218°, 1070 m asl, 17.7.2013, leg. T. Hertach (paratypes 8, 9 and 10, coll. Hertach); N Mezzana Torre, BASI, I, 40.0054°/16.1681°, 1030 m asl, 18.7.2013, leg. T. Hertach (paratype 13, coll. NMBE); N Marsico Nuovo, BASI, I, 40.4659°/15.7380°, 1130 m asl, 20.7.2013, leg. T. Hertach (paratypes 14 and 15, coll. Hertach); N Morra de Sanctis, CAMP, I, 40.9365°/15.2410°, 820 m asl, 22.7.2013, leg. T. Hertach (paratype 17, coll. ETHZ). **Paratypes males, light morph:** Timpa Falascoso, Viggianello, BASI, I, 40.0184°/16.1107°, 950 m asl, 7.7.2010, leg. T. Hertach (paratype 1, coll. Hertach); Serra Alberigo, Viggianello, BASI, I, 40.0014°/16.1171°, 1020 m asl, 7.7.2010, leg. T. Hertach (paratype 3, coll. ETHZ); W Acerno, Monti Picentini, CAMP, I, 40.7545°/15.0406°, 600 m asl, 14.7.2010, leg. T. Hertach (paratype 5, coll. NMBE); N Morra de Sanctis, CAMP, I, 40.9365°/15.2410°, 820 m asl, 22.7.2013, leg. T. Hertach (paratype 18, coll. Hertach, putative hybrid with *C. sibillae*). **Paratypes females:** Serra Alberigo, Viggianello, BASI, I, 39.9980°/16.1218°, 1070 m asl, 17.7.2013, leg. T. Hertach (paratype 11, coll. ETHZ); N Marsico Nuovo, BASI, I, 40.4659°/15.7380°, 1130 m asl, 20.7.2013, leg. T. Hertach (paratype 16, coll. Hertach).

Morphology

Diagnosis

Cicadetta anapaistica lucana occurs in two differently coloured morphs present in the same local populations. The dark-coloured morph resembles all other described *C. montana* complex species. The vast majority of *C. a. lucana* specimens, like *C. a. anapaistica*, are separated from *C. cerdaniensis* s. str. by the predominantly dark basal junction of the anal veins (100% for *C. a. lucana* dark morph and *C. a. anapaistica*, 94.4% for *C. a. lucana* versus 5.0% for *C. cerdaniensis* s. str.). From *C. montana* s. str., many specimens are distinguished by the outer rim of costa darker than the inner rim and the radial/subcostal veins (77.8 versus 9.5%, chi-square contingency test: $\chi^2 = 27.9$, $p < 0.001$ for *C. a. lucana* and 89.5% versus 9.5%, $\chi^2 = 51.1$, $p < 0.001$ for *C. anapaistica*). *Cicadetta anapaistica lucana* have on average a stockier habitus in comparison with *C. sibillae*, with significantly broader wings (**Fig 2.5C**, Wilcoxon-Mann-Whitney rank sum test: $W = 270$, $p = 0.009$) and broader abdomen (**Fig 2.5D**, Wilcoxon-Mann-Whitney rank sum test: $W = 299$, $p = 0.002$). Additionally, in *C. anapaistica* the pronotal collar is frontally often broader in relation to the head than in *C. sibillae*, caused by a more distinct convexity (**Fig 2.5B**, Wilcoxon-Mann-Whitney rank sum test: $W = 586.5$, $p < 0.001$, for *C. anapaistica* versus *C. sibillae* and $W = 279.5$, $p = 0.010$, for *C. a. lucana* versus *C. sibillae*). The light-coloured morph is probably separated from any other described species. Closest to *C. fangoana*, an endemic species of Corsica, it seems to be distinguishable by the more yellowish or ochre than reddish markings on the thorax and by the ochre colouration of the central suture and the frontal margin of the pronotum.

Description

Two colour morphs exist, with 78% dark (including holotype and females) and 22% light. Dark morphs were captured in four and light ones in three local populations.

Measurements: Body length: 18.3 mm in holotype specimen, 17.7 ± 0.7 mm (mean ± SD) in male dark morph paratypes, 17.7 ± 1.0 mm in male light morph paratypes, 20.2 mm and 19.0 mm in female paratypes. Body width (abdomen, tergite II): 6.5 mm in holotype specimen, 5.9 ± 0.2 mm in male dark morph paratypes, 5.8 ± 0.3 mm in male light morph paratypes, 6.1 mm and 6.2 mm in female paratypes. Fore wing length: 19.9 mm in holotype specimen, 20.0 ± 0.8 mm in male dark morph paratypes, 19.4 ± 0.4 mm in male light morph paratypes, 22.5 and 20.3 mm in female paratypes. Fore wing width: 8.7 mm in holotype specimen, 8.2 ± 0.4 mm in male dark morph paratypes, 8.0 ± 0.3 mm in male light morph paratypes, 9.5 and 8.8 mm in female paratypes.

Male holotype (**Fig 2.4B**): The holotype specimen of *C. a. lucana* fits the detailed description of the holotype of *C. a. anapaistica* (Chapter 1 = Hertach, 2011) with the following differences: On the head, mentum dark brown (as in some paratypes of *C. a. anapaistica*). On the thorax, narrow brownish band at posterior margin (as in some paratypes of *C. a. anapaistica*) and lateral depressions of cruciform elevation posterior with an ochre spot, meracanthus directed caudally not laterally (as in some recently captured specimens of *C. a. anapaistica*). On the abdomen, sternites and caudal margins of tergites red brown (as in some paratypes of *C. a. anapaistica*). On fore wings, basal median vein ochre up to the crossveins and the node (as in some paratypes of *C. a. anapaistica*). Basal junction of anal veins dark or black, as in *C. a. anapaistica*, but not reported there. In the genitalia, basal lobe of pygofer brown.

Male paratypes of dark morph: Dark male paratypes differ from the holotype of *C. a. lucana* and/or *C. a. anapaistica* (with holotype and paratypes), as follows. On the head, postclypeus rarely almost completely black. On the thorax, lateral depressions of cruciform elevation posterior normally without an ochre spot, as in *C. a. anapaistica*, meracanthus variable in shape and size. Rarely, lateral margin of pronotal collar frontal to the angles scarcely convex in shape and not clearly recessed (compare **Fig 2.5B**). On fore wing, colour combinations of costal and radial/subcostal veins differ sporadically, especially the exterior rim of costa and radius/subcosta of same colour. Rarely, cubitus anterior vein and cubitus posterior vein/first anal vein almost black. Pterostigma sometimes brownish. Median and cubitus anterior vein fused on both sides for approximately 1 mm in two paratypes. Distal veins rarely with seven or nine apical cells instead of eight. In genitalia, upper lobes of pygofer rarely more angled.

Male paratypes of light morph (**Fig 2.4B and G**): Contrary to the dark morph several parts of the body are ochre or yellowish in colour, instead of black. On the head, postclypeus towards the frontoclypeal suture and the anteclypeus, as well as sometimes the surrounding of the compound eyes, ochre. On the pronotum, central suture, frontal margin and pronotal collar appearing as broad ochre bands. On the mesonotum, two triangular, ochre markings central to the lateral sigilla (in one paratype mesonotum completely ochre except for the submedian and lateral sigillae and the scutal depressions). Cruciform elevation and its lateral depressions predominantly ochre. Ventral side of thorax generally lighter (in one paratype almost completely yellowish, including opercula). On the abdomen, variable ochre fasciae at the tergites in addition to the red brown margins. Legs with light portions more dominant. One paratype with basal junction of anal veins ochre instead of dark at fore wing.

Female paratypes (**Fig 2.4B**): Colouration does not differ from the dark morph described above and is consequently slightly darker than the females of *C. a. anapaistica*. Ratio of body length to ovipositor length (including sheath) 3.1 and 2.9.

Fig 2.10. Italian and Swiss habitats. (A) Habitats of *Cicadetta sibillae* in Tiglieto (Liguria) and (B) at the Monte San Giorgio (Ticino), (C) most important location of *Cicadetta anapaistica lucana* in the mountainous Pollino National Park. (D) Threatened core population habitat of *Cicadetta anapaistica anapaistica* in the Madonie Mountains as a result of overgrazing and soil erosion.

Acoustic behaviour

Diagnosis

The calling song of *C. a. lucana* is intermediate to the songs of *C. cerdaniensis* s. str. and *C. sibillae* on the one hand and *C. a. anapaistica* on the other hand. It is distinguished by the characteristically grouped short echemes in the third phrase (**Fig 2.6D**). *Cicadetta sibillae* and *C. cerdaniensis* s. str. emit evenly distributed (ungrouped) single echemes with no exceptions. The calling song of *C. a. anapaistica* also contains grouped echemes, but normally with a longer faint echeme at the end (**Fig 2.6E**).

Composition of calling song

Similar to *C. sibillae*, recordings of *C. a. lucana* were analysed in detail for a perch temperature range from 23-28 °C (eight individuals, T_{mean} = 24.1 °C), and for some song variables in a broader range (another 43 individuals). They were compared in detail with the data set presented for *C. sibillae* and *C. cerdaniensis* s. str., as well as with ten older recordings of *C. a. anapaistica* (that probably match the same temperature range; **Appendix S2.1**) and another 46 recordings in some variables (most of which included perch temperature measurements).

A phrase 1 pattern as in *C. cerdaniensis* s. str. (Puissant & Boulard, 2000) was never recorded, and similar to *C. a. anapaistica* (Chapter 1 = Hertach, 2011), seems not to be prominent. Phrase 2 (PH_2) consists of a longer series of echemes composed of a low intensity part (FP_2) and a completely connected loud short part (SP_2), which is comparable with the main slow phrases in *C. cantilatrix*, *C. cerdaniensis* s. str., *C. a. anapaistica* and *C. sibillae*. Phrase 3 (PH_3) is intermediate to *C. sibillae*/*C. cerdaniensis* s. str. and *C. a. anapaistica*. It consists of fast repetitions of grouped short echemes. The echemes are grouped normally in pairs of two (E_{3_1} and E_{3_2}) in *C. a. lucana* (pattern 'luca_norm'; **Figs 2.6D and 2.11**). Phrase 2 and phrase 3 are alternating, often for several minutes. The song never starts or ends with phrase 3.

Measurements of the calling song characters in *C. a. lucana* within the 23-28 °C temperature range are reported in **Table 2.2**. Phrase 2 echemes are longer on average in this subspecies than in *C. sibillae* and *C. a. anapaistica.* ED_2 and IED_2 are positively correlated in some individuals, but not in others. G_3 is slightly longer than in *C. a. anapaistica*, although the group does not contain a longer final echeme. Differences in the number of syllables forming the short echemes of phrase 3 are marginal between the two subspecies: 6.42 ± 1.10 (E_{3_1}) and 5.14 ± 0.74 (E_{3_2}) syllables in *C. a. lucana* (mean values of 31 individuals) versus 6.05 ± 1.28 (E_{3_1}) and 5.35 ± 0.79 (E_{3_2}) in *C. a. anapaistica* (mean values of 36 individuals). The dependency on the perch temperature is less obvious in both subspecies of *C. anapaistica* than in *C. sibillae.* Interestingly, this effect seems mainly to be caused by the ability of *C. a. lucana* and especially *C. a. anapaistica* to move the timbals faster than *C. sibillae*, mainly at low temperatures [**Fig 2.12A**, ANCOVA, model with interaction slightly significant for *C. a. lucana* and *C. sibillae*: $F_{species}$ (1, 81) = 16.1, $p_{species} < 0.001$, $F_{temp\ x\ species}$ (1, 81) = 5.1, $p_{temp\ x\ species} = 0.026$; model without interaction for *C. a. anapaistica* and *C. sibillae*: $F_{species}$ (1, 76) = 35.3, $p_{species} < 0.001$]. Syllable rates are approximately 15-20% faster at a perch temperature of 20 °C and form a geographical disruption between the two species (**Fig 2.12B**).

Echeme power (EP) is clearly reduced from phrase 2 (SP_2) to phrase 3 (E_3) in both subspecies [2.3 ± 1.5 dB for *C. a. lucana* (mean values of 27 individuals) and 3.6 ± 1.6 dB for *C. a. anapaistica* (mean values of 40 individuals); see also Chapter 1 = Hertach, 2011]. This power reduction is significantly higher even for *C. a. lucana* compared with *C. sibillae* (Wilcoxon-Mann-Whitney rank sum test: W = 735, p = 0.006). The carrier frequency domain (EF) is broad and not a suitable delimitation criterion for the taxa investigated here (**Table 2.2**).

The song of *C. a. lucana* is variable in qualitative aspects. Two aberrations must be classified. 'Luca_comp' is a more complicated pattern, with three or even four short echemes instead of a pair in phrase 3, produced by a minority of individuals in almost all local populations (**Fig 2.11**). 'Luca_simp' tends to the other direction. In addition to some double echemes a majority of single, ungrouped echemes are emitted. One individual in the Morra de Santis population (Campania) at the northern edge of the distribution area even sang '*sibillae*'-typical ungrouped echemes for several sequences and one 'luca_simp' sequence. Simplified song structures are also known for *C. a. anapaistica* (Chapter 1 = Hertach, 2011) and become more and more equal to the *C. a. lucana* pattern ('anap_simp', 'luca_comp' and 'luca_norm'). They are found in all *C. a. anapaistica* populations. The percentage of individuals simplifying the structure increases by trend from central Sicily to northern Calabria; however, the transition of local populations capable of emitting the final longer faint echeme is abrupt and correlated with topography. The gradient is consequently not a typical cline over the whole species range. Populations capable of producing a longer faint echeme in phrase 3 must be assigned to *C. a. anapaistica*, whereas populations capable of producing solely grouped short echemes are belonging to the new subspecies *C. a. lucana*.

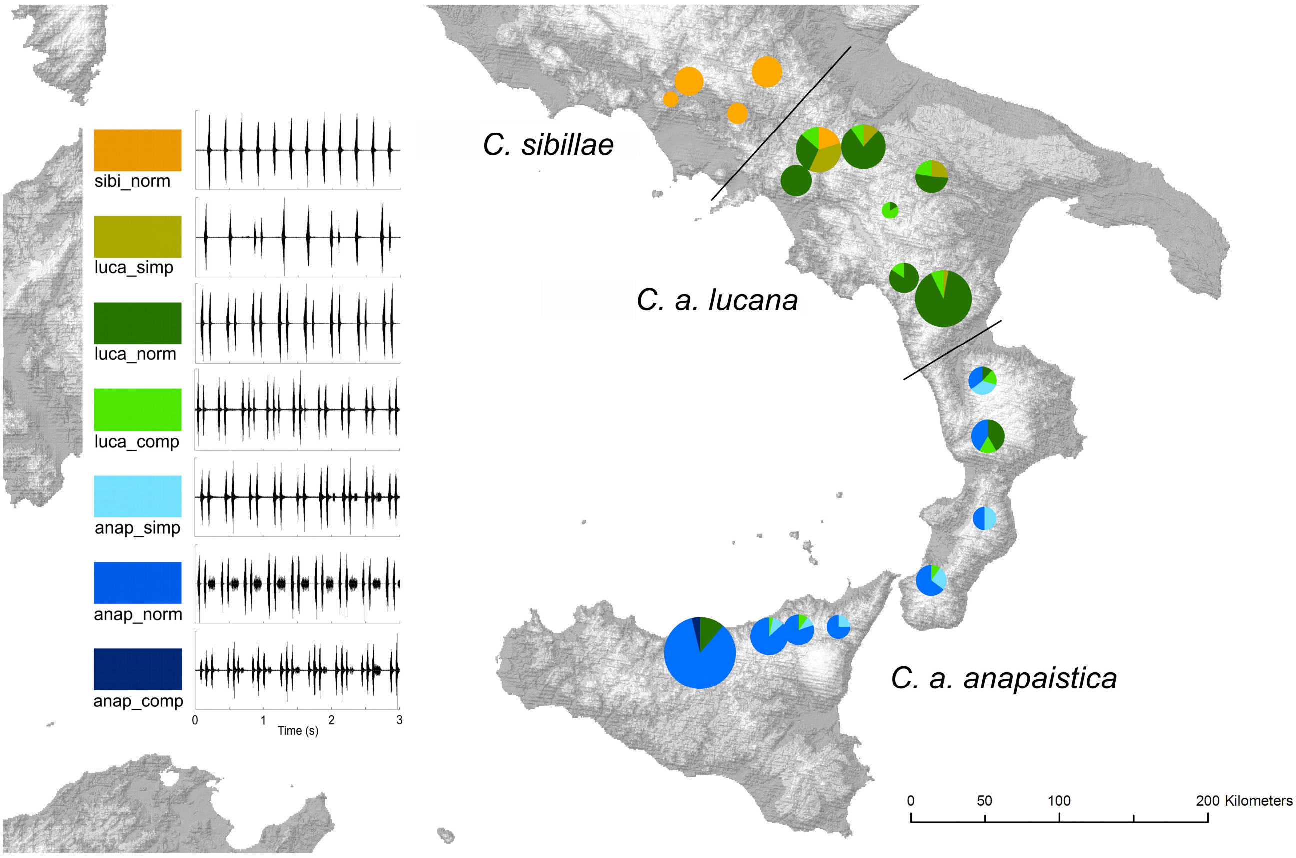

Fig 2.11. Relative portions from the northern simpler to the southern more complex song patterns in phrase 3 in southern Italy. The map includes the contact zones from *Cicadetta sibillae* to *Cicadetta anapaistica lucana* and from *Cicadetta anapaistica lucana* to *Cicadetta anapaistica anapaistica*. The size of the circles relative to the phrases counted ($n_{max} = 111$, $n_{min} = 5$), with a maximum of ten per specimen. Sibi_norm (*C. sibillae*, no variability): 100% single (ungrouped) echemes; luca_simp (simplified *C. a. lucana*): > 50% single (ungrouped) echemes, < 50% grouped double short echemes; luca_norm (normal *C. a. lucana*): > 50% grouped double short echemes; luca_comp (complicated *C. a. lucana*): more than two short echemes per group occurring; anap_simp (simplified *C. a. anapaistica*): < 50% of groups with final longer faint echemes; anap_norm (normal *C. a. anapaistica*): > 50% of groups with double short echemes and final longer faint echemes; anap_comp (complicated *C. a. anapaistica*): > 50% of groups with more than two short echemes and final longer faint echemes. Note: All populations north of the illustrations with typical *C. sibillae* pattern without exception (orange).

Etymology

The majority of all observations of *C. a. lucana* were made in the historical region 'Lucania', which is still vernacular in use and is mainly congruent with the current political region 'Basilicata'. The specific epithet '*lucana*' is the corresponding adjective in female declination and gives the name to this subspecies.

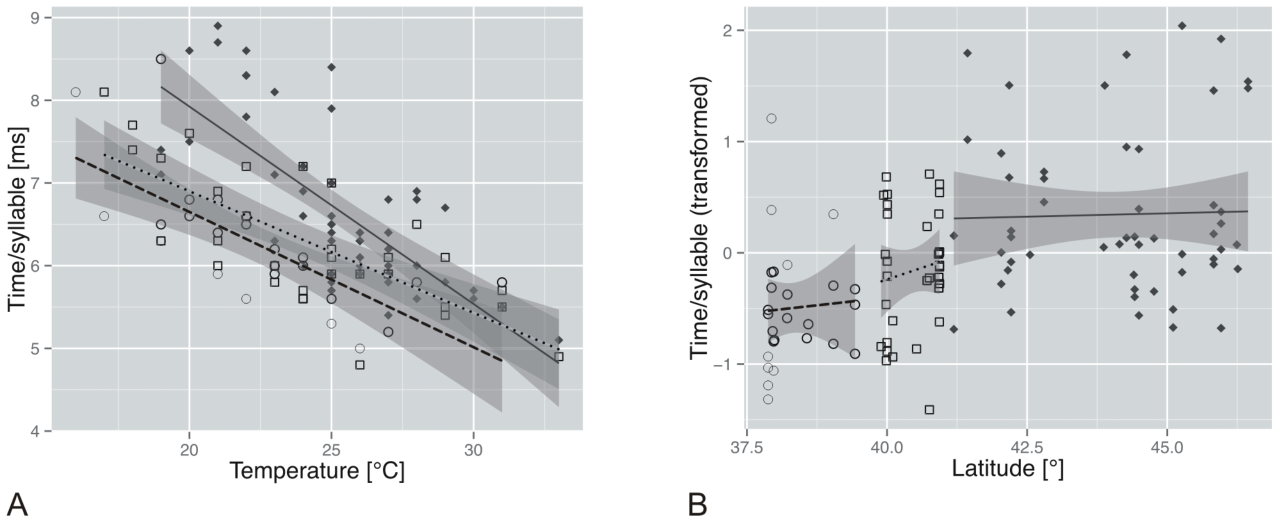

Fig 2.12. Duration of syllables measured at short echemes of phrase 3. *Cicadetta sibillae* (dark diamonds, continuous line), *Cicadetta anapaistica lucana* (transparent squares, dotted line) and *Cicadetta anapaistica anapaistica* (transparent circles, broken line): (A) Dependency on temperature with linear regression trend lines and standard errors (grey areas) and (B) visualisation of the disruption between the two species along the geographical latitude after controlling for temperature (residuals from the global temperature/time trend line).

Distribution and ecology

Cicadetta anapaistica lucana is a subspecies endemic to a southern Italian region of approximately 150 km in length and, at most, 100 km wide (**Fig 2.9**). Local populations reach the southern part of Pollino National Park in the south, the Picentini and Cilento Mountains in the west, the Ofanto River drainage basin in the north and the Difesa Grande Forest near Gravina in Puglia in the east. The distribution is mainly situated in the Basilicata region, but also touches Calabria, Apulia and Campania. Pollino National Park (San Severino Lucano/Viggianello) contained the most important local population by far (**Fig 2.10C**). Additional significant populations were found at Monte Cupolicchio east of Potenza, at Monte Sirino, at Monticchio Lakes, at Morra de Sanctis and at Acerno in the Picentini Mountains.

The altitudinal range goes from 250 m asl (Bosco di Monte Orsino, Potenza) to 1960 m asl (Serra di Crispo, Pollino) with a peak of abundance between 800 and 1100 m asl. The maximum altitude observed was exceptionally high for European cicadas. This subspecies was never found near the coast and we only detected very few individuals below 550 m asl. Favourite habitats are mesophilous, extensively used pastures with various bushes and forest edges or sparse woods (*Quercus* spp. or *Fagus sylvatica*) with a well-developed understory. In the first habitat type, the new subspecies was regularly observed singing in the herb layer, whereas in the second habitat type it can sing high up in the canopy. Many habitats are fragmented by deforestation, especially in the lower north-eastern parts. Some populations at higher altitudes are threatened by overgrazing, for example in Cilento National Park. At lower altitudes, *C. a. lucana* occurs syntopically with up to six different species, but not with any other *Cicadetta* species. At higher altitudes, it is often the single representative of the Cicadidae family. Within the *C. montana* complex only *C. montana* s. str. shares the distribution area. This new subspecies was observed in full activity during the first half of July.

Cicadetta anapaistica anapaistica is not restricted to Sicily, as previously stated (Chapter 1 = Hertach, 2011). Small and isolated local populations were recently found in the Aspromonte Mountains, in the Calabrian Serre and in the Sila Mountains (**Fig 2.9**). They inhabit a narrow ecological niche of more mesophilous habitats than visited before 2013. Additional populations were also found in Sicily, such as in the eastern part of the Nebrodi Mountains and in the Peloritani Mountains. The distribution is significantly larger than expected.

Song-based key for the *Cicadetta cerdaniensis* group

(**compare Figs 2.6 and 2.11**)

1 Main song element composed by the repetition of echemes with a longer low intensity part and a completely connected loud short part, all echeme types shorter than 1.5 s ……………………………… *Cicadetta cerdaniensis* group **2**
\- No echemes existing with a low- and connected high-intensity part or echeme duration longer than 1.5 s …………… ……………………………………………………………………………………………… cicada not belonging to the *C. cerdaniensis* group

2 No fast phrase existing in calling song (but in rarely emitted courtship song) ……………………………………… ***C. cantilatrix***
\- Phrase with fast repetitions of echemes existing in calling song ………………………………………………………………………… **3**

3 Fast-repetition phrase composed by evenly distributed (ungrouped) single echemes ………………………………………… **4**
\- Fast-repetition phrase characterised by echeme groups ………………………………………………………………………………… **5**

4 Central part of fast-repetition phrase composed by echemes with fewer than 6.0 syllables ….. ***C. cerdaniensis* s. str.**
\- Central part of fast-repetition phrase composed by echemes with more than 6.5 syllables ……………………. ***C. sibillae***

5 Fast-repetition phrase characterised by echeme groups of solely short echemes (valid at local population level only) …… ***C. anapaistica lucana***
\- Fast-repetition phrase characterised by echeme groups finished by a longer faint echeme (valid at local population level only) ……***C. anapaistica anapaistica***

DISCUSSION

Taxonomy and song evolution

Disagreement in species delimitation among different data sources is common in invertebrates. For example, only 147 (41%) of 353 sets of arthropod taxa studied showed broad agreement in delimitation among multiple data sources (Schlick-Steiner *et al.*, 2010). A multidisciplinary approach became more and more popular in the late 20th century, particularly for acoustically active groups, such as Hemiptera, Orthoptera or Neuroptera (e.g. Alexander & Moore, 1962; Dugdale & Fleming, 1978; Howard & Furth, 1986; Henry, 1993). Nevertheless, in cicadas many descriptions of new species are still based solely on morphology. Sueur (2001) estimated that songs of only 12% of the known cicada species have been published worldwide. The *Cicadetta cerdaniensis* group studied here is an excellent example of a complex of closely related cryptic species where taxonomic considerations based on any single method such as morphology, molecular systematics or even qualitative song structure are ambiguous or misleading (**Table 2.5**). In addition, incomplete knowledge of distribution patterns and regional within-taxon variability can lead to confusion. By using a combination of data, we have been able to better elucidate the taxonomy of this group.

Table 2.5. Limits of species delimitation using single or multiple lines of evidence for the newly described taxa.

	Misleading or ambiguous species classification with some standard methods				
Final classification	**Colouration**	**mtDNA**	**Colouration + mtDNA**	**Acoustic field determination**	**Acoustic analyses (qual. + quant.)**
Cicadetta sibillae	Several taxa, but not *C. cerdaniensis* s. str.	*C. brevipennis* (or *C. a. anapaistica*)	*C. brevipennis* (or *C. a. anapaistica*)	*C. cerdaniensis* s. str.	*C. cerdaniensis* s. str. or new taxon
Cicadetta a. lucana	Several taxa for dark morph; new species for light morph	*C. brevipennis* (or *C. a. anapaistica*)	*C. brevipennis* (or *C. a. anapaistica*)	*C. a. anapaistica* or new (sub)species	New subspecies

For more than 200 years, taxonomists have had problems finding a convincing systematic understanding of the large *Cicadetta montana* complex (*C. montana* s. l.) based on morphological characters. Only a very few of these old descriptions are assignable to different song patterns (Boulard, 1995; Gogala & Trilar, 2004). Colouration and morphometry seem to provide the best characters when examined in light of song and genetic data. The same is true for the *C. cerdaniensis* subgroup discussed here. In this song group, no morphological feature is truly diagnostic, but several are significantly different (**Figs 2.4 and 2.5**). On the other hand, obvious colouration discrepancies in *C. anapaistica lucana* Hertach, 2015 must be interpreted as dimorphism without taxonomic relevance. Similarly, with an isolated acoustic approach, the song differences between *C. cerdaniensis* s. str. and *C. sibillae* Hertach & Trilar, 2015 would have been interpreted as widespread within-species variability rather than an indication of species status (**Figs 2.6B-C and 2.7**; **Table 2.2**). The splitting in several spatially isolated metapopulations, the high temperature dependency of the song duration variables and intermediate song patterns increased species delimitation difficulties. An isolated interpretation of the molecular phylogenetic results is also misleading (**Fig 2.3**). Although *C. cerdaniensis* s. str. is clearly distinct from the other taxa, *C. sibillae* and *C. anapaistica lucana* are genetically indistinguishable, positioned in a polytomy with a well-supported monophyletic *C. a. anapaistica* clade. Large bursts of evolution (trees with polytomies) characterise several recent studies of acoustically defined cicada species complexes (Buckley *et al.*, 2006; Marshall *et al.*, 2011; Nunes *et al.*, 2014; Popple, 2013). They are the rule rather than the exception and are not surprising given the dramatic climatic and biotic fluctuations of the past 2 million years.

Species delimitation is dependent on the species concept used. We chose the ‘Unified Species Concept’ (De Queiroz, 2007) that defines species as “separately evolving metapopulation lineages” and permits hybridisation and introgression among species (e.g. as described for cicadas in Marshall *et al.*, 2011). In our delimitation of the *C. cerdaniensis* species group, we assigned the least weight to the morphological results and the most weight to the qualitative song differences in relation to the chorological patterns. There is no evidence that cicadas learn songs or match songs of other species, and patterns function as inherited, fine-tuned, specific mate recognition systems (SMRSs; Paterson, 1985). Qualitative song differences in cicadas are normally assigned to species level, even when not supported by molecular investigations (e.g. Nunes *et al.*, 2014; Popple, 2013). Solely quantitative song characters have been accepted as main species delimitation criteria in other European cicadas, such as *Cicada cretensis* Quartau & Simões, 2005 or *Lyristes gemellus* Boulard, 1988, but dependency on the ambient, perch or body temperature was not tested (Boulard, 1988; Quartau & Simões, 2005) in these larger and presumably less sensitive species (compare Popov, 1975; Sanborn *et al.*, 2011). Schlick-Steiner *et al.* (2010) found that the failure rate in multivariate species delimitation processes was lowest for behavioural characters (8%) such as acoustics and was remarkably higher for morphology (23%), mitochondrial DNA (mtDNA, 33%) and nuclear DNA (nDNA, 28%), for 184 published arthropod studies.

Our discovery of reciprocally monophyletic mtDNA clades for *C. sibillae* (and relatives) and *C. cerdaniensis* s. str. in the mitochondrial phylogeny (**Fig 2.3**) prompted us to search for species-delimiting acoustic and morphological characters to exclude artefacts caused, for example, by introgression of *C. brevipennis* mtDNA. We discovered temporal differences in songs that were influenced by the perch temperature. Difficulties in locating individuals caused temperature measurement problems. In addition, body temperature and perch temperature can differ significantly, for example as a result of heat production by the active sonorous system (Fonseca & Revez, 2002; Sanborn *et al.*, 2011). Nevertheless, using regression and ANCOVA, we demonstrated that these differences are significantly specific and well correlated (**Fig 2.8A**). Hence, the number of syllables forming the echemes of the third phrase provides an unusual and surprisingly constant character, which is not significantly temperature dependent (**Fig 2.8B**). European cicada species have never been delimited based on the number of syllables of an echeme before, but this has been recently suggested for Australian cicadas (Popple, 2013). Sueur & Aubin (2003) used the rate of syllables per second instead of the number of syllables as a useful tool for the acoustic distinction of long-lasting songs in the genus *Tibicina*. Conversely, in grasshoppers (Orthoptera) the number of syllables per echeme are more traditionally used for species delimitation (e.g. *Chorthippus biguttulus* versus *Chorthippus eisentrauti*; Ingrisch, 1995), which are deduced from the more obvious leg or wing movements. Whereas the duration of syllables seems scarcely affected by the temperature in *Tibicina*, it is strongly influenced in smaller cicadas (Fonseca & Revez, 2002), which are not or are only partially able to regulate their body temperature (Sanborn, 2006). Nevertheless, we demonstrate that the duration of syllables is a helpful tool to distinguish species (**Fig 2.12**).

Quantitative acoustic characters, mtDNA, colouration of the basal junction of anal veins and a gap in the known distribution of 450 km suggested that *C. sibillae* is no longer evolving in the same metapopulation lineage as *C. cerdaniensis* s. str. The five metapopulations of *C. sibillae* are scarcely distinguishable in all data sets, with the exception of one gradually changing character (ED_{1+2}; **Table 2.4**); our data suggest that they function as one taxonomic unit. Important qualitative song differences distinguish *C. sibillae* from *C. anapaistica*. A small contact zone is characterised by song transitions produced by a minority of males in some local *C. a. lucana* populations, but not in *C. sibillae* populations (**Fig 2.11**). The presence of intermediate song characters in an area of contact is good evidence for hybridisation (Marshall *et al.*, 2011). We suspect unidirectional and local gene flow from *C. sibillae* to *C. a. lucana* populations. As the majority of males, with the exception of the Morra de Sanctis population, sing typically, reproductive barriers must exist between *C. sibillae* and *C. a. lucana*. Simplified song patterns in *C. a. lucana* populations (‘luca_simp’) may appear incomplete and less attractive for females. The complexity of songs is expected to be selected disproportionately as a more attractive phenotype (Bailey, 2006). *Cicadetta sibillae* immigrants may appear only sporadically in *C. a. lucana* populations because of their separation by distances of 50 km and more; however, interloper hybrid males could promote the persistence of simplified patterns, even in the absence of immigration. As observed in other cicada genera (Cooley & Marshall, 2001; Sueur, 2003; Puissant, 2005) and once in a pure *C. sibillae* population, interloper males attempt to intercept females attracted to nearby calling males. In such a system incomplete songs can be passed to the next generation.

Separately evolving metapopulation lineages are also expected for *C. sibillae* and *C. anapaistica* according to two basic properties of sound production. First, *C. anapaistica* has the ability to produce syllable rates that are up to 20% faster at low perch temperatures than *C. sibillae* (**Fig 2.12**). These differences reflect the kinetics of the timbal apparatus (Fonseca & Revez, 2002) and are also present in the cicada genera *Tettigettalna* (under the name *Tettigetta*; Fonseca & Revez, 2002), *Diceroprocta* (Sanborn & Phillips, 2010) and *Pauropsalta* (Popple, 2013), but it was surprising to find them in such closely related taxa. It indicates independent selection for fine neurological and morphological adaptations. This ability seems to be linked with singing activity at temperatures as low as 14 °C for *C. a. anapaistica* (max. 31 °C), which is remarkably lower than the minimum observed for *C. sibillae* at 19 °C (max. 33 °C). The body temperature range of singing cicadas has been suggested to separate sympatric species, including sibling ectothermic pairs (Sanborn *et al.*, 2002). Calling at low temperatures could provide an advantage in acoustic competition where *C. anapaistica* co-occurs with the omnipresent and persistently singing *Tettigettula pygmea* (Olivier, 1790), which favours higher temperatures. Secondly, the two species tend to be driven by contrasting evolutionary forces (Butlin, 1995), with *C. sibillae* being representative of the recognition model (Paterson, 1985) and *C. anapaistica* being representative of the competition model (West-Eberhard, 1984). *Cicadetta sibillae* songs are very stable over all metapopulations in qualitative and quantitative aspects and seem solely affected by the ambient or perch temperature (with the exception of ED_{1+2}). By contrast, in *C. anapaistica* songs are more variable between individuals than is found in any other European cicada (**Fig 2.11**). Recognition of individuals is possible in many cases, especially in *C. a. anapaistica* (Chapter 1 = Hertach, 2011). The higher complexity of the song allows the species to be identified, despite the variation. *Cicadetta anapaistica*, which is characterised by low genetic divergence within each subspecies, appears to be under fluctuating natural selection not only with respect to complex rhythms but also with respect to dimorphic colouration. The rhythms are to a certain extent also influenced by the stimulation of the calling male (song start, restart, calling activity duration, population density; Chapter 1 = Hertach, 2011).

Within *C. anapaistica* (*C. a. lucana* and *C. a. anapaistica*), separately evolving metapopulation lineages are less well defined, but we argue here that the two lineages are valid taxa at the subspecific level. The mtDNA recognises *C. a. anapaistica* as a monophyletic group that is nested within polytomous *C. a. lucana* and *C. sibillae* (**Fig 2.3**). We hypothesise that the lack of distinction between the mtDNA of *C. sibillae* and *C. a. lucana* is a result of hybridisation and introgression: invasive *C. sibillae* mtDNA was passed to *C. a. lucana* and captured. Mitochondrial capture in the absence of song introgression has been seen in other cicada taxa (e.g. Marshall *et al.*, 2011). The lack of *C. sibillae* mtDNA in the more southerly located *C. a. anapaistica* suggests that introgression did not reach the southern subspecies, which is at present separated by a distance of 50 km. Song patterns in the two *C. anapaistica* subspecies are heterogeneous and show overlap when combined with detailed chorological data (**Fig 2.11**). Normal *C. a. lucana* song traits are found in two *C. a. anapaistica* populations close to their mutual contact zone and oddly in the most distant *C. a. anapaistica* populations sampled. A few individuals in almost all *C. a. anapaistica* populations do not or only rarely produce the subspecific final echemes.

Initially, we also considered an alternative scenario where the specimens of *C. a. lucana* are hybrids between *C. sibillae* and *C. a. anapaistica*, rather than the situation described above. This alternative scenario was suggested by the geographically intermediate position of the new subspecies, the qualitatively intermediate song pattern and its mitochondrial similarity to *C. sibillae*; however, biogeographical and song-related evidence argues against a hybrid origin for *C. a. lucana*. First, the 'luca_norm' song pattern is dominant and pure. Second, many quantitative song variables do not show intermediate values in *C. a. lucana*, compared with *C. sibillae* and *C. a. anapaistica* (e.g. **Table 2.2**). In the variables where values are intermediate, the similarity between *C. sibillae* and *C. a. lucana* is generally smaller than the similarity between *C. a. anapaistica* and *C. a. lucana* (e.g. **Fig 2.12B**). Third, the most parsimonious scenario for the evolution of the song patterns in the Apenninian clade makes a hybrid origin of *C. a. lucana* unlikely (compare below). A hybrid origin would also imply that the ability of *C. a. anapaistica* to reduce power for a faint (final) echeme was completely lost in hybrid populations. Finally, palaeogeological considerations, specifically the occurrence of periodic marine inundation in the area between the two *C. anapaistica* subspecies, support our decision (see also below).

The *C. cerdaniensis* group forms an interesting example of song evolution in cicadas. Patterns within this group show stepwise increases in complexity geographically (from north to south). Each step is characterised by the addition of one echeme type in fast phrase 3 [**Fig 2.6**; *C. cantilatrix*, no echeme types; *C. sibillae*, one echeme type; *C. a. lucana*, two echeme types ('luca_norm'); *C. a. anapaistica*, three echeme types ('anap_norm')]. As echeme types are connected with different power and carrier frequency ranges, complexity also increases in these domains. We suggest that species-specific information must be encoded in the time and not in the frequency domain, and that the time domain of phrase 3 played a key role in the speciation process. It is obvious that song elements have changed their function: *C. cantilatrix* is also able to produce a fast series of short echemes, close to the song patterns of *C. cerdaniensis* s. str. and *C. sibillae*. In *C. cantilatrix*, this song pattern is not part of the calling song, but is part of a special, rarely emitted courtship song (Trilar *et al.*, 2006; Hertach, 2007; Chapter 1 = Hertach, 2011). Based on the synthesis of our results above, we suggest a song-evolution scenario for the Apenninian clade in which the common ancestor of *C. sibillae*, *C. a. lucana* and *C. a. anapaistica* had ungrouped echemes. Further evolution in the *C. anapaistica* lineage resulted in the common ancestor of *C. a. lucana* and *C. a. anapaistica* possessing grouped echemes; later the *C. a. anapaistica* lineage alone evolved the ability to reduce power for a longer final echeme.

Distribution and biogeographical aspects

The *Cicadetta cerdaniensis* group, now containing four species and one subspecies, is widely distributed in Europe from the Pyrenees in the west (Puissant, 2006), Germany and Poland in the north (Trilar *et al.*, 2006; Meineke, 2012), Hungary, Romania and Bulgaria in the east (Trilar & Gogala, 2012; Trilar & Gogala, 2008, T. Trilar & M. Gogala, unpubl. data) and Macedonia and Sicily in the south (Gogala *et al.*, 2005; Chapter 1 = Hertach, 2011). The eastern limit is the least studied. *Cicadetta cerdaniensis* s. str., *C. a. anapaistica* and *C. a. lucana* are closely restricted endemics of exclusively or mainly mountainous habitats. *Cicadetta sibillae* and especially *C. cantilatrix* have a wider distribution, including hilly lowlands. *Cicadetta cerdaniensis* s. str. is the only member of the group that at present is clearly allopatric to the other four taxa, which occur in parapatry (or at least close allopatry; **Fig 2.9**).

All three zones where parapatric distribution areas meet are currently characterised by scattered local populations and small population sizes of one of the taxa: *C. cantilatrix* in the south-eastern Italian Alps, *C. a. anapaistica* in Calabria and *C. sibillae* in Campania. Current intraspecific spatial isolation is greater for the two metapopulations of *C. a. anapaistica* separated by the sea and for several metapopulations of *C. sibillae*, than for neighbouring taxa in Italy (**Fig 2.9**).

The Mediterranean biogeographical area is known for historically intricate interactions of taxa, which favoured speciation and hybridisation (Blondel *et al.*, 2010). In the Pleistocene, dramatic climate changes resulted in severe reductions of population sizes for some species (Taberlet *et al.*, 1998; Hewitt, 2000; 2001). European cicadas form a generally thermophilous group connected with temperate forests, and as a result were very likely displaced to southern refugia by ice sheets and tundra during glacial periods. Our data suggest that divergence and speciation processes have resulted in widely separated refugia, as seen in the genus *Cicada* (Pinto-Juma *et al.*, 2009). Typically, small genetic distances among taxa (0.009-0.029; **Table 2.1**) indicate that evolution in this group was relatively recent, roughly within the last 1 million years (Pleistocene), based on a strict clock analysis using both the Brower (1994) and Papadopoulou *et al.* (2010) calibrations. We presume that speciation events in the *C. cerdaniensis* group are linked with the three main European glacial refugia: the Balkan, Iberian and Apenninian peninsulas. Within the Apenninian peninsula, our data suggest glacial refugia for the ancestors of *C. anapaistica* in the south (Calabria/Sicily and Basilicata) and for the ancestors of *C. sibillae* further northwards (eventually Liguria/Tuscany; see Médail & Diadema, 2009; Vega *et al.*, 2010). *Cicadetta cerdaniensis* s. str. populations may have speciated and survived in the Iberian peninsula and *C. cantilatrix* in the Balkans. The speciation events within the Apenninian lineages are obviously more complex than between the three main refugia, and probably took place during more recent glacial cycles. Secondary contact and possible hybridisation would have then occurred after expansion from separate refugia during interglacial periods and since the last glacial maximum (Reinig, 1969; Hewitt, 2000; 2001). Typically, the presence of the Alpine barrier prohibited Apenninian lineages such as *C. sibillae* from colonizing more northern areas (Taberlet *et al.*, 1998; Hewitt, 2000; 2001). Conversely, Balkan lineages or cicadas may have immigrated far to the north and colonised Central Europe, as suggested by the fact that the current distribution pattern of *C. cantilatrix* shows similarities with those of the European Tree Frog (*Hyla arborea*; Stöck *et al.*, 2012) or the grasshoppers *Chorthippus biguttulus* (Ingrisch, 1995) and *Chorthippus parallelus* (Taberlet *et al.*, 1998; Hewitt, 2000; 2001) for which a Balkan refuge has been proposed.

Cicadetta cantilatrix is currently separated from *C. sibillae* by a mountain ridge with unsuitable habitats in the Dolomites and Lessini mountains in north-eastern Italy. This region separates many eastern-western fauna elements (Baroni Urbani *et al.*, 1977; Hellrigl, 1996; Minelli *et al.*, 2006), and the south-eastern Alps appear as a suture zone where expanding lineages from the Apenninian peninsula and the Balkans glacial refugia meet. *Cicadetta sibillae* and *C. a. lucana* are disjunct in the Avellino-Benevento-Foggia region, where the Apennine Mountain ridge decreases to lower altitudes. Confounding present-day analyses is the fact that deforestation is high in this region, except for a belt that theoretically connects local populations between the Picentini Mountains and the Taburno-Camposauro massif. In fact, the forests in this belt seem to have no *C. montana* complex species and scarcely any other cicadas. The region also functions as a barrier for the cicadas *Cicadetta brevipennis* and *Tibicina steveni* (Krynicki, 1837) (T. Hertach, unpubl. data) and classically divides the Apenninian fauna into a central and a southern sector, where the percentage of northern elements significantly changes (Baroni Urbani *et al.*, 1977; Minelli *et al.*, 2006). There are parapatric pairs of taxa published that are of special interest to our findings: Lenk & Wüster (1999) found two cryptic snake species (*Zamenis* spp., under the name *Elaphe*) and Canestrelli *et al.* (2006b) found two salamander species (*Salamandrina* spp.) separated slightly north of our distribution limits. The two salamanders of ancient lineages form a hybrid zone (Hauswaldt *et al.*, 2011), similar in location to *C. sibillae* and *C. a. lucana*. Even more challenging is the case of the subspecies in the snake *Vipera aspis* (Linnaeus, 1758). Barbanera *et al.* (2009) not only reported hybrids between the northern *Vipera aspis francisciredi* and the southern *Vipera aspis hugyi*, but also '*francisciredi*'-like mtDNA introgressed in *V. a hugyi*. Hence, mitochondrial DNA of *V. a. hugyi* from Basilicatian populations is more closely related to *V. a. francisciredi* from the central Apennine than to *V. a. hugyi* from southern Calabria and Sicily. We seem to have exactly the same phylogeographic patterns in the *C. cerdaniensis* group, with *C. sibillae* equivalent to *V. a. francisciredi*, *C. a. lucana* matching the pattern of the introgressed *V. a. hugyi* and *C. a. anapaistica* matching pure *V. a. hugyi*. The Crati-Sibari plain separating the *C. anapaistica* subspecies is known for intense tectonic activity and was repeatedly marine-flooded during the Plio-Pleistocene, separating the Italian peninsula from the Calabrian and Sicilian palaeoarchipelago. It has been shown in different amphibians and in a small mammal that it acted as an effective barrier to dispersal (Canestrelli *et al.*, 2006a; Canestrelli *et al.*, 2007; Vega *et al.*, 2010; Canestrelli *et al.*, 2012). We propose that *C. anapaistica* or its ancestors were also affected by this discontinuity. Sicily and the Italian peninsula were connected by land bridges during glacial maxima in the Late-Middle and Late Pleistocene, which facilitated the movement of northern species onto the island (Bonfiglio *et al.*, 2002; Canestrelli *et al.*, 2007). During these periods *C. a. anapaistica* or its ancestors presumably colonised Sicily. Such immigrant species are nowadays often restricted to the elevated areas from the Peloritani to the Madonie Mountains (Minelli *et al.*, 2006), which exactly matches the cicada distribution.

With respect to the isolation of the five *C. sibillae* metapopulations, we suggest that more recent factors have played a critical role. Between the Insubrian and the northern Apenninian metapopulations, suitable habitats were rare and restricted to small refuges. Apenninian metapopulations of *C. sibillae* are best explained to have become established by interspecific competition with strictly parapatric *C. brevipennis*.

Ecology and threat

The *C. cerdaniensis* group taxa prefer ecotone habitats between extensively used meadows or pastures and sparse woods, which are not too dry. Habitat preferences vary remarkably within the two more widespread species, *C. cantilatrix* and *C. sibillae*, in relation to the regionally present plant communities. *Cicadetta montana* s. str. and *C. brevipennis* are often found in drier habitats, *C. montana* s. str. is also typically found on rocky ridges. Whereas *C. montana* s. str. tends to sing in higher trees and *C. brevipennis* tends to sing in lower bushes or in the herb layer, the *C. cerdaniensis* group inhabits an intermediate vertical niche range.

Individuals of the *C. cerdaniensis* group usually change their perch after a few song sequences (Puissant & Boulard, 2000) and males often call in small groups for long periods of time. Phrases 1 and 2 contain important inter-echeme pauses, which conspecific neighbouring males use to duet. Interestingly, *C. cantilatrix* emerge in cooler northern Switzerland two to three weeks earlier than *C. sibillae* in the warmer more southern parts (Ticino).

Cicadetta anapaistica anapaistica has already been proposed as an endangered species because of the small Mediterranean mountainous ecosystems in which it occurs (Chapter 1 = Hertach, 2011). These habitats are among the most vulnerable areas in the world with respect to climate change (Regato & Salman, 2008). This assessment does not change with the discovery of a substantially wider distribution area because the Calabrian populations are isolated, stenoecious and small. Moreover, the grazing of cattle in the Sicilian core populations, in the Madonie Mountains as well as in the Nebrodi Mountains, was very intense during our second stay after a dry spring with eroding soils, resulting in few individuals singing (**Fig 2.10D**). Soil degradation presumably negatively affects the subterrestrial nymphal development.

As a result of the fact that Italian and Swiss populations are no longer considered to belong to *C. cerdaniensis* s. str., this species is likely to be rare. Puissant (2006 and pers. comm.) has reported only four local populations. Additional fieldwork should be conducted to clarify the conservation status of this species. We also consider *C. a. lucana* to be threatened. Its habitats, especially in the north-eastern part of its range, are often separated by large distances of deforested areas, normally used for intensive agriculture. The exchange between those local populations is interrupted or hindered, and only a few locations have many individuals present. In *C. sibillae* the distribution range is still rather small and fragmented, but only peripheral metapopulations seem to be vulnerable with the western Alpine occurrences that are at first priority for conservation.

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REFERENCES CHAPTER 2

Alexander RD, Moore TE. 1962. The evolutionary relationships of 17-year and 13-year cicadas, and three new species (Homoptera, Cicadidae, *Magicicada*). *Miscellaneous Publications of the Museum of Zoology, University of Michigan* 121: 1-59.

Bailey WJ. 2006. Insect songs – The evolution of signal complexity. In: Drosopoulos S, Claridge M, eds. *Insect sounds and communication: Physiology, behaviour, ecology and evolution.* Boca Raton: CRC Press Taylor & Francis Group, 127-136.

Barbanera F, Zuffi MAL, Guerrini M, Gentilli A, Tofanelli S, Fasola M, Dini F. 2009. Molecular phylogeography of the asp viper *Vipera aspis* (Linnaeus, 1758) in Italy: Evidence for introgressive hybridization and mitochondrial DNA capture. *Molecular Phylogenetics and Evolution* 52: 103-114.

Baroni Urbani C, Ruffo S, Vigna Taglianti A. 1977. Materiali per una biogeografia italiana fondata su alcuni generi di coleotteri Cicindelidi, Carabidi e Crisomelidi. *Memorie della Società Entomologica Italiana* 56: 35-92.

Blondel J, Aronson J, Bodiou JY, Boeuf G. 2010. *The Mediterranean region: Biological diversity in space and time.* 2nd edition. New York: Oxford University Press, 376 pp.

Bonfiglio L, Mangano G, Marra AC, Masini F, Pavia M, Petruso D. 2002. Pleistocene Calabrian and Sicilian bioprovinces. *Geobios* 35: 29-39.

Boulard M. 1988. Les *Lyristes* d'Asie Mineur (Homoptera Cicadidae). I. Sur deux formes éthospécifiques syntopiques et description de deux espèces nouvelles. *L'Entomologiste* 44(3): 153-167.

Boulard M. 1995. Postures de cymbalisation, cymbalisations et cartes d'identité acoustique des cigales. Généralités et espèces méditerranéennes (Homoptera, Cicadoidea). *Ecole pratique des hautes Etudes, Biologie et Evolution des Insectes* 7/8: 1-72.

Bremi H. 1849. Uebersicht der schweizerischen Rhynchoten. *Mittheilungen der Naturforschenden Gesellschaft in Zürich* 34: 325-339.

Brower AVZ. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences USA* 91: 6491-6495.

Brua C, Hugel S. 2008. Présence des cigales *Cicadetta montana* (Scopoli, 1772) et *Cicadetta cantilatrix* Sueur & Puissant, 2007 en Alsace (Hemiptera, Cicadidae). *Bulletin de la Société Entomologique de Mulhouse* 64(3): 49-52.

Buckley TR, Cordeiro M, Marshall DC, Simon C. 2006. Differentiating between hypotheses of lineage sorting and introgression in New Zealand alpine cicadas (*Maoricicada* Dugdale). *Systematic Biology* 55(3): 411-425.

Buckley TR, Simon C, Chambers GK. 2001. Phylogeography of the New Zealand cicada *Maoricicada campbelli* based on mitochondrial DNA sequences: Ancient clades associated with Cenozoic environmental change. *Evolution* 55(7): 1395-1407.

Butlin RK. 1995. Genetic variation in mating signals and responses. In: Lambert DM, Spencer HG, eds. *Speciation and the recognition concept*. Baltimore, Maryland: Johns Hopkins University Press, 327-366.

Canestrelli D, Cimmaruta R, Costantini V, Nascetti G. 2006a. Genetic diversity and phylogeography of the Apennine yellow-bellied toad *Bombina pachypus*, with implications for conservation. *Molecular Ecology* 15: 3741-3754.

Canestrelli D, Cimmaruta R, Nascetti G. 2007. Phylogeography and historical demography of the Italian treefrog, *Hyla intermedia*, reveals multiple refugia, population expansions and secondary contacts within peninsular Italy. *Molecular Ecology* 16: 4808-4821.

Canestrelli D, Sacco F, Nascetti G. 2012. On glacial refugia, genetic diversity, and microevolutionary processes: deep phylogenetical structure in the endemic newt *Lissotriton italicus*. *Biological Journal of the Linnean Society* 105: 42-55.

Canestrelli D, Zangari F, Nascetti G. 2006b. Genetic evidence for two distinct species within the Italian endemic *Salamandrina terdigitata* (Bonnaterre, 1789) (Amphibia: Urodela: Salamandridae). *Herpetological Journal* 16: 221-227.

Cooley JR, Marshall DC. 2001. Sexual signalling in periodical cicadas, *Magicicada* spp. (Hemiptera: Cicadidae). *Behaviour* 138: 827-855.

De Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* 56(6): 879-886.

Dugdale JS, Fleming CA. 1978. New Zealand cicadas of the genus *Maoricicada* (Homoptera: Tibicinidae). *New Zealand Journal of Zoology* 5(2): 295-340.

Evenhuis NL. 2014. *Abbreviations for insect and spider collections of the world.* Available at: http://hbs.bishopmuseum.org/codens/codens-inst.html (accessed 20 May 2015).

Fonseca PJ. 1991. Characteristics of the acoustic signals in nine species of cicadas (Homoptera, Cicadidae). *Bioacoustics* 3: 173-182.

Fonseca PJ, Revez MA. 2002. Temperature dependence of cicada songs (Homoptera, Cicadoidea). *Journal of Comparative Physiology A* 187: 971-976.

Gogala M. 2013. *On the trail of mountain cicadas*. Ljubljana: Slovenian Academy of Sciences and Arts and Royal Entomological Society, 193 pp.

Gogala M, Trilar T. 2004. Bioacoustic investigations and taxonomic considerations on the *Cicadetta montana* species complex (Homoptera: Cicadoidea: Tibicinidae). *Anais da Academia Brasileira de Ciências* 76(2): 316-324.

Gogala M, Trilar T, Drosopoulos S. 2011. Two new species and a new genus of Cicadettini (Hemiptera, Cicadidae) from the Greek island of Evia. *Deutsche Entomologische Zeitschrift* 58(1): 105-117.

Gogala M, Trilar T, Krpač VT. 2005. Fauna of singing cicadas (Auchenorrhyncha: Cicadoidea) of Macedonia – a bioacoustic survey. *Acta Entomologica Slovenica* 13(2): 103-126.

Hanski I, Gilpin M. 1991. Metapopulation dynamics: brief history and conceptual domain. *Biological Journal of the Linnean Society* 42: 3-16.

Hauswaldt JS, Angelini C, Pollok A, Steinfartz S. 2011. Hybridization of two ancient salamander lineages: molecular evidence for endemic spectacled salamanders on the Apennine peninsula. *Journal of Zoology* 284: 248-256.

Hellrigl K. 1996. *Die Tierwelt Südtirols.* Bozen: Naturmuseum Südtirol, 831 pp.

Henry CS. 1993. *Chrysoperla johnsoni* (Neuroptera: Chrysopidae): acoustic evidence for full species status. *Annals of the Entomological Society of America* 86: 14-25.

Hertach T. 2007. Three species instead of only one: Distribution and ecology of the *Cicadetta montana* species complex (Hemiptera: Cicadoidea) in Switzerland. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 80: 37-61.

Hertach T. 2011. Spectacular song pattern from the Sicilian Mountains: The new cicada species *Cicadetta anapaistica* sp. n. (Hemiptera: Cicadidae). *Zootaxa* 2771: 25-40.

Hertach T, Nagel P. 2013. Cicadas in Switzerland: a scientific overview of the historic and current knowledge of a popular taxon (Hemiptera: Cicadidae). *Revue suisse de Zoologie* 120(2): 229-269.

Hertach T, Pollini Paltrinieri L. 2012. Le cicale (Hemiptera: Cicadidae) del Cantone Ticino (Svizzera). *Bollettino della Società ticinese di scienze naturali* 100: 43-51.

Hewitt GM. 2000. The genetic legacy of the Quaternary ice ages. *Nature* 405: 907-913.

Hewitt GM. 2001. Speciation, hybrid zones and phylogeography – or seeing genes in space and time. *Molecular Ecology* 10: 537-549.

Hill KBR, Marshall DC, Simon C, Chambers GK. 2009. Surviving glacial ages within the Biotic Gap: phylogeography of the New Zealand cicada *Maoricicada campbelli*. *Journal of Biogeography* 36(4): 675-692.

Howard DJ, Furth DG. 1986. Review of the *Allonemobius fasciatus* (Orthoptera: Gryllidae) complex with the description of two new species separated by electrophoresis, songs, and morphometrics. *Annals of the Entomological Society of America* 79: 472-481.

Ingrisch S. 1995. Evolution of the *Chorthippus biguttulus* group (Orthoptera, Acrididae) in the Alps, based on morphology and stridulation. *Revue suisse de Zoologie* 102: 475-535.

Lanfear R, Calcott B, Ho SY, Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695-1701.

Lenk P, Wüster W. 1999. A multivariate approach to the systematics of Italian Rat Snakes of the *Elaphe longissima* complex (Reptilia, Colubridae): Revalidation of Camerano's *Callopeltis longissimus* var. *lineata*. *Herpetological Journal* 9: 153-162.

Marshall DC, Hill KBR, Cooley JR, Simon C. 2011. Hybridization, mitochondrial DNA phylogeography, and prediction of the early stages of reproductive isolation: Lessons from New Zealand cicadas (Genus *Kikihia*). *Systematic Biology* 60: 482-502.

Marshall DC, Slon K, Cooley JR, Hill KBR, Simon C. 2008. Steady Plio-Pleistocene diversification and a 2-million-year sympatry threshold in a New Zealand cicada radiation. *Molecular Phylogenetics and Evolution* 48: 1054-1066.

Médail F, Diadema K. 2009. Glacial refugia influence plant diversity patterns in the Mediterranean Basin. *Journal of Biogeography* 36: 1333-1345.

Meineke T. 2012. Bergsingzikaden *Cicadetta cantilatrix* Sueur & Puissant, 2007, *Cicadetta brevipennis* Fieber, 1876 und *Cicadetta montana* s. str. (Scopoli, 1772) im mittleren Deutschland (Auchenorrhyncha, Cicadidae, Cicadettinae). *Entomologische Nachrichten und Berichte* 56(2): 133-142.

Minelli A, Ruffo S, Vigna Taglianti A. 2006. The Italian faunal provinces. In: Ruffo S, Stoch F, eds. *Checklist and distribution of the Italian fauna – 10,000 terrestrial and inland water species.* Memorie del Museo Civico di Storia Naturale di Verona, 2. Serie, Sezione Scienza della Vita 17, 37-39.

Moulds MS. 2005. An appraisal of the higher classification of cicadas (Hemiptera: Cicadoidea) with special reference to the Australian fauna. *Records of the Australian Museum* 57: 375-446.

Nunes VL, Mendes R, Marabuto E, Novais BM, Hertach T, Quartau JA, Seabra SG, Paulo OS, Simões PC. 2014. Conflicting patterns of DNA barcoding and taxonomy in the cicada genus *Tettigettalna* from southern Europe (Hemiptera: Cicadidae). *Molecular Ecology Resources* 14(1): 27-38.

Papadopoulou A, Anastasiou I, Vogler AP. 2010. Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. *Molecular Biology and Evolution* 27: 1659-1672.

Paterson HEH. 1985. The recognition concept of species. In: Vrba ES, ed. *Species and Speciation.* Pretoria: Transvaal Museum, Monograph No. 4, 21-29.

Pinto-Juma GA, Quartau JA, Bruford MW. 2009. Mitochondrial DNA variation and the evolutionary history of the Mediterranean species of *Cicada* L. (Hemiptera, Cicadoidea). *Zoological Journal of the Linnean Society* 155: 266-288.

Popov AV. 1975. The structure of the tymbals and characteristic of sound signals of singing cicadas (Homoptera Cicadidae) from the southern regions of the USSR, *Entomological Review of Washington* 54(2): 7-35.

Popple LW. 2013. A revision of the *Pauropsalta annulata* Goding & Froggatt species group (Hemiptera: Cicadidae) based on morphology, calling songs and ecology, with investigations into calling song structure, molecular phylogenetic relationships and a case of hybridisation between two subspecies. *Zootaxa* 3730(1): 1-102.

Puissant S. 2005. Taxonomy, distribution and first eco-ethological data of *Melampsalta varipes* (Waltl, 1837), an unrecognized cicada (Hemiptera, Cicadidae). *Insect Systematics and Evolution* 36(3): 301-316.

Puissant S. 2006. *Contribution à la connaissance des cigales de France: Géonémie et écologie des populations (Hemiptera, Cicadidae).* Bédeilhac/Aynat: Association pour la Caractérisation et l'Etude des Entomocénoses, 193 pp.

Puissant S, Boulard M. 2000. *Cicadetta cerdaniensis,* espèce jumelle de *Cicadetta montana* décryptée par l'acoustique (Auchenorrhyncha, Cicadidae, Tibicinidae). *Ecole pratique des hautes Etudes, Biologie et Evolution des Insectes* 13: 111-117.

Puissant S, Sueur J. 2010. A hotspot for Mediterranean cicadas (Insecta: Hemiptera: Cicadidae): new genera, species and songs from southern Spain. *Systematics and Biodiversity* 8(4): 555-574.

Quartau JA, Simões PC. 2005. *Cicada cretensis* sp. n. (Hemiptera, Cicadidae) from southern Greece. *Biologia, Bratislava* 60(5): 489-494.

Quartau JA, Simões PC. 2006. Acoustic evolutionary divergence in cicadas: the species of *Cicada* L. in Southern Europe. In: Drosopoulos S, Claridge M, eds. *Insect sounds and communication: Physiology, behaviour, ecology and evolution.* Boca Raton: CRC Press Taylor & Francis Group, 227-237.

R Development Core Team. 2008. *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

Regato P, Salman R. 2008. *Mediterranean Mountains in a Changing World: Guidelines for developing action plans*. Malaga: IUCN Centre for Mediterranean Cooperation, xii + 88 pp.

Reinig WF. 1969. Bastardierungszonen und Mischpopulationen bei Hummeln (*Bombus*) und Schmarotzerhummeln (*Psithyrus*) (Hymenopt., Apidae). *Mitteilungen der Münchner Entomologischen Gesellschaft* 59: 1-89.

Sanborn AF. 2006. Acoustic signals and temperature. In: Drosopoulos S, Claridge M, eds. *Insect sounds and communication: Physiology, behaviour, ecology and evolution.* Boca Raton: CRC Press Taylor & Francis Group, 111-125.

Sanborn AF, Breitbarth JH, Heath JE, Heath MS. 2002. Temperature responses and habitat sharing in two sympatric species of *Okanagana* (Homoptera: Cicadoidea). *Western North American Naturalist* 62: 437-450.

Sanborn AF, Phillips PK. 2010. Reevalution of the *Diceroprocta texana* species complex (Hemiptera: Cicadoidea: Cicadidae). *Annals of the Entomological Society of America* 103(6): 860-865.

Sanborn AF, Simões PC, Phillips PK, Quartau JA. 2011. Thermoregulation and the influence of body temperature on calling song parameters in *Cicada orni* (Hemiptera: Cicadidae). *European Journal of Entomology* 108(3): 365-369.

Schlick-Steiner B, Steiner FM, Seifert B, Stauffer C, Christian E, Corzier RH. 2010. Integrative taxonomy: A multisource approach to exploring biodiversity. *Annual Review of Entomology* 55: 421-438.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671-675.

Simões PC, Quartau JA. 2007. On the dispersal of males of *Cicada orni* in Portugal (Hemiptera: Cicadidae). *Entomologia Generalis* 30(3): 245-252.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Floors P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87: 651-701.

Sueur J. 2001. Audiospectrographical analysis of cicada sound production: a catalogue (Hemiptera, Cicadidae). *Mitteilungen aus dem Museum für Naturkunde Berlin, Deutsche Entomologische Zeitschrift* 48(1): 33-51.

Sueur J. 2003. Indirect and direct acoustic aggression in cicadas: first observations in the Palaearctic genus *Tibicina* Amyot (Hemiptera: Cicadomorpha: Cicadidae). *Journal of Natural History* 37: 2931-2948.

Sueur J, Aubin T. 2003. Specifity of cicada calling songs in the genus *Tibicina* (Hemiptera, Cicadidae). *Systematic Entomology* 28: 481-492.

Sueur J, Aubin T, Simonis-Sueur C. 2008. Seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics* 18(2): 213-226.

Sueur J, Puissant S. 2007. Similar look but different song: a new *Cicadetta* species in the *montana* complex (Insecta, Hemiptera, Cicadidae). *Zootaxa* 1442: 55-68.

Sukumaran J, Holder MT. 2010. DendroPy: a Python library for phylogenetic computing. *Bioinformatics* 26(12): 1569-1571.

Stöck M, Dufresnes C, Litvinchuk SN, Lymberakis P, Biollay S, Berroneau M, Borzée A, Ghali K, Ogielska M, Perrin N. 2012. Cryptic diversity among Western Palearctic tree frogs: Postglacial range expansion, range limits, and secondary contacts of three European tree frog lineages (*Hyla arborea* group). *Molecular Phylogenetics and Evolution* 65: 1-9.

Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* 7: 453-464.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731-2739.

Trilar T, Gogala M. 2008. New data on singing cicadas (Hemiptera: Cicadidae) of Romania. *Entomologica Romanica* 13: 29-33.

Trilar T, Gogala M. 2012. Three species of Mountain Cicadas (*Cicadetta montana* complex) found in Hungary with bioacoustic methods (Hemiptera: Cicadidae). *Folia Entomologica Hungarica* 73: 9-14.

Trilar T, Gogala M, Szwedo J. 2006. Pyrenean Mountain Cicada *Cicadetta cerdaniensis* Puissant & Boulard (Hemiptera: Cicadomorpha: Cicadidae) found in Poland. *Polskie Pismo Entomologiczne* 75(2): 313-320.

Trilar T, Hertach T. 2008. Three species of Mountain Cicadas *Cicadetta montana* (sensu lato) found in northern Italy. *Bulletin of Insectology* 61(1): 185-186.

Trilar T, Holzinger WE. 2004. Bioakustische Nachweise von drei Arten des *Cicadetta montana*-Komplexes aus Österreich (Insecta: Hemiptera: Cicadoidea). *Linzer biologische Beiträge* 36(2): 1383-1386.

Vega R, Amori G, Aloise G, Cellini S, Loy A, Searle JB. 2010. Genetic and morphological variation in a Mediterranean glacial refugium: evidence from Italian pygmy shrews, *Sorex minutus* (Mammalia: Soricomorpha). *Biological Journal of the Linnean Society* 100: 774–787.

West-Eberhard MJ. 1984. Sexual selection, social communication, and species specific signals in insects. In: Lewis T, ed. *Insect Communication*. London: Academic Press, 283-324.

Wickham H. 2009. *ggplot2: Elegant graphics for data analysis.* New York: Springer, VIII + 213 pp.

Zwickl D. 2006. *GARLI: genetic algorithm for rapid likelihood inference.* URL http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html

APPENDIXES

Appendix S2.1. Additional comments on the selection of recordings and the measurements in the song analyses.

Appendix S2.2. Spatial origin of the data analysed acoustically.

Appendix S2.3. Database of the records of *Cicadetta sibillae*, *Cicadetta anapaistica lucana* and *Cicadetta anapaistica anapaistica*.

Appendix S2.1. Additional comments on the selection of recordings and the measurements in the song analyses.

The delimitation of the taxa belonging to the *Cicadetta cerdaniensis* species group was a long process. Impressions from the field work and preliminary results of the analyses on computer induced new, adapted or more precise methods several times. Some metapopulations have been visited again in order to generate comparable recordings. Several reasons resulted in the fact that some recordings were used to investigate only a few variables while others contributed for the whole set of variables (**Appendix S2.2**).

Measuring methods should be reproducible. Therefore, we give some examples on important or difficult variables. We also present our results of a small and initial indoor experiment with *Cicadetta cantilatrix* in order to understand the syllable structures.

Remarks with respect to the perch temperature

Song recordings have been made without measuring temperature before 2010. Then it turned out that the surface of the perch, where the individual was expected to sing (perch temperature), could have an important influence. Variation in perch temperature impacted the speed of the song pattern but also could act as a key to distinguish between sibling species. Hence, for the analyses of *C. cerdaniensis* s. str. and *C. sibillae* only recordings from 2010 onwards could be integrated for the majority of variables. We defined a medium temperature range between 23-28 °C for which we described the typical species-specific values. This temperature range was characterised by a high activity of the investigated taxa. Recordings with a cooler or warmer perch temperature have been used to describe the temperature dependency.

With respect to the distinction of *C. anapaistica anapaistica* qualitative aspects are more important than quantitative which are less or not temperature dependent. Nevertheless, we were looking for an approach to calibrate and integrate the comprehensive data set recorded in 2009 and published in 2011 (Hertach, 2011). Therefore, we visited the populations again in 2013, but only recorded shorter song fragments of a quality as high as possible and with different perch temperatures. These recordings were useful to investigate the syllable numbers and syllable durations. The species-specific syllable duration was then used to calibrate former recordings in order to decide whether they have been made with high, moderate or low probability within the temperature range of 23-28 °C. Out of the 16 recordings based on the former description of the time domain in *C. a. anapaistica* six turned out to belong with high, four with moderate and six with low probability to the defined temperature range. Finally, we also included the four recordings with moderate probability to the analysed data set since two were by trend too cold and two too hot and the species-specific medium values were rather resistant against elimination or integration.

Quality of the recordings

Besides the temperature, mainly three qualities of a field recording were considered for the selection of variables to investigate. Most important, research on the syllable numbers and durations were only possible in very clean recordings. Background noise could be filtered when it is at different frequency ranges than the animal song. Other disturbance, such as echo effects by leaves, can relevantly distort the structure of the syllables. Secondly, longer recordings were preferred. Detailed analyses within the 23-28 °C temperature range were normally three or more minutes long in order to have a typical sample of each individual with several repetitions of the different phrases included. Long recordings were also preferred for the measuring of the phrase durations whereas at least two phrases were required for the analyses of the phrase 3 and of the syllables. Thirdly, a more or less constant sound level of the recording was essential for the measurement of the power differences between phrases 2 and phrases 3.

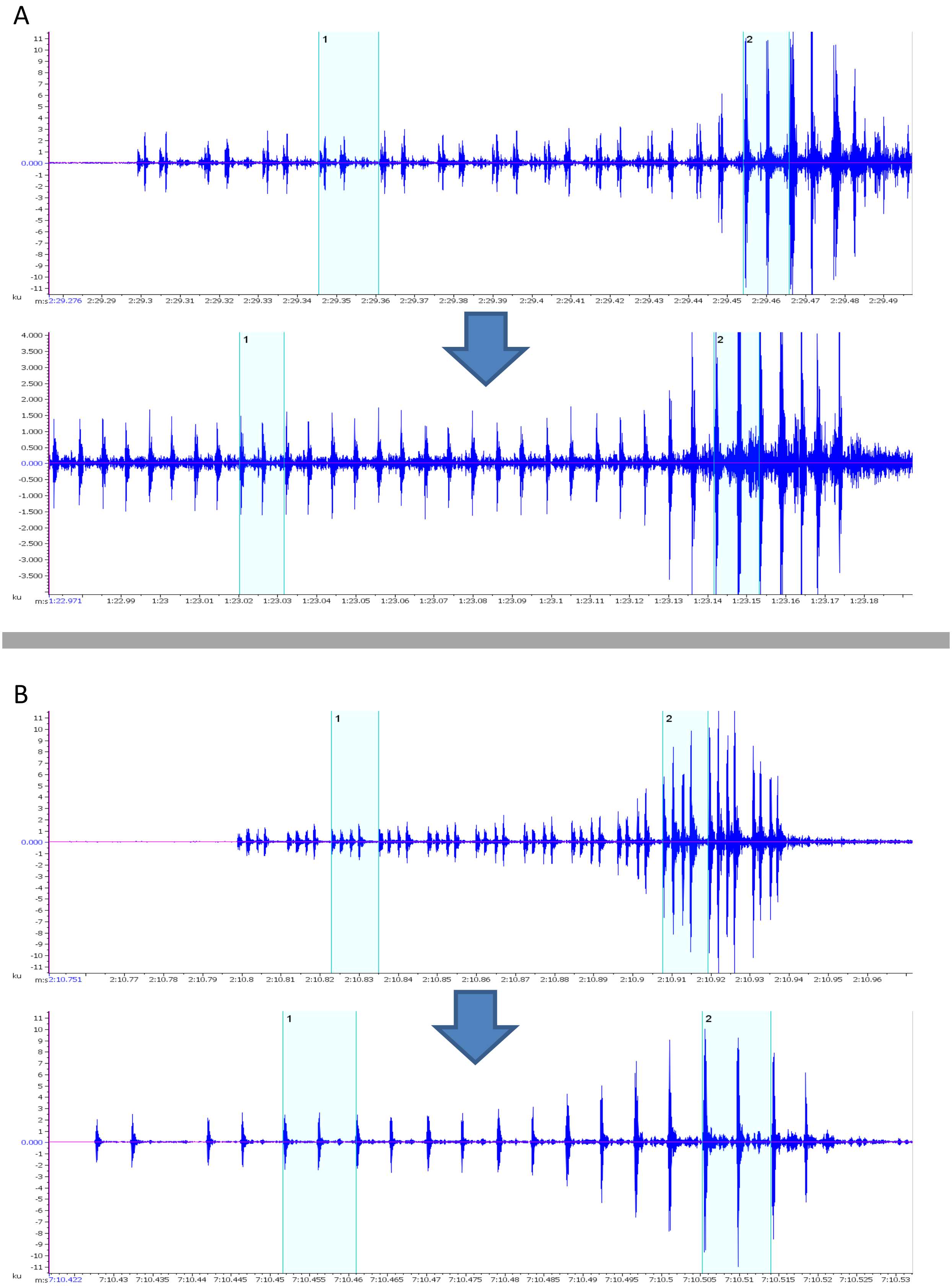

Fig S2.1.1. Timbal movement types of two *Cicadetta cantilatrix* specimens kept indoors. (A) Specimen 1 showing almost synchronous movements of both timbals: both timbals intact (top) and one inactivated (below). (B) Specimen 2 showing alternating movements of both timbals: both timbals intact (top) and one inactivated (below). Selections fit with one syllable duration (blue, in Raven Pro 1.4). Note: Time scale on x-axes constant (≈ 0.11 s), but room temperature slightly variable.

Indoor experiments syllable structure

In order to understand the syllable structures, two *Cicadetta cantilatrix* specimens from the Swiss Jura Mountains have been kept indoors in a cage. Specimens were rarely but spontaneously singing with the aid of a warming lamp. First, recordings were made with intact sound production systems, then one timbal was inactivated and the specimens recorded again (Fonseca, 1991; Sueur & Aubin, 2003). We present the different patterns discovered in **Fig S2.1.1**. They confirm the (almost) synchronous and the clearly alternating movement types. They also demonstrate that complete syllables consist of four pulses. The syllable duration was significantly longer in dependency of the temperature than predicted from the field work (average room temperature 22 °C), indicating that the missing solar radiation has a high impact on the speed of the timbals.

Measuring the phrase 3 temperature dependency, number of syllables within phrase 3 and power difference between phrases 1/2 and phrase 3

For the phrase 3 variables, a series of ten echemes have been chosen *from the centre* of the phrase (**Fig S2.1.2**). This procedure was repeated with a second series originating from a distant song part of the same recording. The last five precedent echemes and the first five following echemes of the adjoining phrases 1/2 were compared with the phrase 3 values for the power (**Fig S2.1.2**, Raven function: Max Power). The power difference was then calculated between the medium values of both phrase types.

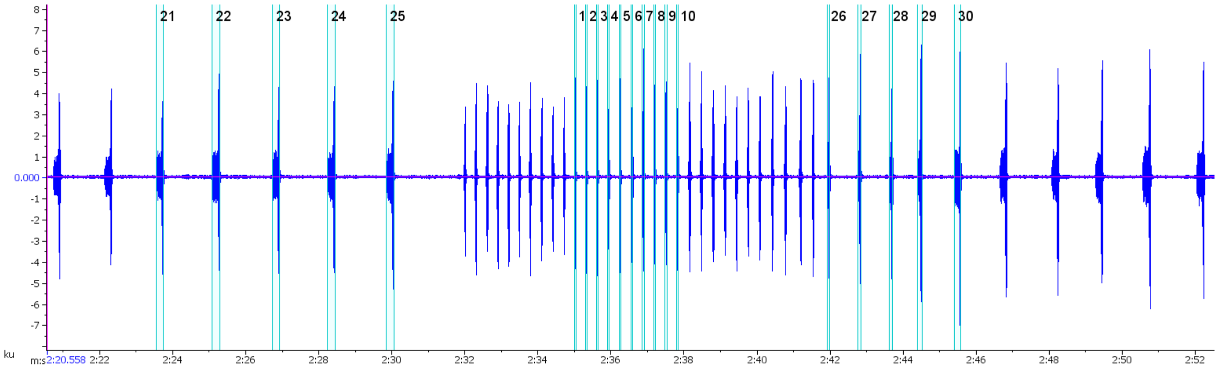

Fig S2.1.2. Oscillogram of a 2–3–2 phrases section with typical selections of the measured echemes (blue) in Raven Pro 1.4.

Measuring the first and second part of E_2

First and second parts of E_2 are without interval and consequently completely connected. The transitions are smooth. Therefore, we want to give examples how we have divided the two sections (**Fig S2.1.3**). We tried to separate the echeme parts exactly when the imaginary envelope curve of the amplitude turns from convex to concave shapes or when the amplitude starts to enlarge abruptly.

E_1 and E_3 echemes were so short that we had difficulties to distinguish first and second parts as had been done by Sueur & Puissant (2007). Therefore, we did not measure parts within these two echeme types.

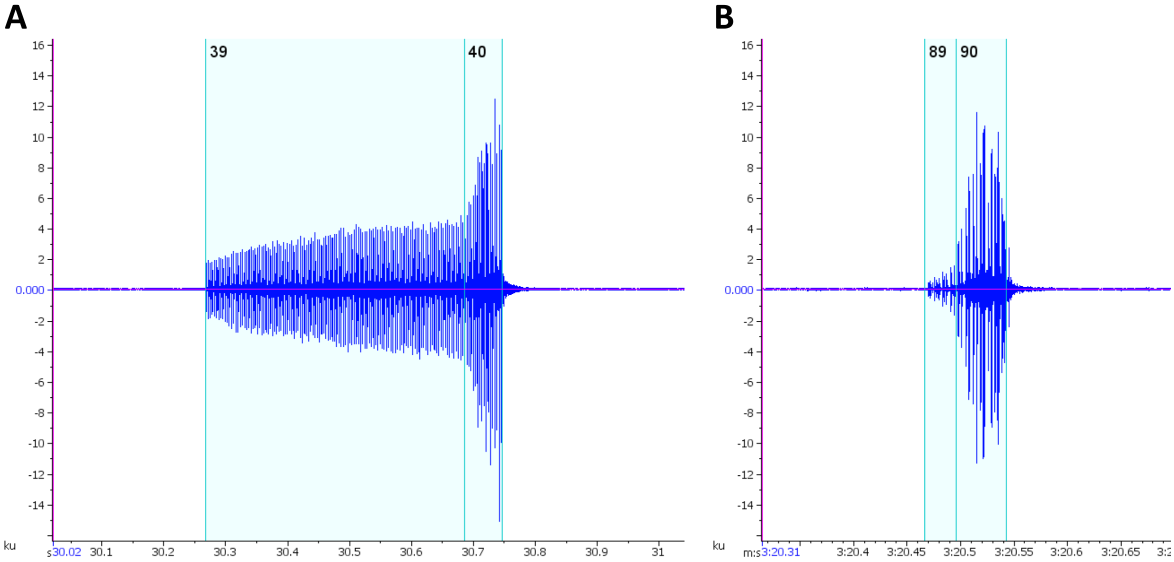

Fig S2.1.3. Oscillograms of opposed but typical E_2 echemes in (A) *Cicadetta anapaistica lucana* and (B) *C. cerdaniensis* s. str. Examples of the division into the first low intensity and the completely connected high intensity parts (blue) in Raven Pro 1.4.

References Appendix S2.1

Fonseca PJ. 1991. Characterstics of the acoustic signals in nine species of cicadas (Homoptera, Cicadidae). *Bioacoustics* 3: 173-182.

Sueur J, Aubin T. 2003. Specifity of cicada calling songs in the genus *Tibicina* (Hemiptera, Cicadidae). *Systematic Entomology* 28: 481-492.

Sueur J, Puissant S. 2007. Similar look but different song: a new *Cicadetta* species in the *montana* complex (Insecta, Hemiptera, Cicadidae). *Zootaxa* 1442: 55-68.

Appendix S2.2. Spatial origin of the data analysed acoustically.

(ordered by taxon name → latitude; sheet 1)

							Number of recordings analysed							
Taxon	**Location, region**	**Metapopulation**	**Altitude [m asl]**	**Lat.**	**Long.**	**Date**	All variables (23-28 °C)	Qualitative aspects phrase 3	Temperature dependency phrase 3	Syllables/E 3	Syllable duration	Delta power phrases, EP_{1+2} - EP_3	Phrase durations, PH_2, PH_3	Recordings analysed, total
Cicadetta anapaistica anapaistica	Serra d. Castagna, Nebrodi	Sicilian	980	37.8305	14.5797	4.7.2009	2	2		2		2	4	4
Cicadetta anapaistica anapaistica	Vallone Madonna degli Angeli, Madonie	Sicilian	1580	37.8397	14.0230	10.7.2009	2	1		1		1	4	4
Cicadetta anapaistica anapaistica	Portella di Colla, Madonie	Sicilian	1350	37.8623	14.0758	7.7.2013		2	2	2	2			2
Cicadetta anapaistica anapaistica	Monte Daino-Monte Mufara, Madonie	Sicilian	1470	37.8638	14.0459	12.7.2009		3		2		3	3	3
Cicadetta anapaistica anapaistica	Portella la Colla-Piano Zucchi, Madonie	Sicilian	1300	37.8781	14.0081	7/9/11.7.2009	5	6		10		10	11	15
Cicadetta anapaistica anapaistica	Portella la Colla-Piano Zucchi, Madonie	Sicilian	1300	37.8781	14.0081	7.7.2013		4	4	4	4	3		4
Cicadetta anapaistica anapaistica	Portella Calacudera, Nebrodi	Sicilian	1570	37.9248	14.6666	4.7.2009	1	2		2		2	1	2
Cicadetta anapaistica anapaistica	Portella Balestra-Contrada Botti, Nebrodi	Sicilian	1420	37.9386	14.7543	10.7.2013		3	3	3	3	3		3
Cicadetta anapaistica anapaistica	Bosco Mangalaviti, Nebrodi	Sicilian	1310	37.9561	14.7694	10.7.2013		2	1	1	1			2
Cicadetta anapaistica anapaistica	Pizzo Luminaria, Nebrodi	Sicilian	1160	37.9587	14.5042	3.7.2009							1	1
Cicadetta anapaistica anapaistica	Pizzo Luminaria, Nebrodi	Sicilian	1160	37.9587	14.5042	9.7.2013		1	1	1	1	1		1
Cicadetta anapaistica anapaistica	Portella Mandrazzi, Monti Peloritani	Sicilian	1120	37.9761	15.1387	11.7.2013		3	3	3	3	3		3
Cicadetta anapaistica anapaistica	Portella Frascianelle, Nebrodi	Sicilian	1300	37.9895	14.8794	10.7.2013		1	1	1	1	1		1
Cicadetta anapaistica anapaistica	SW Delianuova, Aspromonte	Calabrian	980	38.2174	15.8801	13.7.2013		1	1	1	1	1		1
Cicadetta anapaistica anapaistica	Bosco di Moio, Delianuova, Aspromonte	Calabrian	900	38.2227	15.8917	13.7.2013		2	2	2	2	2		2
Cicadetta anapaistica anapaistica	NW Elce della Vecchia, Le Serre	Calabrian	1000	38.5691	16.4022	14.7.2013		1	1	1	1	1		1
Cicadetta anapaistica anapaistica	NW Serra San Bruno, Le Serre	Calabrian	800	38.5950	16.2892	14.7.2013		2	1	1	1	1		2
Cicadetta anapaistica anapaistica	SW Carlopoli, Sila	Calabrian	730	39.0425	16.4311	15.7.2013		3	3	3	3	3		3
Cicadetta anapaistica anapaistica	NE Trearie, Sila	Calabrian	1120	39.1298	16.4227	15.7.2013		1						1
Cicadetta anapaistica anapaistica	Serra Todaro, Acri, Sila	Calabrian	1180	39.4286	16.4207	16.7.2013		4	3	3	3	3		4
Cicadetta anapaistica lucana	SW Colle del Dragone, Pollino	Basilicatian	1380	39.8935	16.0806	17.7.2013	1	1	1	1	1	1	1	1
Cicadetta anapaistica lucana	Piano di Iannace, Pollino	Basilicatian	1680	39.9398	16.1966	8.7.2010		1	1	1	1	1		1
Cicadetta anapaistica lucana	Acqua Tremola, Pollino	Basilicatian	1430	39.9701	16.2184	8.7.2010		1	1	1	1	1		1
Cicadetta anapaistica lucana	Salice-Mezzana Torre, Pollino	Basilicatian	970	39.9871	16.1793	7.7.2010	1	3	2	2	2	2	3	3
Cicadetta anapaistica lucana	Serra Alberigo - Polito, Pollino	Basilicatian	950	40.0002	16.1110	7.7.2010	2	2	2	2	2	2	3	3
Cicadetta anapaistica lucana	Salice-Mezzana Torre, Pollino	Basilicatian	1030	40.0054	16.1681	18.7.2013		7	6	6	6	3	3	7
Cicadetta anapaistica lucana	Monte Sirino	Basilicatian	930	40.1024	15.8564	18.7.2013	1	3	2	2	2	2	2	3
Cicadetta anapaistica lucana	La Sellata, Apennino Lucano	Basilicatian	1220	40.5266	15.7862	20.7.2013	1	1	1	1	1	1	1	1
Cicadetta anapaistica lucana	Bosco di M. Orsino, E Potenza	Basilicatian	250	40.7151	16.1456	5.7.2010		4	2	2	2	2	1	4
Cicadetta anapaistica lucana	W Acerno, Monti Picentini	Basilicatian	620	40.7566	15.0396	14.7.2010	1	7	3	3	3	2	3	7
Cicadetta anapaistica lucana	E Laghi di Monticchio, Vulture	Basilicatian	830	40.9198	15.6214	15.7.2010		1	1	1	1		1	1
Cicadetta anapaistica lucana	San Vito, Monticchio, Vulture	Basilicatian	580	40.9249	15.5635	15.7.2010		3	3	3	3	3	3	3
Cicadetta anapaistica lucana	Laghi di Monticchio, Vulture	Basilicatian	660	40.9337	15.6092	22.7.2013		4	2	2	2	2	1	4
Cicadetta anapaistica lucana	N Morra de Sanctis	Basilicatian	820	40.9365	15.2410	22.7.2013	1	9	6	6	6	3	1	9
Cicadetta anapaistica lucana	N Morra de Sanctis	Basilicatian	820	40.9365	15.2410	14.7.2010		2	1	2		2	2	2
Cicadetta cerdaniensis s. str.	Cami del Puig, Osséja, Pyrénées-Orientales	Pyrenean	1290	42.4066	1.9884	11/12.6.2011	3	9	9	8	8	5	5	9
Cicadetta cerdaniensis s. str.	Le Veinat-la Soulaine, Py, Pyrénées-Orientales	Pyrenean	1080	42.4996	2.3526	14/15.6.2011	5	15	15	13	13	9	10	15

Appendix S2.2. Spatial origin of the data analysed acoustically.

(ordered by taxon name → latitude; sheet 2)

							Number of recordings analysed							
Taxon	Location, region	Metapopulation	Altitude [m asl]	Lat.	Long.	Date	All variables (23-28 °C)	Qualitative aspects phrase 3	Temperature dependency phrase 3	Syllables/E 3	Syllable duration	Delta power phrases, EP_{1+2} - EP_3	Phrase durations, PH_2, PH_3	Recordings analysed, total
Cicadetta sibillae	W Monte Pentime, Taburno-Camposauro	Campanian	630	41.1919	14.5965	20.7.2010	2	2	2	2	2	2	2	2
Cicadetta sibillae	Campagnola, Roccamonfina	Campanian	270	41.3041	14.0560	22.7.2010		1	1					1
Cicadetta sibillae	Valle Agricola, Monti del Matese	Campanian	360	41.4090	14.2150	22.7.2010		4						4
Cicadetta sibillae	N Sella Canala, Monti del Sannio	Campanian	810	41.4362	14.8613	20.7.2010		4	3	2	2			4
Cicadetta sibillae	Verecchie, Monti Simbruini	Central Apenninian	1010	42.0384	13.2459	8.7.2011	1	6	6	3	3	1	1	6
Cicadetta sibillae	Tolfa-La Bianca, Monti della Tolfa	Central Apenninian	590	42.1510	11.9172	14.7.2011	1	2	2	1	1		1	2
Cicadetta sibillae	N Tolfa, Monti della Tolfa	Central Apenninian	420	42.1566	11.9304	14.7.2011		1		1		1		1
Cicadetta sibillae	Monte Calvi, Monti Sabatini	Central Apenninian	450	42.1796	12.2382	16.7.2011	1	3	3	2	2	1	1	3
Cicadetta sibillae	Rocca di Cambio-Rocca di Mezzo, Sirente-Velino	Central Apenninian	1390	42.2140	13.4812	7.7.2011	2	4	4	4	4	2	2	4
Cicadetta sibillae	Val Carpineto, Leonessa, Monti Reatini	Central Apenninian	990	42.5517	13.0158	10.7.2011		3	3	2	1			3
Cicadetta sibillae	N Pretare, Monti Sibillini	Central Apenninian	1050	42.8016	13.2918	12.7.2011	1	2	2	2	2	1	1	2
Cicadetta sibillae	Monte Vadiglione, Casentino-Falterona	Northern Apen.	740	43.8666	11.6181	3.7.2012		1	1	1	1			1
Cicadetta sibillae	N Campigna, Casentino-Falterona	Northern Apen.	950	43.8854	11.7567	3.7.2012		1	1	1	1			1
Cicadetta sibillae	Monte Gaginara, Cinqueterre, Liguria	Northern Apen.	650	44.1333	9.7150	16.7.2007		2				2		2
Cicadetta sibillae	Monte Faggiola, Valsenio	Northern Apen.	800	44.1481	11.4917	2.7.2012	1	1	1	1	1	1	1	1
Cicadetta sibillae	Valle Grande, Moneglia	Northern Apen.	300	44.2483	9.4583	13.7.2007		1				1		1
Cicadetta sibillae	Castelnuovo di Bisano, Bologna	Northern Apen.	480	44.2727	11.3691	2.7.2012	1	2	2	2	2	1	1	2
Cicadetta sibillae	Cargedolo, Frignano	Northern Apen.	860	44.2742	10.5677	16.7.2012	1	2	2	1	1	1	1	2
Cicadetta sibillae	SW San Nicolo, Camogli, Liguria	Northern Apen.	250	44.3283	9.1567	14.7.2007		1				1		1
Cicadetta sibillae	Traverde, Pontremoli	Northern Apen.	510	44.4033	9.8714	23.6.2012	1	1	1	1	1	1	1	1
Cicadetta sibillae	SW Pontinevra, Savona	Northern Apen.	570	44.4191	8.4201	20.6.2012	1	3	3	3	3	1	1	3
Cicadetta sibillae	Lottulo, Valle Maira	Western Alpine	780	44.4944	7.2351	18.6.2012	2	4	4	4	4	2	2	4
Cicadetta sibillae	N Pregola, Monte Lesima	Northern Apen.	1120	44.7548	9.2915	18.7.2012	1	3	3	3	3	1	1	3
Cicadetta sibillae	W Serralunga di Crea, Monferrato	Western Alpine	240	45.1055	8.2661	28.7.2013		2	2	2	2	2		2
Cicadetta sibillae	Monte Momello, Lanzo	Western Alpine	660	45.2658	7.4646	15.6.2012	2	3	3	3	3	1	2	3
Cicadetta sibillae	Valle Toscolano, Lago di Garda	Insubrian	500	45.6834	10.6245	17.6.2010		1		1				1
Cicadetta sibillae	Lodrino, Val Trompia	Insubrian	900	45.7050	10.2810	18.6.2010		2		2				2
Cicadetta sibillae	Monte Barro, Lecco	Insubrian	750	45.8283	9.3783	16.6.2007		1				1		1
Cicadetta sibillae	Monte Barro, Lecco	Insubrian	670	45.8290	9.3790	2.7.2013	2	5	5	5	5	5	2	6
Cicadetta sibillae	Monte Chiusarella, Varese	Insubrian	650	45.8650	8.8133	14.6.2007		2				2		2
Cicadetta sibillae	Monte San Giorgio, Ticino	Insubrian	1000	45.9100	8.9503	27.6.2007		2				2		2
Cicadetta sibillae	Sasso delle Parole, Barbengo, Ticino	Insubrian	450	45.9565	8.9064	16.7.2008		2				2		2
Cicadetta sibillae	Monte Caslano, Ticino	Insubrian	470	45.9612	8.8801	22.6.2013	2	5	5	5	5	2	2	5
Cicadetta sibillae	I Gan, San Vittore, Mesocco	Insubrian	460	46.2421	9.1106	21.6.2013		1	1	1	1	1		1
Cicadetta sibillae	Crap de Maria, Castaneda, Mesocco	Insubrian	760	46.2592	9.1263	21.6.2013		1	1	1	1	1		1
Cicadetta sibillae	Brez, Cloz, Trentino	Insubrian	860	46.4419	11.1122	17.7.2011		3	3	2	2			3
						sum	48	201	146	157	133	110	86	218

Appendix S2.3. Database of the records of *Cicadetta sibillae, Cicadetta anapaistica lucana* and *Cicadetta anapaistica anapaistica* .

(ordered by taxon name → latitude; sheet 1)

Taxon	♂	♀	Location	Political Region	Country	Altitude [m asl]	Lat.	Long.	Observer	Year	Month	Day
Cicadetta a. anapaistica	1		above Petralia Sottana	SICI	Italy	1080	37.8075	14.0972	Hertach Thomas	2009	7	12
Cicadetta a. anapaistica	10		Serra di Castagna, Nebrodi	SICI	Italy	980	37.8305	14.5797	Hertach Thomas	2009	7	4
Cicadetta a. anapaistica	10	2	Vallone Madonna degli Angeli, Madonie	SICI	Italy	1580	37.8397	14.0230	Hertach Thomas	2009	7	10
Cicadetta a. anapaistica	3		Vallone Madonna degli Angeli, Madonie	SICI	Italy	1530	37.8405	14.0238	Hertach Thomas	2009	7	10
Cicadetta a. anapaistica	8		Casa Gorgonero, Petralia	SICI	Italy	1170	37.8424	14.0968	Hertach Thomas	2009	7	7
Cicadetta a. anapaistica	3		Vallone Madonna degli Angeli, Madonie	SICI	Italy	1460	37.8436	14.0210	Hertach Thomas	2009	7	10
Cicadetta a. anapaistica	4		Quacella, Madonie	SICI	Italy	1330	37.8484	14.0161	Hertach Thomas	2009	7	10
Cicadetta a. anapaistica	2		Monte dell'Abate, Cesaro, Nebrodi	SICI	Italy	1270	37.8542	14.7062	Hertach Thomas	2009	7	4
Cicadetta a. anapaistica	5	2	Piano di Farina, Madonie	SICI	Italy	1330	37.8582	14.0688	Hertach Thomas	2009	7	12
Cicadetta a. anapaistica	7		Piano di Farina, Madonie	SICI	Italy	1380	37.8622	14.0783	Hertach Thomas	2009	7	12
Cicadetta a. anapaistica	10		Portella di Colla, Madonie	SICI	Italy	1350	37.8623	14.0758	Hertach Thomas	2013	7	7
Cicadetta a. anapaistica	5		Piano di Farina, Madonie	SICI	Italy	1390	37.8624	14.0754	Hertach Thomas	2009	7	7
Cicadetta a. anapaistica	5		Monte Daino, Madonie	SICI	Italy	1470	37.8638	14.0459	Hertach Thomas	2009	7	12
Cicadetta a. anapaistica	5		Monte Daino, Madonie	SICI	Italy	1500	37.8647	14.0388	Hertach Thomas	2009	7	7
Cicadetta a. anapaistica	5		Monte Daino, Madonie	SICI	Italy	1490	37.8652	14.0395	Hertach Thomas	2009	7	7
Cicadetta a. anapaistica	4		Monte Mufara, Madonie	SICI	Italy	1530	37.8653	14.0320	Hertach Thomas	2009	7	12
Cicadetta a. anapaistica	2		Monte Daino, Madonie	SICI	Italy	1500	37.8655	14.0398	Hertach Thomas	2009	7	12
Cicadetta a. anapaistica	1		Monte Daino, Madonie	SICI	Italy	1470	37.8662	14.0417	Hertach Thomas	2009	7	12
Cicadetta a. anapaistica	1		Monte Mufara, Madonie	SICI	Italy	1550	37.8679	14.0304	Hertach Thomas	2009	7	12
Cicadetta a. anapaistica	3		Portella la Colla, Madonie	SICI	Italy	1410	37.8683	14.0068	Hertach Thomas	2009	7	9
Cicadetta a. anapaistica	30	1	Portella la Colla, Madonie	SICI	Italy	1450	37.8697	14.0086	Hertach Thomas	2009	7	7
Cicadetta a. anapaistica	15		Portella la Colla, Madonie	SICI	Italy	1430	37.8697	14.0097	Hertach Thomas	2013	7	7
Cicadetta a. anapaistica	3		Portella la Colla, Madonie	SICI	Italy	1470	37.8711	14.0107	Hertach Thomas	2009	7	9
Cicadetta a. anapaistica	2		Portella la Colla, Madonie	SICI	Italy	1470	37.8711	14.0107	Hertach Thomas	2009	7	11
Cicadetta a. anapaistica	3		Pizzo Colla, Madonie	SICI	Italy	1450	37.8720	13.9951	Hertach Thomas	2009	7	9
Cicadetta a. anapaistica	5		Portella la Colla - Portella Arena, Madonie	SICI	Italy	1430	37.8732	14.0126	Hertach Thomas	2013	7	7
Cicadetta a. anapaistica	2		Hotel Baita del Faggio, Madonie	SICI	Italy	1360	37.8737	14.0062	Hertach Thomas	2009	7	11
Cicadetta a. anapaistica	4		Pizzo Colla, Madonie	SICI	Italy	1540	37.8741	13.9917	Hertach Thomas	2009	7	9
Cicadetta a. anapaistica	3		Pizzo Colla, Madonie	SICI	Italy	1550	37.8742	13.9903	Hertach Thomas	2009	7	9
Cicadetta a. anapaistica	10	1	Hotel Baita del Faggio, Madonie	SICI	Italy	1380	37.8742	14.0070	Hertach Thomas	2009	7	11
Cicadetta a. anapaistica	4		Rifugio Ostello Gioventu, Madonie	SICI	Italy	1560	37.8772	14.0169	Hertach Thomas	2009	7	7
Cicadetta a. anapaistica	3		Hotel Baita del Faggio, Madonie	SICI	Italy	1290	37.8778	14.0081	Hertach Thomas	2013	7	7
Cicadetta a. anapaistica	3		Hotel Baita del Faggio, Madonie	SICI	Italy	1330	37.8781	14.0081	Hertach Thomas	2009	7	11
Cicadetta a. anapaistica	1		Mandria Castiglia, Madonie	SICI	Italy	1300	37.8800	14.0099	Hertach Thomas	2009	7	11
Cicadetta a. anapaistica	3		Pizzo Manca Badia, Nebrodi	SICI	Italy	1160	37.8807	14.4972	Hertach Thomas	2013	7	9
Cicadetta a. anapaistica	3		Contrada Cicogna, Nebrodi	SICI	Italy	1330	37.8809	14.6600	Hertach Thomas	2009	7	4
Cicadetta a. anapaistica	1		Piano Zucchi, Madonie	SICI	Italy	1180	37.8839	14.0002	Hertach Thomas	2013	7	7
Cicadetta a. anapaistica	1		Mandria Castiglia, Madonie	SICI	Italy	1300	37.8846	14.0088	Hertach Thomas	2009	7	11
Cicadetta a. anapaistica	1		Valle di Zotta Fonda, Madonie	SICI	Italy	1750	37.8850	14.0381	Hertach Thomas	2009	7	8
Cicadetta a. anapaistica	1		Piano Zucchi, Madonie	SICI	Italy	1150	37.8856	14.0016	Hertach Thomas	2013	7	7
Cicadetta a. anapaistica	10		Contrada Radica, Nebrodi	SICI	Italy	1400	37.8924	14.6533	Hertach Thomas	2009	7	4
Cicadetta a. anapaistica	10	1	Pizzo Stefano, Madonie	SICI	Italy	1550	37.8945	14.0583	Hertach Thomas	2009	7	8
Cicadetta a. anapaistica	15	2	Pizzo Stefano, Madonie	SICI	Italy	1580	37.8952	14.0563	Hertach Thomas	2009	7	8
Cicadetta a. anapaistica	15	1	Portella Creta, Nebrodi	SICI	Italy	1500	37.9058	14.5121	Hertach Thomas	2009	7	3
Cicadetta a. anapaistica	3		Portella Femmina Morta, Nebrodi	SICI	Italy	1530	37.9197	14.6544	Hertach Thomas	2009	7	4
Cicadetta a. anapaistica	3		Portella Calacudéra, Nebrodi	SICI	Italy	1570	37.9248	14.6666	Hertach Thomas	2009	7	4
Cicadetta a. anapaistica	2	1	Portella Calacudéra, Nebrodi	SICI	Italy	1560	37.9249	14.6668	Hertach Thomas	2009	7	4
Cicadetta a. anapaistica	3		Portella Calacudéra, Nebrodi	SICI	Italy	1540	37.9299	14.6746	Hertach Thomas	2009	7	4
Cicadetta a. anapaistica	1		Portella Calacudéra, Nebrodi	SICI	Italy	1500	37.9322	14.6752	Hertach Thomas	2009	7	4
Cicadetta a. anapaistica	3		Portella Calacudéra, Nebrodi	SICI	Italy	1490	37.9349	14.6744	Hertach Thomas	2009	7	4
Cicadetta a. anapaistica	1		Pizzo di Bella Fontana, Nebrodi	SICI	Italy	1320	37.9357	14.5108	Hertach Thomas	2009	7	3
Cicadetta a. anapaistica	6		Portella Balestra-Contrada Botti, Longi	SICI	Italy	1420	37.9386	14.7543	Hertach Thomas	2013	7	10
Cicadetta a. anapaistica	1		Portella Balestra-Contrada Botti, Longi	SICI	Italy	1470	37.9411	14.7546	Hertach Thomas	2013	7	10
Cicadetta a. anapaistica	5		Bosco Mangalaviti, Longi	SICI	Italy	1310	37.9561	14.7694	Hertach Thomas	2013	7	10
Cicadetta a. anapaistica	2		Pizzo Luminaria, Nebrodi	SICI	Italy	1180	37.9572	14.5036	Hertach Thomas	2009	7	3
Cicadetta a. anapaistica	4		Pizzo Luminaria, Nebrodi	SICI	Italy	1160	37.9587	14.5042	Hertach Thomas	2013	7	9
Cicadetta a. anapaistica	1		Pizzo Castellano, Nebrodi	SICI	Italy	1080	37.9595	14.4989	Hertach Thomas	2013	7	9
Cicadetta a. anapaistica	2		Pizzo Castellano, Nebrodi	SICI	Italy	1040	37.9599	14.4927	Hertach Thomas	2009	7	3
Cicadetta a. anapaistica	3		Cozzo Canfuto, Nebrodi	SICI	Italy	920	37.9668	14.4874	Hertach Thomas	2009	7	3
Cicadetta a. anapaistica	10		Portella Mandrazzi, Monti Peloritani	SICI	Italy	1120	37.9761	15.1387	Hertach Thomas	2013	7	11
Cicadetta a. anapaistica	1		Portella Pertusa, Monti Peloritani	SICI	Italy	1010	37.9804	15.1442	Hertach Thomas	2013	7	11
Cicadetta a. anapaistica	7		Portella Frascianelle, Nebrodi	SICI	Italy	1300	37.9895	14.8794	Hertach Thomas	2013	7	10
Cicadetta a. anapaistica	1		Pizzo di Verna, Monti Peloritani	SICI	Italy	900	38.0087	15.2293	Hertach Thomas	2013	7	11
Cicadetta a. anapaistica	7		Portella Rinazzo, Nebrodi	SICI	Italy	1030	38.0195	14.8713	Hertach Thomas	2013	7	10
Cicadetta a. anapaistica	3		SW Delianuova	CALA	Italy	980	38.2174	15.8801	Hertach Thomas	2013	7	13
Cicadetta a. anapaistica	1		Bosco di Moio, Delianuova	CALA	Italy	900	38.2227	15.8917	Hertach Thomas	2013	7	13
Cicadetta a. anapaistica	5		NW Elce della Vecchia	CALA	Italy	1000	38.5691	16.4022	Hertach Thomas	2013	7	14
Cicadetta a. anapaistica	5		NW Serra San Bruno	CALA	Italy	780	38.5906	16.2942	Hertach Thomas	2013	7	14
Cicadetta a. anapaistica	1		NW Serra San Bruno	CALA	Italy	800	38.5950	16.2892	Hertach Thomas	2013	7	14
Cicadetta a. anapaistica	8		SW Carlopoli	CALA	Italy	730	39.0425	16.4311	Hertach Thomas	2013	7	15
Cicadetta a. anapaistica	5		NE Trearie	CALA	Italy	1120	39.1298	16.4227	Hertach Thomas	2013	7	15
Cicadetta a. anapaistica	6		Serra Todaro, Sila	CALA	Italy	1180	39.4286	16.4207	Hertach Thomas	2013	7	16
Cicadetta a. lucana	1		above San Donato di Nineo	CALA	Italy	1250	39.7316	16.0347	Hertach Thomas	2010	7	9
Cicadetta a. lucana	4		NE Morano Calabro	CALA	Italy	1020	39.8602	16.0953	Hertach Thomas	2010	7	9
Cicadetta a. lucana	5		Timpone Rotondella, Pollino	CALA	Italy	980	39.8606	16.0953	Hertach Thomas	2013	7	17
Cicadetta a. lucana	1		SW Colle del Dragone	CALA	Italy	1380	39.8935	16.0806	Hertach Thomas	2013	7	17
Cicadetta a. lucana	2		Bosco Cerviero, Pollino	CALA	Italy	900	39.9115	16.0426	Hertach Thomas	2010	7	9
Cicadetta a. lucana	1		Bosco Cerviero, Pollino	CALA	Italy	830	39.9175	16.0340	Hertach Thomas	2010	7	9
Cicadetta a. lucana	1		Serra di Crispo, Pollino	BASI	Italy	1960	39.9290	16.2119	Hertach Thomas	2010	7	8
Cicadetta a. lucana	2		Piano di Iannace, Pollino	BASI	Italy	1680	39.9398	16.1966	Hertach Thomas	2010	7	8
Cicadetta a. lucana	5		S Madonna di Pollino	BASI	Italy	1590	39.9456	16.1814	Hertach Thomas	2010	7	8
Cicadetta a. lucana	1		Timpane Valle Dellauze, Pollino	BASI	Italy	1320	39.9462	16.1457	Hertach Thomas	2010	7	7
Cicadetta a. lucana	10		S Madonna di Pollino	BASI	Italy	1540	39.9467	16.1784	Hertach Thomas	2010	7	8
Cicadetta a. lucana	2		Impieso, Pollino	BASI	Italy	1260	39.9614	16.1839	Hertach Thomas	2010	7	8

Appendix S2.3. Database of the records of *Cicadetta sibillae, Cicadetta anapaistica lucana* and *Cicadetta anapaistica anapaistica* .

(ordered by taxon name → latitude; sheet 2)

Taxon	♂	♀	Location	Political Region	Country	Altitude [m asl]	Lat.	Long.	Observer	Year	Month	Day
Cicadetta a. lucana	2		Acqua Tremola, Pollino	BASI	Italy	1420	39.9654	16.2059	Hertach Thomas	2010	7	8
Cicadetta a. lucana	4		Acqua Tremola, Pollino	BASI	Italy	1430	39.9701	16.2184	Hertach Thomas	2010	7	8
Cicadetta a. lucana	1		S Timpone di Balsamano, Pollino	BASI	Italy	1110	39.9736	16.2724	Hertach Thomas	2010	7	8
Cicadetta a. lucana	1		above Viggianello	BASI	Italy	760	39.9809	16.1064	Hertach Thomas	2013	7	18
Cicadetta a. lucana	1		NE Terranova di Pollino	BASI	Italy	990	39.9816	16.3073	Hertach Thomas	2010	7	8
Cicadetta a. lucana	1		Cozzo Prinicipessa, Pollino	BASI	Italy	1110	39.9826	16.1413	Hertach Thomas	2010	7	7
Cicadetta a. lucana	4		Salice, Pollino	BASI	Italy	970	39.9868	16.1803	Hertach Thomas	2013	7	17
Cicadetta a. lucana	6		Mezzana Torre, Pollino	BASI	Italy	970	39.9871	16.1793	Hertach Thomas	2010	7	7
Cicadetta a. lucana	5		Serra Alberigo, Pollino	BASI	Italy	890	39.9953	16.1105	Hertach Thomas	2013	7	18
Cicadetta a. lucana	15	1	Serra Alberigo - Polito, Pollino	BASI	Italy	1070	39.9980	16.1218	Hertach Thomas	2013	7	17
Cicadetta a. lucana	5		Serra Alberigo, Pollino	BASI	Italy	950	40.0002	16.1110	Hertach Thomas	2010	7	7
Cicadetta a. lucana	5		Serra Alberigo - Polito, Pollino	BASI	Italy	1040	40.0012	16.1170	Hertach Thomas	2013	7	17
Cicadetta a. lucana	30		Serra Alberigo, Pollino	BASI	Italy	1020	40.0014	16.1171	Hertach Thomas	2010	7	7
Cicadetta a. lucana	10		N Mezzana Torre, Pollino	BASI	Italy	1040	40.0054	16.1677	Hertach Thomas	2010	7	7
Cicadetta a. lucana	15		N Mezzana Torre, Pollino	BASI	Italy	1030	40.0054	16.1681	Hertach Thomas	2013	7	18
Cicadetta a. lucana	1		E Castelluccio	BASI	Italy	620	40.0102	15.9484	Hertach Thomas	2013	7	18
Cicadetta a. lucana	1		San Severino Lucano	BASI	Italy	970	40.0171	16.1469	Hertach Thomas	2010	7	8
Cicadetta a. lucana	15		Timpa Falascoso, Pollino	BASI	Italy	950	40.0184	16.1107	Hertach Thomas	2010	7	7
Cicadetta a. lucana	1		Bosco Magnano, San Severino Lucano	BASI	Italy	630	40.0509	16.1258	Hertach Thomas	2010	7	7
Cicadetta a. lucana	1		N Lauria	BASI	Italy	830	40.0766	15.8601	Hertach Thomas	2010	7	10
Cicadetta a. lucana	20		E Monte Sirino	BASI	Italy	900	40.0975	15.8573	Hertach Thomas	2010	7	10
Cicadetta a. lucana	1		NE Lauria	BASI	Italy	880	40.0978	15.8574	Hertach Thomas	2013	7	18
Cicadetta a. lucana	5		Monte Sirino	BASI	Italy	930	40.1024	15.8564	Hertach Thomas	2013	7	18
Cicadetta a. lucana	15		Monte Sirino	BASI	Italy	970	40.1171	15.8666	Hertach Thomas	2013	7	18
Cicadetta a. lucana	2		E Monte Sirino	BASI	Italy	1110	40.1401	15.8747	Hertach Thomas	2010	7	10
Cicadetta a. lucana	1		E Monte Sirino	BASI	Italy	1220	40.1448	15.8658	Hertach Thomas	2010	7	10
Cicadetta a. lucana	3		Monte Sirino	BASI	Italy	1230	40.1492	15.8615	Hertach Thomas	2013	7	19
Cicadetta a. lucana	1		Monte Sirino	BASI	Italy	1270	40.1492	15.8539	Hertach Thomas	2013	7	19
Cicadetta a. lucana	2		Monte Gurmara	BASI	Italy	1040	40.1586	15.7734	Hertach Thomas	2010	7	10
Cicadetta a. lucana	3		SW Fontana d'Eboli	BASI	Italy	1200	40.1592	15.8466	Hertach Thomas	2013	7	19
Cicadetta a. lucana	6		S Fontana d'Eboli	BASI	Italy	910	40.1782	15.8744	Hertach Thomas	2013	7	19
Cicadetta a. lucana	1		Monti di Vallivona, Cilento	CAMP	Italy	1180	40.2553	15.4771	Hertach Thomas	2010	7	11
Cicadetta a. lucana	2		Vallivona, Monti Cervati, Cilento	CAMP	Italy	1160	40.2634	15.4642	Hertach Thomas	2010	7	11
Cicadetta a. lucana	1		N Marsico Nuovo	BASI	Italy	1010	40.4513	15.7338	Hertach Thomas	2013	7	20
Cicadetta a. lucana	3		Bosco Monte Piano, Dolomiti Lucane	BASI	Italy	1050	40.4545	16.1367	Hertach Thomas	2010	7	6
Cicadetta a. lucana	2		Bosco Monte Piano, Dolomiti Lucane	BASI	Italy	1090	40.4595	16.1225	Hertach Thomas	2010	7	6
Cicadetta a. lucana	10	1	N Marsico Nuovo	BASI	Italy	1130	40.4659	15.7380	Hertach Thomas	2013	7	20
Cicadetta a. lucana	3		La Sellata	BASI	Italy	1220	40.5266	15.7862	Hertach Thomas	2013	7	20
Cicadetta a. lucana	1		S Pignola	BASI	Italy	1110	40.5438	15.7856	Hertach Thomas	2013	7	20
Cicadetta a. lucana	15		Monte Cupolicchio	BASI	Italy	750	40.6494	16.0554	Hertach Thomas	2010	7	5
Cicadetta a. lucana	10		Monte Cupolicchio	BASI	Italy	710	40.6619	16.0538	Hertach Thomas	2010	7	5
Cicadetta a. lucana	2		Bosco di Monte Orsino	BASI	Italy	250	40.7151	16.1456	Hertach Thomas	2010	7	5
Cicadetta a. lucana	1		W Acerno	CAMP	Italy	580	40.7478	15.0420	Hertach Thomas	2010	7	13
Cicadetta a. lucana	15	1	W Acerno	CAMP	Italy	600	40.7545	15.0406	Hertach Thomas	2010	7	14
Cicadetta a. lucana	7		W Acerno	CAMP	Italy	620	40.7566	15.0396	Hertach Thomas	2010	7	14
Cicadetta a. lucana	1		NE Avigliano	BASI	Italy	1010	40.7634	15.7629	Hertach Thomas	2013	7	20
Cicadetta a. lucana	1		Bosco Difesa Grande, Gravina in Puglia	PUGL	Italy	330	40.7681	16.4097	Hertach Thomas	2010	7	4
Cicadetta a. lucana	5		E Laghi di Monticchio	BASI	Italy	830	40.9198	15.6214	Hertach Thomas	2010	7	15
Cicadetta a. lucana	15		San Vito, Monticchio	BASI	Italy	580	40.9249	15.5635	Hertach Thomas	2010	7	15
Cicadetta a. lucana	15		Laghi di Monticchio	BASI	Italy	650	40.9306	15.6138	Hertach Thomas	2010	7	14
Cicadetta a. lucana	7		Laghi di Monticchio	BASI	Italy	660	40.9337	15.6092	Hertach Thomas	2013	7	22
Cicadetta a. lucana	3		Laghi di Monticchio	BASI	Italy	650	40.9346	15.6109	Hertach Thomas	2010	7	14
Cicadetta a. lucana	1		Laghi di Monticchio	BASI	Italy	680	40.9359	15.6092	Hertach Thomas	2010	7	14
Cicadetta a. lucana	8		N Morra de Sanctis	CAMP	Italy	820	40.9365	15.2410	Hertach Thomas	2010	7	14
Cicadetta a. lucana	20		N Morra de Sanctis	CAMP	Italy	820	40.9365	15.2410	Hertach Thomas	2013	7	22
Cicadetta a. lucana	2		SE Bisaccio	CAMP	Italy	830	40.9932	15.4042	Hertach Thomas	2010	7	14
Cicadetta sibillae	6		W Monte Pentime	CAMP	Italy	630	41.1919	14.5965	Hertach Thomas	2010	7	20
Cicadetta sibillae	1		below Campolattaro	CAMP	Italy	450	41.2933	14.7311	Hertach Thomas	2010	7	20
Cicadetta sibillae	1		above Campagnola, Roccamonfina	CAMP	Italy	420	41.3026	14.0273	Hertach Thomas	2010	7	22
Cicadetta sibillae	7		Campagnola, Roccamonfina	CAMP	Italy	270	41.3041	14.0560	Hertach Thomas	2010	7	22
Cicadetta sibillae	10		Terme di Suio	LAZI	Italy	20	41.3231	13.8833	Hertach Thomas	2010	7	23
Cicadetta sibillae	10		Valle Agricola	CAMP	Italy	360	41.4090	14.2150	Hertach Thomas	2010	7	22
Cicadetta sibillae	6		N Sella Canala	MOLI	Italy	810	41.4362	14.8613	Hertach Thomas	2010	7	20
Cicadetta sibillae	1		N Sella Canala	MOLI	Italy	790	41.4378	14.8588	Hertach Thomas	2010	7	20
Cicadetta sibillae	1		Monte Sambuco	PUGL	Italy	890	41.5311	15.0825	Hertach Thomas	2011	6	28
Cicadetta sibillae	4		N Cerasuolo	LAZI	Italy	780	41.5953	14.0225	Hertach Thomas	2011	7	3
Cicadetta sibillae	1		NE San Vito Romano	LAZI	Italy	690	41.8973	12.9808	Hertach Thomas	2010	7	25
Cicadetta sibillae	5		S Vallepietra	LAZI	Italy	700	41.9102	13.2231	Hertach Thomas	2011	7	9
Cicadetta sibillae	4		NE Vallepietra	LAZI	Italy	900	41.9250	13.2485	Hertach Thomas	2011	7	9
Cicadetta sibillae	1		NE Vallepietra	LAZI	Italy	1010	41.9353	13.2503	Hertach Thomas	2011	7	9
Cicadetta sibillae	1		Sant. della Santissima Trinità, Vallepietra	LAZI	Italy	1320	41.9546	13.2491	Hertach Thomas	2011	7	9
Cicadetta sibillae	1		Ville di Fano	ABRU	Italy	1410	41.9904	13.2710	Hertach Thomas	2011	7	8
Cicadetta sibillae	10		Verecchie	ABRU	Italy	1010	42.0384	13.2459	Hertach Thomas	2011	7	8
Cicadetta sibillae	3		N Poggio Cinolfo	LAZI	Italy	770	42.1222	13.0595	Hertach Thomas	2011	7	9
Cicadetta sibillae	2		SE Collalto Sabino	LAZI	Italy	790	42.1298	13.0596	Hertach Thomas	2011	7	9
Cicadetta sibillae	10		Tolfa - La Bianca	LAZI	Italy	590	42.1510	11.9172	Hertach Thomas	2011	7	14
Cicadetta sibillae	3		Tolfa	LAZI	Italy	530	42.1534	11.9278	Hertach Thomas	2011	7	14
Cicadetta sibillae	10		N Tolfa	LAZI	Italy	420	42.1566	11.9304	Hertach Thomas	2011	7	14
Cicadetta sibillae	1		NE Tolfa	LAZI	Italy	440	42.1573	11.9428	Hertach Thomas	2011	7	14
Cicadetta sibillae	8		E San Lorenzo nähe Nespolo	LAZI	Italy	770	42.1663	13.0534	Hertach Thomas	2011	7	9
Cicadetta sibillae	5		Monte Calvi, Monti Sabatini	LAZI	Italy	450	42.1796	12.2382	Hertach Thomas	2011	7	16
Cicadetta sibillae	2		SE Rocca di Mezzo	ABRU	Italy	1210	42.1825	13.5809	Hertach Thomas	2011	7	8

Appendix S2.3. Database of the records of *Cicadetta sibillae, Cicadetta anapaistica lucana* and *Cicadetta anapaistica anapaistica* .

(ordered by taxon name → latitude; sheet 3)

Taxon	♂	♀	Location	Political Region	Country	Altitude [m asl]	Lat.	Long.	Observer	Year	Month	Day
Cicadetta sibillae	1		SE Rocca di Mezzo	ABRU	Italy	1330	42.1831	13.5649	Hertach Thomas	2011	7	8
Cicadetta sibillae	1		Ricetto-Tonnicoda	LAZI	Italy	930	42.1917	13.0771	Hertach Thomas	2011	7	9
Cicadetta sibillae	5		Monte Calvi, Monti Sabatini	LAZI	Italy	350	42.2059	12.2402	Hertach Thomas	2011	7	16
Cicadetta sibillae	1		Tonnicoda	LAZI	Italy	670	42.2062	13.0884	Hertach Thomas	2011	7	9
Cicadetta sibillae	6		Rocca di Cambio-Rocca di Mezzo	ABRU	Italy	1390	42.2140	13.4812	Hertach Thomas	2011	7	7
Cicadetta sibillae	2		N Campolano	LAZI	Italy	550	42.2329	13.0843	Hertach Thomas	2011	7	9
Cicadetta sibillae	5		N Teglieto	LAZI	Italy	550	42.2572	13.0648	Hertach Thomas	2011	7	9
Cicadetta sibillae	5		S Capradosso	LAZI	Italy	710	42.3127	13.0355	Hertach Thomas	2011	7	9
Cicadetta sibillae	1		E Poggio Bustone	LAZI	Italy	920	42.5042	12.9029	Hertach Thomas	2011	7	10
Cicadetta sibillae	1		SW Lago di Provvidenza	ABRU	Italy	1150	42.5043	13.3996	Hertach Thomas	2011	7	11
Cicadetta sibillae	1		S Lago di Campotosto	ABRU	Italy	1300	42.5071	13.3590	Hertach Thomas	2011	7	11
Cicadetta sibillae	1		S Lago di Campotosto	ABRU	Italy	1300	42.5083	13.3610	Hertach Thomas	2011	7	11
Cicadetta sibillae	2		Albaneto-Posta	LAZI	Italy	1060	42.5306	13.0603	Hertach Thomas	2011	7	10
Cicadetta sibillae	1		NE Montereale	ABRU	Italy	900	42.5462	13.2650	Hertach Thomas	2011	7	11
Cicadetta sibillae	6		Val Carpineto, Leonessa	LAZI	Italy	990	42.5517	13.0158	Hertach Thomas	2011	7	10
Cicadetta sibillae	6		N Aringo	ABRU	Italy	1100	42.5693	13.2694	Hertach Thomas	2011	7	11
Cicadetta sibillae	1		W Leonessa	LAZI	Italy	990	42.5725	12.9420	Hertach Thomas	2011	7	10
Cicadetta sibillae	1		W Morice	ABRU	Italy	870	42.7268	13.4434	Hertach Thomas	2011	7	11
Cicadetta sibillae	1		NE San Martino near Acquasanta Terme	MARC	Italy	820	42.7373	13.4533	Hertach Thomas	2011	7	11
Cicadetta sibillae	1		San Martino near Acquasanta Terme	MARC	Italy	880	42.7381	13.4500	Hertach Thomas	2011	7	11
Cicadetta sibillae	7		above Pito near Acquasanta Terme	MARC	Italy	930	42.7509	13.4360	Hertach Thomas	2011	7	11
Cicadetta sibillae	1		SE Acquasanta Terme	MARC	Italy	840	42.7543	13.4455	Hertach Thomas	2011	7	11
Cicadetta sibillae	3		N Pretare, Monti Sibillini	MARC	Italy	1050	42.8016	13.2918	Hertach Thomas	2011	7	12
Cicadetta sibillae	5		N Pretare, Monti Sibillini	MARC	Italy	1160	42.8102	13.3070	Hertach Thomas	2011	7	12
Cicadetta sibillae	13		W Rigo	MARC	Italy	1080	42.8200	13.3180	Hertach Thomas	2011	7	12
Cicadetta sibillae	3		Valle Orsara, Monti Sibillini	MARC	Italy	940	42.8305	13.3239	Hertach Thomas	2011	7	12
Cicadetta sibillae	1		Castro, Montegallo	MARC	Italy	820	42.8485	13.3274	Hertach Thomas	2011	7	12
Cicadetta sibillae	3		W Cornaloni	MARC	Italy	890	42.8636	13.3331	Hertach Thomas	2011	7	12
Cicadetta sibillae	3		W Cornaloni	MARC	Italy	970	42.8682	13.3422	Hertach Thomas	2011	7	12
Cicadetta sibillae	1		S Passo la Calla	TOSC	Italy	640	43.8197	11.7425	Hertach Thomas	2012	7	3
Cicadetta sibillae	5		Valico Croce a Mori	TOSC	Italy	840	43.8457	11.6452	Hertach Thomas	2012	7	3
Cicadetta sibillae	2		S Passo la Calla	TOSC	Italy	1000	43.8498	11.7506	Hertach Thomas	2012	7	3
Cicadetta sibillae	1		Valico Croce a Mori	TOSC	Italy	880	43.8565	11.6323	Hertach Thomas	2012	7	3
Cicadetta sibillae	3		Fornace, Londa	TOSC	Italy	500	43.8590	11.5987	Hertach Thomas	2012	7	3
Cicadetta sibillae	2		Monte Vadiglione	TOSC	Italy	610	43.8638	11.6104	Hertach Thomas	2012	7	3
Cicadetta sibillae	15		Monte Vadiglione	TOSC	Italy	740	43.8666	11.6181	Hertach Thomas	2012	7	3
Cicadetta sibillae	10	1	N Campigna	EMIL	Italy	950	43.8854	11.7567	Hertach Thomas	2012	7	3
Cicadetta sibillae	1		Corniolo, Casentino-Falterona	EMIL	Italy	530	43.9011	11.7850	Hertach Thomas	2012	7	3
Cicadetta sibillae	3		Razzuolo-Ronta	TOSC	Italy	440	44.0202	11.4494	Hertach Thomas	2012	7	3
Cicadetta sibillae	10		above Razzuolo	TOSC	Italy	720	44.0362	11.4701	Hertach Thomas	2012	7	3
Cicadetta sibillae	1		above Razzuolo	TOSC	Italy	840	44.0401	11.4819	Hertach Thomas	2012	7	3
Cicadetta sibillae	1		Acquetico above Pieve di Teco	LIGU	Italy	340	44.0579	7.8761	Hertach Thomas	2012	6	19
Cicadetta sibillae	3		SW Castelnuovo di Garfagnana	TOSC	Italy	460	44.0691	10.3295	Hertach Thomas	2012	6	25
Cicadetta sibillae	2		Passo Sambuco	TOSC	Italy	1020	44.0719	11.4872	Hertach Thomas	2012	7	3
Cicadetta sibillae	3		Passo Sambuco	TOSC	Italy	900	44.0736	11.4972	Hertach Thomas	2012	7	3
Cicadetta sibillae	1		SW Castelnuovo di Garfagnana	TOSC	Italy	360	44.0889	10.3774	Hertach Thomas	2012	6	25
Cicadetta sibillae	5		Piedimonti-Passo Sambuco	TOSC	Italy	650	44.0910	11.5183	Hertach Thomas	2012	7	3
Cicadetta sibillae	10		Castelpoggio-Gragnana	TOSC	Italy	430	44.1125	10.0788	Hertach Thomas	2012	6	25
Cicadetta sibillae	5		S Ponte di Nava	LIGU	Italy	780	44.1151	7.8676	Hertach Thomas	2012	6	19
Cicadetta sibillae	4		above Volastra, Cinqueterre	LIGU	Italy	570	44.1217	9.7283	Hertach Thomas	2007	7	16
Cicadetta sibillae	4		Monte Capri, Cinqueterre	LIGU	Italy	730	44.1250	9.7417	Hertach Thomas	2007	7	16
Cicadetta sibillae	2		Monte Cuna, Cinqueterre	LIGU	Italy	730	44.1267	9.7367	Hertach Thomas	2007	7	16
Cicadetta sibillae	40	1	Monte Gaginara, Cinqueterre	LIGU	Italy	650	44.1333	9.7150	Hertach Thomas	2007	7	16
Cicadetta sibillae	1		Bibbiana	TOSC	Italy	690	44.1358	11.5098	Hertach Thomas	2012	7	2
Cicadetta sibillae	5		Monte Faggiola	TOSC	Italy	800	44.1481	11.4917	Hertach Thomas	2012	7	2
Cicadetta sibillae	1		Tirli	TOSC	Italy	650	44.1529	11.4801	Hertach Thomas	2012	7	2
Cicadetta sibillae	1		Castiglione di Garfagnana	TOSC	Italy	620	44.1536	10.4147	Hertach Thomas	2012	6	25
Cicadetta sibillae	7		Nasago, Ormea	PIEM	Italy	640	44.1596	7.9572	Hertach Thomas	2012	6	19
Cicadetta sibillae	1		N Coniale	TOSC	Italy	690	44.1709	11.4476	Hertach Thomas	2012	7	2
Cicadetta sibillae	2		W Roccapelago	EMIL	Italy	1070	44.2001	10.5868	Hertach Thomas	2012	7	15
Cicadetta sibillae	2		Colle di Canda	TOSC	Italy	810	44.2017	11.3950	Hertach Thomas	2012	7	2
Cicadetta sibillae	3		N Pievepelago	EMIL	Italy	700	44.2123	10.6258	Hertach Thomas	2012	6	26
Cicadetta sibillae	3		N Frassineta	EMIL	Italy	440	44.2246	11.3554	Hertach Thomas	2012	7	2
Cicadetta sibillae	1		Piandelagotti	EMIL	Italy	1110	44.2349	10.5216	Hertach Thomas	2012	7	16
Cicadetta sibillae	1		Moneglia, Smeraldo	LIGU	Italy	200	44.2417	9.4733	Hertach Thomas	2007	7	13
Cicadetta sibillae	10		E Montecreto	EMIL	Italy	840	44.2472	10.7297	Hertach Thomas	2012	6	26
Cicadetta sibillae	2		Moneglia, Valle Grande	LIGU	Italy	300	44.2483	9.4583	Hertach Thomas	2007	7	13
Cicadetta sibillae	1		Piandelagotti	EMIL	Italy	980	44.2499	10.5389	Hertach Thomas	2012	7	16
Cicadetta sibillae	1		W Magliano	TOSC	Italy	270	44.2532	10.0567	Hertach Thomas	2012	6	24
Cicadetta sibillae	2		S Licciana	TOSC	Italy	260	44.2586	10.0376	Hertach Thomas	2012	6	24
Cicadetta sibillae	3		Ponte di Strettara	EMIL	Italy	530	44.2622	10.7123	Hertach Thomas	2012	6	26
Cicadetta sibillae	1		S Ponte Scoltenna	EMIL	Italy	310	44.2639	10.8627	Hertach Thomas	2012	6	26
Cicadetta sibillae	10		Castelnuovo di Bisano	EMIL	Italy	480	44.2727	11.3691	Hertach Thomas	2012	7	2
Cicadetta sibillae	2		Cargedolo	EMIL	Italy	860	44.2742	10.5677	Hertach Thomas	2012	7	16
Cicadetta sibillae	8		Castelnuovo di Bisano	EMIL	Italy	460	44.2747	11.3705	Hertach Thomas	2012	7	2
Cicadetta sibillae	1		NE Licciana	TOSC	Italy	240	44.2797	10.0607	Hertach Thomas	2012	6	24
Cicadetta sibillae	1		Codevilla, Mallare	LIGU	Italy	480	44.2857	8.2757	Hertach Thomas	2012	6	20
Cicadetta sibillae	7		Bocca dei Ravari	EMIL	Italy	730	44.2968	11.0191	Hertach Thomas	2012	6	26
Cicadetta sibillae	5		above Castello di Comano	TOSC	Italy	770	44.3039	10.1152	Hertach Thomas	2012	6	24
Cicadetta sibillae	3		above San Fruttuoso, Portofino	LIGU	Italy	200	44.3183	9.1700	Hertach Thomas	2007	7	14
Cicadetta sibillae	2		S Zena	EMIL	Italy	270	44.3194	11.3643	Hertach Thomas	2012	7	2
Cicadetta sibillae	6		Semaforo nuovo, Portofino	LIGU	Italy	250	44.3200	9.1683	Hertach Thomas	2007	7	14
Cicadetta sibillae	25		SW San Nicolo, Camogli	LIGU	Italy	250	44.3283	9.1567	Hertach Thomas	2007	7	14

Appendix S2.3. Database of the records of *Cicadetta sibillae, Cicadetta anapaistica lucana* and *Cicadetta anapaistica anapaistica* .

(ordered by taxon name → latitude; sheet 4)

Taxon	♂	♀	Location	Political Region	Country	Altitude [m asl]	Lat.	Long.	Observer	Year	Month	Day
Cicadetta sibillae	10		N Altare	LIGU	Italy	450	44.3499	8.3434	Hertach Thomas	2012	6	20
Cicadetta sibillae	3		SE Montepastore	EMIL	Italy	590	44.3599	11.1332	Hertach Thomas	2012	6	26
Cicadetta sibillae	3		Monte Tramonto	EMIL	Italy	690	44.3648	11.1569	Hertach Thomas	2012	6	26
Cicadetta sibillae	2		SW Medelana	EMIL	Italy	660	44.3686	11.1636	Hertach Thomas	2012	6	26
Cicadetta sibillae	2		Valditapca	EMIL	Italy	1040	44.3851	10.0901	Hertach Thomas	2012	6	24
Cicadetta sibillae	1		Rimagna	EMIL	Italy	900	44.3856	10.1259	Hertach Thomas	2012	6	24
Cicadetta sibillae	5		above Traverde	TOSC	Italy	510	44.4033	9.8714	Hertach Thomas	2012	6	23
Cicadetta sibillae	5		below Passo del Righedo	TOSC	Italy	560	44.4149	9.9005	Hertach Thomas	2012	6	24
Cicadetta sibillae	15		SW Pontinevra	LIGU	Italy	570	44.4191	8.4201	Hertach Thomas	2012	6	20
Cicadetta sibillae	8		C. del Ben, Pontinvrea	LIGU	Italy	600	44.4250	8.4267	Hertach Thomas	2008	8	1
Cicadetta sibillae	5		SW Pontinevra	LIGU	Italy	570	44.4261	8.4130	Hertach Thomas	2012	6	20
Cicadetta sibillae	2		Nugareto	EMIL	Italy	180	44.4504	11.2471	Hertach Thomas	2012	6	26
Cicadetta sibillae	2		Cavassolo, Doria	LIGU	Italy	130	44.4506	9.0311	Hertach Thomas	2013	7	4
Cicadetta sibillae	1		E Carpineti	EMIL	Italy	580	44.4536	10.5503	Hertach Thomas	2012	7	16
Cicadetta sibillae	5		Passo del Brattello	TOSC	Italy	860	44.4574	9.8210	Hertach Thomas	2012	6	23
Cicadetta sibillae	1		S Aquabianca	LIGU	Italy	940	44.4592	8.6451	Hertach Thomas	2013	7	27
Cicadetta sibillae	7		above Trensasco	LIGU	Italy	400	44.4600	8.9470	Hertach Thomas	2013	7	4
Cicadetta sibillae	1		E Valdena	EMIL	Italy	750	44.4601	9.8061	Hertach Thomas	2012	6	23
Cicadetta sibillae	5		S Passo della Sillara	EMIL	Italy	1040	44.4711	10.0179	Hertach Thomas	2012	6	24
Cicadetta sibillae	1		Passo del Faiallo	LIGU	Italy	700	44.4786	8.7108	Hertach Thomas	2013	7	27
Cicadetta sibillae	3		S Urbe	LIGU	Italy	630	44.4797	8.5827	Hertach Thomas	2012	6	20
Cicadetta sibillae	5		N Passo della Sillara	EMIL	Italy	950	44.4913	9.9973	Hertach Thomas	2012	6	24
Cicadetta sibillae	4		above S. Damiano, Maira	PIEM	Italy	800	44.4913	7.2581	Hertach Thomas	2012	6	18
Cicadetta sibillae	5		Lottulo-Stroppo	PIEM	Italy	700	44.4923	7.2330	Hertach Thomas	2012	6	18
Cicadetta sibillae	5		Laccio	LIGU	Italy	600	44.4935	9.1327	Hertach Thomas	2013	7	4
Cicadetta sibillae	1		SE Bedonia	EMIL	Italy	420	44.4936	9.6630	Hertach Thomas	2012	6	23
Cicadetta sibillae	5		Lottulo-Stroppo	PIEM	Italy	730	44.4942	7.2071	Hertach Thomas	2012	6	18
Cicadetta sibillae	20		Lottulo	PIEM	Italy	780	44.4944	7.2351	Hertach Thomas	2012	6	18
Cicadetta sibillae	5		Lottulo-Stroppo	PIEM	Italy	820	44.5018	7.1542	Hertach Thomas	2012	6	18
Cicadetta sibillae	5		Berceto	EMIL	Italy	810	44.5110	9.9975	Hertach Thomas	2012	6	24
Cicadetta sibillae	5		Serra - Montebruno	LIGU	Italy	700	44.5198	9.2304	Hertach Thomas	2013	7	4
Cicadetta sibillae	1		above Anzola	EMIL	Italy	810	44.5250	9.5512	Hertach Thomas	2012	6	23
Cicadetta sibillae	15		Tiglieto	LIGU	Italy	420	44.5277	8.6104	Hertach Thomas	2012	6	20
Cicadetta sibillae	5		Tiglieto	LIGU	Italy	420	44.5277	8.6104	Hertach Thomas	2013	7	27
Cicadetta sibillae	5		above Tiglieto	LIGU	Italy	620	44.5300	8.6269	Hertach Thomas	2012	6	21
Cicadetta sibillae	6		Monte Oliveto, Campo Ligure	LIGU	Italy	640	44.5366	8.7273	Hertach Thomas	2012	6	21
Cicadetta sibillae	3		Passo delle Lepre	EMIL	Italy	1290	44.5374	9.4765	Hertach Thomas	2012	6	23
Cicadetta sibillae	5		Ponteceno	EMIL	Italy	710	44.5379	9.5941	Hertach Thomas	2012	6	23
Cicadetta sibillae	5		Monte Calvo, Tiglieto	LIGU	Italy	590	44.5387	8.6276	Hertach Thomas	2012	6	21
Cicadetta sibillae	2		Capanna di Marcarolo	LIGU	Italy	760	44.5446	8.7705	Hertach Thomas	2012	6	21
Cicadetta sibillae	5		N Monte Calvo, Tiglieto	LIGU	Italy	540	44.5500	8.6300	Hertach Thomas	2012	6	21
Cicadetta sibillae	1		above Rocco Stefano	LIGU	Italy	1230	44.5520	9.4753	Hertach Thomas	2012	6	22
Cicadetta sibillae	5		Ponte Nespole, Torrente Gorzente	LIGU	Italy	510	44.5728	8.7889	Hertach Thomas	2012	6	21
Cicadetta sibillae	2		W Cozzano	EMIL	Italy	760	44.5747	10.1712	Hertach Thomas	2012	7	16
Cicadetta sibillae	5		S Molare	PIEM	Italy	360	44.5769	8.6035	Hertach Thomas	2013	7	27
Cicadetta sibillae	10		Monte Tugello - Monte Tobbio	LIGU	Italy	540	44.5775	8.7869	Hertach Thomas	2012	6	21
Cicadetta sibillae	4		S Fragnolo	EMIL	Italy	680	44.5801	10.1607	Hertach Thomas	2012	7	16
Cicadetta sibillae	5		E Camarza	LIGU	Italy	490	44.5861	8.9979	Hertach Thomas	2012	6	21
Cicadetta sibillae	5		Monte Tugello - Monte Tobbio	LIGU	Italy	550	44.5863	8.7912	Hertach Thomas	2012	6	21
Cicadetta sibillae	4		N Rovegno	LIGU	Italy	550	44.5910	9.2825	Hertach Thomas	2013	7	4
Cicadetta sibillae	5		above Calestano	EMIL	Italy	560	44.5910	10.1479	Hertach Thomas	2012	7	16
Cicadetta sibillae	5		S Langhirano	EMIL	Italy	340	44.5924	10.2651	Hertach Thomas	2012	7	16
Cicadetta sibillae	12		Orezzoli	EMIL	Italy	970	44.5930	9.3808	Hertach Thomas	2012	6	22
Cicadetta sibillae	1		Vallecalda, Capanne di Marcarolo	LIGU	Italy	460	44.5990	8.9107	Hertach Thomas	2012	6	21
Cicadetta sibillae	3		I Piani, Vallenzona	PIEM	Italy	1090	44.6048	9.1027	Hertach Thomas	2012	6	21
Cicadetta sibillae	10		I Piani, Vallenzona	LIGU	Italy	870	44.6063	9.0946	Hertach Thomas	2012	6	21
Cicadetta sibillae	5		NE Vobbia	LIGU	Italy	520	44.6074	9.0495	Hertach Thomas	2012	6	21
Cicadetta sibillae	7		E Cipollina	LIGU	Italy	600	44.6181	8.9253	Hertach Thomas	2012	6	21
Cicadetta sibillae	1		Dova Inferiore - Dova Supperiore	PIEM	Italy	940	44.6326	9.1194	Hertach Thomas	2012	6	21
Cicadetta sibillae	1		Cariseto	EMIL	Italy	950	44.6469	9.3717	Hertach Thomas	2012	6	22
Cicadetta sibillae	1		N Cariseto	EMIL	Italy	900	44.6645	9.3727	Hertach Thomas	2012	6	22
Cicadetta sibillae	3		below Montaldo di Cosola	PIEM	Italy	620	44.6668	9.1382	Hertach Thomas	2012	6	22
Cicadetta sibillae	1		Capanne di Cosola, Monte Lesima	EMIL	Italy	1420	44.6679	9.2052	Hertach Thomas	2012	6	22
Cicadetta sibillae	2		Capanne di Cosola, Monte Lesima	EMIL	Italy	1410	44.6759	9.2031	Hertach Thomas	2012	6	22
Cicadetta sibillae	10		above Montaldo di Cosola	PIEM	Italy	1160	44.6773	9.1804	Hertach Thomas	2012	6	22
Cicadetta sibillae	5		Cerignale	EMIL	Italy	600	44.6783	9.3402	Hertach Thomas	2012	6	22
Cicadetta sibillae	5		Cerignale	EMIL	Italy	810	44.6791	9.3586	Hertach Thomas	2012	6	22
Cicadetta sibillae	1		Passo del Giovà	EMIL	Italy	1270	44.6827	9.2215	Hertach Thomas	2012	7	18
Cicadetta sibillae	5		Ponte Organasco	EMIL	Italy	410	44.6876	9.3039	Hertach Thomas	2013	7	4
Cicadetta sibillae	6		Ponte Organasco	EMIL	Italy	460	44.6900	9.3100	Hertach Thomas	2012	6	22
Cicadetta sibillae	1		S Casali	EMIL	Italy	900	44.6937	9.7572	Hertach Thomas	2012	7	17
Cicadetta sibillae	2		Lama, Brallo	LOMB	Italy	660	44.7021	9.3092	Hertach Thomas	2012	6	22
Cicadetta sibillae	1		above Lama	EMIL	Italy	570	44.7024	9.2970	Hertach Thomas	2013	7	4
Cicadetta sibillae	5		above Corbesassi	LOMB	Italy	1000	44.7077	9.2653	Hertach Thomas	2013	7	4
Cicadetta sibillae	1		Cima di Colletta	LOMB	Italy	1310	44.7094	9.2598	Hertach Thomas	2012	6	22
Cicadetta sibillae	3		Cima di Colletta	LOMB	Italy	1310	44.7094	9.2598	Hertach Thomas	2012	7	18
Cicadetta sibillae	4		SE Morfasso	EMIL	Italy	680	44.7161	9.7253	Hertach Thomas	2012	7	17
Cicadetta sibillae	5		N Corbesassi	LOMB	Italy	940	44.7179	9.2722	Hertach Thomas	2013	7	4
Cicadetta sibillae	10		N Negruzzo	LOMB	Italy	1180	44.7196	9.2243	Hertach Thomas	2012	7	18
Cicadetta sibillae	7		W Negruzzo	LOMB	Italy	1260	44.7211	9.2186	Hertach Thomas	2012	7	18
Cicadetta sibillae	1		Monte Santa Cristina	EMIL	Italy	530	44.7263	9.8908	Hertach Thomas	2012	7	17
Cicadetta sibillae	1		E Bore	EMIL	Italy	780	44.7285	9.8001	Hertach Thomas	2012	7	17
Cicadetta sibillae	1		above Brallo	LOMB	Italy	1060	44.7324	9.2725	Hertach Thomas	2012	7	18

Appendix S2.3. Database of the records of *Cicadetta sibillae, Cicadetta anapaistica lucana* and *Cicadetta anapaistica anapaistica* .

(ordered by taxon name → latitude; sheet 5)

Taxon	♂	♀	Location	Political Region	Country	Altitude [m asl]	Lat.	Long.	Observer	Year	Month	Day
Cicadetta sibillae	20		SW Brallo di Pregola	LOMB	Italy	970	44.7341	9.2759	Hertach Thomas	2013	7	4
Cicadetta sibillae	2		Cegni	LOMB	Italy	940	44.7391	9.2280	Hertach Thomas	2012	7	18
Cicadetta sibillae	10		Brallo di Pregola	LOMB	Italy	900	44.7401	9.2931	Hertach Thomas	2012	6	22
Cicadetta sibillae	1		Pregola	LOMB	Italy	950	44.7420	9.2848	Hertach Thomas	2012	7	18
Cicadetta sibillae	1		SE Bettola	EMIL	Italy	850	44.7489	9.6405	Hertach Thomas	2012	7	17
Cicadetta sibillae	15		N Pregola	LOMB	Italy	1120	44.7548	9.2915	Hertach Thomas	2012	7	18
Cicadetta sibillae	3		Cima di Valle Scura	EMIL	Italy	1100	44.7641	9.2855	Hertach Thomas	2012	7	18
Cicadetta sibillae	5		W Cella	PIEM	Italy	540	44.7785	9.1625	Hertach Thomas	2012	7	19
Cicadetta sibillae	3		N Cella	LOMB	Italy	650	44.7802	9.1869	Hertach Thomas	2012	7	18
Cicadetta sibillae	4		W Bettola	EMIL	Italy	610	44.7822	9.5857	Hertach Thomas	2012	7	17
Cicadetta sibillae	5		N Cella	LOMB	Italy	640	44.7824	9.1897	Hertach Thomas	2012	7	19
Cicadetta sibillae	5		N Cella	LOMB	Italy	610	44.7841	9.1913	Hertach Thomas	2012	7	18
Cicadetta sibillae	1		Passo del Cerro	EMIL	Italy	750	44.7908	9.5471	Hertach Thomas	2012	7	17
Cicadetta sibillae	4		S Perino	EMIL	Italy	390	44.7909	9.5220	Hertach Thomas	2012	7	17
Cicadetta sibillae	2		W Passo del Penice	LOMB	Italy	1080	44.8007	9.3135	Hertach Thomas	2012	7	18
Cicadetta sibillae	5		Monte d'Alpe	LOMB	Italy	1050	44.8022	9.3149	Hertach Thomas	2013	7	3
Cicadetta sibillae	5		E Passo del Penice	EMIL	Italy	1020	44.8030	9.3371	Hertach Thomas	2012	7	18
Cicadetta sibillae	2		E Passo del Penice	EMIL	Italy	940	44.8064	9.3473	Hertach Thomas	2012	7	18
Cicadetta sibillae	5		Monte d'Alpe	LOMB	Italy	1010	44.8087	9.3186	Hertach Thomas	2013	7	3
Cicadetta sibillae	5		S Romagnese	LOMB	Italy	680	44.8272	9.3240	Hertach Thomas	2013	7	3
Cicadetta sibillae	5		S Romagnese	LOMB	Italy	650	44.8327	9.3265	Hertach Thomas	2013	7	3
Cicadetta sibillae	6		E Zavattarello	LOMB	Italy	470	44.8685	9.2858	Hertach Thomas	2013	7	3
Cicadetta sibillae	5		Sacro Monte di Crea	PIEM	Italy	380	45.0952	8.2700	Hertach Thomas	2013	7	27
Cicadetta sibillae	5		W Serralunga di Crea	PIEM	Italy	240	45.1055	8.2661	Hertach Thomas	2013	7	27
Cicadetta sibillae	10		W Serralunga di Crea	PIEM	Italy	240	45.1055	8.2661	Hertach Thomas	2013	7	28
Cicadetta sibillae	2		above Caprie, Susa	PIEM	Italy	400	45.1192	7.3204	Hertach Thomas	2012	6	16
Cicadetta sibillae	10		E Celle, Susa	PIEM	Italy	870	45.1304	7.3548	Hertach Thomas	2012	6	16
Cicadetta sibillae	3		Il Dazio, Viù	PIEM	Italy	620	45.2374	7.4263	Hertach Thomas	2012	6	15
Cicadetta sibillae	5		Monte Momello, Lanzo	PIEM	Italy	660	45.2658	7.4646	Hertach Thomas	2012	6	15
Cicadetta sibillae	4		S Rozello	PIEM	Italy	600	45.2660	7.4410	Hertach Thomas	2012	6	15
Cicadetta sibillae	2		Passo Tre Termini	LOMB	Italy	680	45.6608	10.1030	Hertach Thomas	2010	6	18
Cicadetta sibillae	6		Valle Toscolano	LOMB	Italy	500	45.6834	10.6245	Hertach Thomas	2010	6	17
Cicadetta sibillae	1		Valle Toscolano	LOMB	Italy	470	45.6956	10.6117	Hertach Thomas	2010	6	17
Cicadetta sibillae	30		Lodrino	LOMB	Italy	900	45.7050	10.2810	Hertach Thomas	2010	6	18
Cicadetta sibillae	25		Monte Barro, Lecco	LOMB	Italy	750	45.8283	9.3783	Hertach T./Trilar T.	2007	6	16
Cicadetta sibillae	35		Monte Barro, Lecco	LOMB	Italy	670	45.8290	9.3790	Hertach Thomas	2013	7	2
Cicadetta sibillae	80		Monte Chiusarella, Rasa	LOMB	Italy	650	45.8650	8.8133	Hertach T./Trilar T.	2007	6	14
Cicadetta sibillae	10		Poncione d'Arzo, Costa di Prabello	TICINO	Switzerland	700	45.8849	8.9432	Hertach Thomas	2006	5	28
Cicadetta sibillae	3		Roncaia, Monte	TICINO	Switzerland	890	45.8852	9.0232	Monnerat Christian	2009	6	12
Cicadetta sibillae	25		Poncione d'Arzo, Costa di Prabello	TICINO	Switzerland	750	45.8877	8.9407	Hertach Thomas	2006	5	28
Cicadetta sibillae	1		Poncione d'Arzo, Pre Sacco	TICINO	Switzerland	775	45.8877	8.9355	Hertach Thomas	2006	5	28
Cicadetta sibillae	6		Giura, Meride	TICINO	Switzerland	660	45.8929	8.9524	Hertach Thomas	2004	7	11
Cicadetta sibillae	6		Monte San Giorgio	TICINO	Switzerland	870	45.9037	8.9501	Hertach Thomas	2005	6	5
Cicadetta sibillae	35		Monte San Giorgio	TICINO	Switzerland	1000	45.9100	8.9503	Hertach Thomas	2007	6	27
Cicadetta sibillae	1		Monte San Giorgio	TICINO	Switzerland	960	45.9100	8.9503	Hertach Thomas	2013	6	3
Cicadetta sibillae	50		Monte San Giorgio	TICINO	Switzerland	960	45.9109	8.9516	Hertach Thomas	2005	6	5
Cicadetta sibillae	25		Monte San Giorgio	TICINO	Switzerland	1040	45.9109	8.9516	Hertach Thomas	2006	5	27
Cicadetta sibillae	12		Monte San Giorgio	TICINO	Switzerland	960	45.9118	8.9504	Hertach Thomas	2004	7	11
Cicadetta sibillae	25		Monte San Giorgio	TICINO	Switzerland	980	45.9119	8.9491	Hertach Thomas	2005	6	6
Cicadetta sibillae	7		Sasso delle Parole, Barbengo	TICINO	Switzerland	410	45.9556	8.9051	Hertach Thomas	2007	6	28
Cicadetta sibillae	7		Sasso delle Parole, Barbengo	TICINO	Switzerland	420	45.9556	8.9064	Hertach Thomas	2008	7	15
Cicadetta sibillae	10		Sasso delle Parole, Barbengo	TICINO	Switzerland	450	45.9565	8.9064	Hertach Thomas	2008	7	16
Cicadetta sibillae	2		Sasso delle Parole, Barbengo	TICINO	Switzerland	470	45.9570	8.9065	Hertach Thomas	2008	7	15
Cicadetta sibillae	4		Monte Caslano	TICINO	Switzerland	460	45.9604	8.8795	Hertach Thomas	2005	5	27
Cicadetta sibillae	5		Monte Caslano	TICINO	Switzerland	460	45.9604	8.8795	Hertach Thomas	2006	6	5
Cicadetta sibillae	8		Monte Caslano	TICINO	Switzerland	460	45.9604	8.8795	Hertach Thomas	2007	6	26
Cicadetta sibillae	12		Monte Caslano	TICINO	Switzerland	460	45.9604	8.8795	Hertach Thomas	2010	6	24
Cicadetta sibillae	4		Monte Caslano	TICINO	Switzerland	350	45.9604	8.8799	Hertach Thomas	2010	6	24
Cicadetta sibillae	20	2	Monte Caslano	TICINO	Switzerland	470	45.9612	8.8801	Hertach Thomas	2013	6	22
Cicadetta sibillae	1		San Vitale, Arogno	TICINO	Switzerland	680	45.9622	8.9840	Hertach Thomas	2010	6	25
Cicadetta sibillae	3		San Vitale, Arogno	TICINO	Switzerland	720	45.9630	8.9839	Hertach Thomas	2010	6	23
Cicadetta sibillae	2		San Vitale, Arogno	TICINO	Switzerland	730	45.9643	8.9827	Hertach Thomas	2010	6	23
Cicadetta sibillae	1		San Evasio, Pugerna	TICINO	Switzerland	590	45.9700	8.9808	Hertach Thomas	2010	6	23
Cicadetta sibillae	1		San Salvatore	TICINO	Switzerland	860	45.9780	8.9477	Hertach Thomas	2009	7	24
Cicadetta sibillae	1		San Salvatore-Lugano Paradiso	TICINO	Switzerland	820	45.9793	8.9483	Hertach Thomas	2000	7	14
Cicadetta sibillae	8		San Salvatore	TICINO	Switzerland	820	45.9793	8.9483	Hertach Thomas	2004	7	10
Cicadetta sibillae	1		Oliveto, San Salvatore	TICINO	Switzerland	320	45.9805	8.9551	Hertach Thomas	2010	6	23
Cicadetta sibillae	1		Bocchetta di Nava, Tremezzo	LOMB	Italy	810	46.0018	9.2133	Hertach Thomas	2013	6	4
Cicadetta sibillae	4		Castagnola-Gandria	TICINO	Switzerland	280	46.0039	8.9929	Hertach Thomas	2007	6	17
Cicadetta sibillae	1		Castagnola-Gandria	TICINO	Switzerland	290	46.0039	8.9968	Hertach Thomas	2007	6	28
Cicadetta sibillae	1		Castagnola	TICINO	Switzerland	430	46.0040	8.9878	Hertach Thomas	2006	7	27
Cicadetta sibillae	10		Ruvigliana-Gandria	TICINO	Switzerland	460	46.0066	8.9956	Hertach Thomas	2005	7	13
Cicadetta sibillae	1		Passo Streccione, Denti della Vecchia	LOMB	Italy	1430	46.0520	9.0279	Hertach Thomas	2009	6	12
Cicadetta sibillae	1		Passo Streccione, Denti della Vecchia	TICINO	Switzerland	1400	46.0538	9.0279	Hertach Thomas	2009	6	12
Cicadetta sibillae	1		Gudo, Malacarne	TICINO	Switzerland	320	46.1775	8.9318	Hertach Thomas	2005	7	15
Cicadetta sibillae	1		Saleggi, Tegna	TICINO	Switzerland	225	46.1825	8.7493	Hertach Thomas	2005	7	20
Cicadetta sibillae	1		I Gan, San Vittore	GRISONS	Switzerland	460	46.2421	9.1106	Hertach Thomas	2013	6	21
Cicadetta sibillae	1		Crap de Maria, Castaneda	GRISONS	Switzerland	670	46.2578	9.1267	Hertach Thomas	2013	6	21
Cicadetta sibillae	1		Crap de Maria, Castaneda	GRISONS	Switzerland	760	46.2592	9.1263	Hertach Thomas	2013	6	21
Cicadetta sibillae	1		Crap de Maria, Castaneda	GRISONS	Switzerland	783	46.2595	9.1246	Juillerat Laurent	2010	7	10
Cicadetta sibillae	1		NW Revo	ALTO	Italy	850	46.3991	11.0452	Hertach Thomas	2011	7	17
Cicadetta sibillae	6		Brez, Cloz	ALTO	Italy	860	46.4419	11.1122	Hertach Thomas	2011	7	17
Cicadetta sibillae	2		Monte Ori, Castelfondo	ALTO	Italy	1110	46.4631	11.1193	Hertach Thomas	2010	6	4

Chapter 3: Molecular species-delimitation methods recover most song-delimited cicada species in the European *Cicadetta montana* complex

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ABSTRACT

Molecular species delimitation is increasingly being used to discover and illuminate species level diversity, and a number of methods have been developed. Here, we compare the ability of two molecular species delimitation methods to recover song-delimited species in the *Cicadetta montana* cryptic species complex throughout Europe. Recent bioacoustics studies of male calling songs (premating reproductive barriers) have revealed cryptic species diversity in this complex. Maximum likelihood and Bayesian phylogenetic analyses were used to analyse the mitochondrial genes *COI* and *COII* and the nuclear genes *EF1α* and *period* for thirteen European *Cicadetta* species as well as the closely related monotypic genus *Euboeana*. Two molecular species delimitation methods, general mixed Yule-coalescent (GMYC) and Bayesian phylogenetics and phylogeography, identified the majority of song-delimited species and were largely congruent with each other. None of the molecular delimitation methods were able to fully recover a recent radiation of four Greek species.

INTRODUCTION

Species identification and delimitation is an integral part of biodiversity assessment, along with other forms of ecological, phylogenetic and speciation research (Hey *et al.*, 2003; Coyne & Orr, 2004; Buckley *et al.*, 2006; Fujita *et al.*, 2012). The process of speciation is often gradual, leading to difficulty in distinguishing closely related species (Dobzhansky, 1940; Coyne & Orr, 2004). As species persist over time, differences accumulate that make them easier to identify. Integrated approaches to species delimitation are critical to accurately understand species boundaries between morphologically similar species while maintaining stable taxonomy (Marshall *et al.*, 2006; Fujita *et al.*, 2012). Multiple sources of data (e.g. morphology, ecology, behaviour, biochemistry, DNA) are often used for species delimitation with recent emphasis on the molecular data (Knowles & Carstens, 2007; Carstens & Dewey, 2010; Yang & Rannala, 2010; Pons *et al.*, 2011). Each of these types of data can be plagued by confounding factors such as convergent selection, ascertainment bias or inaccurate modelling (Hey *et al.*, 2003; Marshall *et al.*, 2006; Barraclough, 2010; Solís-Lemus *et al.*, 2015).

Incomplete lineage sorting, gene flow, recombination, stochastic coalescence events and sex-biased dispersal rates can lead to gene trees that do not reflect species trees (Irwin, 2002; Carstens & Knowles, 2007; Knowles & Carstens, 2007; Petit & Excoffier, 2009). However, even when hybridisation and misleading lineage sorting are absent, molecular phylogenies can be discordant compared to species designations. For cryptic taxa, molecular phylogenies are sometimes discordant with species designations based on morphology, ecology and behaviour because either the molecular markers have not had enough time since divergence to accumulate sufficient changes, or the number of taxa has been underestimated using morphological characters (Hickerson *et al.*, 2006; De Queiroz, 2007; Butlin *et al.*, 2008; Monaghan *et al.*, 2009; Hey & Pinho, 2012). Gene tree conflicts can also result from phylogenetic inference errors rather than biological processes (Yang & Rannala, 2010).

Discordance between different species delimitation data types is problematic for understanding the diversity of life. However, it is equally problematic if only molecular species delimitation is used because this is shown to be misleading in many studies (Smadja & Butlin, 2008; Marshall *et al.*, 2011; Carstens *et al.*, 2013; Solís-Lemus *et al.*, 2015). Conversely, molecular species delimitation may reveal additional cryptic taxa or hybrids that were not apparent with other types of data (Marshall *et al.*, 2011). When this happens, preliminary molecular phylogenies have been used as a basis for further study with additional types of data that corroborate the discovery of these previously unrecognised species or hybrids (Bickford *et al.*, 2007; Pfenninger & Schwenk, 2007; Wiens, 2007).

Species delimitation is a major focus of evolutionary research and more methods of assessing species boundaries based on molecular data are becoming available in large part because molecular data are becoming easier to obtain (Pritchard *et al.*, 2000; Marshall *et al.*, 2006; Pons *et al.*, 2006; Yang & Rannala, 2010; Ence & Carstens, 2011). Molecular species delimitation methods are often used as preliminary assessment of biodiversity in the absence of other data or as part of comprehensive species delimitation studies with multiple types of data (Esselstyn *et al.*, 2012; Harrington & Near, 2012). They are also quite useful when species delimitation based on morphology, behaviour or biochemistry is equivocal or inconclusive (e.g. Marshall *et al.*, 2011).

Molecular species delimitation methods

Early molecular species delimitation methods were tree-based and employed genetic distance thresholds to identify groups of individuals belonging to monophyletic clades on gene trees (Hebert *et al.*, 2003; Pons *et al.*, 2006; De Queiroz, 2007). More recently, coalescent methods have become available (Edwards *et al.*, 2007; Knowles & Carstens, 2007; Kubatko *et al.*, 2009; Yang & Rannala, 2010). Increasingly, the general mixed Yule-coalescent (GMYC) method is used with either a single gene tree or on a concatenated gene tree (Pons *et al.*, 2006; Monaghan *et al.*, 2009; Papadopoulou *et al.*, 2009). Coalescent-based methods require multiple genetic markers and include the following: 1) species delimitation using species tree estimation via maximum likelihood – SpeDeSTEM (Kubatko *et al.*, 2009; Ence & Carstens, 2011), 2) Bayesian phylogenetics and phylogeography – BPP (Yang & Rannala, 2010; Rannala & Yang, 2013) and 3) approximate Bayesian computation – ABC (Knowles & Carstens, 2007; Knowles, 2009). In addition the species tree estimating programmes *BEAST (Bayesian Evolutionary Analysis by Sampling Trees; Heled & Drummond, 2010; Drummond *et al.*, 2012) and BEST (Bayesian Estimation of Species Trees; Edwards *et al.*, 2007; Liu, 2008) can be used in conjunction with species delimitation methods. All of these programmes have been used in empirical studies (Fujita *et al.*, 2012). We chose to use GMYC because of its intuitive biologically meaningful appeal and BPP because, unlike GMYC, it takes into account phylogenetic uncertainty.

Pons *et al.*’s (2006) GMYC method identifies species based on the break in diversification between interspecific and intraspecific splitting patterns of a phylogenetic tree. It is a commonly used likelihood method based on lineage through time plots made with an ultrametric tree and compared to a null model with a constant rate of branching. This method does not require the designation of species a priori and only examines one fully bifurcating phylogenetic tree. This method does not take into account phylogenetic inference uncertainty or gene tree conflicts.

Bayesian phylogenetics and phylogeography (Yang & Rannala, 2010) is a Bayesian coalescent programme that uses multiple gene trees and incorporates the uncertainty of the phylogeny using the posterior probability distribution to assess the species tree. This method assumes that discordance in gene trees is due to phylogenetic uncertainty or incomplete lineage sorting. BPP requires a priori assignment of individuals to the most subdivided groups based on prior biological knowledge and requires a starting guide phylogeny to limit parameter space. It then estimates population sizes and time to last common ancestor for each split of the species tree. Splits with low probability are identified and represent groups of individuals that should be considered one species and not multiple species.

The study group

Cicadas (Hemiptera: Cicadidae) are a widespread insect family found on every continent except Antarctica. The continued discovery of new species and collection of new specimens of these insects for acoustic, morphological and molecular analyses is important for understanding cicada evolution and for conservation (Gogala & Trilar, 2004; Moulds, 2005; Quartau & Simões, 2006; Gogala *et al.*, 2008a; Marshall *et al.*, 2009; 2011; Puissant & Sueur, 2010; Gogala *et al.*, 2012; Popple, 2013; Nunes *et al.*, 2014; Chapter 2 = Hertach *et al.*, 2015). Song characters are especially powerful for species delimitation. They are important premating cues that facilitate assortative mating in many species of insects, amphibians and birds (Wells & Henry, 1998). Male cicadas produce airborne calling songs that signal mating availability to females. Calling songs thus function as species specific mate recognition signals (Dugdale & Fleming, 1978; Marshall & Cooley, 2000; Cooley & Marshall, 2001; Gogala & Trilar, 2004; Sueur & Aubin, 2004; Marshall *et al.*, 2008; Gogala, 2013) that can be used to separate species faster than morphological or molecular differences (Popple *et al.*, 2008; Marshall *et al.*, 2011; Popple, 2013; Nunes *et al.*, 2014; Chapter 2 = Hertach *et al.*, 2015). Song differences often precede morphological differentiation (Hill *et al.*, 2009; Marshall *et al.*, 2009). Correct classification of specimens to species is essential for assessing their phylogenetic relationships, biogeography, life history and global diversity.

Cicadetta montana Scopoli 1772 is a small cicada (15-23 mm body length) that is generally black with yellow to reddish brown markings. The species description was based on a type specimen from Slovenia (Gogala, 2013). *Cicadetta montana* is also the type species for the genus *Cicadetta* Kolenati, 1857 (Sanborn, 2014). The genus *Cicadetta* has historically been a catch-all taxon (reviewed in Moulds, 2012) and gradually species are being removed or revised as additional morphological, molecular and/or bioacoustics studies are conducted (e.g. Lee *et al.*, 2002; Lee, 2008; Moulds, 2012; Qi *et al.*, 2015).

Originally, the distribution of the species *C. montana* was predicted to reach from Western Europe to the Far East (Nast, 1972; Kudryasheva, 1979). Recently, *C. montana* from Europe was recognised as a group of cryptic species distinguished primarily by their distinct high frequency songs – some with very complex song structure (Gogala & Trilar, 1999; 2004; Sueur & Puissant, 2007; Chapter 1 = Hertach, 2011; Gogala, 2013; Chapter 2 = Hertach *et al.*, 2015). There are only two species that have obvious distinguishing morphological features, most species appear morphologically indistinguishable. There are currently thirteen described European species in this complex [*Cicadetta montana* s. str. (Scopoli, 1772), *Cicadetta concinna* Germar, 1821, *Cicadetta brevipennis* Fieber, 1876, *Cicadetta fangoana* Boulard, 1976, *Cicadetta macedonica* Schedl, 1999, *Cicadetta cerdaniensis* Puissant & Boulard, 2000, *Cicadetta cantilatrix* Sueur & Puissant, 2007, *Cicadetta hannekeae* Gogala, Drosopoulos & Trilar, 2008, *Cicadetta olympica* Gogala, Drosopoulos & Trilar, 2009, *Cicadetta kissavi* Gogala, Drosopoulos & Trilar, 2009, *Cicadetta anapaistica* Hertach, 2011, *Cicadetta dirfica* Gogala, Trilar & Drosopoulos, 2011 and *Cicadetta sibillae* Hertach & Trilar, 2015] and one species described from Korea, *Cicadetta abscondita* Lee, 2008 (Gogala & Trilar, 2004; Lee, 2008; Gogala *et al.*, 2011; Chapter 1 = Hertach, 2011; Chapter 2 = Hertach *et al.*, 2015) that is not studied here. Based on the similarity of song characters, there are three main groups (**Fig 3.1**): (A) '*Montana*' which includes *C. montana* s. str., *C. olympica*, *C. brevipennis* and *C. fangoana* and is characterised by relatively simple, long-lasting echemes or one long echeme and a single short echeme, (B) '*Macedonica*' which includes *C. macedonica*, *C. concinna*, *C. hannekeae*, *C. dirfica* and *C. kissavi* and is characterised by sequences of many short echemes combined with longer echemes and (C) '*Cerdaniensis*' which includes *C. cantilatrix*, *C. cerdaniensis*, *C. sibillae* and *C. anapaistica* and is characterised by double echemes modulated in power (Gogala, 2013; Chapter 2 = Hertach *et al.*, 2015). The taxon *Euboeana castaneivaga* (Gogala *et al.*, 2011) is morphologically very close to the *C. montana* complex but has very peculiar calling song without any similarities with the three acoustic groups mentioned above (Gogala *et al.*, 2011). Many of these species are found sympatrically or parapatrically, and their species distributions vary greatly in area. Habitat selection is significantly different between some species (Hertach, 2007; Gogala & Trilar, 2014). Prior to our study, phylogenetic relationships were unknown for European species, except for the *C. cerdaniensis* song group (Chapter 2 = Hertach *et al.*, 2015).

Here, we present gene and species phylogenies based on mitochondrial and nuclear genes to understand the evolutionary history of currently described song-delimited species of the European *Cicadetta montana* cryptic species complex.

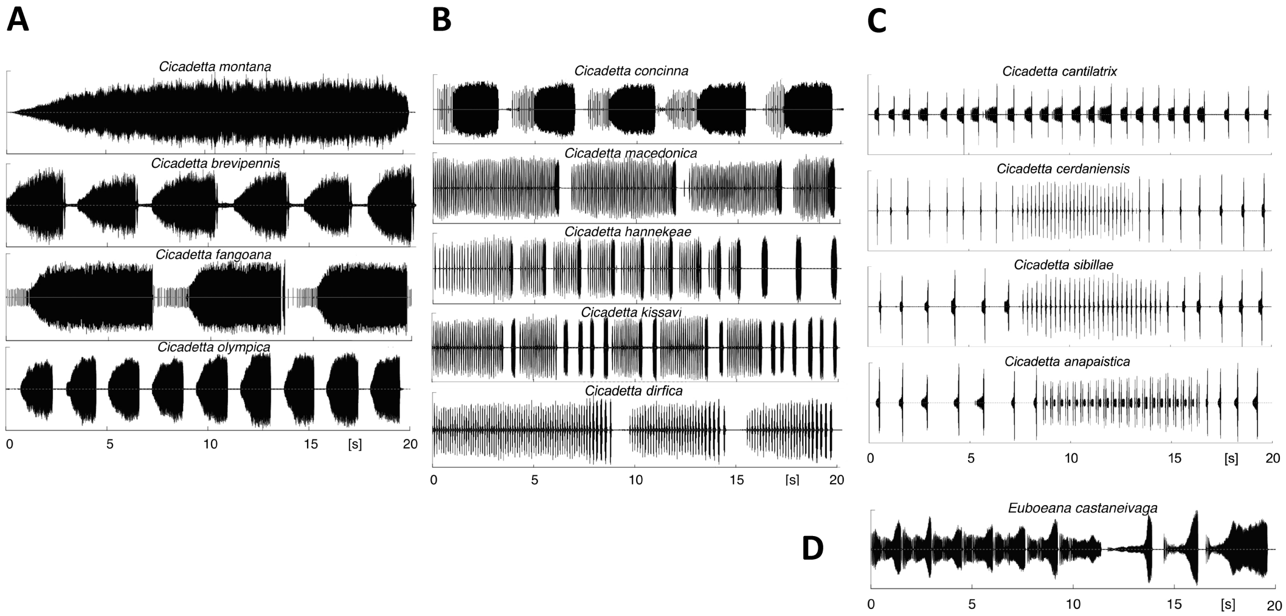

Fig 3.1. Oscillograms (time versus amplitude) of the calling songs of *Cicadetta montana* complex species, grouped according to similarities in song patterns. Duration of song selections is for easier comparison in all species 20 s. As the duration of the complete song is normally longer than this, we selected some of the shortest examples (e.g. *Cicadetta montana* s. str., *Cicadetta hannekeae*) or just a part of the song (e.g. *C. kissavi, Euboeana castaneivaga*). (A) *'Montana'* group, (B) *'Macedonica'* group, (C) *'Cerdaniensis'* group, (D) *Euboeana*, which is according to the song pattern completely different and not related to any of the groups mentioned above.

MATERIALS AND METHODS

Specimen collection and sequencing methods

Collected cicada specimens were identified based on songs, which were recorded when possible. Songs were also recorded from individuals not caught but present in the collecting localities. A total of 87 specimens from thirteen described European species of *Cicadetta* and three specimens from the monotypic genus *E. castaneivaga* were collected from 61 localities (**Fig 3.2 and Appendix S3.1**). Specimens from the subspecies *C. anapaistica lucana* Hertach, 2015 were not included in these analyses, but were incorporated in Chapter 2 (= Hertach *et al.*, 2015). The newly described subspecies of *C. concinna arachnocepta* Gogala, Trilar & Krpač, 2015 was not distinguished from *C. c. concinna*. Sampling was guided by preliminary phylogenetic analyses and song type distributions. Specimens of *Tettigettula pygmea* (Olivier, 1790) and *Hilaphura varipes* (Waltl, 1837) were used in preliminary analyses as out-group taxa because they were

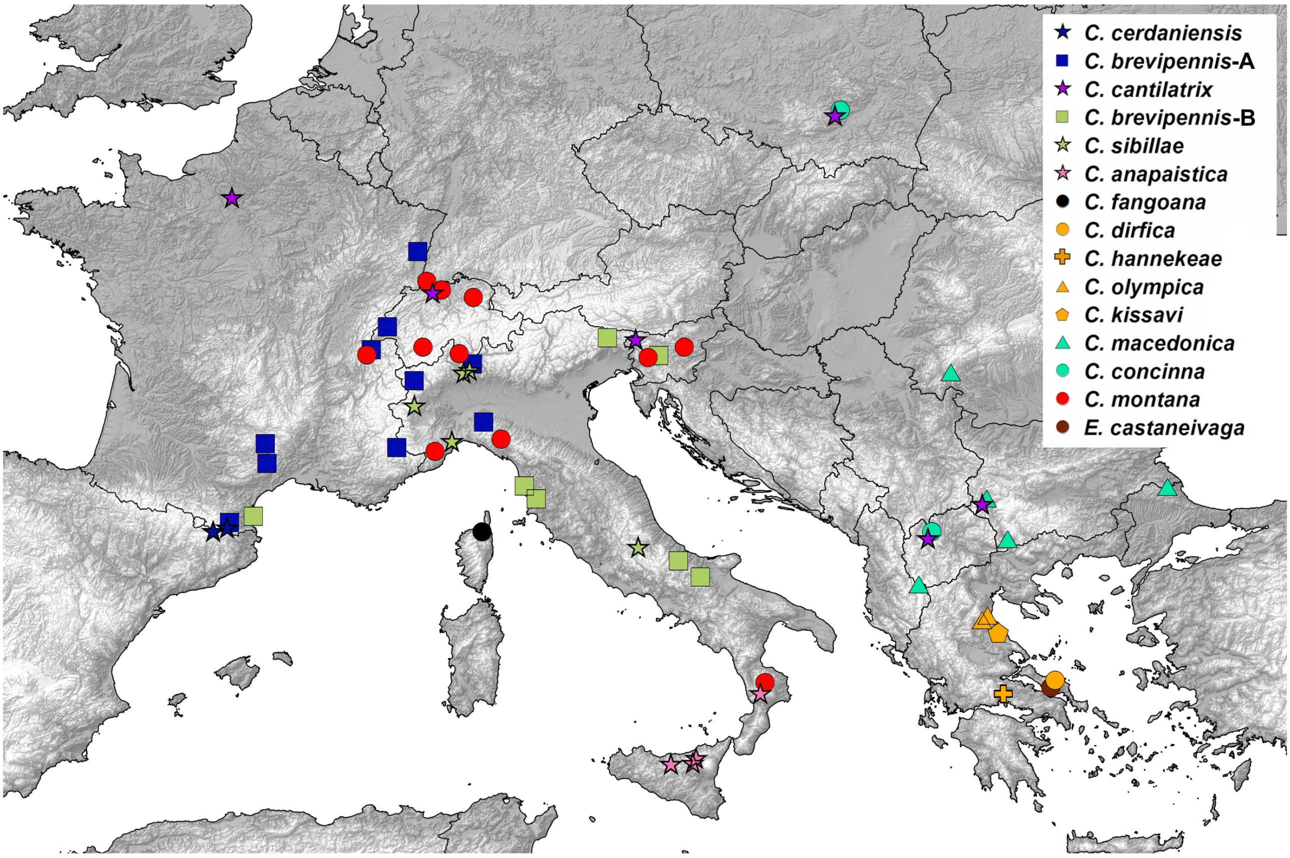

Fig 3.2. Map of all European collecting locations of *Cicadetta* and *Euboeana* specimens used in this study. Colours and order correspond to **Fig 3.3A**, *'cerdaniensis'* song group with star icon.

determined to be the closest relatives of the *Cicadetta montana* species group based on phylogenetic analyses of the tribe Cicadettini (Marshall *et al.*, 2016).

Genomic DNA was extracted using the Qiagen DNeasy Blood & Tissue kit (Qiagen, USA) and Clontech Nucleospin Tissue kits (Clontech, USA) according to the manufacturer's instructions except the digestion, which was conducted over 8-18 h at 54 °C. DNA was extracted from legs, which were kept in ethanol and stored at -20 °C or obtained from pinned specimens. Standard polymerase chain reaction (PCR) methods using the Takara ExTaq kit were used to amplify four genes. The mitochondrial genes *cytochrome oxidase subunit I* (*COI*: 810 bp of the 30 end) and *cytochrome oxidase subunit II* (*COII*: 693 bp) were amplified using the following primers: *COI* – either C1-J-2195 (Simon *et al.*, 1994) or C1-J-1490 (Folmer *et al.*, 1994) and TL2-N-3014 (Simon *et al.*, 1994) with an annealing temperature of 56 and 45 °C, respectively, for the forward primer; *COII* – TL2-J-3034 and TK-N-3786 (Simon *et al.*, 1994) with an annealing temperature of 52 °C. A large section of the nuclear gene *elongation factor-1 alpha* (*EF1α*) encompassing five exons and four introns was amplified in three overlapping fragments (2167 bp): segment 1) EF1-F001-cicada and EF1-R752-cicada (Arensburger *et al.*, 2004), segment 2) EF1-f650-ambig (Lee & Hill, 2010) and EF1-N1419 (Sueur *et* *al.*, 2007) and segment 3) DV-EF-F1 (Marshall *et al.*, 2008) and EF-R114-cicada (Arensburger *et al.*, 2004) with a touchdown cycling programme: 94 °C for 2 min, (94 °C for 45 s, 55 °C to 45 °C for 45 s decreasing 0.5 °C degrees/cycle, 72 °C for 1 min) for 20 cycles, (94 °C for 45 s, 45 °C for 45 s, 72 °C for 1:15 min) for 10 cycles, followed by 72 °C for 5 min. A section of intron from the nuclear gene *period* (356 bp) was amplified using the primers Per-491-For and Per1201-Rev (Buckley *et al.*, 2006) using the touchdown cycle programme given above. All gene segments were sequenced in both directions.

Amplified products were purified using the Qiagen PCR Purification Kit (Qiagen) or ExoSAP-It (Affymetrix, USA) at one-fifth the recommended concentration. Cleaned PCR product was cycle-sequenced with Big Dye Terminator Ready Reaction Kit 1/8 to 1/4 scale reaction volumes and purified using Sephadex filtration (GE Healthcare, USA). Sequences were obtained from an ABI 3100 capillary sequencer (Applied Biosystems, USA), analysed using ABI PRISM sequencing software (Applied Biosystems) and edited in Sequencher 3.1 (Gene Codes Corp., USA) or Geneious (Biomatters Ltd., New Zealand). Gene sequences were deposited in GenBank with the following accession numbers: *COI*: KT901699 – KT901780, *COII*: KT901473 – KT901554, *EF1α*: KT901555 – KT901624, *period*: KT901625 – KT901698. Gene alignments were deposited at Dryad: doi: 10.5061/dryad.7db58.

Model selection and phylogenetic analyses

Sequences were aligned in Geneious or Mesquite (Maddison & Maddison, 2007) and checked by eye. Ambiguous base calls (e.g. due to heterozygosity at nuclear loci) were coded as ambiguities in later analyses. *COI*, *COII* and *period* had no gaps or length variants so alignment was unambiguous. *EF1α* was aligned in Geneious using ClustalW with a gap-open penalty of 15, gap extension penalty of 5, and adjusted by eye.

Alternative combinations of partitioning schemes and partition-specific substitutions models were tested using the 'greedy' algorithm in the programme PartitionFinder 1.0.1 (Lanfear *et al.*, 2012). This programme requires the data set to be as subdivided as possible and finds the best data set grouping. The BIC criterion was used to determine the best model for each partition and the best partition scheme, which was estimated with branch lengths linked. PartitionFinder was run estimating all possible models of evolution on mitochondrial and nuclear data sets separately. The mitochondrial genes *COI* and *COII* were concatenated and divided by gene and codon position. The five *EF1α* exons were separated and divided by codon, and the four introns were separated for a total of 19 partitions. The best model of evolution was determined separately for the intron of *period*, which was not partitioned.

To assess gene tree discordance, the concatenated mitochondrial genes and each individual nuclear gene tree were estimated with maximum likelihood analysis performed in GARLI 2.0 (Zwickl, 2006) using the models of evolution and partitioning schemes selected by PartitionFinder. One hundred bootstrap pseudoreplicates were performed with the models of each partition unlinked and summarised on the ML tree with SumTrees, which is part of the DendroPy 3.12.0 package (Sukumaran & Holder, 2010).

Bayesian inference for the concatenated mitochondrial genes and individual nuclear genes was performed using BEAST 1.7.5 (Drummond *et al.*, 2012) which was run for 30 million generations with a discarded burn-in of 10% using PartitionFinder partitions and models of evolution. Model parameters and partition rate multipliers were estimated separately for each partition. BEAST analyses were run using a strict clock with a random starting tree and a Yule speciation tree prior. Relative rate parameters for each partition were set to an exponential distribution with mean of 1.0. All other priors were left at the default values in BEAUti 1.8.0. Convergence of model parameters and posterior distributions were visualised with Tracer 1.5. TreeAnnotator 1.7.5, distributed with the BEAST package, was used to produce the maximum clade credibility trees.

Pairwise genetic divergence

Uncorrected genetic distances and ML model-corrected distances using the Tajima-Nei model (Tajima & Nei, 1984) were estimated with MEGA 6 (Tamura *et al.*, 2013) on the *COI* and *COII* concatenated data set for within and between song-delimited species. Specimens were grouped according to described species except for *C. brevipennis* which was grouped into A- and B-clades identified by phylogenetic analyses.

Phylogeny-based species delimitation

We tested for the threshold between interspecific and intraspecific diversification using the generalised mixed Yule-coalescent model (GMYC) of Pons *et al.* (2006) implemented in the R package SPLITS (Fontaneto *et al.*, 2007; Monaghan *et al.*, 2009). An ultrametric tree of the four genes produced by BEAST was used. The GMYC analysis was run with an interval of 0, 10. A range of intervals was tested with no apparent change in results.

Coalescent species delimitation

We used the coalescent programme BPP 2.1 (Yang & Rannala, 2010) to analyse all four sequenced genes. This programme requires individuals to be a priori assigned to the most subdivided population groupings that make biological sense. The guide tree, which helps reduce parameter space, was provided based on the concatenated *COI* and *COII* ML tree produced with GARLI. The three species *C. olympica*, *C. kissavi* and *C. hannekeae* were combined in this analysis because there were only a few sequences of each species present in the *EF1α* data set.

Bayesian phylogenetics and phylogeography assumes no admixture after speciation and estimates ancestral population size (ϑ) and root age (τ_0). BPP was run for 500,000 steps, sampling every 5 with a discarded burning of 10,000 steps. Species delimitation was performed with rjMCMC algorithms 0 ($\varepsilon = 15$) and algorithm 1 ($\alpha = 2$, $m = 1$). The gamma-distributed prior distributions for the parameters ϑ and τ were varied to reflect potential speciation histories: G($\alpha = 1$, $\beta = 10$) for both priors reflects a history of 'large population size and deep divergence'; G($\alpha = 2$, $\beta = 2000$) for both priors reflects a history of 'small population size and shallow divergence'; G($\alpha = 1$, $\beta = 10$) for τ and G($\alpha = 2$, $\beta = 2000$) for ϑ reflects 'small population size and deep divergence' (Leaché & Fujita, 2010; Yang & Rannala, 2010). The remaining divergence time parameters were assigned the Dirichlet prior by BPP (Yang & Rannala, 2010). Rates among loci were allowed to vary according to a Dirichlet distribution with $\alpha = 2$, and the heredity scalars were set to 1 for nuclear loci and 0.25 for the concatenated mitochondrial genes.

RESULTS

Model selection and gene tree analyses

The optimal partitioning scheme and models for each partition are given in **Appendix S3.2**. The two mitochondrial genes, *COI* and *COII*, were concatenated and partitioned together by codon position with third positions the most variable. As expected, the nuclear genes *EF1α* and *period* have a slower rate of evolution and are characterised by a lower number of variable and parsimony-informative sites with introns being the most variable. The *EF1α* introns had more variable sites than the *period* intron but appear to contain a lot more ambiguity as illustrated in the phylogenies.

Phylogenies produced with maximum likelihood (ML) using GARLI and Bayesian inference (BI) using BEAST were similar. However, some differences in tree topology and branch support were present in the mitochondrial phylogeny estimation. Preliminary analyses (not shown) included specimens of *T. pygmea* and *H. varipes* as out-group taxa to the *C. montana* species complex and supported the position of *E. castaneivaga* within the *Cicadetta montana* group. The mitochondrial phylogeny was rooted based on BI using BEAST with out-group taxa included in the analyses. This resulted in the same topology as mid-point rooting the ML phylogeny of the in-group taxa, so the out-group taxa were dropped in all subsequent analyses to improve the estimation of model parameters and branch supports. The *EF1α* and *period* phylogenies were midpoint rooted.

The mitochondrial phylogeny (**Fig 3.3A**) strongly supports the monophyly of *C. montana* s. str. in both ML and BI analyses. *Euboeana castaneivaga*, *C. dirfica*, *C. cantilatrix* and *C. anapaistica* are also shown to be monophyletic, not including specimens of the newly described *C. anapaistica lucana* subspecies (Chapter 2 = Hertach *et al.*, 2015). Only one specimen of *C. fangoana* was used in this study; however, given its distinctive song and its geographic separation (on the island of Corsica, France, in the absence of any other *Cicadetta* species), it is clearly differentiated and presumed to be monophyletic. *Cicadetta hannekeae*, *C. olympica* and *C. kissavi* are polyphyletic and closely related to *C. dirfica*. All four of these species are found in Greece and have geographically highly restricted distributions except for *C. hannekeae* (Gogala *et al.*, 2008a; 2009; 2011). *Cicadetta macedonica* and *C. concinna* are polyphyletic and sister to the four Greek species.

An unexpected result of the mitochondrial phylogeny was the polyphyly of two species with very different songs: *C. cerdaniensis* and *C. brevipennis*. Specimens from these two species were mixed and divided into A- and B-clades (**Fig 3.3A**). Multiple specimens from both species were resequenced to rule out contamination and none was found. Preliminary phylogenetic analyses led to further investigation into the *C. cerdaniensis* species which resulted in the description and recognition of a new species, *C. sibillae* (Chapter 2 = Hertach *et al.*, 2015) based on song, morphology and DNA data. Specimens of *C. cerdaniensis* s. str. and *C. brevipennis*-A form a clade with no differentiation between the two song-delimited species. Similarly, *C. sibillae* and *C. brevipennis*-B form a monophyletic group (that also includes *C. anapaistica*) also with no differentiation between these two song-delimited species. *Cicadetta cantilatrix* is placed as sister to the *C. cerdaniensis/C. brevipennis*-A clade; however, this branch is not well supported (0.63 bootstrap support) and was not reconstructed in BI analyses. The *EF1α* and *period* gene tree phylogenies (**Fig 3.3B-C**) were not as well resolved as the mitochondrial phylogeny and although the *period* gene tree found the major subdivisions in the complex, it was not strongly supported (**Fig 3.3C**). The *EF1α* phylogeny (**Fig 3.3B**)

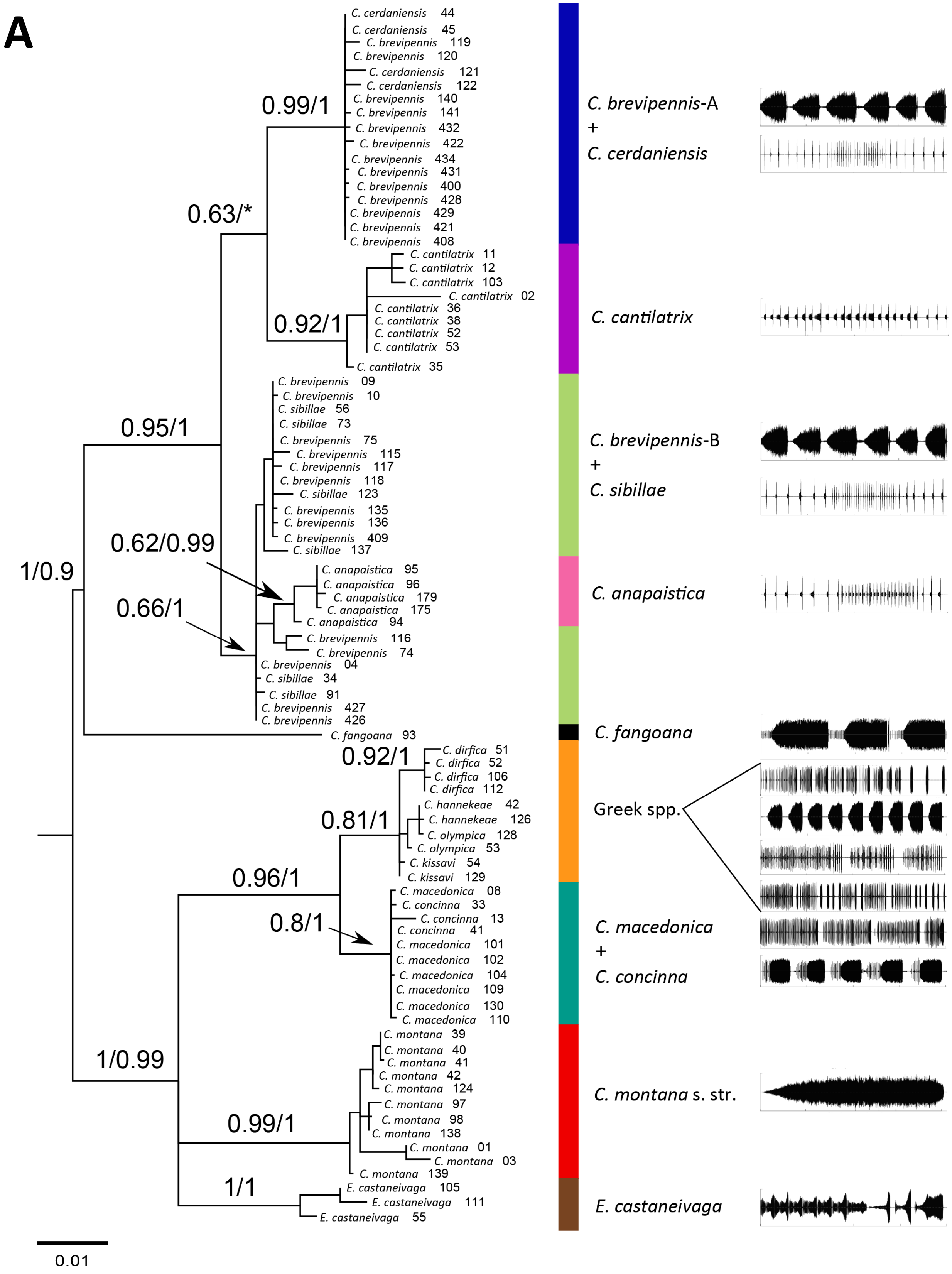

Fig 3.3. Mitochondrial and nuclear phylogenies of *Cicadetta montana* cryptic species complex and *Euboeana castaneivaga*. Song-delimited species names and specimen identification numbers are given. Maximum likelihood (ML) phylogenies are shown with nodal support from ML bootstrap replicates analyses in the programme GARLI followed by posterior probability supports from Bayesian analysis in the programme BEAST. Nodes with less than 50% support have been collapsed. (A) *COI* and *COII*, (B) *EF1α* nuclear gene phylogeny, (C) *period* nuclear gene phylogeny. See next pages.

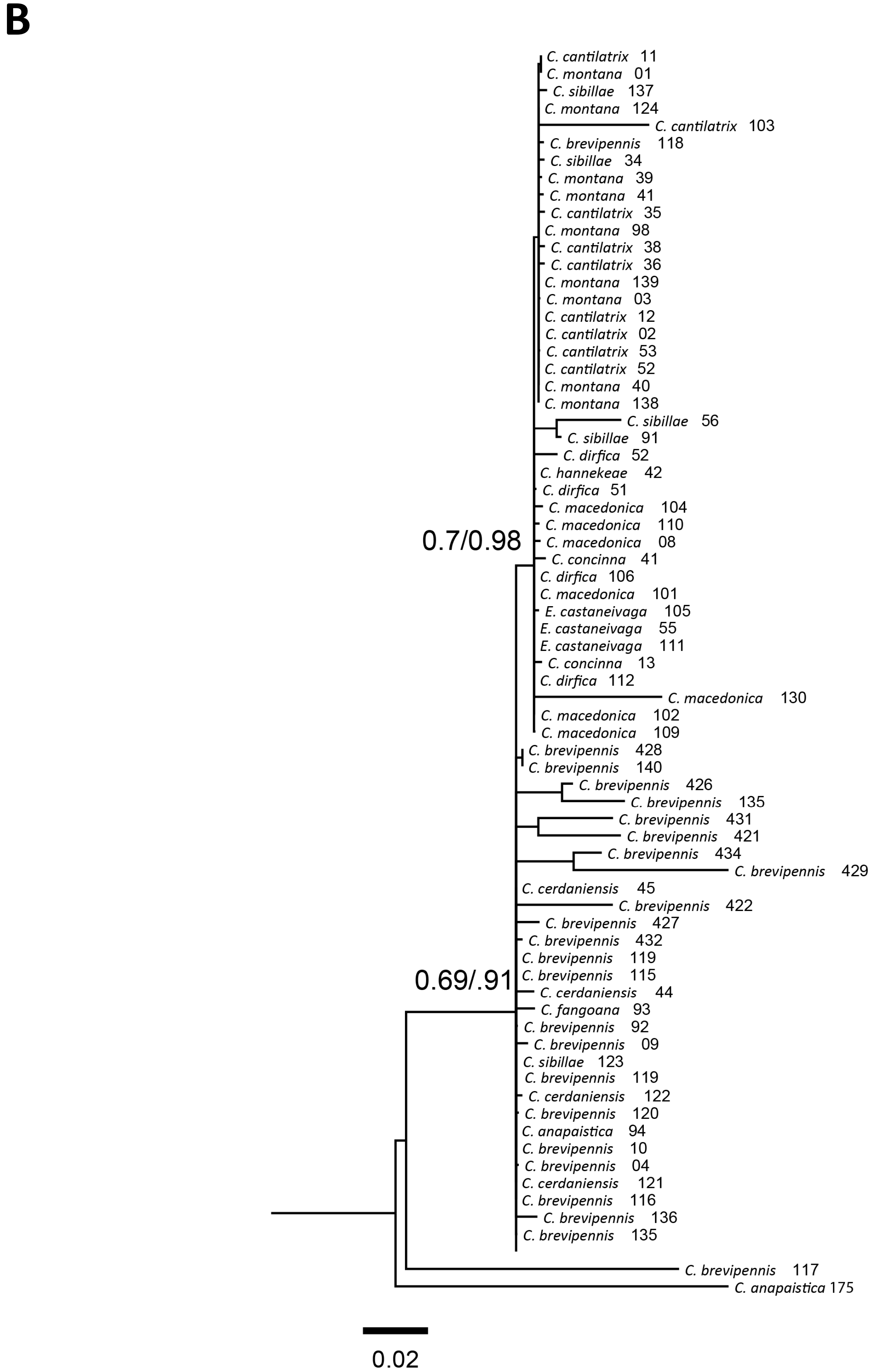

Fig 3.3. Continued

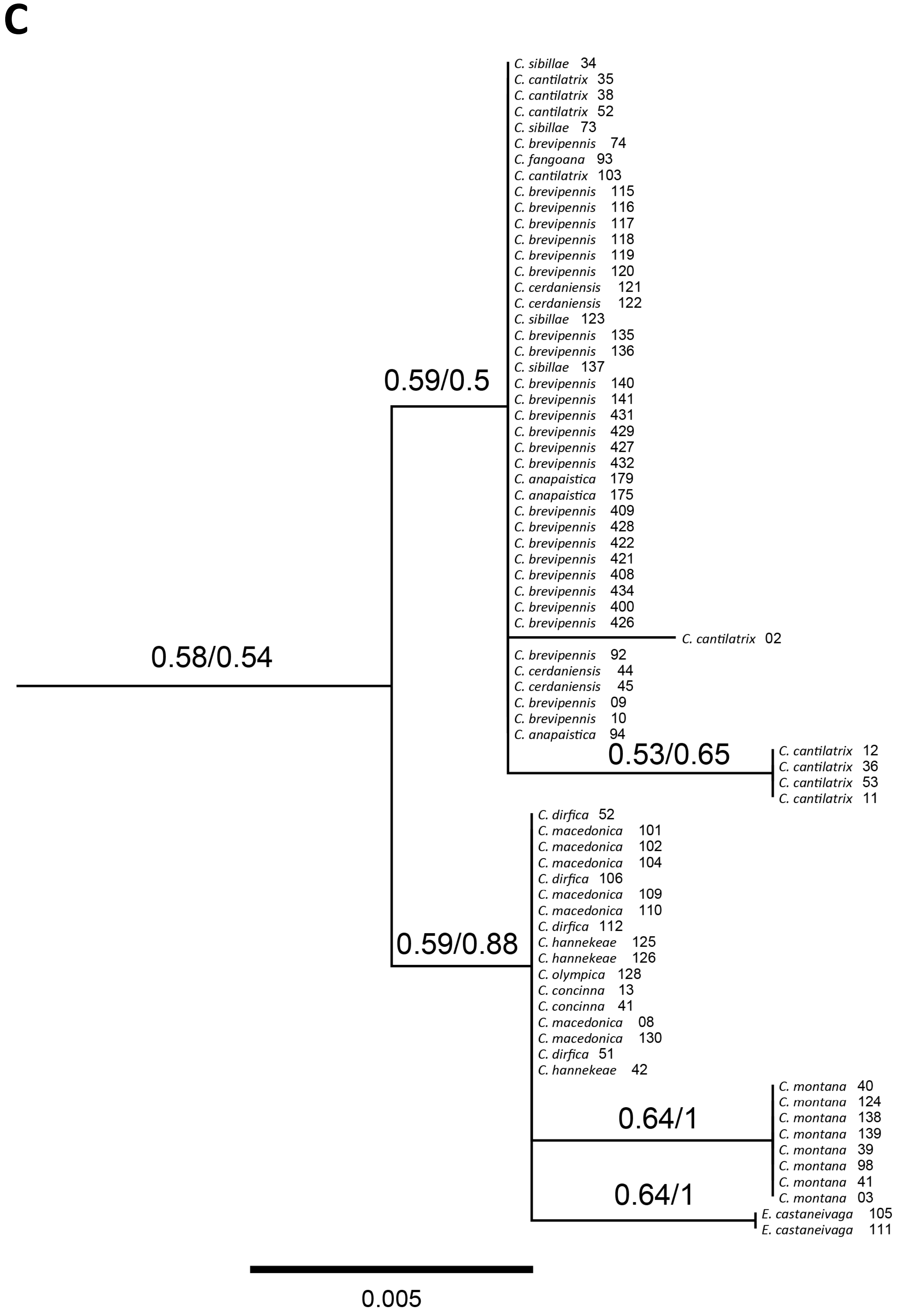

Fig 3.3. Continued

did not recover any species as monophyletic. The *period* gene tree (**Fig 3.3C**) recovered only *C. montana* s. str. and *E. castaneivaga* as monophyletic.

Pairwise genetic divergence

Intraspecific mitochondrial divergence of song-delimited species is low (**Table 3.1**) even with specimens collected from very distant localities (up to 1750 km). All song-delimited species had a corrected genetic diversity of ≤ 0.008 substitutions/sites within species. Estimates of pairwise mitochondrial genetic divergence among species (**Table 3.2**) indicate that the six species *C. macedonica*, *C. concinna*, *C. hannekeae*, *C. dirfica*, *C. olympica* and *C. kissavi* are all < 2% diverged from each other. As expected from the phylogenies, *C. brevipennis*-A + *C. cerdaniensis* and *C. brevipennis*-B + *C. sibillae* + *C. anapaistica* are < 1% diverged from each other. All other species range between 0.021 and 0.051 substitutions per site (2.0-5.1% uncorrected divergence). *Euboeana castaneivaga* is not more divergent than any other *C. montana* complex species; this along with its position on the phylogenetic trees supports the close relationship of this species with the rest of the group.

Table 3.1. Within-species genetic divergence for mtDNA data. Uncorrected p-distances and ML distances calculated over sequence pairs.

Species	p-distance	ML
C. anapaistica	0.002	0.002
C. brevipennis-A	0.001	0.001
C. brevipennis-B	0.004	0.004
C. cantilatrix	0.006	0.006
C. cerdaniensis	0	0
C. concinna	0.001	0.001
C. dirfica	0.001	0.001
C. fangoana	n/a	n/a
C. hannekeae	0.001	0.001
C. kissavi	0	0
C. macedonica	0.001	0.001
C. montana s. str.	0.006	0.006
C. olympica	0.003	0.003
C. sibillae	0.005	0.005
E. castaneivaga	0.008	0.008

Table 3.2. Average pairwise genetic distances for *Cicadetta* and *Euboeana* song-delimited species for the mtDNA data. ML pairwise distances (substitutions/site) are below the diagonal and uncorrected p-distances (pairwise percentage/100) are above the diagonal.

	C. anapaistica	***C. brevipennis*-A**	***C. brevipennis*-B**	***C. cantilatrix***	***C. cerdaniensis***	***C. concinna***	***C. dirfica***	***C. fangoana***	***C. hannekeae***	***C. kissavi***	***C. macedonica***	***C. montana* s. str.**	***C. olympica***	***C. sibillae***	***E. castaneivaga***
C. anapaistica		0.020	0.009	0.023	0.019	0.043	0.041	0.035	0.040	0.041	0.043	0.039	0.041	0.010	0.039
***C. brevipennis*-A**	0.020		0.019	0.025	0.001	0.051	0.048	0.042	0.046	0.048	0.049	0.044	0.047	0.021	0.043
***C. brevipennis*-B**	0.009	0.019		0.024	0.018	0.043	0.042	0.037	0.041	0.042	0.043	0.040	0.042	0.005	0.040
C. cantilatrix	0.024	0.026	0.024		0.025	0.039	0.038	0.041	0.037	0.038	0.040	0.039	0.037	0.024	0.037
C. cerdaniensis	0.020	0.001	0.018	0.025		0.050	0.047	0.041	0.046	0.047	0.049	0.043	0.047	0.020	0.042
C. concinna	0.044	0.053	0.045	0.041	0.053		0.012	0.044	0.014	0.011	0.002	0.036	0.014	0.044	0.033
C. dirfica	0.043	0.050	0.044	0.039	0.049	0.012		0.043	0.002	0.000	0.012	0.039	0.003	0.042	0.036
C. fangoana	0.036	0.043	0.039	0.043	0.043	0.045	0.045		0.042	0.043	0.045	0.048	0.043	0.038	0.045
C. hannekeae	0.041	0.048	0.042	0.038	0.048	0.014	0.002	0.043		0.003	0.015	0.037	0.002	0.041	0.035
C. kissavi	0.043	0.050	0.044	0.040	0.050	0.011	0.000	0.045	0.003		0.012	0.039	0.003	0.042	0.036
C. macedonica	0.044	0.052	0.045	0.042	0.051	0.002	0.012	0.046	0.015	0.012		0.038	0.015	0.044	0.034
***C. montana* s. str.**	0.040	0.046	0.041	0.040	0.045	0.038	0.040	0.051	0.039	0.040	0.039		0.037	0.041	0.035
C. olympica	0.042	0.050	0.043	0.039	0.049	0.014	0.003	0.044	0.002	0.003	0.015	0.039		0.042	0.035
C. sibillae	0.010	0.022	0.005	0.025	0.021	0.046	0.044	0.039	0.042	0.044	0.046	0.043	0.044		0.040
E. castaneivaga	0.041	0.045	0.042	0.039	0.044	0.034	0.037	0.047	0.036	0.038	0.035	0.036	0.036	0.042	

Phylogeny-based species delimitation

Phylogeny-based species delimitation using the GMYC method on all four genes was based on a fully bifurcating ultrametric mitochondrial starting tree produced by BEAST. The threshold between interspecific and intraspecific coalescent events was identified by GMYC (**Fig 3.4**), and the null hypothesis of a constant diversification rate was rejected ($p = 0.018$). Seven ML entities (i.e. species) were recovered with a confidence interval of 5-16 entities. One entity (*C. fangoana*) was a single lineage and the rest are groups of taxa. This analysis recovered the song-delimited species *C. montana* s. str., *C. cantilatrix* and *E. castaneivaga* as monophyletic clades. This analysis grouped *C. brevipennis*-B, *C. anapaistica* and *C. sibillae* into one ML entity and also grouped *C. brevipennis*-A and *C. cerdaniensis* into a second ML entity. In addition, the four Greek species (*C. dirfica*, *C. olympica*, *C. kissavi* and *C. hannekeae*) were grouped with *C. macedonica* and *C. concinna* into one entity.

Coalescent species delimitation

The Bayesian coalescent species delimitation programme BPP strongly supported eight of the twelve taxa that were tested (**Fig 3.5**). BPP used gene trees from each of the three loci sequenced (*COI* + *COII*, *EF1α* and *period*) to assess the likelihood that each of the a priori designated putative species were supported as species. Similar to the GMYC results, the BPP analyses resulted in strong support (> 0.95 posterior probability) for species designation of *C. montana* s. str., *E. castaneivaga*, *C. fangoana* and *C. cantilatrix*. Unlike GMYC, BPP did differentiate the *C. concinna* + *C. macedonica* versus *C. dirfica* + the three remaining Greek species *C. kissavi*, *C. hannekeae* and *C. olympica* which were a priori grouped due to a low number of sequences for the two nuclear genes used. Posterior probabilities did not support the division of *C. concinna* and *C. macedonica*, but under the 'large and shallow' and 'small and shallow' priors did support *C. dirfica* as a separate lineage to the other three Greek species. Interestingly, the best tree topology (**Table 3.3**) for both the 1 and 0 algorithms under all three combinations of priors supported the existence of all of the nodes 1-6 (**Fig 3.5**), which suggests that there is support for these lineages as species.

The BPP analyses also recognised the same groupings as GMYC of *C. cerdaniensis* + *C. brevipennis*-A + *C. cantilatrix* (node 9) and *C. anapaistica* + *C. brevipennis*-B + C. sibillae. There was low support using both algorithms with different priors for the split between *C. brevipennis*-A and *C. cerdaniensis* (node 10) instead suggesting a possible trifurcation including *C. cantilatrix* (node 9). Only when BPP was run with a ϑ prior for a 'large population' and a τ prior for a 'shallow divergence', the best tree topology resulted in this node (10)

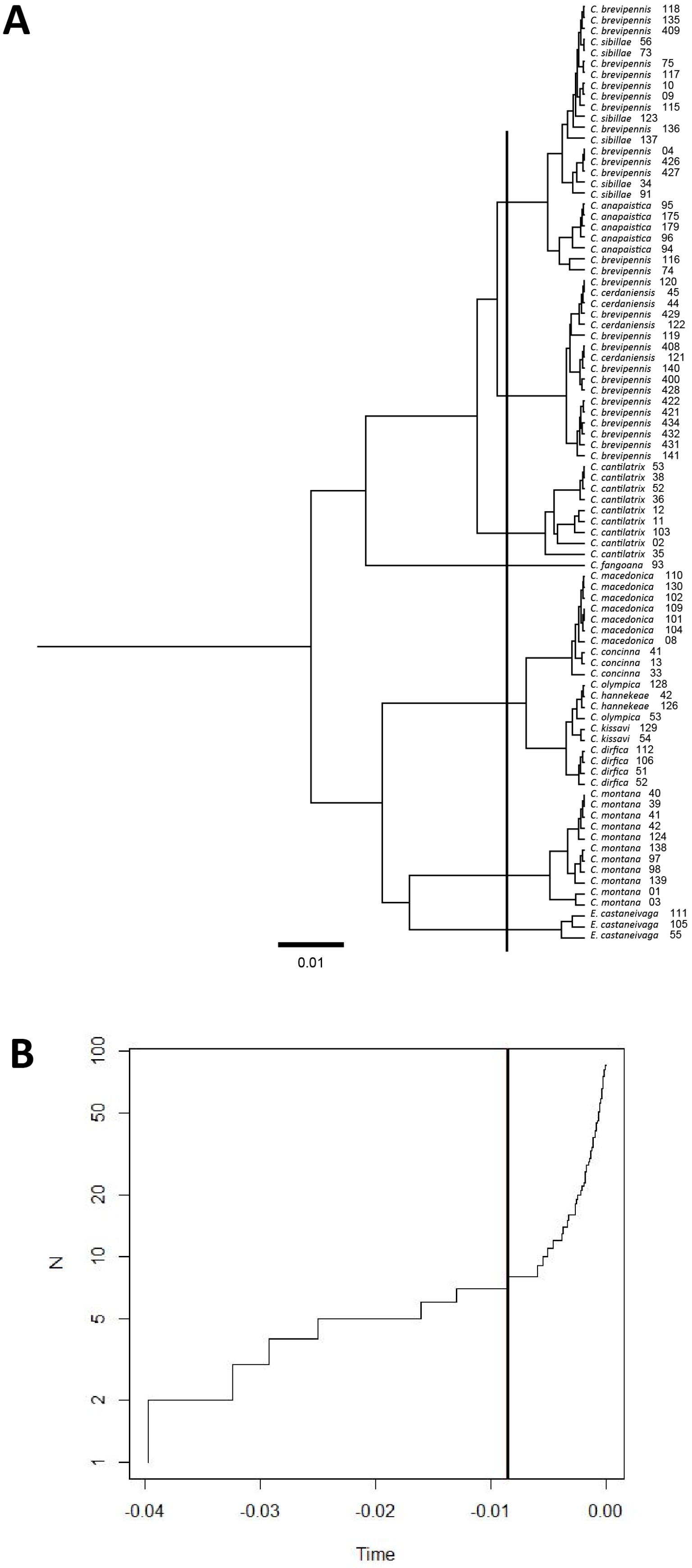

Fig 3.4. General mixed Yule-coalescent identified taxonomic units on BEAST phylogeny. Ultrametric tree produced by BEAST using all four genes. Specimen names are the song-delimited species names and specimen identification numbers. (A) Single threshold results with vertical line marking the clades to the right which represent species clusters. (B) Lineage through time plot with vertical line showing inter- versus intraspecific diversification.

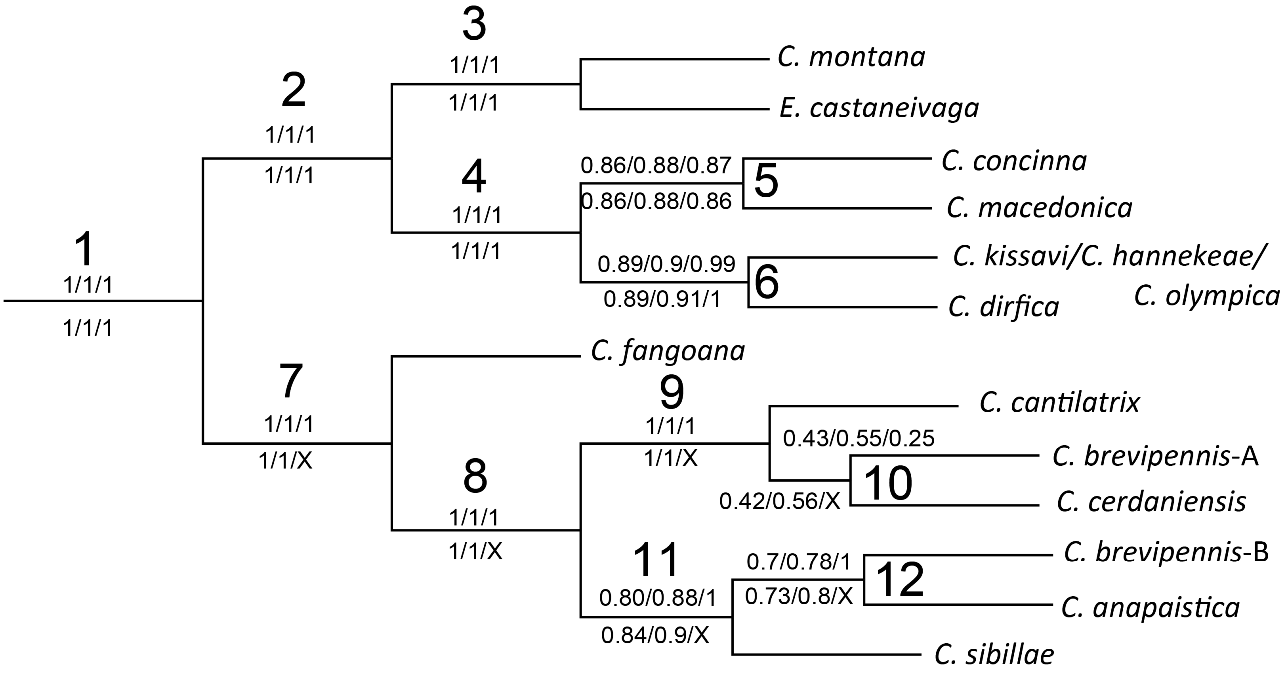

Fig 3.5. Bayesian phylogenetics and phylogeography species tree derived from the mitochondrial genes *COI* and *COII* and the nuclear genes *EF1α* and *period*. The guide tree based on the ML mitochondrial phylogeny produced by the programme GARLI is provided with speciation probabilities indicated on each node. *Cicadetta kissavi, Cicadetta hannekeae* and *Cicadetta olympica* were grouped together for analyses. *Cicadetta brevipennis* was divided in to A- and B-lineages. Speciation probabilities from rjMCMC algorithm 0 are indicated at each node above the line and rjMCMC algorithm 1 below the line. For each algorithm, speciation probabilities are given for different ϑ and τ priors reflecting 'large populations and deep divergence'/'large populations and shallow divergence'/'small populations and shallow divergence'.

being supported (**Table 3.3**). This is the least supported split in the analysis. Similarly, the splitting of *C. brevipennis*-B and *C. anapaistica* into two taxa rather than one is not well supported by any of the BPP analyses, although the best tree topology ('small-population size/shallow divergence') does recover this node (12) using the rjMCMC algorithm 0. The difference between *C. sibillae* and *C. brevipennis*-B + *C. anapaistica* (node 11) is similarly well supported only in the 'small-population size/shallow divergence' analysis using the rjMCMC algorithm 0.

Multiple user-provided guide trees were tested (data not shown) including guide trees using *E. castaneivaga* as an out-group, *C. cantilatrix* sister to the *C. brevipennis* + *C. anapaistica*, *C. sibillae* clade, and different arrangements of *C. brevipennis*-A and -B, *C. cerdaniensis*, *C. sibillae* and *C. anapaistica*. All of the song-delimited species that were highly supported using the guide tree shown in **Fig 3.5** were supported using different guide trees.

Both rjMCMC algorithms 0 and 1 were implemented in BPP with three different sets of priors for ϑ and τ for each algorithm (**Table 3.3**), and the results were mostly congruent. Only algorithm 1 with priors set for a ‘small population and shallow divergences’ resulted in relatively different posterior probabilities and best tree topologies. In this analysis, the entire clade of *C. fangoana*, *C. cantilatrix*, *C. cerdaniensis*, *C. brevipennis*, *C. anapaistica* and *C. sibillae* would be collapsed into one taxon.

Table 3.3. Bayesian phylogenetics and phylogeography tree topology frequencies under different algorithm and priors on ϑ and τ_0. Gamma priors are (α, β). Tree topology is based on nodes in the guide tree where 1 represents a resolved node and 0 represents a collapsed node with nodes labelled corresponding to tree nodes 1-11 in **Fig 3.5**. Tree frequency is based on how many times a tree was found to be the best. Mean $\ln_L$: mean log likelihood.

			Best tree toplogy													
Algorithm	**ϑ prior**	**τ prior**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Tree frequency**	**Mean $\ln_L$**
0	(1,10)	(1,10)	1	1	1	1	1	1	1	1	1	0	1	1	0.302	-12820.89
0	(1,10)	(2, 2000)	1	1	1	1	1	1	1	1	1	1	1	1	0.340	-12812.68
0	(2, 2000)	(2, 2000)	1	1	1	1	1	1	1	1	1	0	1	1	0.658	-12878.62
1	(1,10)	(1,10)	1	1	1	1	1	1	1	1	1	0	1	1	0.328	-12819.77
1	(1,10)	(2, 2000)	1	1	1	1	1	1	1	1	1	1	1	1	0.358	-12820.32
1	(2, 2000)	(2, 2000)	1	1	1	1	1	1	0	0	0	0	0	0	0.858	-12879.92

DISCUSSION

Phylogenetic and coalescent species delimitation methods

Molecular species delimitation methods and species tree estimation did not recover all of the fourteen song-delimited species we investigated here. However, both GMYC and BPP recognised many species, and a combination of acoustic and multiple molecular methods is preferable for understanding species diversity (Esselstyn *et al.*, 2012; Carstens *et al.*, 2013; Solís-Lemus *et al.*, 2015). Four song-delimited species were consistently recognised in all methods: *C. montana* s. str., *E. castaneivaga*, *C. fangoana* and *C. cantilatrix*. Two clades of species were consistently underestimated: 1) *C. macedonica* and *C. concinna*, despite obvious differences in morphology, and 2) the four Greek species *C. dirfica*, *C. olympica*, *C. kissavi* and *C. hannekeae*. The lack of resolution in these two clades is likely due to either recent gene flow or recent radiations. According to song patterns, *C. olympica* is more closely related to *C. montana* s. str. and *C. brevipennis* (**Fig 3.1A**).

The most surprising finding of this study is the close relationship between *C. brevipennis* mtDNA haplotypes and mtDNA haplotypes of both *C. cerdaniensis* and *C. sibillae* which was previously not recognised in morphological, ecological and song data. Neither GMYC nor BPP were able to recognise these song-delimited species but did recognise the clades of *C. brevipennis*-A + *C. cerdaniensis* and *C. brevipennis*-B + *C. sibillae*.

Analysis of song-delimited species with the GMYC method demonstrated the reliance of this method on a single predefined phylogenetic tree with no incorporation of phylogenetic reconstruction estimation as part of the method. GMYC underestimated the number of species present in this group to a greater extent than BPP (**Figs 3.4 and 3.5**). Although multiple loci were used, information is lost because concatenation of data even with partitioning and accurate modelling has been shown to be insufficient (Degnan & Rosenberg, 2006; Carstens & Knowles, 2007; Kubatko & Degnan, 2007). Locus selection and phylogeny estimation are particularly important with this method because GMYC can only identify species that are monophyletic (Pons *et al.*, 2006). Complete taxon sampling affects all species delimitation methods; GMYC has been shown to be inaccurate because of the underlying assumption of constant speciation (Lohse, 2009; Papadopoulou *et al.*, 2009; Esselstyn *et al.*, 2012; Fujisawa & Barraclough, 2013).

Unlike GMYC, molecular species delimitation using BPP incorporates tree construction into the analysis (starting with a guide tree). BPP (**Fig 3.5**) identified more of the song-delimited species of cicadas than GMYC. This is in large part because BPP does not require species to have monophyletic gene tree clades and these are lacking in many of the species studied here. This method was more difficult to use because uncertainty in the effects of the different rjMCMC algorithms and the effects of different priors on ϑ and τ needed to be tested (Yang & Rannala, 2010; Camargo *et al.*, 2012). However, both rjMCMC algorithms with three different sets of priors for ϑ and τ resulted in consistent speciation probabilities on the guide tree, but different best tree topologies. The user-provided guide tree must be used with caution because the placement of disparate lineages together is more likely to support their designation as species and could result in misleading results (Leaché & Fujita, 2010; Yang & Rannala, 2010; Camargo *et al.*, 2012; Fujita *et al.*, 2012). Although BPP takes the uncertainty of different gene trees into account due to its reliance on coalescent theory, it is not possible to partition the genes and apply appropriate models of evolution to different data subsets (Yang & Rannala, 2010). This method also requires a priori assignment of specimens to species, which will limit the ability to discover additional cryptic taxa if reliable species defining characters like songs are not available. Individual gene tree analysis and examination are recommended prior to implementing this method to help guide the assignment of specimens to species being tested. It is possible to use this method to test clearly defined competing hypotheses of species groups (Harrington &

Near, 2012), but this also assumes that the user has identified and has adequate data for any possible cryptic species.

Both phylogenetic and coalescent-based species delimitation methods are susceptible to incomplete taxon sampling, and coalescent-based methods would also benefit from additional loci (Lohse, 2009; Papadopoulou *et al.*, 2009; Huang *et al.*, 2010; Esselstyn *et al.*, 2012). The identification of easily amplified nuclear genes without pseudogenes is difficult in non-model organisms. Increased use of next-generation sequencing technologies like ultraconserved elements and anchored phylogenomics (Lemmon *et al.*, 2012; McCormack *et al.*, 2012) will certainly change this, but they are still relatively expensive for population phylogenetics compared to sequencing a few loci for many individuals. It is also clear that increased molecular data are not sufficient in some groups for clear species delimitation (Buckley *et al.*, 2006; Marshall *et al.*, 2006; Ross *et al.*, 2010; Dupuis *et al.*, 2012).

Cicadetta montana cryptic species complex evolution

The phylogeny of the *C. montana* cryptic species complex supports the description of many species based primarily on male calling songs. *Cicadetta montana* species have been grouped into three main divisions based on song similarity (Gogala *et al.*, 2008b; Gogala, 2013). However, none of these song groups are supported by any of the phylogenetic analyses (**Fig 3.3A**).

The monophyly of the type species of the genus, *C. montana* s. str., is strongly supported by all molecular analyses. This species remains distinct despite its sympatric and parapatric distributions with other species of this cryptic species complex. It is interesting that this species does not occur in Greece south of 40° north latitude line. Further to the south, it is replaced by *C. hannekeae* and other endemic species (Gogala *et al.*, 2008a).

The close genetic relationship between *C. concinna* and *C. macedonica* is surprising given the clear morphological differences in the wing colouration of *C. concinna*, which was described by Germar in 1821. The geographical distributions of both species are very different and are only found overlapping with recently described subspecies *C. c. arachnocepta* (Gogala *et al.*, 2014) in a small region of northern Macedonia; however, their songs are somewhat similar (**Fig 3.1B**). This may be a result of a recent speciation or hybridisation, but additional population genetic analyses would be required to determine which is more likely.

The close relationships of *C. dirfica*, *C. hannekeae*, *C. olympica* and *C. kissavi* may also reflect hybridisation or a recent radiation. These four species are allopatric and all of them except *C. hannekeae*, which is widely distributed throughout Greece, are isolated from each other on mountains (on slopes at elevations above 800 m asl to tree line). No intermediate individuals have been detected between any of these species. Increased sampling may help to resolve these species. Mountain habitats are commonly associated with an increase of insect biodiversity and endemism due to geographical isolation (Buckley *et al.*, 2001; Knowles, 2001; Knowles & Richards, 2005; Pryke & Samways, 2010; Garrick, 2011).

All of the molecular analyses support the inclusion of the monotypic genus *Euboeana* within the *C. montana* species complex. Acoustic and morphological differences between *E. castaneivaga* and *C. dirfica* distinguish these two sympatric species (Gogala *et al.*, 2011). However, given the close molecular association, it appears *E. castaneivaga* should be placed within the genus *Cicadetta*. Given the clear genetic difference between *C. dirfica* and *E. castaneivaga* it is unlikely that *E. castaneivaga* is part of the recent Greek radiation or hybridisation discussed above. *Euboeana castaneivaga* is distributed on the island Euboea and some Kikladan islands (Andros, Naxos). It is not limited to localities of higher elevation (Gogala & Trilar, 2014 and unpublished data).

The most striking finding of this phylogenetic study was the complicated relationships between *C. cerdaniensis*, *C. brevipennis*, *C. sibillae* and *C. anapaistica* (**Fig 3.3**). Preliminary phylogenies of *COI* and *COII* data showed polyphyletic relationships between *C. brevipennis* and *C. cerdaniensis* with *C. anapaistica* placed sister to two *C. brevipennis* specimens. Subsequent analyses showed significant differences between *C. cerdaniensis* specimens and resulted in the description of *C. sibillae* (Chapter 2 = Hertach *et al.*, 2015). Song patterns clearly group *C. cantilatrix*, *C. cerdaniensis*, *C. sibillae* and *C. anapaistica* (Sueur & Puissant, 2007; Chapter 1 = Hertach, 2011; Gogala, 2013; Chapter 2 = Hertach *et al.*, 2015). *Cicadetta cerdaniensis* was described in 2000; however, *C. cantilatrix* was not described until 2007 (Sueur & Puissant, 2007). *Cicadetta cantilatrix* is strongly supported in all the analyses as a monophyletic species. *Cicadetta anapaistica* was discovered in 2009 in southern Italy (Chapter 1 = Hertach, 2011) and *C. sibillae* was recently described and is found in central and northern Italy and southern Switzerland (Chapter 2 = Hertach *et al.*, 2015). The song group *C. cantilatrix*, *C. cerdaniensis*, *C. anapaistica* + *C. sibillae* is not supported in any of the analyses presented; however, there is a pattern of evolution from the simplest male songs (*C. cantilatrix*) to the most complex (*C. anapaistica*) and all taxa together show parapatric or allopatric distributions.

The song of *Cicadetta brevipennis* is characteristically different from all other species in the *Cicadetta montana* complex (Gogala & Trilar, 2004; Sueur & Puissant, 2007; Gogala *et al.*, 2011), except *C. fangoana* to which it was expected to be closely related. The lack of reciprocal monophyly in *C. brevipennis*-A and -B, *C. sibillae*, *C. anapaistica* and *C. cerdaniensis* cryptic species was indeed so surprising to us that we went back and resequenced many of the specimens and collected specimens from additional localities. However, these species remained strikingly intermingled. Analysis of *C. brevipennis*-A and -B is ongoing to further understand the division of this taxon.

Despite the fact that *C. brevipennis* may be two species, the unusual pattern of phylogenetically intermixed *C. brevipennis*-A and *C. cerdaniensis* (morphologically cryptic but very different in song) is difficult to explain. This is made even more striking by the lack of differentiation between *C. brevipennis*-B and *C. sibillae* which are intermixed in a similar way. It may be a result of a combination of gene flow, mitochondrial capture, incomplete lineage sorting, selection or convergence of songs. The nuclear genes examined have too little information to address this question. Additional nuclear gene data will be necessary to determine the cause. At present, we can only speculate. This pattern could have occurred by past hybridisation or incomplete lineage sorting so that both *C. cerdaniensis* and *C. brevipennis* had similar genomes but distinct songs. This may have been followed by the division of *C. brevipennis*/*C. cerdaniensis* populations into two geographically isolated lineages during glaciation in an Iberian and an Apenninian refuge (Chapter 2 = Hertach *et al.*, 2015). Several hundred individuals have been heard and recorded from throughout the distributions of these species, and particularly within areas of sympatry and parapatry, and only two individuals have emitted an intermediate song. This pattern could also have been produced if one mitochondrial clade is derived from the ancestral *C. brevipennis* clade and the second represents the ancestral *C. cerdaniensis* clade. Genetic introgression could then have occurred from one species into the other in the Apenninian clade and the opposite genetic introgression pattern in the Iberian clade. Alternatively, songs could be controlled by one or a few polymorphic loci, which give rise to both song types depending on genotypic combinations. This is highly unlikely given this pattern has never been observed in any other cicada species and the song type of *C. sibillae* and *C. cerdaniensis* is similar to *C. cantilatrix* which is clearly monophyletic.

The genetic distances and biogeographical patterns suggest that the *C. montana* species complex diverged roughly during the Plio-Pleistocene but more work is needed to understand the complete history of the complex. *Cicadetta montana* s. l. was originally described as having a Palearctic distribution (Kudryasheva, 1979). In addition to the cryptic species discovered in Europe, *C. abscondita* was described from Korea (Lee, 2008); it had originally been included within *C. montana* and may indeed be part of this complex. Given the cryptic diversity in Europe and the lack of exploration in central Asia, it is very likely there are additional cryptic *C. montana* complex species in Asia that have not yet been discovered (Gogala, 2013). Detailed morphological, acoustic and phylogenetic/molecular clock analyses should be conducted in the future that include all possible members of the *Cicadetta montana* complex: the European species (studied here), the Korean species and any other related species that may be present in central Asia.

CONCLUSIONS

Despite the fact that *C. montana* was described in 1772, it was not until the last two decades that the majority of cryptic species were described from Europe. This new biodiversity discovery is due to the application of in-depth acoustic analyses (Puissant & Boulard, 2000; Gogala & Trilar, 2004; Hertach, 2007; Chapter 1 = Hertach, 2011; Gogala *et al.*, 2008a; 2008b; 2009; 2011; Gogala, 2013; Chapter 2 = Hertach *et al.*, 2015).

Although in-depth acoustic analyses are more successful than molecular species delimiting algorithms in identifying cryptic species in the *C. montana* complex, phylogenetic and species delimitation analyses revealed cryptic lineages within the *C. cerdaniensis* s. l. and *C. brevipennis* song groups. An approach combining acoustic, phylogenetic, chorological, morphological and ecological data is necessary for understanding the evolution of the complex and essential for making wise taxonomic decisions.

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REFERENCES CHAPTER 3

Arensburger P, Buckley TR, Simon C, Moulds MS, Holsinger KE. 2004. Biogeography and phylogeny of the New Zealand cicada genera (Hemiptera: Cicadidae) based on nuclear and mitochondrial DNA data. *Journal of Biogeography*. 31: 557-569.

Barraclough TG. 2010. Evolving entities: towards a unified framework for understanding diversity at the species and higher levels. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365: 1801-1813.

Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution* 22: 148-155.

Buckley TR, Cordeiro M, Marshall DC, Simon C. 2006. Differentiating between hypotheses of lineage sorting and introgression in New Zealand alpine cicadas (*Maoricicada* Dugdale). *Systematic Biology* 55(3): 411-425.

Buckley TR, Simon C, Chambers GK. 2001. Phylogeography of the New Zealand cicada *Maoricicada campbelli* based on mitochondrial DNA sequences: Ancient clades associated with Cenozoic environmental change. *Evolution* 55(7): 1395-1407.

Butlin RK, Galindo J, Grahame JW. 2008. Sympatric, parapatric or allopatric: the most important way to classify speciation? *Philosophical Transactions of the Royal Society B: Biological Sciences* 363: 2997-3007.

Camargo A, Morando M, Avila L, Sites JW. 2012. Species delimitation with ABC and other coalescent-based methods in lizards of the *Liolaemus darwinii* complex (Squamata: Liolaemidae). *Evolution* 66: 2834-2849.

Carstens BC, Dewey TA. 2010. Species delimitation using a combined coalescent and information-theoretic approach: an example from North American *Myotis* bats. *Systematic Biology* 59: 400-414.

Carstens BC, Knowles LL. 2007. Estimating species phylogeny from gene-tree probabilities despite incomplete lineage sorting: an example from *Melanoplus* grasshoppers. *Systematic Biology* 56: 400-411.

Carstens BC, Pelletier TA, Reid NM, Satler JD. 2013. How to fail at species delimitation. *Molecular Ecology* 22: 4369-4383.

Cooley JR, Marshall DC. 2001. Sexual signalling in periodical cicadas, *Magicicada* spp. (Hemiptera: Cicadidae). *Behaviour* 138: 827-855.

Coyne JA, Orr HA. 2004. *Speciation.* Sunderland, MA: Sinauer Associates, 545 pp.

De Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* 56(6): 879-886.

Degnan JH, Rosenberg NA. 2006. Discordance of species trees with their most likely gene trees. *PLoS Genetics* 2: e68.

Dobzhansky T. 1940. Speciation as a stage in evolutionary divergence. *American Naturalist*: 312-321.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969-1973.

Dugdale JS, Fleming CA. 1978. New Zealand cicadas of the genus *Maoricicada* (Homoptera: Tibicinidae). *New Zealand Journal of Zoology* 5(2): 295-340.

Dupuis JR, Roe AD, Sperling FA. 2012. Multi-locus species delimitation in closely related animals and fungi: one marker is not enough. *Molecular Ecolology* 21: 4422-4436.

Edwards SV, Liu L, Pearl DK. 2007. High-resolution species trees without concatenation. *Proceedings of the National Academy of Sciences* 104: 5936-5941.

Ence DD, Carstens BC. 2011. SpedeSTEM: a rapid and accurate method for species delimitation. *Molecular Ecology Resources* 11: 473-480.

Esselstyn JA, Evans BJ, Sedlock JL, Khan FAA, Heaney LR. 2012. Single-locus species delimitation: a test of the mixed Yule-coalescent model, with an empirical application to Philippine round-leaf bats. *Proceedings of the Royal Society B: Biological Sciences* 279: 3678-3686.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294-299.

Fontaneto D, Herniou EA, Boschetti C, Caprioli M, Melone G, Ricci C, Barraclough TG. 2007. Independently evolving species in asexual bdelloid rotifers. *PLoS Biology* 5: e87.

Fujisawa T, Barraclough TG. 2013. Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent approach: a revised method and evaluation on simulated data sets. *Systematic Biology* 62: 707-724.

Fujita MK, Leaché AD, Burbrink FT, McGuire JA, Moritz C. 2012. Coalescent-based species delimitation in an integrative taxonomy. *Trends in Ecology & Evolution* 27: 480-488.

Garrick RC. 2011. Montane refuges and topographic complexity generate and maintain invertebrate biodiversity: recurring themes across space and time. *Journal of Insect Conservation* 15: 469-478.

Gogala M. 2013. *On the trail of mountain cicadas*. Ljubljana: Slovenian Academy of Sciences and Arts and Royal Entomological Society, 193 pp.

Gogala M, Drosopoulos S, Trilar T. 2008a. *Cicadetta montana* complex (Hemiptera, Cicadidae) in Greece a new species and new records based on bioacoustics. *Deutsche Entomologische Zeitschrift* 55: 91-100.

Gogala M, Drosopoulos S, Trilar T. 2008b. Present status of mountain cicadas *Cicadetta montana* (sensu lato) in Europe. *Bulletin of Insectology* 61(1): 123-124.

Gogala M, Drosopoulos S, Trilar T. 2009. Two mountains, two species: new taxa of the *Cicadetta montana* species complex in Greece (Hemiptera: Cicadidae). *Acta entomologica slovenica* 17: 13-28.

Gogala M, Drosopoulos S, Trilar T. 2012. *Cicadivetta goumenissa*, a new cicada species from Peloponesos, Greece (Hemiptera: Cicadidae). *Acta entomologica slovenica* 20: 5-16.

Gogala M, Trilar T. 1999. The song structure of *Cicadetta montana macedonica* Schedl with remarks on songs of related singing cicadas (Hemiptera: Auchenorrhyncha: Cicadomorpha: Tibicinidae). *Reichenbachia* 33: 91-97.

Gogala M, Trilar T. 2004. Bioacoustic investigations and taxonomic considerations on the *Cicadetta montana* species complex (Homoptera: Cicadoidea: Tibicinidae). *Anais da Academia Brasileira de Ciências* 76(2): 316-324.

Gogala M, Trilar T. 2014. Distribution of endemic cicadas (Hemiptera: Cicadidae) on Evia and adjacent islands in Greece. *Acta entomologica slovenica* 22: 45-58.

Gogala M, Trilar T, Drosopoulos S. 2011. Two new species and a new genus of Cicadettini (Hemiptera, Cicadidae) from the Greek island of Evia. *Deutsche Entomologische Zeitschrift* 58(1): 105-117.

Gogala M, Trilar T, Krpač V. 2014. New subspecies *Cicadetta concinna arachnocepta* (Hemiptera: Cicadidae) from Macedonia. *Acta entomologica slovenica* 22(2): 101-114.

Harrington RC, Near TJ. 2012. Phylogenetic and coalescent strategies of species delimitation in snubnose darters (Percidae: Etheostoma). *Systematic Biology* 61: 63-79.

Hebert PD, Ratnasingham S, de Waard SR. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B: Biological Sciences* 270 (Suppl. 1): S96-S99.

Heled J, Drummond AJ. 2010. Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution* 27: 570-580.

Hertach T. 2007. Three species instead of only one: Distribution and ecology of the *Cicadetta montana* species complex (Hemiptera: Cicadoidea) in Switzerland. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 80: 37-61.

Hertach T. 2011. Spectacular song pattern from the Sicilian Mountains: The new cicada species *Cicadetta anapaistica* sp. n. (Hemiptera: Cicadidae). *Zootaxa* 2771: 25-40.

Hertach T, Trilar T, Wade EJ, Simon C, Nagel P. 2015. Songs, genetics, and morphology: revealing the taxonomic units in the European *Cicadetta cerdaniensis* cicada group, with a description of new taxa (Hemiptera: Cicadidae). *Zoological Journal of the Linnean Society* 173(2): 320-351.

Hey J, Pinho C. 2012. Population genetics and objectivity in species diagnosis. *Evolution* 66: 1413-1429.

Hey J, Waples RS, Arnold ML, Butlin RK, Harrison RG. 2003. Understanding and confronting species uncertainty in biology and conservation. *Trends in Ecology & Evolution* 18: 597-603.

Hickerson MJ, Meyer CP, Moritz C. 2006. DNA barcoding will often fail to discover new animal species over broad parameter space. *Systematic Biology* 55: 729-739.

Hill KBR, Marshall DC, Simon C, Chambers GK. 2009. Surviving glacial ages within the Biotic Gap: phylogeography of the New Zealand cicada *Maoricicada campbelli*. *Journal of Biogeography* 36(4): 675-692.

Huang H, He Q, Kubatko LS, Knowles LL. 2010. Sources of error inherent in species-tree estimation: impact of mutational and coalescent effects on accuracy and implications for choosing among different methods. *Systematic Biology* 59: 573-583.

Irwin DE. 2002. Phylogeographic breaks without geographic barriers to gene flow. *Evolution* 56: 2383-2394.

Knowles LL. 2001. Did the Pleistocene glaciations promote divergence? Tests of explicit refugial models in montane grasshoppers. *Molecular Ecology* 10: 691-701.

Knowles LL. 2009. Statistical phylogeography. *Annual Review of Ecology, Evolution, and Systematics* 40: 593-612.

Knowles LL, Carstens BC. 2007. Delimiting species without monophyletic gene trees. *Systematic Biology* 56: 887-895.

Knowles LL, Richards CL. 2005. Importance of genetic drift during Pleistocene divergence as revealed by analyses of genomic variation. *Molecular Ecology* 14: 4023-4032.

Kubatko LS, Carstens BC, Knowles LL. 2009. STEM: species tree estimation using maximum likelihood for gene trees under coalescence. *Bioinformatics* 25: 971-973.

Kubatko LS, Degnan JH. 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. *Systematic Biology* 56: 17-24.

Kudryasheva JV. 1979. *Larvae of singing cicadas (Homoptera, Cicadidae) of the Fauna of SSSR.* Moskva: Nauka, 159 pp. (in Russian)

Lanfear R, Calcott B, Ho SY, Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695-1701.

Leaché AD, Fujita MK. 2010. Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*). *Proceedings of the Royal Society B: Biological Sciences* 277: 3071-3077.

Lee YJ. 2008. Revised synonymic list of Cicadidae (Insecta: Hemiptera) from the Korean Peninsula, with the description of a new species and some taxonomic remarks. *Proceedings of the Biological Society of Washington* 121: 445-467.

Lee YJ, Choe HJ, Lee ML, Woo KS. 2002. A phylogenetic consideration of the Far Eastern Palaearctic species of the genus *Cicadetta* Kolenati (Homoptera, Cicadidae) based on the mitochondrial *COI* gene sequences. *Journal of Asia-Pacific Entomology* 5: 3-11.

Lee YJ, Hill KBR. 2010. Systematic revision of the genus *Psithyristria* Stål (Hemiptera: Cicadidae) with seven new species and a molecular phylogeny of the genus and higher taxa. *Systematic Entomology* 35: 277-305.

Lemmon AR, Emme SA, Lemmon EM. 2012. Anchored hybrid enrichment for massively high-throughput phylogenomics. *Systematic Biology* 61: 727-744.

Liu L. 2008. BEST: Bayesian estimation of species trees under the coalescent model. *Bioinformatics* 24: 2542-2543.

Lohse K. 2009. Can mtDNA barcodes be used to delimit species? A Response to Pons *et al.* (2006). *Systematic Biology* 58: 439-442.

Maddison W, Maddison D. 2007. *Mesquite: a modular system for evolutionary analysis*. Version 2.75. 2011. URL http://mesquiteproject.org.

Marshall DC, Cooley JR. 2000. Reproductive character displacement and speciation in periodical cicadas, with description of a new species, 13-year *Magicicada neotredecim*. *Evolution* 54: 1313-1325.

Marshall DC, Hill KBR, Cooley JR, Simon C. 2011. Hybridization, mitochondrial DNA phylogeography, and prediction of the early stages of reproductive isolation: Lessons from New Zealand cicadas (Genus *Kikihia*). *Systematic Biology* 60: 482-502.

Marshall DC, Hill KBR, Fontaine KM, Buckley TR, Simon C. 2009. Glacial refugia in a maritime temperate climate: cicada (*Kikihia subalpina*) mtDNA phylogeography in New Zealand. *Molecular Ecology* 18: 1995-2009.

Marshall DC, Hill KBR, Moulds MS, Vanderpool D, Cooley JR, Mohagan AB, Simon C. 2016. Inflation of molecular clock rates and dates: Molecular phylogenetics, biogeography, and diversification of a global cicada radiation from Australasia (Hemiptera: Cicadidae: Cicadettini). *Systematic Biology* 65(1): 16-34.

Marshall DC, Slon K, Cooley JR, Hill KBR, Simon C. 2008. Steady Plio-Pleistocene diversification and a 2-million-year sympatry threshold in a New Zealand cicada radiation. *Molecular Phylogenetics and Evolution* 48: 1054-1066.

Marshall JC, Arevalo E, Benavides E, Sites JL, Sites JW. 2006. Delimiting species: comparing methods for Mendelian characters using lizards of the *Sceloporus grammicus* (Squamata: Phrynosomatidae) complex. *Evolution* 60: 1050-1065.

McCormack JE, Faircloth BC, Crawford NG, Gowaty PA, Brumfield RT, Glenn TC. 2012. Ultraconserved elements are novel phylogenomic markers that resolve placental mammal phylogeny when combined with species-tree analysis. *Genome Research* 22: 746-754.

Monaghan MT, Wild R, Elliot M, Fujisawa T, Balke M, Inward DJ, Lees DC, Ranaivosolo R, Eggleton P, Barraclough TG. 2009. Accelerated species inventory on Madagascar using coalescent-based models of species delineation. *Systematic Biology* 58: 298-311.

Moulds MS. 2005. An appraisal of the higher classification of cicadas (Hemiptera: Cicadoidea) with special reference to the Australian fauna. *Records of the Australian Museum* 57: 375-446.

Nast J. 1972. *Plaearctic Auchenorrhyncha (Homoptera). An annotated check list.* Warszawa: Polish scientific publishers, 550 pp.

Nunes VL, Mendes R, Marabuto E, Novais BM, Hertach T, Quartau JA, Seabra SG, Paulo OS, Simões PC. 2014. Conflicting patterns of DNA barcoding and taxonomy in the cicada genus *Tettigettalna* from southern Europe (Hemiptera: Cicadidae). *Molecular Ecology Resources* 14(1): 27-38.

Papadopoulou A, Monaghan MT, Barraclough TG, Vogler AP. 2009. Sampling error does not invalidate the Yule-coalescent model for species delimitation. A response to Lohse (2009). *Systematic Biology* 58: 442-444.

Petit RJ, Excoffier L. 2009. Gene flow and species delimitation. *Trends in Ecology & Evolution* 24: 386-393.

Pfenninger M, Schwenk K. 2007. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology* 7: 121.

Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* 55: 595-609.

Pons J, Fujisawa T, Claridge EM, Anthony Savill R, Barraclough TG, Vogler AP. 2011. Deep mtDNA subdivision within Linnean species in an endemic radiation of tiger beetles from New Zealand (genus *Neocicindela*). *Molecular Phylogenetics and Evolution* 59: 251-262.

Popple LW. 2013. A revision of the *Pauropsalta annulata* Goding & Froggatt species group (Hemiptera: Cicadidae) based on morphology, calling songs and ecology, with investigations into calling song structure, molecular phylogenetic relationships and a case of hybridisation between two subspecies. *Zootaxa* 3730(1): 1-102.

Popple LW, Walter G, Raghu S. 2008. The structure of calling songs in the cicada *Pauropsalta annulata* Goding & Froggatt (Hemiptera: Cicadidae): evidence of diverging populations? *Evolutionary Ecology* 22: 203-215.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

Pryke J, Samways M. 2010. Significant variables for the conservation of mountain invertebrates. *Journal of Insect Conservation* 14: 247-256.

Puissant S, Boulard M. 2000. *Cicadetta cerdaniensis,* espèce jumelle de *Cicadetta montana* décryptée par l'acoustique (Auchenorrhyncha, Cicadidae, Tibicinidae). *Ecole pratique des hautes Etudes, Biologie et Evolution des Insectes* 13: 111-117.

Puissant S, Sueur J. 2010. A hotspot for Mediterranean cicadas (Insecta: Hemiptera: Cicadidae): new genera, species and songs from southern Spain. *Systematics and Biodiversity* 8(4): 555-574.

Qi S, Hayashi M, Wei C. 2015. A review of the cicada genus *Kosemia* Matsumura (Hemiptera: Cicadidae). *Zootaxa* 3911: 451-492.

Quartau JA, Simões PC. 2006. Acoustic evolutionary divergence in cicadas: the species of *Cicada* L. in Southern Europe. In: Drosopoulos S, Claridge M, eds. *Insect sounds and communication: Physiology, behaviour, ecology and evolution.* Boca Raton: CRC Press Taylor & Francis Group, 227-237.

Rannala B, Yang Z. 2013. Improved reversible jump algorithms for Bayesian species delimitation. *Genetics* 194: 245-253.

Ross KG, Gotzek D, Ascunce MS, Shoemaker DD. 2010. Species delimitation: a case study in a problematic ant taxon. *Systematic Biology* 59: 162-184.

Sanborn AF. 2014. *Catalogue of the Cicadoidea (Hemiptera: Auchenorrhyncha).* London: Academic Press/Elsevier, 1002 pp.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Floors P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87: 651-701.

Smadja C, Butlin R. 2008. On the scent of speciation: the chemosensory system and its role in premating isolation. *Heredity* 102: 77-97.

Solís-Lemus C, Knowles LL, Ané C. 2015. Bayesian species delimitation combining multiple genes and traits in a unified framework. *Evolution* 69: 492-507.

Sueur J, Aubin T. 2004. Acoustic signals in cicada courtship behaviour (order Hemiptera, genus *Tibicina*). *Journal of Zoology* 262: 217-224.

Sueur J, Puissant S. 2007. Similar look but different song: a new *Cicadetta* species in the *montana* complex (Insecta, Hemiptera, Cicadidae). *Zootaxa* 1442: 55-68.

Sueur J, Vanderpool D, Simon C, Ouvrard D, Bourgoin T. 2007. Molecular phylogeny of the genus *Tibicina* (Hemiptera, Cicadidae): rapid radiation and acoustic behaviour. *Biological Journal of the Linnean Society* 91: 611-626.

Sukumaran J, Holder MT. 2010. DendroPy: a Python library for phylogenetic computing. *Bioinformatics* 26(12): 1569-1571.

Tajima F, Nei M. 1984. Estimation of evolutionary distance between nucleotide sequences. *Molecular Biology and Evolution* 1: 269-285.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725-2729.

Wells MM, Henry CS. 1998. Songs, reproductive isolation, and speciation in cryptic species of insects. In: Howard D, Berlocher S, eds. *Endless Forms: Species and Speciation.* New York: Oxford University Press, 217-233.

Wiens JJ. 2007. Species delimitation: new approaches for discovering diversity. *Systematic Biology* 56: 875-878.

Yang Z, Rannala B. 2010. Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences* 107: 9264-9269.

Zwickl D. 2006. *GARLI: genetic algorithm for rapid likelihood inference.* URL http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html

APPENDIXES

Appendix S3.1. Specimen collection data.

Appendix S3.2. Estimated dataset optimal partitioning schemes and substitution models.

Appendix S3.1. Specimen collection data.

C = *Cicadetta*, E = *Euboeana*, Lat. = latitude, Long. = longitude (arranged in alphabetical order)

Specimen	Country	Lat.	Long.	Location
C. anapaistica.94	Italy	37.84	14.02	Sicily, Madonie
C. anapaistica.95	Italy	37.83	14.58	Sicily, Nebrodi, Cerami
C. anapaistica.96	Italy	37.92	14.67	Sicily, Nebrodi, Cesaro
C. anapaistica.175	Italy	37.86	14.08	Sicily, Madonie
C. anapaistica.179	Italy	39.13	16.42	Calabria, Sila Piccola, Trearie
C. brevipennis.04	France	42.76	3.02	Eastern Pyrenees, Torreilles
C. brevipennis.09	Slovenia	46.02	14.39	Osrednjeslovenska, Lukovica
C. brevipennis.10	Slovenia	46.02	14.39	Osrednjeslovenska, Lukovica
C. brevipennis.74	Italy	41.53	15.08	Apulia, Monti della Daunia
C. brevipennis.75	Italy	43.51	10.43	Tuscany, Livorno
C. brevipennis.92	Switzerland	45.91	8.95	Ticino, Monte San Giorgio
C. brevipennis.115	Italy	41.53	15.08	Apulia, Monti della Daunia
C. brevipennis.116	Italy	41.55	15.10	Apulia, Monti della Daunia
C. brevipennis.117	Italy	41.88	14.53	Abruzzi, Torrebruna
C. brevipennis.118	Italy	41.88	14.53	Abruzzi, Torrebruna
C. brevipennis.119	Switzerland	46.19	6.03	Geneva, Aire-la-Ville
C. brevipennis.120	France	42.57	2.36	Eastern Pyrenees, Fuilla
C. brevipennis.135	Italy	43.25	10.75	Tuscany, Foresta di Monterufoli
C. brevipennis.136	Italy	43.25	10.75	Tuscany, Foresta di Monterufoli
C. brevipennis.140	Switzerland	46.69	6.52	Vaud, Arnex-sur-Orbe
C. brevipennis.141	Italy	45.63	7.33	Aosta, Cogne, Epinel
C. brevipennis.400	France	44.27	6.89	Maritime Alps, S.-Étienne-de-Tinée
C. brevipennis.408	France	48.21	7.36	Alsace, Bergheim
C. brevipennis.409	Italy	46.43	12.92	Friuli-Venezia Giulia, Avaglio
C. brevipennis.421	Switzerland	45.91	8.95	Ticino, Monte San Giorgio
C. brevipennis.422	Switzerland	45.91	8.95	Ticino, Monte San Giorgio
C. brevipennis.426	France	42.76	3.04	Eastern Pyrenees, Torreilles
C. brevipennis.427	France	42.76	3.04	Eastern Pyrenees, Torreilles
C. brevipennis.428	France	44.22	3.22	Aveyron, Mostuéjouls
C. brevipennis.429	France	43.83	3.31	Hérault, Saint-Félix-de-l'Héras
C. brevipennis.431	Italy	44.80	9.31	Lombardy, Passo del Penice
C. brevipennis.432	Italy	44.80	9.31	Lombardy, Passo del Penice
C. brevipennis.434	Italy	44.80	9.31	Lombardy, Passo del Penice
C. cantilatrix.02	Slovenia	46.37	13.74	Goriška, Trenta
C. cantilatrix.11	Poland	50.47	20.47	Świętokrzyskie, Pińczów, Polana Polichno
C. cantilatrix.12	Macedonia	41.89	21.26	Skopje, Suva Planina
C. cantilatrix.35	Switzerland	47.42	7.82	Basel-Land, Diegten
C. cantilatrix.36	Switzerland	47.41	8.01	Aargau, Erlinsbach
C. cantilatrix.38	Switzerland	47.41	8.01	Aargau, Erlinsbach
C. cantilatrix.52	France	49.08	1.67	Val-d'Oise, Haute-Isle
C. cantilatrix.53	France	49.08	1.67	Val-d'Oise, Haute-Isle
C. cantilatrix.103	Bulgaria	42.37	22.83	Kjustendil, Mt. Konyavska, Carvenjano

Appendix S3.1. Specimen collection data.

C = *Cicadetta*, E = *Euboeana*, Lat. = latitude, Long. = longitude (arranged in alphabetical order)

Specimen	**Country**	**Lat.**	**Long.**	**Location**
C. cerdaniensis.44	France	42.50	2.35	Eastern Pyrenees, Py
C. cerdaniensis.45	France	42.41	1.99	Eastern Pyrenees, Osséja
C. cerdaniensis.121	France	42.41	1.99	Eastern Pyrenees, Osséja
C. cerdaniensis.122	France	42.50	2.35	Eastern Pyrenees, Py
C. concinna.13	Macedonia	41.89	21.26	Skopje, Suva Planina
C. concinna.33	Poland	50.45	20.56	Świętokrzyskie, Pińczów, Krzyżanowice Dolne
C. concinna.41	Poland	50.45	20.56	Świętokrzyskie, Pińczów, Krzyżanowice Dolne
C. dirfica.51	Greece	38.60	23.85	Euboea, Mt. Dirfis
C. dirfica.52	Greece	38.60	23.85	Euboea, Mt. Dirfis
C. dirfica.106	Greece	38.60	23.85	Euboea, Mt. Dirfis
C. dirfica.112	Greece	38.60	23.85	Euboea, Mt. Dirfis
C. fangoana.93	France	42.60	9.26	Corsica, Santo-Pietro-di-Tenda
C. hannekeae.42	Greece	38.55	22.55	Dytiki Ellada, Parnassos
C. hannekeae.126	Greece	38.55	22.55	Dytiki Ellada, Parnassos
C. kissavi.54	Greece	39.80	22.66	Thessalia, Larissa, Mt. Ossa
C. kissavi.129	Greece	39.76	22.66	Thessalia, Larissa, Mt. Ossa
C. macedonica.08	Macedonia	40.98	20.89	Resen, Galičica
C. macedonica.101	Romania	45.00	22.53	Caraș-Severin, Băile Herculane
C. macedonica.102	Bulgaria	42.40	22.88	Kjustendil, Mt. Konyavska, Uglyartsi
C. macedonica.104	Bulgaria	41.96	27.66	Burgas, Strandzha, Slivarovo
C. macedonica.109	Bulgaria	42.40	22.88	Kjustendil, Mt. Konyavska, Uglyartsi
C. macedonica.110	Bulgaria	42.40	22.88	Kjustendil, Mt. Konyavska, Uglyartsi
C. macedonica.130	Bulgaria	41.41	23.59	Blagoewgrad, Mt. Slavyanka, Livadi
C. montana.01	Slovenia	45.99	14.05	Goriška, Idrija, Ljubevč
C. montana.03	Slovenia	46.14	15.11	Zasavska, Hrastnik
C. montana.39	Switzerland	47.43	8.04	Aargau, Küttigen
C. montana.40	Switzerland	47.58	7.68	Basel, Bettingen
C. montana.41	Switzerland	47.30	9.03	St. Gallen, Mosnang, Alplispitz
C. montana.42	Switzerland	46.30	7.57	Valais, Salgesch
C. montana.97	Switzerland	46.17	8.61	Ticino, Centovalli, Lionza
C. montana.98	Italy	39.33	16.55	Calabria, Sila, Lago di Ariamacina
C. montana.124	France	46.14	5.99	Upper Savoy, Humilly
C. montana.138	Italy	44.21	7.95	Piedmont, Colle di Casotto
C. montana.139	Italy	44.46	9.81	Emilia-Romagna, Passo del Bratello
C. olympica.53	Greece	40.10	22.45	Kendriki Makedonia, Mt. Olympus
C. olympica.128	Greece	40.11	22.46	Kendriki Makedonia, Mt. Olympus
C. sibillae.34	Switzerland	45.91	8.95	Ticino, Monte San Giorgio
C. sibillae.56	Italy	45.87	8.82	Lombardy, Varese, Mt. Chiusarella
C. sibillae.73	Italy	44.43	8.43	Liguria, Pontinvrea
C. sibillae.91	Switzerland	45.91	8.95	Ticino, Monte San Giorgio
C. sibillae.123	Italy	42.21	13.48	Abruzzi, Rocca di Cambio
C. sibillae.137	Italy	45.13	7.35	Piedmont, Val di Susa, Celle
E. castaneivaga.55	Greece	38.60	23.85	Euboea, Mt. Dirfis
E. castaneivaga.105	Greece	38.59	23.85	Euboea, Mt. Dirfis
E. castaneivaga.111	Greece	38.59	23.85	Euboea, Mt. Dirfis

Appendix S3.2. Estimated dataset optimal partitioning schemes and substitution models.

N Sites: number of base pairs, VS: variable sites, PI: parsimony informative sites. Model All and Model MrBayes are the best substitution models predicated based on all possible models and models implementable in MrBayes, respectively.

Partition	N Sites	VS	PI	Model_All	Model_MrBayes
COI+*COII*_codon1	501	21	21	HKY+I	HKY+I
COI+*COII*_codon2	501	4	4	F81	F81
COI+*COII*_codon3	501	138	138	K81uf	GTR
EF1α p1	875	13	13	F81+G	F81+G
EF1α p2	437	4	4	HKY+I	HKY+I
EF1α p3	839	48	48	K81uf+G	HKY+G
EF1α p4				JC	JC
period intron	396	6	5	F81	F81

Chapter 4: Complex within a complex: Integrative taxonomy reveals hidden diversity in *Cicadetta brevipennis* (Hemiptera: Cicadidae) and unexpected relationships with a song divergent relative

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ABSTRACT

Multiple sources of data in combination are essential for species delimitation and classification of difficult taxonomic groups. Here we investigate a cicada taxon with unusual cryptic diversity and we attempt to resolve seemingly contradictory data sets. Cicada songs act as species-specific premating barriers and have been used extensively to reveal hidden taxonomic diversity in morphologically similar species. The Palaearctic *Cicadetta montana* species complex is an excellent example where distinct song patterns have disclosed multiple recently described species. Indeed, two taxa turned out to be especially diverse in that they form a 'complex within the complex': the *Cicadetta cerdaniensis* song group (four species studied previously) and *Cicadetta brevipennis* (examined in details here).

Based on acoustic, morphological, molecular, ecological and spatial data sampled throughout their broad European distribution, we find that *Cicadetta brevipennis* s. l. comprises five lineages. The most distinct lineage is identified as *Cicadetta petryi* Schumacher, 1924, which we re-assign to the species level. *Cicadetta brevipennis litoralis* Puissant & Hertach ssp. n.* and *Cicadetta brevipennis hippolaidica* Hertach ssp. n.* are new to science. The latter hybridises with *Cicadetta brevipennis brevipennis* Fieber, 1876 at a zone inferred from intermediate song patterns. The fifth lineage requires additional investigation.

The *C. cerdaniensis* and the *C. brevipennis* song groups exhibit characteristic, clearly distinct basic song patterns that act as reproductive barriers. However, they remain completely intermixed in the Bayesian and maximum likelihood *COI* and *COII* mitochondrial DNA phylogenies. The closest relative of each of the four *cerdaniensis* group species is a *brevipennis* group taxon. In our favoured scenario the phylogenetic pairs originated in common Pleistocene glacial refuges where the taxa speciated and experienced sporadic inter-group hybridisation leading to extensive introgression and mitochondrial capture.

INTRODUCTION

Species delimitation and classification are among the most enduring contributions to science, and knowledge of species and subspecies boundaries is essential for conservation and management decisions. However, taxonomists are still far from a consensus on the definition of ‘species’ (Hey, 2006), and many species concepts are conflicting or impractical to apply. In this study we investigate a European cicada complex (Insecta: Hemiptera: Cicadidae) that illustrates many problems raised in the species concept/species delimitation debates. From previous studies, we know that described species in this complex are morphologically almost indistinguishable and molecularly interdigitated. Behaviour, represented by acoustic song patterns, is similar in cases with distinct mitochondrial haplotypes; but song patterns are clearly different in some taxa that possess closely related or identical haplotypes (Chapter 2 = Hertach *et al.*, 2015; Chapter 3 = Wade *et al.*, 2015). In this complicated system, we attempt to revise the taxonomy in light of modern species concepts and address the question of how such a strange phylogenetic pattern might have evolved.

Species concepts and integrative taxonomy

De Queiroz (2007) recognised the primary defining criterion of species as “separately evolving metapopulation lineages” and suggested that evidence for speciation could come from one or more sources (e.g. reproductive isolation, diagnosability, monophyly). Increasingly, new studies indicate that

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*The new subspecies names are not yet available at the submission date of the present thesis.

speciation can occur in the presence of intermittent or continuous gene flow between diverging populations (e.g. Mallet, 2008; Pinho & Hey, 2010; Smadja & Butlin, 2011; Sullivan *et al.*, 2014) and that species contain genes assembled from multiple semi-independently evolving lineages (Martin *et al.*, 2013). A useful new way of thinking about speciation was suggested by Butlin *et al.* (2008). In this view, reproductive isolation takes place in three phases: initiation, strengthening and completion. During each of these phases diverging populations can experience gene flow on a continuum from zero to high and can intermittently be allopatric, parapatric, or sympatric in no particular order. Thus gene flow can start and stop at various stages of the speciation process resulting in complex genetic and geographic patterns.

As noted by De Queiroz (2007), species delimitation and conceptualisation are two different issues. The variety of methods used to delimit species has rapidly increased with molecular based methods and models (Wiens, 2007; Carstens *et al.*, 2013; Solís-Lemus *et al.*, 2015). Recently, species boundaries have been estimated on genetic data exclusively (e.g. Hebert *et al.*, 2003; Pons *et al.*, 2006). Raising concern over purely genetic definitions of species, a number of studies have demonstrated that gene trees do not reflect species boundaries (e.g. Berthier *et al.*, 2006; Carstens & Knowles, 2007; Warren *et al.*, 2012; Nicholls *et al.*, 2012) due to incomplete lineage sorting and ongoing gene flow (as reviewed in Funk & Omland, 2003).

Integrative taxonomy combines data from multiple sources such as genetics, morphology and ecology and has been increasingly applied to difficult groups (Schlick-Steiner *et al.*, 2010; Yeates *et al.*, 2011). It goes beyond naming species and provides insight into the speciation processes (Schilthuizen, 2002; Schlick-Steiner *et al.*, 2010) and the recognition of current and past gene flow (Carstens *et al.*, 2013; Solís-Lemus *et al.*, 2015). Results from various data sources conflict more often than generally expected. Only 41% of arthropod taxa from a literature survey showed broad agreement in delimitation among multiple data sets (Schlick-Steiner *et al.*, 2010), thus interpretations seek to understand the source of the conflict. Here we use an integrative approach and combine acoustic, molecular, morphological, spatial and ecological data to understand the evolution of the *Cicadetta brevipennis* group.

Bioacoustic relevance in cicadas

Cicada males produce well known songs with their timbals. These songs are species-specific, act as premating barriers to non-conspecific females, and are an important component of specific-mate recognition systems (SMRS; Paterson, 1985; Den Hollander, 1995; Villet, 1995). Acoustic characters have been used extensively to reveal hidden taxonomic diversity when morphological traits are missing or weak (e.g. Dugdale & Fleming, 1978; Gogala & Trilar, 2004; Quartau & Simões, 2006; Puissant & Sueur, 2010).

Songs provide an excellent medium to study the early stages of reproductive isolation (Marshall *et al.*, 2009; 2011). They are especially useful because even under scenarios of divergence with gene flow, genes involved in species recognition are less likely to cross species boundaries than other genes (Via, 2012; Feder *et al.*, 2013); however although songs are the most probable indicators of species identity, a species-specific song does not guarantee that foreign genes are absent (Marshall *et al.*, 2011).

Cicadetta montana s. l. (Scopoli, 1772) is an ideal model system for bioacoustic research. As recently as the turn of the millennium (Gogala & Trilar, 1999; 2004; Puissant & Boulard, 2000), it has been successively shown to comprise a species complex of at least 13 morphologically similar but acoustically distinct species and two subspecies (Gogala *et al.*, 2014; Chapter 2 = Hertach *et al.*, 2015). The faint high frequency calling songs possess a remarkable richness and complexity in rhythms.

***Cicadetta brevipennis* and the *Cicadetta cerdaniensis* group**

Cicadetta brevipennis Fieber, 1876 (sensu Gogala & Trilar, 2004) and *Cicadetta cerdaniensis* s. l. Puissant & Boulard, 2000 are widespread taxa belonging to the *Cicadetta montana* species complex. Recent research on the two taxa disclosed hidden diversity and confusing phylogenetic relationships, which we systematically attempted to comprehend with our investigations. Prior to the research presented here, *C. brevipennis* was viewed as one species (e.g. Puissant, 2006; Hertach, 2007; Delorme *et al.*, 2015; Gurcel, 2015) characterised by a distinct song pattern (Gogala & Trilar, 2004): A binary structure of a long swelling echeme, followed after a brief break by a short echeme, is repeated multiple times.

In *Cicadetta cerdaniensis* s. l. some species diversity was more obvious and disclosed by a series of studies. In the first step, *Cicadetta cerdaniensis* s. l. was recognised as a group of different taxa characterised by the group-typical basic repetition of shorter echemes importantly modulated in power and species-specific, additional elements of various complexities (Sueur & Puissant, 2007; Chapter 1 = Hertach, 2011). Two species were described with qualitatively different songs: *Cicadetta cantilatrix* Sueur & Puissant, 2007 and *Cicadetta anapaistica* Hertach, 2011.

In a second step, the addition of molecular studies of the entire *Cicadetta montana* species complex (Chapter 3 = Wade *et al.*, 2015) demonstrated a phylogenetically close relationship between the *Cicadetta cerdaniensis* song group and *Cicadetta brevipennis*. In particular, they comprised two interdigitated (polyphyletic), well supported main clades based on analysis of segments of the mitochondrial genes cytochrome C oxidase subunits I and II (*COI* and *COII*): '*cerdaniensis*-A + *brevipennis*-A' and '*cerdaniensis*-B +

brevipennis-B + *C. anapaistica*' (Chapter 2 = Hertach *et al.*, 2015; Chapter 3 = Wade *et al.*, 2015). Some haplotypes were shared between song-divergent taxa and contamination was ruled out by re-sequencing. Nuclear markers (*elongation factor 1-alpha* and *period*) had a low number of variable and parsimony-informative sites and were informative only at the deeper levels of the tree. Thus molecular species delimitation methods [general mixed Yule-coalescent (GMYC; Pons *et al.*, 2006) and Bayesian phylogenetics and phylogeography (BPP; Yang & Rannala, 2010)] were successful in recognizing some species (e.g. *Cicadetta montana* s. str.) but not the species in the *cerdaniensis-brevipennis* polytomy (Chapter 3 = Wade *et al.*, 2015).

In a third step, in-depth investigations of '*cerdaniensis*-A' and '*cerdaniensis*-B' revealed a new species, *Cicadetta sibillae* Hertach & Trilar, 2015. In addition to having different mitochondrial haplotypes, it is distinguishable by quantitative acoustics, minor colouration characters and geographic location (Chapter 2 = Hertach *et al.*, 2015).

The findings in these second and third steps of our series of studies stimulated the central question of this manuscript: Does *C. brevipennis* also comprises more than one taxon? Or more specifically: Are '*brevipennis*-A' and '*brevipennis*-B' two different species? Alternatively, one or both units could result from gene exchange with *cerdaniensis*-group taxa (e.g. introgression with mtDNA capture; Funk & Omland, 2003; Marshall *et al.*, 2011; Nicholls *et al.*, 2012). '*Brevipennis*-B' could for example be introgressed with its neighbour *C. sibillae* (= former '*cerdaniensis*-B'). Our preliminary results, based on in-depth fieldwork suggested not only the two lineages '*brevipennis*-A' and '*brevipennis*-B' but rather a total of five potentially separately evolving metapopulations lineages (De Queiroz, 2007), subsequently defined as operational taxonomic units (OTUs): brev, hipp, lito, petr and bulg. We treat bulg only tentatively.

We postulate that song differences between *C. brevipennis* s. l. and the *cerdaniensis* song group are such important and effective reproductive barriers that we can focus separately on the details of differentiation inside the *cerdaniensis* group (Chapter 2 = Hertach *et al.*, 2015) and now inside the supposed *C. brevipennis* group. All of the single-copy well developed, nuclear markers available for cicadas (Owen *et al.*, 2015) are more appropriate for older divergences than are examined here. We therefore make use of other relevant nuclear-encoded data such as acoustics and morphology to evaluate the mitochondrial gene phylogeny and interpret the species histories. We study *C. brevipennis* s. l. from central Spain in the west to central Germany in the north, to the Black Sea in the east and to southern Italy in the south. We later combine the *C. brevipennis* and *cerdaniensis* group results and discuss the evolutionary histories based on a biogeographical scenario. We also discuss habitat preferences and potential threats to these taxa.

MATERIALS AND METHODS

Fieldwork

Fieldwork for acoustic, molecular and morphological data collection was conducted in many European countries with a focus on Italy, France, Switzerland and Slovenia (**Appendixes S4.1 and S4.6**). Cicadas were localised with directional microphones. Several authors used ultrasonic detectors (e.g. Pettersson D 200) to enhance their ability to detect high frequency domains (10-20 kHz) over larger distances. Calling songs were recorded with different professional portable recorders (e.g. Marantz PMD 660) under natural conditions and over a wide temperature range (17-33 °C). We measured the temperature of the surface, where the singing individual was presumed to sit, with a TFA ScanTemp 410 infrared thermometer (Chapter 2 = Hertach *et al.*, 2015) because duration of song elements can be strongly affected by temperature (Fonseca & Revez, 2002).

Voucher specimens were collected with a net. Cicadas are not protected by law in the investigated countries (with the exception of Spain); in biological reserves we obtained specific permits to collect specimens. Threatened taxa were collected conservatively, in some cases over several years (Torreilles, Monti della Daunia), so as not to endanger the survival of local populations. One leg was removed from selected fresh specimens and preserved in ethanol for later molecular analysis. All specimens were pinned and dried.

Song analyses

Songs were analysed using Raven Pro 1.4 (Cornell Lab of Ornithology). *Cicadetta brevipennis* s. l. calling songs consist of the repetition of a binary long-short-echeme pattern (e.g. Gogala & Trilar, 2004; **Fig 4.1**). This principle song element we call phrase 1 or main phrase (PH_1). Puissant (2001) described and illustrated a special courtship song with a fast progression of very short and longer echemes (under the name of *C. montana*). We call this part phrase 2 or ‘courtship element’ (PH_2). We use abbreviations for standard song variables (E = echeme, D = duration, F = carrier frequency and P = average power) which can be combined (e.g. ED = echeme duration).

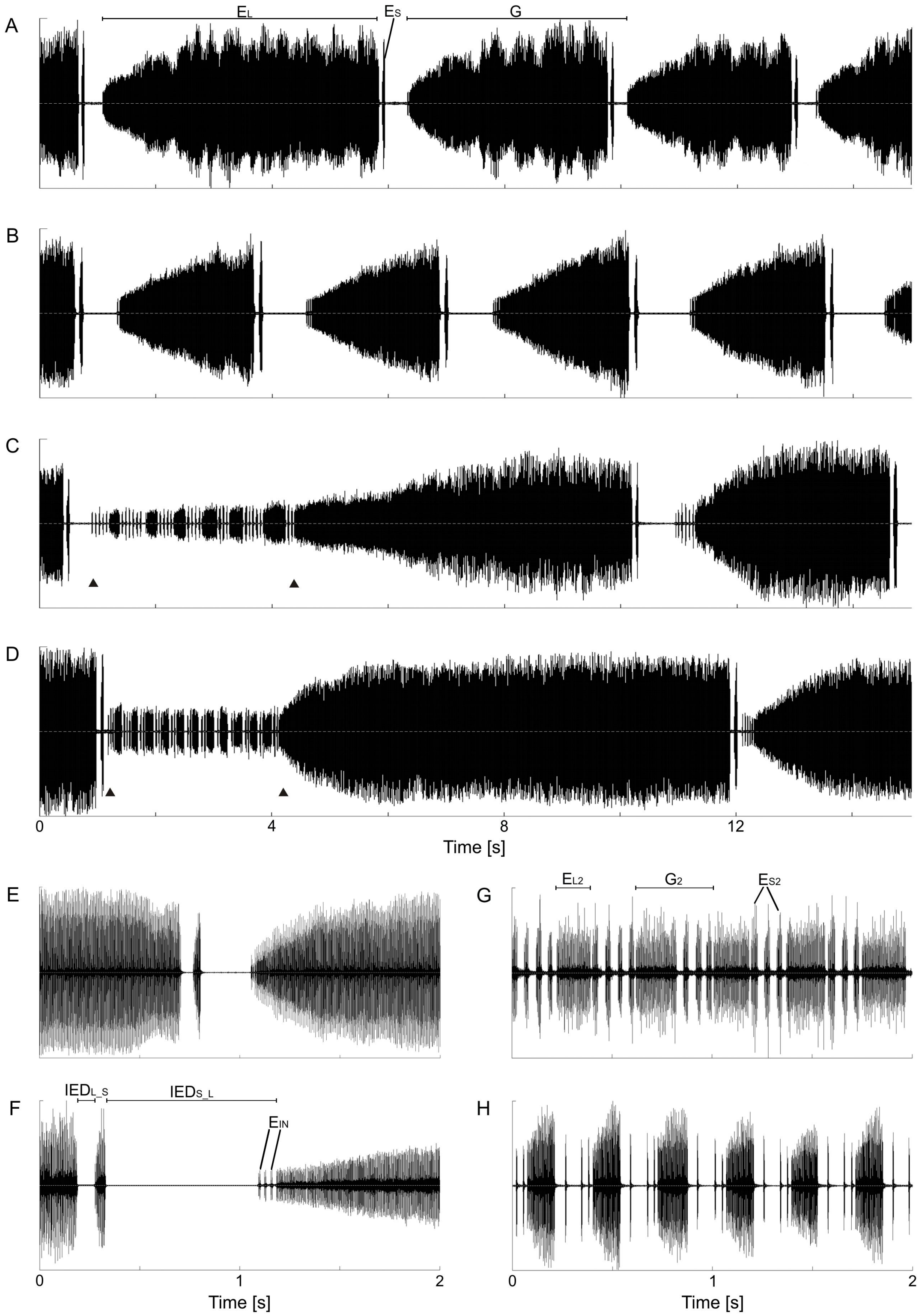

Fig 4.1. Oscillograms (time versus amplitude) of typical calling songs of operational taxonomic units (petr, brev, hipp and lito) with terms of variables. (A)/(E) petr (Ticino, Switzerland/Kyffhäuser, Germany), (B)/(F) brev (central Slovenia), (C)/(G) hipp (Monti della Daunia, Italy), (D) lito (Torreilles, France). (H) *Tettigettula pygmea* (Olivier, 1790) song pattern for comparison to (G). (A-D) 15 s sections, (E-H) 2 s sections. PH_1 = phase 1 (A-F), PH_2 = phrase 2 (C-D, start and end marked with triangles, and G); E_L and E_S = echeme types ‘long’ and ‘short’ of PH_1; IED_{L_S} and IED_{S_L} = inter-echeme durations between echeme types E_L and E_S respectively E_S and E_L; E_{IN} = introductory short echemes in front of the E_L (PH_1); E_{L2} and E_{S2} = echeme types ‘long’ and ‘short’ of PH_2; G and G_2 = echeme/inter-echeme groups.

Acoustic recordings were assorted according to: 1) metapopulation membership, 2) quality of the recording, 3) the number of repeated elements (sample size) and 4) perch temperature. These categories were considered hierarchically for the choice of the next recording to be studied. Thus, we could avoid errors introduced by interesting traits in the song structure being included disproportionately by the analyser. Investigations were conducted on three sets of analyses: 1) The frequency of occurrence of the phrase 2 (163 males), 2) qualitative and quantitative aspects within phrase 1 (55 males) and 3) qualitative and quantitative aspects within the phrase 2 (31 males; all listed in **Appendix S4.1**). For the first analysis, we counted the percentage of long-short echeme groups (PH_1) followed by the phrase 2 (PH_2). The minimal sample size was 10 long-short-echeme groups per individual. For the second analysis, we measured the time and carrier frequency domains of 20 long-short-echeme groups (G). The long echemes (E_L) are characterised by a continuous increase of the power level. We investigated these progressions of power for the same 20 long echemes by splitting the echeme in predefined time steps (0-0.05 s, 0.05-0.15 s, 0.15-0.30 s, 0.30-0.60 s, 0.60-1.50 s, 1.50 s to end) and measuring the average power level for each segment (**Fig 4.2**; Raven function: 'Average Power' in decibel, dB). The 0-0.05 s segment was defined as the baseline and the absolute dB values were set to the relative value 0 (or 0%). The absolute values of the 1.50 s to end segment were used as the maxima and correspondingly set to 1 (or 100%). We were then able to calculate relative power differences for each segment in between with respect to the baseline and maximum values. We obtained comparable results in the programme Audacity calculating the 'Root Mean Square' (RMS). We also investigated the number and duration of syllables (= complete cycles of timbal movements) forming the short echemes (see Chapter 2 = Hertach *et al.*, 2015). For the third analysis, the structure of the phrase 2 (PH_2), we counted the number of short echemes (E_{S2}) in between two neighbouring long echemes (E_{L2}; **Fig 4.1G**), measured some duration characters and compared the power levels of E_{L2} echemes with the following E_L (1.50 s to end segment). The sample size was constant with six echeme/inter-echeme groups (G_2; the forth, third and second last of the phrase) taken from two different PH_2 per individual.

In a very few cases, we randomly concatenated song recordings from different males to create composite individuals in order to reach the minimal sample size of song elements. This is especially true for the Torreilles population in southern France. This practice may equalise extreme song patterns of single specimens towards the taxon-typical medium values but did not influence the final conclusions.

Statistical tests on song variables were conducted in R (R Development Core Team, 2008) between individuals of the two widespread operational taxonomic units (OTUs); i.e. brev and petr. Variables were tested for temperature dependencies between these two OTUs with analysis of covariance (ANCOVA; see Chapter 2 = Hertach *et al.*, 2015). ANCOVAs yielded ambiguous results for the temperature dependencies (several variables close to the significance level 0.05). However, they demonstrated that interactions between the covariate 'temperature' and the categorical factor 'taxon' are not present and rates of change (= slopes) could consequently be regarded as similar between the two OTUs. Therefore, we built up a general lineal model (GLM) to eliminate the supposed but uncertain temperature dependencies for each variable in the form: variable = a + b * temperature + c * OTU where the two OTUs were set to 0 and 1 (R commands: lm(variable~temperature+OTU) for normally distributed variables and glm(…) for Poisson-distributed variables). Values of the partial residuals in the variance of the opposite OTU were scored as failures. Measurements of the remaining OTUs (i.e. hipp and lito) were afterwards assigned for each variable and individual to these variances and evaluated as fitting perfectly to the first or the second OTU or being located in the overlap. A principal component analysis (PCA) was conducted to visualise the power of a combination of a few song characters for separating taxa. We used a correlation matrix implemented in the function prcomp(…) with the option 'scale = TRUE'. Graphs were generated with the package ggplot2 (Wickham, 2009) and illustrations of oscillograms produced with Seewave (Sueur *et al.*, 2008).

The song recordings are preserved in the Slovenian Wildlife Sound Archive of the Slovenian Museum of Natural History Ljubljana. Song examples are presented at the web pages *Songs of European singing cicadas* (http://www.cicadasong.eu). Raw data of acoustic and other analyses were deposited at Figshare: doi: 10.6084/m9.figshare.3168250.

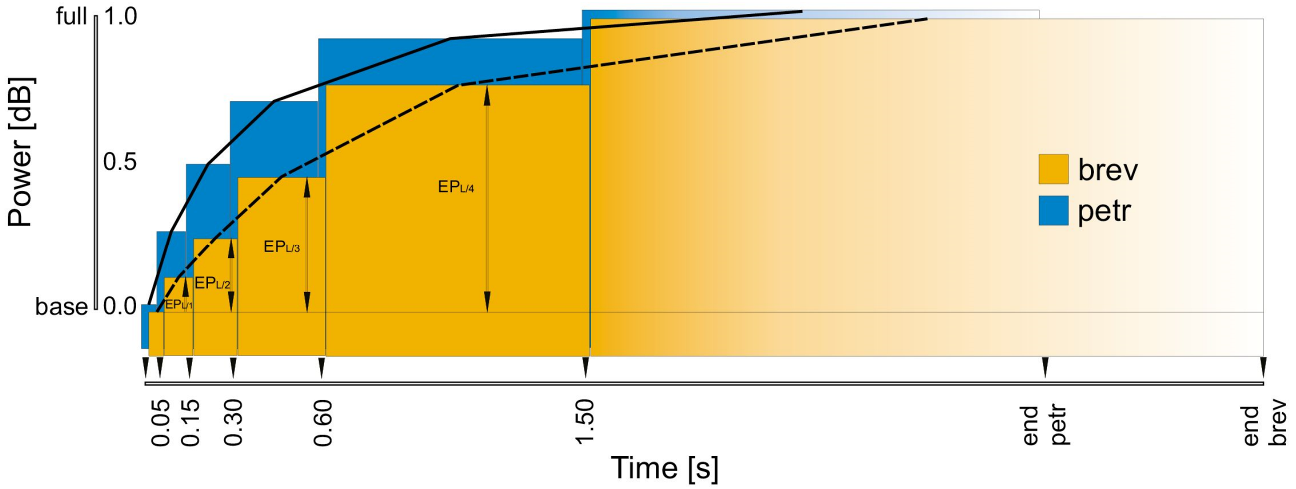

Fig 4.2. Schematic illustration of power levels (P) of the swelling long echemes (E_L). Predefined time sections for average power measurement [base ($EP_{L/0}$) ≔ 0-0.05 s, $EP_{L/1}$ ≔ 0.05-0.15 s, $EP_{L/2}$ ≔ 0.15-0.30 s, $EP_{L/3}$ ≔ 0.30-0.60 s, $EP_{L/4}$ ≔ 0.60-1.50 s, full power ($EP_{L/5}$) ≔ 1.50 s to end]. Values set to 0 for the baseline and 1 for the final time section. Example showing medium values of operational taxonomic units (OTU) brev and petr (n_{ind} = 35).

Molecular phylogenetic analyses

For reconstructing phylogenies, a molecular dataset was assembled of the mitochondrial gene segments from the *COI* and *COII* genes. The dataset improves upon previous work (Chapter 2 = Hertach *et al.*, 2015; Chapter 3 = Wade *et al.*, 2015) by increasing the number of specimens and sampling new geographic regions. The eight newly sequenced specimens of *C. brevipennis* s. l. originate from eastern and northern parts of the distribution and include a type specimen collected in 1916 (*C. montana* f. *petryi* Schumacher, 1924; Natural History Museum of Berlin). The total number of specimens of *C. brevipennis* s. l. is now 33. *Cicadetta montana* s. str. was chosen as an appropriate out-group in all analyses (Chapter 3 = Wade *et al.*, 2015). For each cicada, genomic DNA was extracted from one leg using a Qiagen DNeasy Blood & Tissue Kit, following the manufacturers' instructions, except that DNA digestion was conducted over 24 hours or more. Polymerase chain reaction (PCR) amplification was performed with a PCR Beads kit (GE Healthcare) and the following primers: C1-J-2195 and TL2-N-3014 (*COI*) with an annealing temperature of 56 °C and TL2-J-3034 and TK-N-3786 with an annealing temperature of 53 or 50 °C (*COII*; Simon *et al.*, 1994). PCR products were sequenced using the forward and reverse primers by the Sanger DNA sequencing service of Microsynth AG, Balgach, Switzerland. The complementary sequences were assembled and edited with CodonCode Aligner 4. Sequences were aligned with MUSCLE (Edgar, 2004) in Geneious Pro 6.1.8 (http://www.geneious.com) with default settings. We constructed two alignments, one including all *C. brevipennis* s. l. sequences and one including additionally the closely related *cerdaniensis* song group sequences (67 samples including the out-group). We adopted this strategy to demonstrate relationships within the *C. brevipennis* s. l. and relationships on a broader taxonomic scale. For each alignment codon positions for protein coding genes were determined using TranslatorX (Abascal *et al.*, 2010). Sequences are deposited in GenBank: *COI*: KT901699 – KT901780 and KU679422 – KU679433, *COII*: KT901473 – KT901554 and KU679434 – KU679445.

As in Chapters 2 and 3 (= Hertach *et al.*, 2015; Wade *et al.*, 2015) *COI* and *COII* gene trees were concatenated and PartitionFinder 1.0.1 (Lanfear *et al.*, 2012) was used to determine the best fitting model under the Bayesian information criterion. The programme suggested a total of three partitions (both genes shared the same model for each codon position in each alignment) which were used for Bayesian inference and maximum likelihood (ML) analysis. Bayesian inference was carried out with MrBayes 3.2.1 (Ronquist *et al.*, 2012) employing parallel runs of four simultaneous Markov chains for 10 million generations, sampling every 1,000 generations. Model parameters were independently optimised for each partition. The first million generations were discarded as burn-in, based on stationarity of the log-likelihood tree scores, and whether the effective sample size of all parameters were > 200, evaluated using Tracer 1.5 (Rambaut & Drummond, 2007). We conducted ML analysis with non-parametric bootstrapping in RAxML 8.2

(Stamatakis, 2014) under the same partitioning schemes applied in Bayesian inference. We ran 1,000 bootstrap replicates in ML analysis. Geneious Pro 6.1.8 was used to calculate genetic distances between samples.

Morphological analyses

Song-identified males or females from single-species local populations were included in the morphological analyses, in total 132 dry prepared specimens of *C. brevipennis* s. l. were studied. The terminology of Moulds (2005) is used for the descriptions. Some principal distances (body length, body width, fore wing length, fore wing width) were directly measured with vernier callipers or photographed with a Leica DFC425 camera on a Leica M205 C stereomicroscope (fore wing) or a Keyence VHX 2000 digital photo-microscope (body). These measurements are part of an ongoing comprehensive morphometric study on a larger number of *Cicadetta montana* s. l. taxa. In our current work, they are used to document variability among specimens for the taxonomic descriptions. ImageJ 1.47 (Schneider *et al.*, 2012) provides a simple application to measure distances from the photographs.

Distribution patterns

Maps based on specimen localities were generated with ArcGIS (map source: http://www.worldclim.org and http://www.diva-gis.org). The final distribution map integrates previously published records from the literature and unpublished observations from the databases of the first four authors (**Appendix S4.6**). We define metapopulations as a set of conspecific local populations that presumably interact via individuals moving among populations (Hanski & Gilpin 1991).

Nomenclatural acts

We infer taxonomic conclusions under De Queiroz' (2007) 'Unified Species Concept', which led us to combine data sets from different sources in a practical way. The taxa have been checked for concordance with the numerous old names existing and intermediately regarded as synonyms of *Cicadetta montana* s. l. based on original descriptions, type localities and, whenever available, type specimens. We also critically examined the name *Cicadetta brevipennis* Fieber, 1876 with the aid of published and unpublished original works preserved in the National Museum of Natural History Paris (MNHN; Soulier-Perkins, pers. comm.) and putative type specimens according to the International Code of Zoological Nomenclature (ICZN, 1999).

RESULTS

Data from 78 *Cicadetta brevipennis* s. l. populations from Italy, France, Switzerland, Slovenia, Germany, Austria, Bulgaria, Romania, Croatia, Serbia and Spain were analysed. The operational taxonomic units (OTUs) brev, hipp and lito are subunits of the former '*brevipennis*-B'; petr is identical with '*brevipennis*-A'; bulg is completely new and the data set is not informative enough for conclusions. The five OTUs are colour-coded in the illustrations: brev = orange, hipp = red, lito = pink, petr = blue and bulg = green.

Molecular phylogenetic analyses

The *Cicadetta brevipennis* song group forms three well supported main clades in the Bayesian concatenated *COI* and *COII* phylogeny (posterior probabilities 0.94-1; **Fig 4.3A-B**): petr, bulg and the remaining three OTUs. In maximum likelihood mitochondrial phylogeny petr and bulg have strong support, while the third branch is weaker (bootstrap values 0.76-1; **Fig 4.3A-B and Appendix S4.2**). This clade is again clustered in three subclades containing lito, hipp and brev/hipp (posterior probabilities 0.81-0.99, bootstrap values 0.81-0.89). It appears evident that the hipp unit was partly introgressed by brev since we found both distinct haplotypes in one single population (Monti della Daunia 'MD'). The supposedly original hipp mtDNA is restricted to this location. The lito haplotypes are endemic to coastal southern France (Torreilles 'TO').

Table 4.1. Uncorrected average pairwise distances of mtDNA within the *Cicadetta brevipennis* song group (in %). *COI* above diagonal, *COII* below, within taxa distance in the grey diagonal (*COI*/*COII*). OTU hipp has two clearly different haplotypes.

	brev	hipp 1	hipp 2	hipp (tot)	lito	petr	bulg
brev	0.2/0.1	1.3	0.4	0.8	0.7	2.6	2.6
hipp 1	0.6	0.7/0.3	1.5		0.7	3.0	2.8
hipp 2	0.1	0.7	0.5/0.0		0.8	2.7	3.0
hipp (tot)	0.3			1.1/0.4	0.7	2.9	2.9
lito	0.2	0.8	0.2	0.5	0.0/0.0	2.1	2.4
petr	1.6	1.9	1.6	1.7	1.5	0.2/0.0	2.4
bulg	2.8	3.2	3.0	3.0	3.1	3.0	0.0/0.0

Average uncorrected pairwise genetic distances of the three main clades in the *brevipennis* song group are 2.1 to 3.0% (in *COI*) and 1.5 to 3.2% (in *COII*) (**Table 4.1**). From previous studies we know that genetic distances among any song delimited taxa in the *C. montana* complex are at most 5.1% for the two mitochondrial genes concatenated (Chapter 3 = Wade *et al.*, 2015)

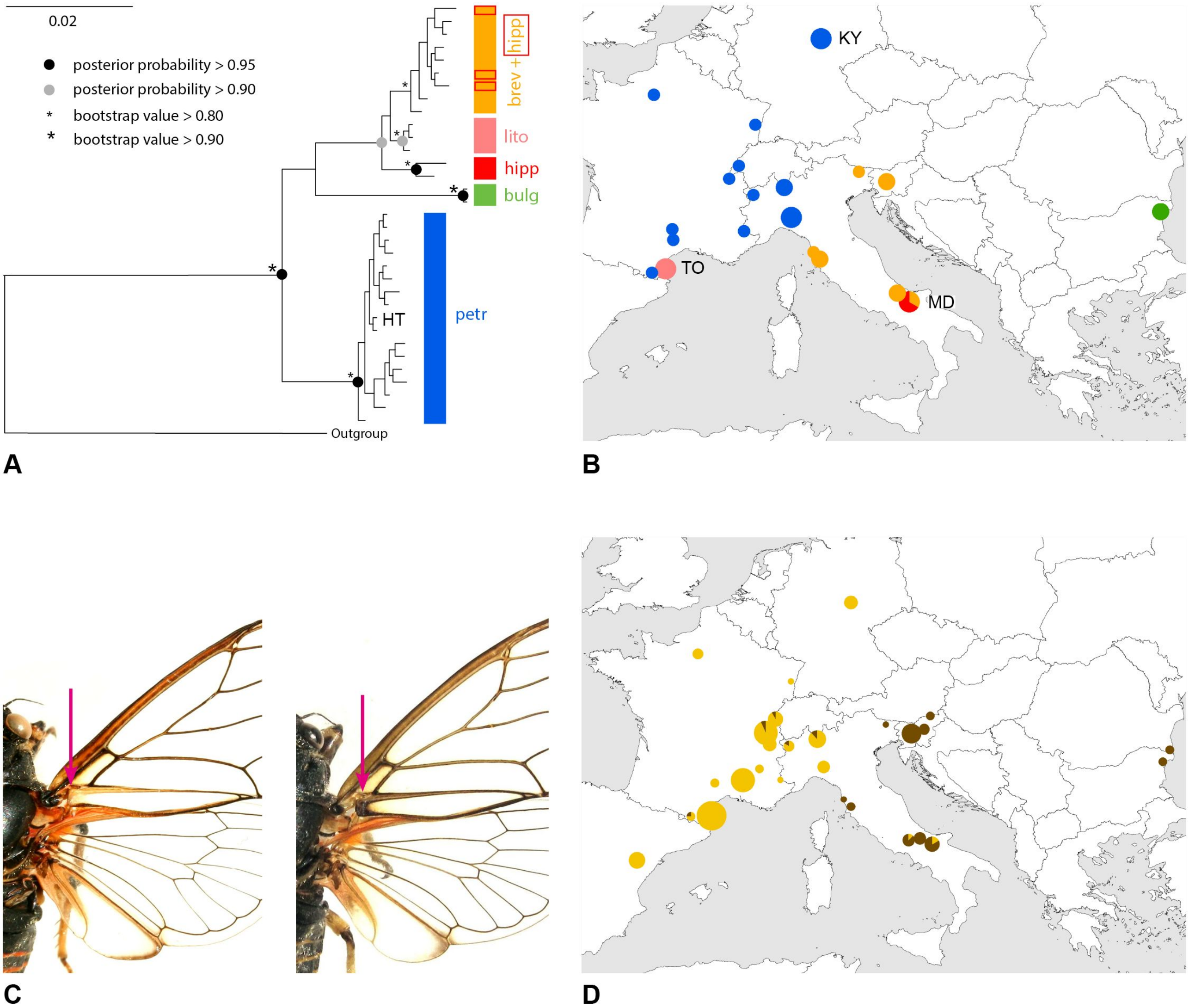

Fig 4.3. Molecular phylogenetic relationships and colouration trait within the *brevipennis* song group. (A) Bayesian mtDNA phylogeny with posterior probabilities (filled circles) and ML bootstrap values (star icons) from RAxML analysis, (B) geographic distribution of colour-coded clades in the phylogenetic tree, (C) dark or yellowish colour of basal junction of anal veins in the fore wing, (D) geographic distribution of this colouration trait. Notes: Supposedly introgressed hipp specimens marked with red square. HT = holotype. Data is pooled for several closely neighbouring local populations in the maps. Size of the circles relative to the number of investigated specimens (n_{max} = 3 resp. 23). Important populations: KY = Kyffhäuser, MD = Monti della Daunia, TO = Torreilles.

Morphology

The majority of species within the *C. montana* species complex exhibit few interspecific morphological traits but remarkably high intraspecific variability. Lineages in the *brevipennis* group can be distinguished from each other with high probability by the predominantly yellowish versus dark basal junction of the anal veins in the fore wing; i.e. for petr (94%, n_{ind} = 72, males and females), lito (100%, n_{ind} = 23), brev (0%, n_{ind} = 19), hipp (11%, n_{ind} = 14) and bulg (0%, n_{ind} = 4; **Fig 4.3C-D**). Only ten of the 132 specimens examined morphologically were ambiguous: one was predominantly dark instead of yellowish; another yellowish instead of dark; and eight specimens were ambivalent dark and yellowish (counted half for each character state).

In hipp, the slightly elevated value of light junctions results from a distinct dimorphism: Some specimens in the south-easternmost Italian Monti Della Daunia (‘MD’) population are generally paler. The two morphs in hipp are not correlated with the two haplotypes mentioned above. The specimens we designate as lito are generally short winged and with light wing venations and are distinguishable from many specimens within the *C. montana* species complex and in particular within the *brevipennis* group.

Song patterns

All songs of the *Cicadetta brevipennis* group are composed of a repeated binary structure. A long echeme (E_L) of approximately 3.5 s duration (but with extremes from 0.7 to 60 s) precedes a short pause (IED_{L_S}) and a short echeme (E_S) both of them lasting no longer than 0.1 s (**Fig 4.1**). In rare cases and more often in the courtship behaviour the E_S can be missing. Duration and carrier frequency measurements and counts are summarised in **Table 4.2**.

Song patterns show at least one obvious qualitative difference among taxa. In brev and petr populations, phrase 2 (PH_2) is used for courtship as originally defined above. It is sung when a female is in the vicinity of the male. In lito and hipp populations, PH_2 is emitted in a clearly elevated frequency of occurrence (**Figs 4.1 and 4.4**). We observed the highest rates in Torreilles (‘TO’, lito), where 27% of the long-short echemes (n = 117) were followed by PH_2, and at Monti Della Daunia (‘MD’, hipp) with a 23% rate (n = 746). In these populations we hypothesise that PH_2 forms part of the calling song to attract females. In addition to the frequency of occurrence, five other facts support this hypothesis: 1) Naturally and artificially completely isolated males sang PH_2. 2) PH_2 was emitted in the first calls in the morning when mating is unusual.
3) Males singing PH_2 often flew to new perches while males that are engaged with females are generally

Table 4.2. Measurements of acoustic variables in the *Cicadetta brevipennis* song group for the entire temperature range. Mean values between individuals ± SD. Interesting values bold and italic. Notes: n_{ind} = number of individuals. Putative hybrids excluded. D = duration, F = frequency, P = power. E_{IN} rate is the portion of long echemes fitting with the following minimal criterion: at least two introductory chirps in front of the long echeme followed by minimal pauses of 0.015 s. For other song variables see **Figs 4.1 and 4.2**.

Phrase 1 (PH$_1$)		**petr (n_{ind} = 19)**	**brev (n_{ind} = 16)**	**hipp (n_{ind} = 12)**	**lito (n_{ind} = 4)**
Perch temperature [°C]		24.7 ± 3.1	26.2 ± 3.0	23.6 ± 2.6	24.0 ± 0.0
Durations [s]	ED$_L$	3.058 ± 1.121	3.798 ± 1.200	2.936 ± 0.839	***8.890 ± 5.985***
	ED$_S$	***0.043 ± 0.006***	0.056 ± 0.008	0.055 ± 0.007	0.056 ± 0.011
	IED$_{L\_S}$	0.064 ± 0.012	0.073 ± 0.012	0.071 ± 0.009	0.080 ± 0.007
	IED$_{S\_L}$	***0.488 ± 0.198***	1.100 ± 0.328	0.956 ± 0.308	***0.311 ± 0.076***
Counts	E$_{IN}$ number	***1.2 ± 0.7***	6.7 ± 5.0	7.4 ± 4.0	3.7 ± 0.5
	E$_{IN}$ rate	***10%***	76%	87%	88%
	Syllables/E$_S$	6.1 ± 0.8	7.2 ± 1.1	7.6 ± 0.9	6.9 ± 0.4
E$_L$ standardised power levels	EP$_{L/1}$	***0.25 ± 0.04***	0.12 ± 0.04	0.14 ± 0.07	0.15 ± 0.04
	EP$_{L/2}$	***0.48 ± 0.07***	0.25 ± 0.07	0.29 ± 0.11	0.33 ± 0.09
	EP$_{L/3}$	***0.70 ± 0.07***	0.46 ± 0.08	0.52 ± 0.12	0.55 ± 0.12
	EP$_{L/4}$	***0.91 ± 0.04***	0.77 ± 0.05	0.83 ± 0.09	0.78 ± 0.13
Frequency [kHz]	EF$_L$ (centre)	14.3 ± 1.0	14.6 ± 0.8	14.4 ± 0.8	14.7 ± 0.2
	EF$_L$ (1. Quartile)	13.4 ± 0.9	13.6 ± 0.7	13.4 ± 0.8	13.9 ± 0.2
	EF$_L$ (3. Quartile)	15.5 ± 1.1	15.8 ± 0.9	15.2 ± 0.9	15.6 ± 0.1
	EF$_S$	14.2 ± 1.0	14.5 ± 0.9	14.3 ± 0.8	14.7 ± 0.3

Phrase 2 (PH$_2$)		**(n_{ind} = 4)**	**(n_{ind} = 4)**	**(n_{ind} = 17)**	**(n_{ind} = 6)**
Durations [s]	ED$_{2L}$	0.220 ± 0.083	0.198 ± 0.071	0.205 ± 0.055	0.199 ± 0.052
	GD$_2$	0.502 ± 0.066	0.446 ± 0.119	0.502 ± 0.107	0.433 ± 0.126
Counts	E$_{2S}$	4.5 ± 0.6	4.5 ± 1.7	4.2 ± 0.9	***3.3 ± 1.1***
Power [dB]	EP$_{L/5}$ - EP$_{2L}$	13.6 ± 1.6	16.4 ± 3.3	***10.6 ± 1.3***	14.4 ± 2.6

known to be more stationary. 4) We demonstrate that in hipp the PH$_2$ is relatively louder in comparison with PH$_1$ than in all other taxa (n_{ind} = 31), which is a good indication of longer distance communication (Wilcoxon rank sum test: W = 12, $p < 0.001$, **Table 4.2 and Appendix S4.4**). 5) These differences hold up over time in the few cases where repeated measurements were made. Nevertheless, it was not possible in every case to infer whether PH$_2$ was part of the courtship behaviour or of the calling song since not many recorded individuals were seen. This fact makes conclusions more difficult, especially in a contact zone between typical brev and typical hipp where the values are intermediate on the population level (**Fig 4.4B**, enlarged part).

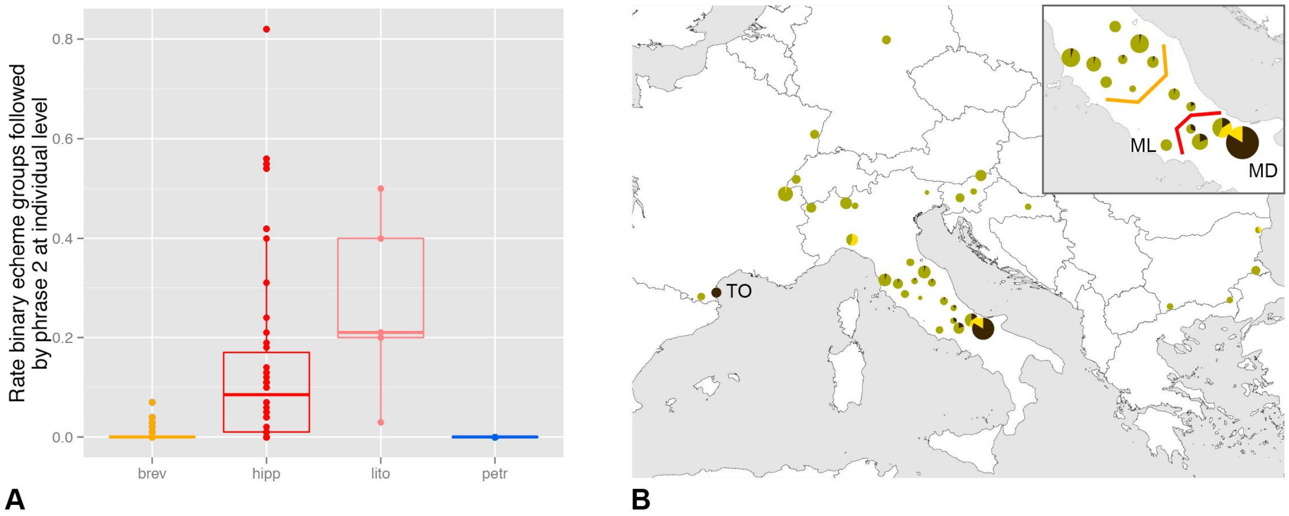

Fig 4.4. Phrase 2 (PH_2) occurrences. Acoustic trait that separates hipp and lito from other OTUs. (A) PH_2 used as calling song rather than courtship at the individual level and (B) as diagnostic trait on population level (dark brown = PH_2 calling song, yellow = PH_2 courtship song, olive = PH_2 not emitted; values relative to the PH_2 rate of the 'MD' population). Orange and red lines mark suggested limits of OTUs brev and hipp, respectively, in central Italy (enlarged part). Notes: Data is pooled for several closely neighbouring local populations. Size of the circles relative to the number of investigated individuals (n_{max} = 27). Populations mentioned in the text: MD = Monti della Daunia, ML = Monti Lepini, TO = Torreilles.

With in-depth observations and analyses, we became aware of additional song characters in the first and main phrases (PH_1) that distinguish between the widespread OTUs brev and petr (**Fig 4.5; Table 4.2**). All these characters have been tested for temperature dependency and significance first with ANCOVA and then with GLM (**Table 4.3**). 1) The progression of the power levels in the E_L is faster in petr populations than is typical for brev, resulting in a more parabolic than linear envelope of the amplitude (**Figs 4.2 and 4.5A-C**). $EP_{L/1}$ and $EP_{L/2}$ are the most distinct. 2) IED_{S_L} (inter-echeme durations between the end of the E_S and the next E_L) are shorter in petr than in brev. The minimum IED_{S_L} value per individual perfectly distinguishes brev from petr in our dataset (**Figs 4.1E-F and 4.5D-F**). 3) OTU brev populations often start the E_L echemes with a series of stammering short chirps (E_{IN}) while these chirps are much rarer in petr (**Figs 4.1E-F and 4.5G-I**). In petr the chirps are somehow an artefact caused by starting problems of the song apparatus (e.g. low temperatures) while in brev they are likely to have become part of the specific-mate recognition systems. Almost as distinct as the number of chirps is the percentage of starts with at least two introductory echemes followed by pauses of at least 0.015 s (= minimum criterion, E_{IN} rate). 4) Sums of short pause and short echeme ($IED_{L\_S} + ED_S$) are significantly longer in brev than in petr. However, the overlap is higher than in the first three characters. 5) The speed of the timbal movements measured by the use of the syllable duration of E_S is significantly faster in petr than in brev. Summarizing, brev is quantitatively always more sluggish than petr.

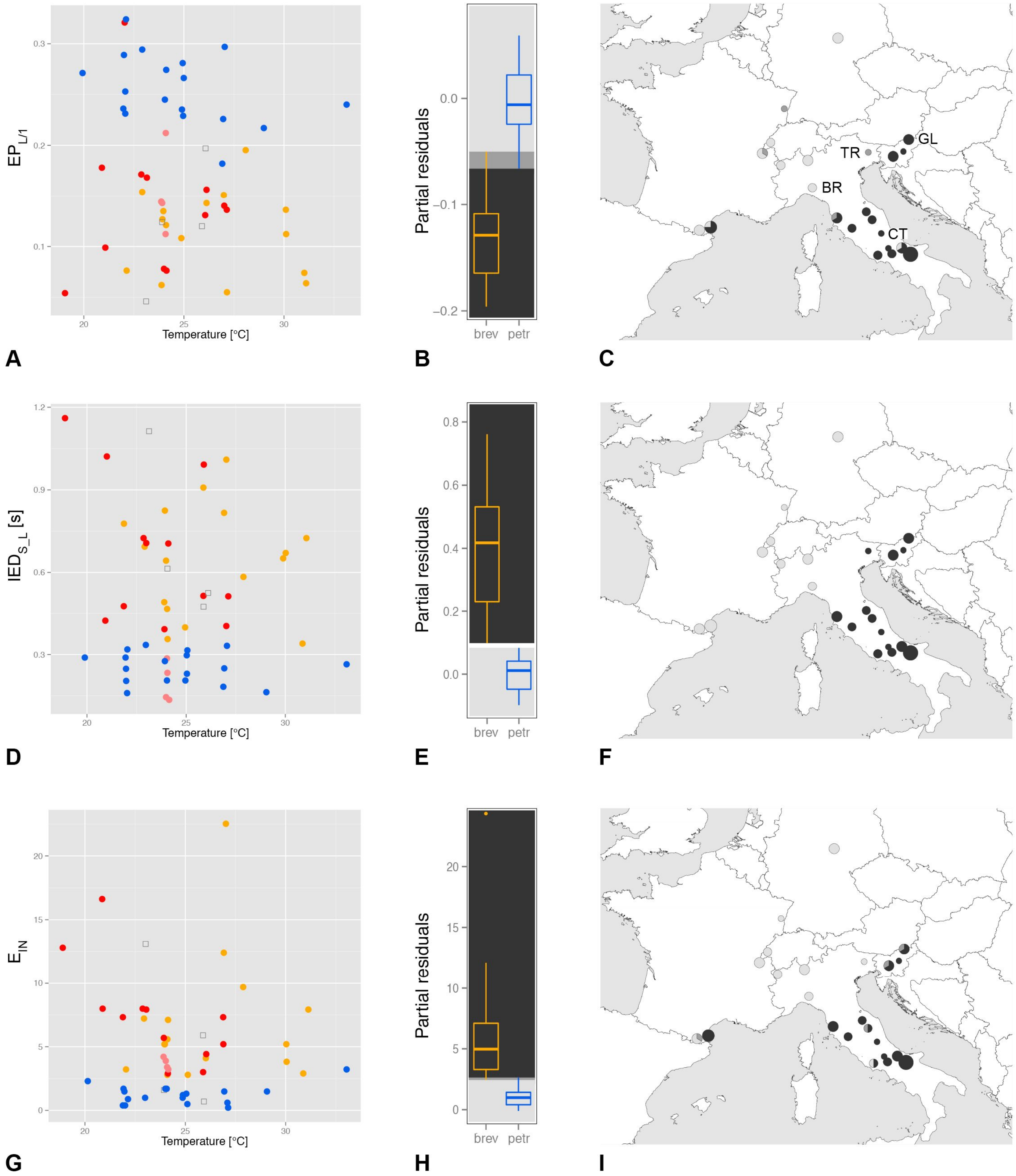

Fig 4.5. Three acoustic traits to distinguish petr from brev. (A-C) standardised power level $EP_{L/1}$, (D-F) minimal duration of the IED_{S_L}, (G-I) number of introductory chirps (E_{IN}). (A)(D)(G) dependencies on the perch temperature for single individuals (blue = petr, orange = brev, red = hipp, pink = lito, hollow squares = potential hybrids; temperature jitter = 0.15), (B)(E)(H) boxplots after controlling for the temperature with general linear models for brev and petr, (C)(F)(I) spatial patterns of temperature controlled values (light grey = typically petr, black = typically brev, dark grey = within the overlap). Notes: Data is pooled for several closely neighbouring local populations. Size of the circles relative to the number of investigated individuals (n_{max} = 6). Populations mentioned in the text: BR = Brallo di Pregola, CT = Campotosto, GL = Glanz an der Weinstrasse and TR = Travesio.

Table 4.3. General linear models (GLM) testing for temperature dependencies and acoustic significances between petr and brev OTUs. Function: variable = a + b * temperature + c * OTU where the two OTUs were set to 0 and 1. Significance and coefficients for 34 individuals. For song variables see **Figs 4.1 and 4.2 and Table 4.2**.

Variable	Model	t/z_{temp}	p_{temp}	t/z_{OTUs}	p_{OTUs}	a	b	c
E_{IN} number	glm	1.22	0.2230	6.88	0.0000	-0.662	0.036	1.622
E_{IN} rate	glm	-0.33	0.7450	2.54	0.0110	-1.490	-0.030	2.011
$EP_{L/1}$	lm	-1.54	0.1350	-10.28	0.0000	0.335	-0.003	-0.134
$EP_{L/2}$	lm	-2.12	0.0418	-9.85	0.0000	0.679	-0.008	-0.224
IED_{S_L} (minimum)	lm	-0.23	0.8230	7.75	0.0000	0.299	-0.002	0.396
$IED_{L\_S} + ED_S$	lm	-2.87	0.0072	5.23	0.0000	0.162	-0.002	0.025
Syllable duration	lm	-4.21	0.0002	3.58	0.0011	0.012	-2.1×10^{-4}	1.1×10^{-3}

Individuals belonging to OTUs hipp and lito have first phrases 1 (PH_1) that are similar to brev; however, a few of the acoustic characters are ambivalent or even closer to petr than brev. Such an exception is the short IED_{S_L} of lito (**Fig 4.5D-F**). With respect to the syllable duration, hipp is significantly faster than brev [GLM: t_{temp} (1, 25) = -4.3, $p_{temp} < 0.001$, t_{OTUs} (1, 25) = 2.9, $p_{OTUs} = 0.007$], but cannot be distinguished from petr [GLM: t_{temp} (1, 27) = -3.9, $p_{temp} < 0.001$, t_{OTUs} (1, 27) = 0.4, $p_{OTUs} = 0.660$]. The within-lito variation is relatively high between the four song samples. Therefore, in the future, some values should be consolidated with more recordings taken at different ambient temperatures. We regard four individuals from zones of potential contacts between OTUs as 'hybrids' [populations Travesio 'TR', Monti Lentini 'ML' (2 individuals), Campotosto 'CT'].

Differences are generally small and it is not surprising that a few individuals fall into the wrong OTU when single characters are examined. However, a combination of the three characters from **Fig 4.5** clearly separates petr from the remaining OTUs, especially brev, in a principal component analysis (PCA, **Fig 4.6**). A few new recordings from remote Serbian (brev) and Spanish populations (petr) were used to roughly test the acoustic traits.

Distribution and ecological traits

The geographical patterns of the different OTUs are unusual but spatially coherent with the exception of southern French lito near Perpignan (e.g. **Figs 4.3-4.5**). Endemic lito is within the brev-hipp clade in the molecular study but isolated over more than 600 km from its nearest relatives. The acoustic investigations support its closeness to brev and especially hipp. We only know two local lito populations and they live in

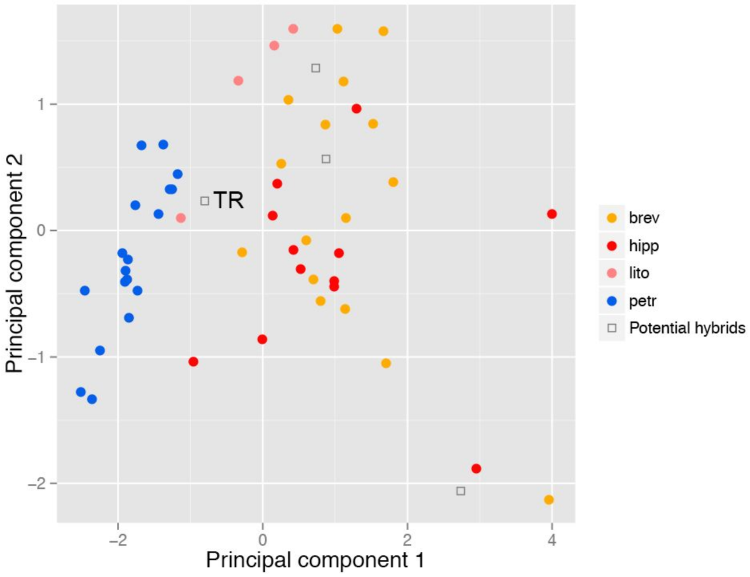

Fig 4.6. Principal component analyses (PCA) among specimens for the three song characters from Fig 4.5. Specimen mentioned in the text: TR = Travesio population.

habitats extraordinary for European cicadas in general and particularly for species of the *C. montana* complex: salt marshes at sea level composed of the Common Glasswort (*Salicornia europaea,* **Fig 4.7E**).

The OTU we designate as brev occurs in two metapopulations. The Apenninian metapopulation reaches from a northern imaginary line between Pisa (Tuscany) and Forlì (Emilia-Romagna) to a southern line between Terni (Umbria) and Ascoli Piceno (the Marches). Habitats are mainly oak forests in a hilly territory (**Fig 4.7C**). Population densities are fairly high for the woody landscapes west of Siena and the main ridge of the Apennine Mountains. The second metapopulation is in Slovenia and southern Austria and probably extends to north-eastern Italy and northern Serbia. We found it here usually on trees and shrubs not far from dry and extensive meadows or other open spaces. The two metapopulations are isolated (over 200 km apart) by the Padan Plain and the Adriatic Sea.

Operational taxonomic unit hipp haplotypes were only found in the Monti Della Daunia population, but we also include in this OTU all populations in the north-west direction at least until the National Parks of Abruzzo and Majella ending in a 120 km to 60 km range (**Fig 4.4B**, red line). These populations form a metapopulation that is characterised by mountainous habitats often composed as ecotones with extensive grazed pastures and a high number of bushes (**Fig 4.7D**). Operational taxonomic unit hipp inhabits a more mountainous ecological niche than brev (Wilcoxon rank sum test for altitude of all records: $W = 351.5$, $p < 0.001$; see also **Fig 4.7F**). A few populations between the Apennine brev and hipp metapopulations were not assigned to one of the two groups because they have intermediate song patterns. Some isolated

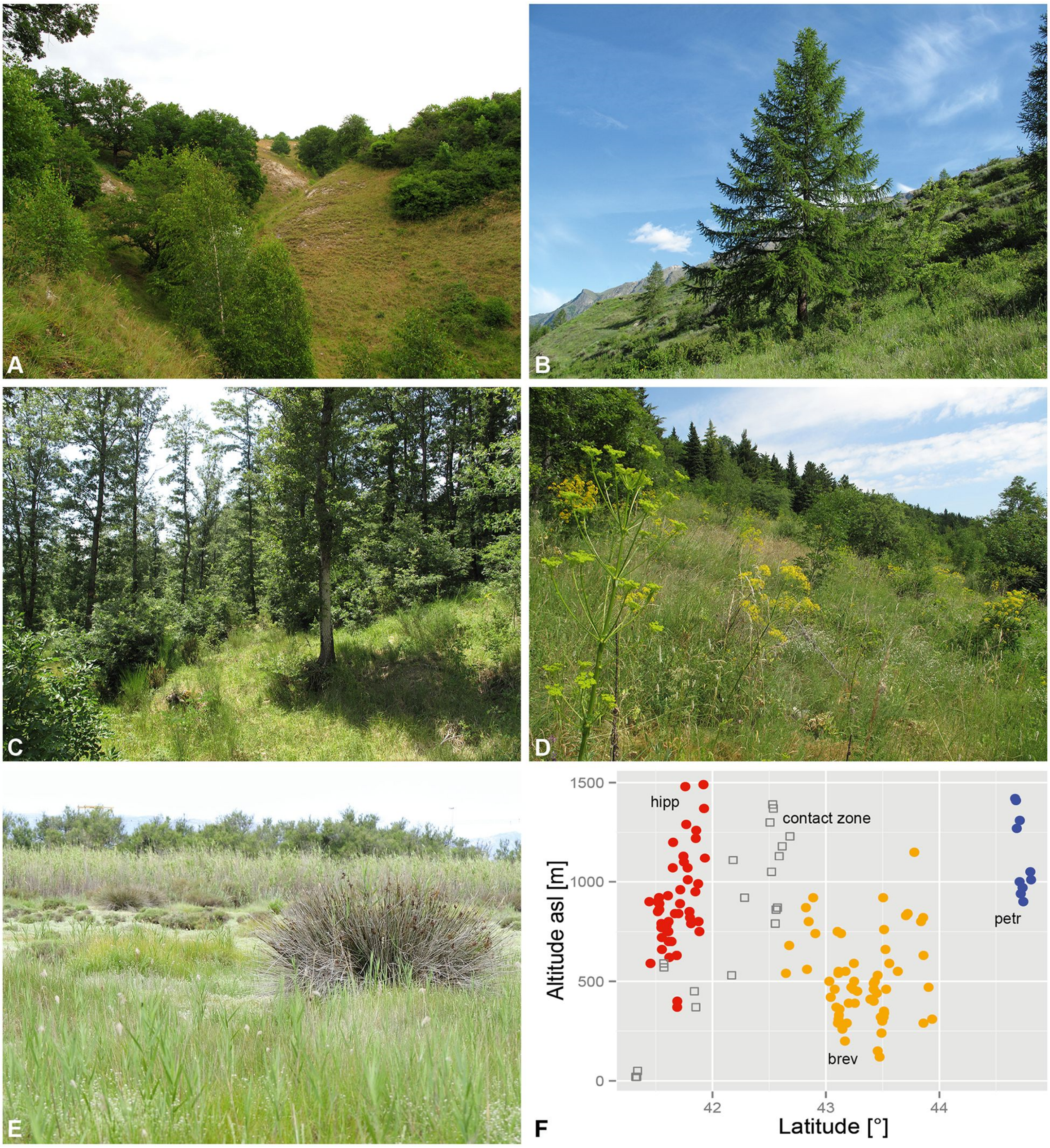

Fig 4.7. Contrasting habitats in the *Cicadetta brevipennis* group. (A) Dry pastures in the Kyffhäuser (Thuringia, Germany), habitat of petr (locus typicus of *C. montana* f. *petryi* Schumacher, 1924), (B) extensive pastures with the European Larch (*Larix decidua*) at 1500 m asl in a south alpine valley (Val de Cogne, Aosta, Italy), extraordinary habitat of petr, (C) oak forests of the foothills (Monte Amiata, Tuscany, Italy), typical habitat of brev in the Apennine Mountains, (D) extensive pastures at higher altitudes in the Monti Della Daunia massif (Apulia, Italy), typical habitat of hipp, (E) salt marsh at sea level at Torreilles (eastern Pyrenees, France), extraordinary but typical habitat of lito, (F) altitudinal niches of petr, brev and hipp in the Apennine Mountains visualised against the geographical latitude.

populations at lower altitudes (e.g. Monti Lepini ‘ML’; **Fig 4.4B**) are in the geographic vicinity of hipp but probably are ecologically and acoustically closer to brev or intermediates.

Operational taxonomic unit petr populations are distributed from central Germany (Kyffhäuser, **Fig 4.7A**) to the Pyrenees and further to western Spain and along the southern slope of the Alps at least to Lake Como, but also in isolated local populations around Brallo di Pregola (‘BR’; e.g. **Fig 4.5**) in the northern Apennine entering another mountain chain. In southern France, petr inhabits Mediterranean and sub-Mediterranean vegetation classes, often composed as ecotones with grassy areas and bushes. Some populations live in forests. North- and eastwards petr is restricted to dry meadows with bushes. Males regularly sing in the herb layer. Exceptional southern alpine habitats were found in the vegetation zone of the European Larch (*Larix decidua*) up to 1750 m asl (**Fig 4.7B**).

Populations of petr and brev are separated by no more than approximately 150 km throughout the Apennine Mountains, and likely the southern Alps. However, the single individual investigated acoustically from the south-eastern Italian Alps is intermediate in song patterns (Travesio ‘TR’; **Figs 4.5 and 4.6**). Operational taxonomic unit bulg was found close to the Black Sea in Bulgaria (**Fig 4.3B**).

Status of operational taxonomic units

Morphological traits (**Fig 4.3C-D**) and song differences (**Figs 4.1, 4.5 and 4.6**) are spatially congruent with the petr and brev-hipp-lito clades of the molecular study (**Fig 4.3A-B**). We thus conclude that the OTU petr is evolving as a separate metapopulation lineage. In contrast, the data suggest that brev and hipp are not evolving separately. We interpret them as two subspecies exhibiting a larger Apenninian contact zone (compare **Figs 4.3B and 4.4B**). The data suggest that lito is a third subspecies. A summary of the taxonomic relevant traits is given in **Table 4.4**. We formally describe these taxa later in this paper as follows: petr = *Cicadetta petryi* Schumacher, 1924 described as a form and now regarded as a proper species, brev = *Cicadetta brevipennis brevipennis* Fieber, 1876, hipp = *Cicadetta brevipennis hippolaidica* Hertach ssp. n., lito = *Cicadetta brevipennis litoralis* Puissant & Hertach ssp. n.

Schumacher (1924) described *Cicadetta montana* f. *petryi* as a distinct colour-morph with a precise type locality in the Kyffhäuser (Germany; “steile Gipshänge der Kattenburg bei Frankenhausen” = steep gypseous slopes at Kattenburg near Frankenhausen). The description was not commented upon until Boulard & Mondon (1995) gave species status for specimens from France coloured with four lighter spots on the mesonotum and light basal fore wing venations. Boulard (1995) described the calling song of these specimens in the *brevipennis* group scheme as having very long lasting E_L. Subsequent authors reduced the status of the taxa again to subspecific level or synonymised them completely with *C. montana* s. l. (e.g. Puissant, 2001) or *C. brevipennis* (e.g. Puissant, 2006). These taxonomic decisions were justified insofar as the acoustic and morphological characters given by Boulard were not constant. Schumacher does not provide any song data, but Meineke (2012) demonstrated with sonograms that the Kyffhäuser population belongs to the *brevipennis* group. We visited the Kyffhäuser population and identified the characters within the petr species ('KY'; **Figs 4.3-4.5**). Finally, we were able to extract mtDNA of the 100-years-old type specimens preserved in the Natural History Museum of Berlin ('HT'; **Figs 4.3A and 4.8A**). The type specimen is clearly in the petr lineage.

Table 4.4. Matrix of taxonomically relevant traits in the *Cicadetta brevipennis* song group. Tolerated failure rates < 10% per trait, double symbols for flawless characters in our dataset. For song variables see **Figs 4.1 and 4.2 and Table 4.2**.

Character	**Data source**	**petr *C. petryi***	**brev *C. b. brevipennis***	**hipp *C. b. hippolaidica* ssp. n.**	**lito *C. b. litoralis* ssp. n.**
Genetic distance of *COI* < 1.5% and *COII* < 1% to *C. cerdaniensis*	Genetics	++	– –	– –	– –
E_L/E_S followed by phrase 2: rate < 0.03 (population level)	Acoustics	++	++	– –	– –
Power level $EP_{L/1}$ > 20% of the total power increase	Acoustics	+	–	–	?
IED_{S_L} minimum < 0.34 s	Acoustics	++	– –	– –	++
E_{IN} number < 2.5	Acoustics	+	–	– –	– –
Basal junction of anal veins of fore wing light	Morphology	+	– –	–	++
Veins of apical cell 8 lateral and frontal predominantly dark	Morphology	+	+	++	–
Postclypeus without yellowish spot on the top of the groove	Morphology	+	+	+	–
Habitats at altitudes > 700-800 m asl	Ecology	+/–	–	+	– –
Habitat not in salt marshes	Ecology	++	++	++	– –

For stability and universality of the nomenclature, we retain the name *Cicadetta brevipennis*. However, in our opinion *Cicadetta montana* var. *brevipennis* sensu Fieber (1876) cannot be identified as a song-defined species (see **Appendix S4.5**). We argue that a putative type specimen (**Fig 4.8B and F**) which was found in the Museum of Natural History Vienna is doubtful (see **Appendix S4.5**). Thus, we intend to propose to the ICZN that they disqualify the doubtful Vienna specimen as a potential type. We favour designating a song-determined neotype from the population at Glanz an der Weinstrasse ('GL') which is only 40 km away from

Graz (Austria; compare Art. 75.3.6; ICZN, 1999). Graz is a locus typicus derived from Fieber's unpublished plates (National Museum of Natural History Paris).

Taxonomic descriptions

Cicadetta petryi Schumacher, 1924

Diagnosis

Song characterised by a binary pattern, phrase 2 only emitted in courtship behaviour. Long echemes starting without or with only few introductory chirps (E_{IN} number < 2.5), more chirps possible at low temperatures. Power increasing rapidly in the long echemes ($EP_{L/1} > 20\%$, $EP_{L/2} > 38\%$). Echeme intervals short, especially the pause between the short and the next long echeme (IED_{S_L} minimum < 0.34 s).

92.7% of the *Cicadetta petryi* males and 100% of the females ($n_{ind} = 72$) have a predominantly light (yellowish) basal junction of anal veins, whereas this wing part is dark in 100% of the *Cicadetta b. brevipennis* ($n_{ind} = 19$). The dark phenotype is also predominant in *C. sibillae* (92.3%, $n_{ind} = 28$), *C. montana* s. str. (91.5%, $n_{ind} = 71$) and *C. anapaistica* (97.3%, $n_{ind} = 38$) among closely related or sympatrically occurring species. From *C. montana* s. str., many specimens are additionally distinguished by the generally lighter basal venation and particularly by the outer rim of the costa darker than the inner rim and darker than the radial/subcostal veins in the fore wing (99.2% males and 77.3% females versus 10.0% males and 2.4% females).

Examined material

We examined 61 males including the holotype and 11 females (**Appendix S4.1**). We describe here the variation of the species in comparison to the holotype description of Schumacher (1924).

Holotype male: Verbatim label information: "10.6.1916/Kattenburg/Kyffh. Geb." (label rectangular, white, handwritten) and "*Cicadetta montana*/Scop./f. *Petryi*/nov./*" (label rectangular, white, handwritten) and "Berlin" (label rectangular, white, handwritten; Natural History Museum of Berlin, **Fig 4.8A**).

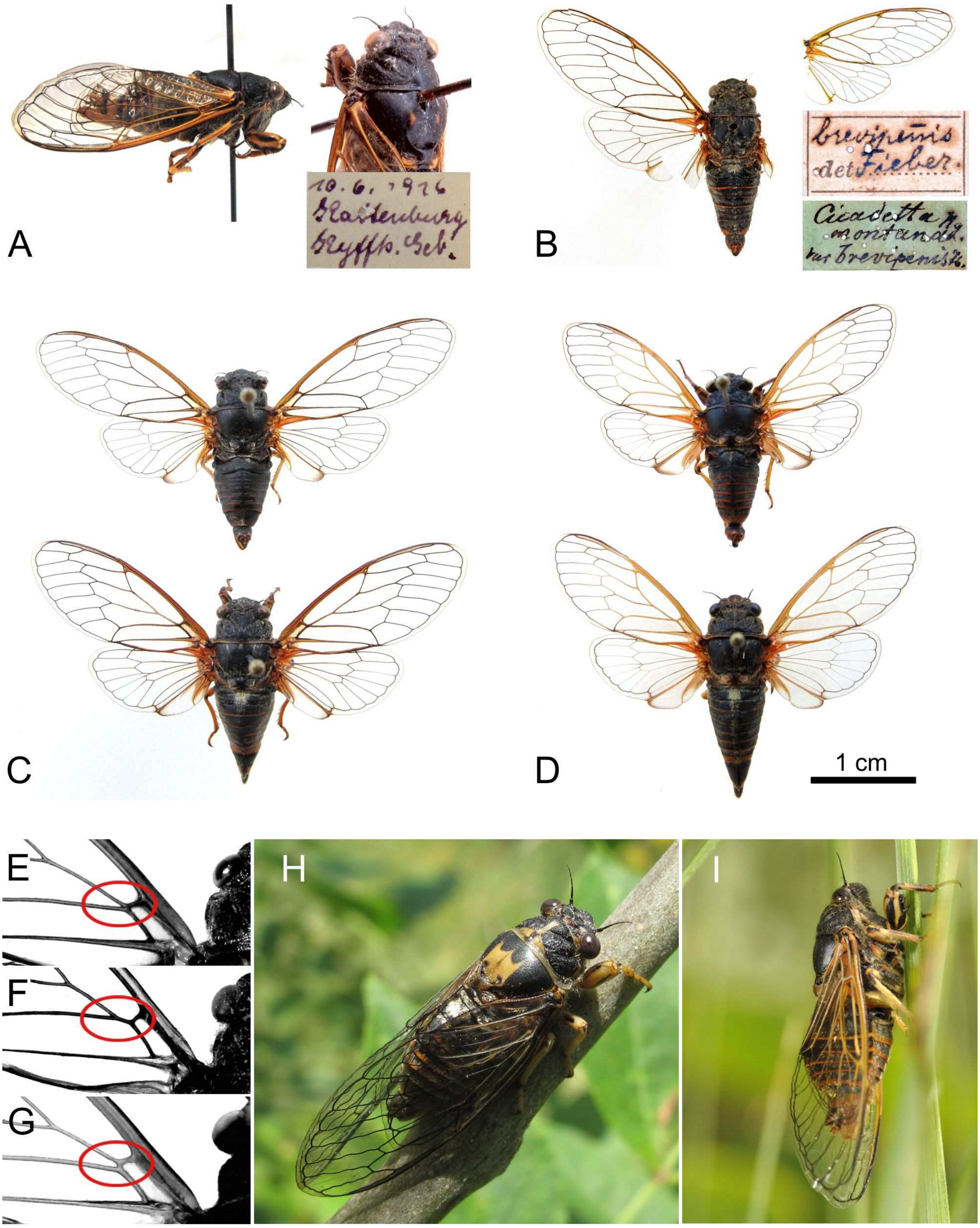

Fig 4.8. *Cicadetta brevipennis* group morphology. (A) *Cicadetta petryi* holotype in lateral and dorsolateral view from the Natural History Museum of Berlin with original label, (B) *Cicadetta montana* var. *brevipennis* Fieber, 1876 putative type from the Museum of Natural History Vienna with broken wings and original labels, (C) *Cicadetta brevipennis hippolaidica* ssp. n. with holotype specimen (above) and female paratype (below), (D) *Cicadetta brevipennis litoralis* ssp. n. with holotype specimen (above) and female paratype (below). (E) Normal, (F) aberrant fused, (G) aberrant shifted compositions of median and cubitus anterior veins arising from the basal cell. (H) *Cicadetta b. hippolaidica* ssp. n. light morph male and (I) singing *Cicadetta b. litoralis* ssp. n in natural conditions.

Morphology

Males' variability: Schumacher (1924) described his colour form mainly based on two yellowish spots on the mesonotum, which he thought to be unique (**Fig 4.8A**, dorsolateral view). In fact, a minority of specimens in all Central European *Cicadetta montana* complex taxa exhibits such smaller or larger yellowish spots. According to our examined material, light markings on the mesonotum are completely lacking in 74.3% of *C. petryi*. The "powerful yellowish basal venation" (Schumacher, 1924) gradually leads to much darker phenotypes; approximately 10% have some basal veins dark brown or even black. In the males we examined, the outer rim of the costa is generally darker than the inner rim of costa and darker than the radial/subcostal vein (with one specimen ambivalent), but colour tints are sometimes different from Schumacher, for example brown for the outer rim of costa and ochre for both other rims.

Schumacher pointed out the stocky and short body of his type. *Cicadetta petryi* tends to be a rather small species within the complex, but with high variability (**Table 4.5**). However, specimens are not particularly stocky (ratio of body length to width measured at tergite II: 3.08 ± 0.11, males only), which is obvious in comparison for example with *C. anapaistica* (2.99 ± 0.08). The wing ratio ("2.5 to 2.6"; Schumacher, 1924) can be specified to a range from 2.31 to 2.62 (males only).

Table 4.5. Body and wing measurements within the *Cicadetta brevipennis* group. Body width measured at tergite II of the abdomen.

Males		***C. petryi*** **(n_{ind} = 61)**	***C. b. brevipennis*** **(n_{ind} = 17)**	***C. b. hippolaidica*** **ssp. n. (n_{ind} = 12)**	***C. b. litoralis*** **ssp. n. (n_{ind} = 21)**
Body length [mm]	Mean ± SD	17.2 ± 0.7	17.6 ± 1.0	16.9 ± 0.7	17.8 ± 0.8
	Maximum	19.2	19.5	18.3	19.5
	Minimum	15.7	15.4	15.9	16.1
Body width [mm]	Mean ± SD	5.6 ± 0.3	5.7 ± 0.2	5.6 ± 0.2	5.7 ± 0.2
	Maximum	6.2	6.2	5.9	6.0
	Minimum	5.0	5.3	5.1	5.3
Fore wing length [mm]	Mean ± SD	18.3 ± 0.8	19.4 ± 0.9	18.7 ± 0.5	18.0 ± 0.8
	Maximum	20.4	21.3	19.4	19.4
	Minimum	16.4	18.0	17.7	16.3
Fore wing width [mm]	Mean ± SD	7.4 ± 0.4	7.7 ± 0.4	7.5 ± 0.3	7.5 ± 0.4
	Maximum	8.5	8.6	8.3	8.2
	Minimum	6.6	7.3	7.1	6.8

Females		***C. petryi*** **(n_{ind} = 11)**	***C. b. brevipennis*** **(n_{ind} = 2)**	***C. b. hippolaidica*** **ssp. n. (n_{ind} = 2)**	***C. b. litoralis*** **ssp. n. (n_{ind} = 2)**
Body length [mm]	Mean ± SD	18.5 ± 0.7	18.6 ± 0.0	17.7 ± 0.3	20.0 ± 0.6
Body width [mm]	Mean ± SD	5.8 ± 0.3	5.9 ± 0.1	5.6 ± 0.3	6.1 ± 0.3
Fore wing length [mm]	Mean ± SD	19.7 ± 0.7	20.8 ± 0.5	19.4 ± 0.0	18.3 ± 0.8
Fore wing width [mm]	Mean ± SD	8.1 ± 0.3	8.0 ± 0.4	8.0 ± 0.1	7.9 ± 0.5

Females' variability: Colouration does not differ significantly from the variability given for males. However, their venation tends to be lighter, which results in more specimens having the outer rim of costa not darker than the two neighboured rims.

Cicadetta brevipennis brevipennis Fieber, 1876

Diagnosis

Song characterised by a binary pattern, phrase 2 only emitted in courtship behaviour. Long echemes starting with a series of introductory chirps (E_{IN} number > 2.5). Power increasing slowly in the long echemes ($EP_{L/1} < 20\%$, $EP_{L/2} < 38\%$). Echeme intervals long, especially the pause between the short and the next long echeme (IED_{S_L} minimum > 0.34 s).

Cicadetta b. brevipennis can be separated with high probability by the predominantly dark basal junction of the anal veins (100%, $n_{ind} = 19$) from *C. petryi* and *C. cerdaniensis* (5%, $n_{ind} = 20$). Again, many specimens are distinguished from *C. montana* s. str. by the outer rim of costa darker than the inner rim and darker than the radial/subcostal veins in the fore wing (89.5% versus 9.5%).

Examined material

We describe the variation of 19 specimens (17 males and 2 females; **Appendix S4.1**) in comparison to Fieber (1876).

Morphology

Males' variability: Fieber's (1876) *C. montana* belongs to the taxa with basal part of the fore wing venations predominantly brownish or yellowish which fits with our material. He describes the varieties *C. montana* var. *brevipennis* and *C. montana* var. *longipennis* with a relatively large number of characters. While a few of the characters are truly ambiguous, others are dependent on the preparation or on the age of the specimen. We omit such characters and concentrate on the remaining ones ["(given in quotation marks)"] and list them in the order provided by Fieber.

The lateral part of the pronotal collar is often “rounded”, but about one third of our specimens have a straight margin. Its angle is usually short, but we would in most specimens not have called the end “almost truncated” as Fieber did. The groove of the clypeus is normally “narrow”; sometimes parts of it are enlarged. The margins of the fore wing are rarely almost “monochrome yellow to red”, but normally trichromatic with the outer rim of costal vein darker than the inner rim and darker than the subcostal/radial vein. All specimens have a dark junction of the anal veins, which unfortunately is not a character listed by Fieber. The big majority of specimens are with genus-typical origin of median and cubitus anterior veins at one point at basal cell (**Fig 4.8E**; but compare **Appendix S4.5**). The yellowish part of the operculum covers, in all specimens of our sample, the apical half rather than “only the apical margin”. This apical margin is normally and in accordance with Fieber “not recessed”. The meracanthus is often slightly bent sidewise and straight, but only rarely “curved and hooked”; and only the base of the meracanthus is “blackish”, but the tip is much lighter. The median lobe of the uncus is variable in shape and length; often it is “short”, sometimes “almost semi-circular”. Sternites III to V frequently possess “dark spots”. These spots have variable, rarely “semi-circular” shapes but are by trend “shrinking”. On sternites VI and VII spots are more often missing than observed by Fieber. The latter is wider than long and, therefore, scarcely “elongated” in the sense of Fieber, but normally “trapezoidal”. Sternite VIII is in fact “elongated and oval, narrower towards the tip”. A minority has – disagreeing with Fieber – a faint central ridge. For body and wing sizes see **Table 4.5**.

Females’ variability: The two females do not differ significantly from the variability given for males. Their frontal margins of the fore wings tend to exhibit less pronounced colour tints.

Cicadetta brevipennis hippolaidica Hertach ssp. n.

Diagnosis

Song characterised by a binary pattern, phrase 2 is regularly or at least from time to time emitted in the calling song (frequency of occurrence > 0.03 on the population level) and relatively loud. Long echemes starting with a series of introductory chirps (E_{IN} number > 2.5). Power increasing slowly in the long echemes ($EP_{L/1} < 20\%$, $EP_{L/2} < 38\%$). Echeme intervals long, especially the pause between the short and the next long echeme (IED_{S_L} minimum > 0.34 s).

Cicadetta brevipennis hippolaidica ssp. n. occurs in two distinct morphs. The dark coloured morph resembles all other described *C. montana* complex species. However, the vast majority of *Cicadetta b. hippolaidica* ssp. n. specimens, like *C. b. brevipennis*, are separated from *C. petryi* by the predominantly dark basal junction of anal veins [95.5% for *C. b. hippolaidica* ssp. n. (dark morphs only), 89.3% for *C. b. hippolaidica* ssp. n. (dark and light morphs) versus 6.2% for *C. petryi*]. From *C. montana* s. str., many specimens are distinguished by the outer rim of costa darker than the inner rim and darker than the radial/subcostal veins (92.9% versus 9.5%). Light coloured morphs are clearly distinguished by large ochre markings on the pronotum and mesonotum from all other described species with the exception of some *C. anapaistica lucana* Hertach, 2015, a taxon exhibiting the same dimorphism (Chapter 2 = Hertach *et al.*, 2015), and some southern French populations of *C. petryi* approaching typical light morphs.

Type series

The type series consists of 12 males and two females representing the whole distribution range but with a clear focus on the Monti Della Daunia population (6 types). The specimens are kept in the Natural History Museum of Basel (NHMB), the Natural History Museum of Bern (NMBE) and one private collection.

Holotype male: Verbatim label information: "Monte Sambuco, PUGL, I/41.5311°/15.0825°, 890 m asl/28.6.2011, leg. Thomas Hertach" (label rectangular, white, printed) and "HOLOTYPUS ♂/*Cicadetta brevipennis hippolaidica* ssp. n./Hertach 2016" (label rectangular, light red with dark red margin, printed; NHMB).

Paratypes: All paratypes with labels "PARATYPUS XX Y, *Cicadetta brevipennis hippolaidica* ssp. n. Hertach 2016" (label rectangular, white with red margin, printed) at which 'XX' is the number of the paratype and 'Y' the sex of the specimen. Number '2' does not exist. **Paratypes males, dark morph**: Monte Sambuco, Monti della Daunia, APUL, I, 41.5312°/15.0830°, 860 m asl, 19.7.2010, leg. T. Hertach (paratype 1, coll. Hertach); Monte Sambuco, Monti della Daunia, APUL, I, 41.5311°/15.0825°, 890 m asl, 28.6.2011, leg. T. Hertach (paratype 4, coll. Hertach); N Torrebruna, ABRU, I, 41.8846°/14.5297°, 750 m asl, 1.7.2011, leg. T. Hertach (paratypes 7 to 9, coll. Hertach); S Pietrabbondante, MOLI, I, 41.7158°/14.3767°, 960 m asl, 2.7.2011, leg. T. Hertach (paratype 10, coll. NMBE); Lago Selva, Cardito, LAZI, I, 41.6071°/13.9758°, 930 m asl, 3.7.2011, leg. T. Hertach (paratype 11, coll. Hertach); Picinisco-Mainarde, LAZI, I, 41.6527°/13.9037°, 930 m asl, 3.7.2011, leg. T. Hertach (paratype 12, coll. Hertach). **Paratypes males, light morph:** Monte Sambuco, Monti della Daunia, APUL, I, 41.5311°/15.0825°, 890 m asl, 28.6.2011, leg. T. Hertach (paratype 3, NMBE); Monte Sambuco, Monti della Daunia, APUL, I, 41.5284°/15.0838°, 920 m asl, 28.6.2011, leg. T. Hertach (paratype 5, coll. Hertach); Monte Sambuco, Monti della Daunia, APUL, I, 41.5490°/15.1036°, 790 m asl, 29.6.2011, leg. T. Hertach (paratype 6, coll. Hertach). **Paratypes females:** N Pescasseroli, ABRU, I, 41.8564°/13.7847°, 1260 m asl, 4.7.2011, leg. T. Hertach (paratype 13, coll. NMBE); N Pescasseroli, ABRU, I, 41.8564°/13.7847°, 1260 m asl, 4.7.2011, leg. T. Hertach (paratype 14, coll. Hertach).

Morphological description

The frequency of the two different coloured morphs in *C. b. hippolaidica* ssp. n. is difficult to estimate over the whole distribution range. Light morphs were so far only caught in the Monti Della Daunia population with a probability of 50%.

Male holotype with remarks on the variability of dark morph paratypes **(Fig 4.8C):** Body length: 17.2 mm, body width (tergite II): 5.7 mm, fore wing length: 19.2 mm, fore wing width: 7.6 mm (for variability in size of the type series see **Table 4.5**).

Head: Black with ochre patch on epicranial suture (in paratypes rarely additionally frontal to posterior margin ochre). Postclypeus with longitudinal narrow groove, black with lateral margins ochre (in paratypes rarely with a yellowish triangle patch at postclypeus towards the frontoclypeal suture), anteclypeus predominantly black. Rostrum reaching mid trochanter, labrum ochre, mentum lateral brown, labium blackish. Antennae dark brown to black with lighter margins of scapes.

Thorax: Pronotum generally black (in paratypes sometimes with a light spot on the central fissure), posterior margin of pronotal collar towards the angles ochre, central interrupted (in one paratype completely black, in others without interruption). Lateral angles of pronotal collar pronounced, pronotal collar frontal with turned up margin and straight in dorsal view (in paratypes sometimes convex in shape). Scutum, cruciform elevation and metanotum black, the latter with yellowish margins (in paratypes rarely with paired lighter spots on the cruciform elevation or on the scutum). Ventral side generally black, except membranes at bases of legs orange to brown. Opercula not overlapping, kidney-shaped with black base and ochre distal part. Meracanthus with straight spike directed slightly laterally (in paratypes rarely bent inward).

Abdomen: Abdomen triangular in cross section. Tergites I and II black (in paratypes rarely tergite II with lighter sections), tergites III through VII frontal black and caudal with small orange to brown bands strongly narrowed to dorsal ridge, tergite VIII light portions more important. Sternite VIII large and long. Sternites III through VII brown with darker sectors lessened towards the end of abdomen (in paratypes rarely spots missing or cloudy). Epipleurites caudally brown, frontally darker. Timbals with three ribs, two long and one shortened, and timbal plate.

Legs: All legs with yellowish and black fasciae and dots, mid and hind tibiae predominantly yellow. Fore femurs holding three spines with decreasing length towards the tibia.

Wings: Fore wing hyaline except for slightly yellowish basal cell and for brownish pterostigma. Basal membrane orange. Median and cubitus anterior vein originating in one point at basal cell (in some paratypes with median and cubitus anterior vein fused for less than 1 mm on one or both sides; **Fig 4.8E-F**). Colouration of basal veins ranging from ochre to dark brown. Exterior rim of costal vein darker than inner rim and radial/subcostal vein (in paratypes rarely all three rims light). Basal junction of anal veins predominantly dark brown to black (**Figs 4.3C and 4.8**). Distal veins almost black with eight apical cells (in two paratypes with one-sided seven cells). Hind wing transparent except for orange base of costal cell, orange to brownish margins of jugum and plaga and dark brown spotted apical vannus margin. Veins dark, especially in distal part, with six apical cells. Cubitus anterior vein lighter than median vein.

Genitalia: Pygofer predominantly dark with lateral parts ochre with rounded dorsal beak and blunt upper lobes. Median lobe of uncus blackish and curved upwards (in some paratypes with yellowish parts), tongue-shaped and rather broad. Claspers hooked and dark brown. Pseudoparameres flattened and with sharp end. Anal tube and anal style reddish to brown orange.

Male paratypes of light morph **(Fig 4.8H):** Contrary to the dark morph several parts of the body are coloured ochre or almost golden instead of black. On the head, postclypeus towards the frontoclypeal suture and sometimes towards the anteclypeus, the anteclypeus itself as well as the surrounding of the compound eyes ochre. On the pronotum, central suture, frontal margin and pronotal collar appearing as broad ochre bands. On the mesonotum, completely ochre except for the submedian and lateral sigillae and the scutal depressions (in two paratypes) or additionally except for the central part (in one paratype). Cruciform elevation and its lateral depressions completely ochre. Ventral side of thorax in one paratype significantly lighter. At the legs, light portions more dominant, especially at the fore leg. One paratype with basal junction of anal veins ochre instead of dark at fore wing.

Female paratypes **(Fig 4.8C):** Both females are perfectly within the variation of the dark morph males. On the pronotum, one female with a yellowish spot on the central fissure, the other with a continuous light posterior margin. Scutum and cruciform elevations completely black. On fore wings, the basal veins relatively light, but still exterior rim of costal vein darker than inner rim and radial/subcostal vein, and basal junction of anal veins dark. Genitalia: Tergite IX dorsal dark abruptly narrowed halfway, lateral brown red to golden. Dorsal beak rounded, anal styles orange. Ovipositor brown orange, tip dark. Ratio of body length to ovipositor length (including sheath) 2.8 and 2.6.

Etymology

The new subspecies *C. b. hippolaidica* ssp. n. is characterised by regular repetitions of phrase 2 in the calling song. This element is close to the song pattern (type 1; Popov *et al.*, 1997; Puissant & Sueur, 2010) of the tiny cicada *Tettigettula pygmea* (Olivier, 1790). The similarity is so striking (**Fig 4.1G-H**) that it seems as if *C. b. hippolaidica* ssp. n. sometimes imitates the numerically dominant syntopic *T. pygmea.* This imitation is unlikely to be real because cicadas are not expected to learn songs from other individuals or species

(Marshall *et al.*, 2011). Nevertheless, we use this amusing phenomenon to name the new subspecies after the tree warbler bird genus *Hippolais* von Baldenstein, 1827 where different European species are excellent imitators of non-conspecific songs.

Cicadetta brevipennis litoralis Puissant & Hertach ssp. n.

Diagnosis

Song characterised by a binary pattern, phrase 2 is regularly emitted in the calling song (frequency of occurrence >> 0.03 on population level). Long echemes starting with a series of introductory chirps (E_{IN} number > 2.5). Power increasing rather slowly in the long echeme. The pause between the short and the next long echeme fast (IED_{S_L} minimum < 0.34 s). Long echeme durations highly variable.

Cicadetta brevipennis litoralis ssp. n. is the taxon within the *C. montana* species complex with the lowest fore wing length to body length ratio and the lightest colour venations known (**Fig 4.8D and I**). A high number of specimens can be distinguished from the other taxa described from Western, Central and Southern Europe. Central hind wing venations are predominantly coloured light brown, yellow, ochre or whitish (93.5%, n_{ind} = 23) whereas the majority of venations are darker (brown or black) in all other taxa (98.9%, n_{ind} = 315). Apical cell 8 (without ambient venation) is surrounded by predominantly light brown, yellow or ochre venations (91.3%) against predominantly darker colouration (close to the tint of the ambient vein) for the other taxa (98.1%). The constantly ochre or yellowish colouration of the top of the postclypeal groove (95.6%) is unusual in comparison to the other *brevipennis* group taxa (3.2%) as is the yellow-tinting of the wings. Fore wing length to body length ratio in males is 1.01 ± 0.04 (min. 0.91, max. 1.11) but for example in *C. petryi* 1.07 ± 0.04, in *C. b. brevipennis* 1.11 ± 0.06 and in *C. b. hippolaidica* ssp. n. 1.11 ± 0.03. Contrary to *C. b. brevipennis* and *C. b. hippolaidica* ssp. n., the basal junction of anal veins is yellowish (100%) as in *C. petryi*.

Type series

The type series consists of 21 males and two females representing the two known local populations near Perpignan. It is kept in the National Museum of Natural History Paris (MNHN), the Slovenian Museum of Natural History Ljubljana (PMSL) and two private collections. For nature conservation reasons we omit verbatim label information and rounded the geographic coordinates.

Holotype male: Torreilles, eastern Pyrenees, F, 42.76°/3.02°, 1 m asl, 19.5.2001, leg. S. Puissant and "*Cicadetta montana*/Dét. S. PUISSANT 2001" (three labels rectangular, white, hand-written) and "HOLOTYPUS ♂/ *Cicadetta brevipennis litoralis* ssp. n./Puissant & Hertach 2016" (label rectangular, light red with dark red margin, printed; MNHN).

Paratypes: All paratypes with labels "PARATYPUS XX Y, *Cicadetta brevipennis litoralis* ssp. n. Puissant & Hertach 2016" (label rectangular, white with red margin, printed) at which 'XX' is the number of the paratype and 'Y' the sex of the specimen. **Paratype males:** Alénya, eastern Pyrenees, F, 42.65°/2.99°, 5 m asl, 31.5.1999, leg. S. Puissant (paratypes 1 and 2, coll. Puissant); Torreilles, eastern Pyrenees, F, 42.76°/3.02°, 1 m asl, 31.5.1999, leg. S. Puissant (paratype 3, coll. Puissant); Torreilles, eastern Pyrenees, F, 42.76°/3.02°, 1 m asl, 5.6.1999, leg. S. Puissant (paratype 4, coll. Puissant); Torreilles, eastern Pyrenees, F, 42.76°/3.02°, 1 m asl, 19.6.1999, leg. S. Puissant (paratypes 5 and 6, coll. Puissant); Torreilles, eastern Pyrenees, F, 42.76°/3.02°, 1 m asl, 19.5.2001, leg. S. Puissant (paratypes 7 and 8, coll. Puissant); Torreilles, eastern Pyrenees, F, 42.76°/3.02°, 1 m asl, 24.5.2001, leg. S. Puissant (paratypes 9 and 10, coll. Puissant); Torreilles, eastern Pyrenees, F, 42.76°/3.02°, 1 m asl, 14.6.2002, leg. S. Puissant (paratypes 13 to 20, coll. PMSL); Torreilles, eastern Pyrenees, F, 42.76°/3.02°, 1 m asl, 4.6.2013, leg. S. Puissant (paratypes 21 and 22, coll. Hertach). **Paratype females:** Torreilles, eastern Pyrenees, F, 42.76°/3.02°, 1 m asl, 24.5.2001, leg. S. Puissant (paratype 11, coll. MNHN); Torreilles, eastern Pyrenees, F, 42.76°/3.02°, 1 m asl, 26.5.2001, leg. S. Puissant (paratype 12, coll. Puissant).

Morphological description

***Male holotype* (Fig 4.8D):** Body length: 16.8 mm, body width (tergite II): 5.4 mm, fore wing length: 17.5 mm, fore wing width: 7.4 mm (for variability in size of the type series see **Table 4.5**).

The holotype specimen of *Cicadetta brevipennis litoralis* ssp. n. fits the detailed description of the holotype of *C. b. hippolaidica* ssp. n. with the following differences: On the head, longitudinal narrow groove of the postclypeus on the top ochre. Supra-antennal plate partly ochre. On the thorax, posterior margin of pronotal collar ochre without central interruption, frontal to the angles convex and clearly recessed in dorsal view (as in some paratypes of *C. b. hippolaidica* ssp. n.). Cruciform elevation brownish, its lateral depressions ochre (as in some light morph paratypes of *C. b. hippolaidica* ssp. n.). Posterior margins of mesonotum and metanotum remarkably light. On the abdomen, sternite VII without darker spot (as in some paratypes of *C. b. hippolaidica* ssp. n.). Wings appear tinted yellowish and not completely colourless. On the fore wings, median and cubitus anterior vein originating at two slightly shifted points at basal cell (**Fig 4.8G**). Basal venation remarkably yellow to whitish ochre including the basal cell and the basal junction of anal veins but with a darker exterior rim of costa and posterior margin with anal veins II and III. Distal veins still light with the exception of the ambient venation and adjoining bases of veins leading away. In particular, apical cells lateral and frontal predominantly light bordered. On the hind wings, the ambient, the cubitus posterior and the anal II veins are dark while the remaining veins are very light yellow to whitish ochre.

Male paratypes: Male paratypes of *C. b. litoralis* ssp. n. differ from the holotype as follows: On the thorax, light colouration at posterior margin of pronotal collar sometimes interrupted, more rarely completely missing (both variants as in some *C. b. hippolaidica* ssp. n.). Frontal margin often also marked lighter, in one paratype with spot on central fissure. Approximately half of the paratypes with more or less straight lateral margins of pronotal collar in dorsal view (as in some *C. b. hippolaidica* ssp. n.). Cruciform elevation often with lighter paired spots, rarely completely light or completely dark. Scutum occasionally with two small spots (as in some *C. b. hippolaidica* ssp. n.). Meracanthus sometimes bent inward (as in some paratypes of *C. b. hippolaidica* ssp. n.). On the abdomen, sternites normally with well pronounced dark spots also on segment VII (as in some *C. b. hippolaidica* ssp. n.). On the fore wings, specimens with genus-typical origin of median and cubitus anterior veins at one point at basal cell are outnumbered against the formation described for the holotype (estimated ratio 1:2). A few paratypes with a short fusion of median and cubitus anterior veins at the base (as in a minority of *C. b. hippolaidica* ssp. n.; **Fig 4.8E-G**). Colouration of the veins also in paratypes light and rather constant. Apical cells lateral or/and frontal rarely darker towards the colour tint of the ambient vein. Hind wing rarely predominantly darker veined, but cubital anterior veins light in all specimens.

***Female paratypes* (Fig 4.8D):** Both females are similar to the majority of males with respect to colouration of body and wings. On the fore wings, one female with median and cubitus anterior vein originating at two slightly shifted points at basal cell, the other with shortly fused alternative. Genitalia: Tergite IX dorsal dark, lateral brown red, dark colouration continuously narrowed to the end. Dorsal beak rounded, anal styles orange. Ovipositor brown orange, tip dark. Both females with long body, but short wings: ratio wing length to body length 0.90 and 0.92 (1.07 ± 0.04 for other *brevipennis* group taxa pooled). Ratio of body length to ovipositor length (including sheath) 3.0 for both specimens.

Etymology

Cicadetta b. litoralis ssp. n. lives in extraordinary habitats influenced by salt-water near the coast (**Fig 4.7E**). '*Litoralis*' is Latin adjective meaning 'coastal' or 'belonging to the coast'.

DISCUSSION

Parallels between the *Cicadetta cerdaniensis* and *Cicadetta brevipennis* song groups

Our work has revealed astonishing parallels between the *cerdaniensis* and the *brevipennis* song groups for different character sets. The most striking result is that the mtDNA identified four inter-group species pairs (**Fig 4.9**). Similar haplotypes are not only present in the pairs *C. petryi*/*C. cerdaniensis* s. str. (blue; former 'A-lineages') and *C. b. brevipennis*/*C. sibillae* (orange; former 'B-lineages'; Chapter 2 = Hertach *et al.*, 2015; Chapter 3 = Wade *et al.*, 2015) but also in bulg and *C. cantilatrix* (green). Moreover, the sister taxon of one *C. b. hippolaidica* ssp. n. haplogroup is *C. a. anapaistica* (red) another taxon of the *cerdaniensis* song group. In the *brevipennis* group as well as in the *cerdaniensis* group central and southern Apenninian (Italy) taxa share mtDNA haplotypes.

Another similarity arises from wing colouration. Yellowish or dark basal junctions of anal veins are not only indicative for the *brevipennis* song group but also for approximately 95% of the specimens to distinguish between *C. cerdaniensis* s. str. on the one hand (yellowish) and *Cicadetta sibillae* and *Cicadetta anapaistica* on the other hand (dark) in the *cerdaniensis* song group (Chapter 2 = Hertach *et al.*, 2015). Thus, Apenninian taxa of both song groups tend to have dark basal junctions of anal veins, while western taxa are normally yellowish (**Figs 4.3C-D and 4.9**). Much lighter coloured morphs occur in southern Apenninian taxa of both song groups, in *C. b. hippolaidica* ssp. n. (**Fig 4.8H**) and *C. a. lucana*; these taxa are separated maximally by 65 km at present.

Even in the clearly distinct song patterns, we can find similarities between the two groups. The southern Apenninian calling song patterns are the most complex in rhythm (*C. anapaistica* and *C. b. hippolaidica* ssp. n.), while the western and the central Apenninian species (*C. cerdaniensis* versus *C. sibillae* and *C. petryi* versus *C. b. brevipennis*) can mainly be separated by quantitative and scarcely by qualitative characters. The central Apenninian taxa (*C. sibillae* and *C. b. brevipennis*) move their timbals slower than western and southern sister taxa (compare Chapter 2 = Hertach *et al.*, 2015). The sum of the parallel traits is so striking, that it could help to better understand the biogeography and phylogeny of both groups.

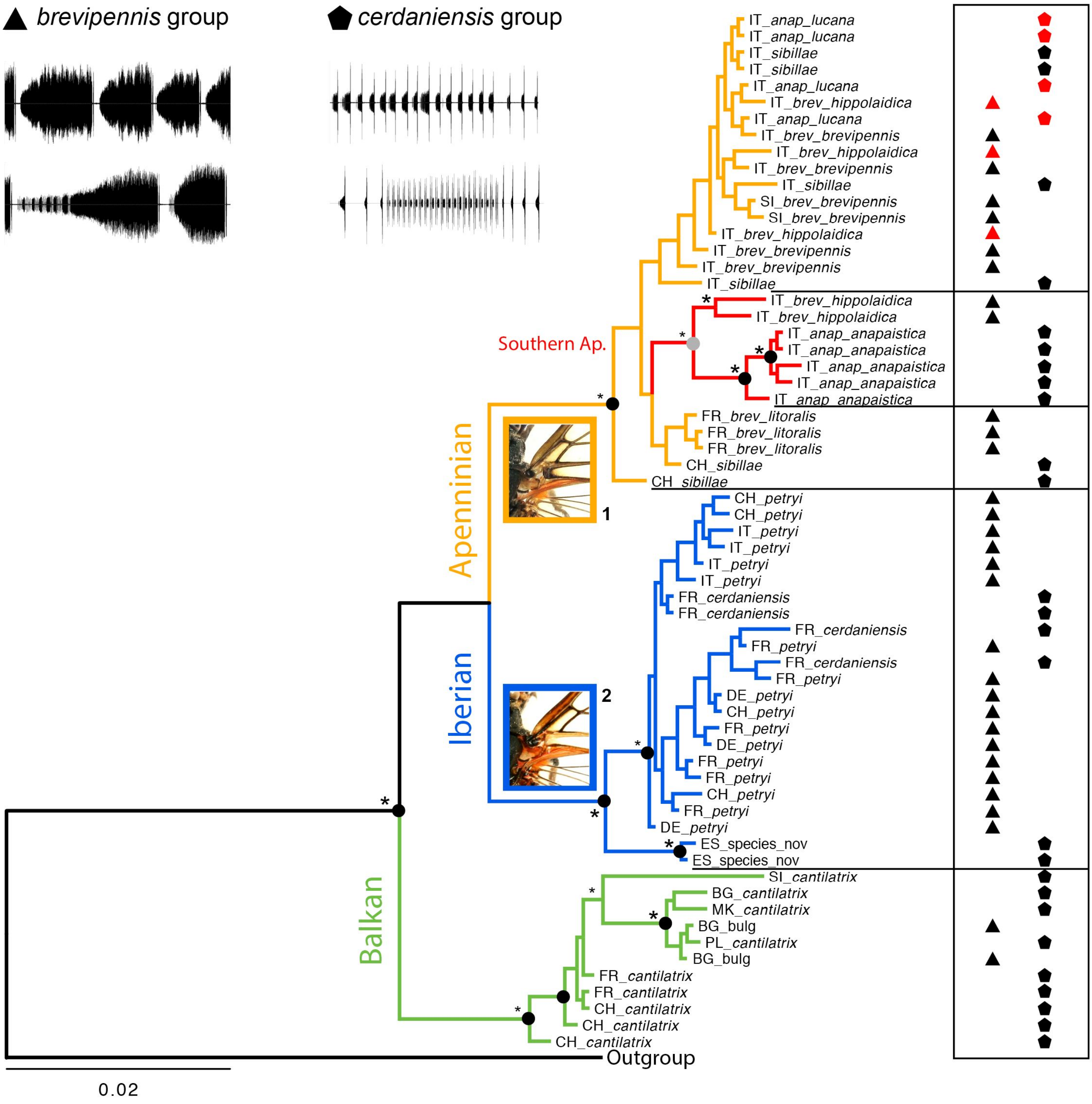

Fig 4.9. *COI* and *COII* concatenated phylogeny of the *Cicadetta brevipennis* and *Cicadetta cerdaniensis* song groups. Data set expanding previous studies (Chapter 2 = Hertach *et al.*, 2015; Chapter 3 = Wade *et al.*, 2015) with eight newly sampled specimens. Bayesian phylogeny showing hypothesised geographic origins of the clades, the song groups of individual specimens and a colouration trait. Notes: Bayesian posterior probabilities (filled circles: black > 0.95, grey > 0.90) and ML bootstrap values (star icons: large > 0.90, small > 0.80) from RAxML analysis (**Appendix S4.3**). Red song-symbols identify specimens with southern Apenninian origin presumably introgressed by central Apenninian taxa. Country codes: BG = Bulgaria, CH = Switzerland, DE = Germany, ES = Spain, FR = France, IT = Italy, MK = Macedonia, PL = Poland, SI = Slovenia. OTU bulg is poorly known. Basic and complicated acoustic examples of both song groups (15 s; *Cicadetta petryi*, top left; *Cicadetta brevipennis hippolaidica* ssp. n., below left; *Cicadetta cantilatrix*, top right; *Cicadetta anapaistica anapaistica*, below right). [1]Colouration trait not valid for endemic *Cicadetta brevipennis litoralis* ssp. n., [2]Spanish undescribed taxon ambivalent dark or light.

Moreover, *Cicadetta b. litoralis* ssp. n. and *C. b. hippolaidica* ssp. n. have evolved phrase 2 to function differently from other taxa (i.e. as a calling song element rather than a courtship element). A similar case is documented for the *cerdaniensis* group where *C. sibillae* and *C. cerdaniensis* emit a phrase in the calling song that is known only as part of the courtship behaviour in *C. cantilatrix* (Chapter 2 = Hertach *et al.*, 2015).

Integrative species delimitation and the meaning of 'species'

The failure to recover the *cerdaniensis* group and the *brevipennis* group as two reciprocally monophyletic clades was surprising, especially to the bioacousticians. Within-song-group patterns are so closely related and among-group differences so complex that these groups should each have one common ancestor; one for the *cerdaniensis* group and one for the *brevipennis* group. In the absence of song, systematists would have concluded the existence of a western, a north-eastern and an Italian taxon, respectively, some supported by colouration traits (**Fig 4.9**). Phylogeny-based species delimitation including both mitochondrial and nuclear genes *elongation factor 1-alpha* and *period* using the GMYC method recovered these three geographic entities (Chapter 3 = Wade *et al.*, 2015). The same result emerged when working with multiple user-provided guides in the Bayesian coalescent species delimitation programme BPP (Chapter 3 = Wade *et al.*, 2015). This kind of delimitation is very strange in repeatedly combining populations that have such distinct songs. A naive observer might describe each mtDNA clade as displaying a song-polymorphism, but the consideration of the combined datasets as a whole plus studies of many other cicada species complexes, argue against this explanation. Songs are innate in cicadas and decisive for the attraction of conspecific females and have been demonstrated to distinguish cryptic species of cicadas (e.g. Puissant & Sueur, 2010; Marshall *et al.*, 2011; Popple, 2013) and many other insect taxa. The *brevipennis* and *cerdaniensis* groups must be regarded as stable units. Among thousands of individuals, we recorded or heard, at most, three times, mixed-group song fragments (always in syntopic populations between *C. sibillae* and *C. petryi*). The presence of intermediate song characters in an area of contact is good evidence for hybridisation (e.g. Drosopoulos *et al.*, 2006; Marshall *et al.*, 2011; Chapter 2 = Hertach *et al.*, 2015). These observations are hints that recent sexual inter-group contacts exist, but at a very low level. Hybrid songs are normally expected to be unattractive and can be regarded as pre-zygotic behavioural barriers to allospecific females (Gottsberger & Mayer, 2007). The inter-group stability can also be demonstrated with respect to the distribution patterns: The *brevipennis* group shows an allopatric to parapatric distribution and the *cerdaniensis* group contains five allopatrically to parapatrically distributed taxa with similar but not exactly the same geographic boundaries (**Fig 4.10**; Chapter 2 = Hertach *et al.*, 2015).

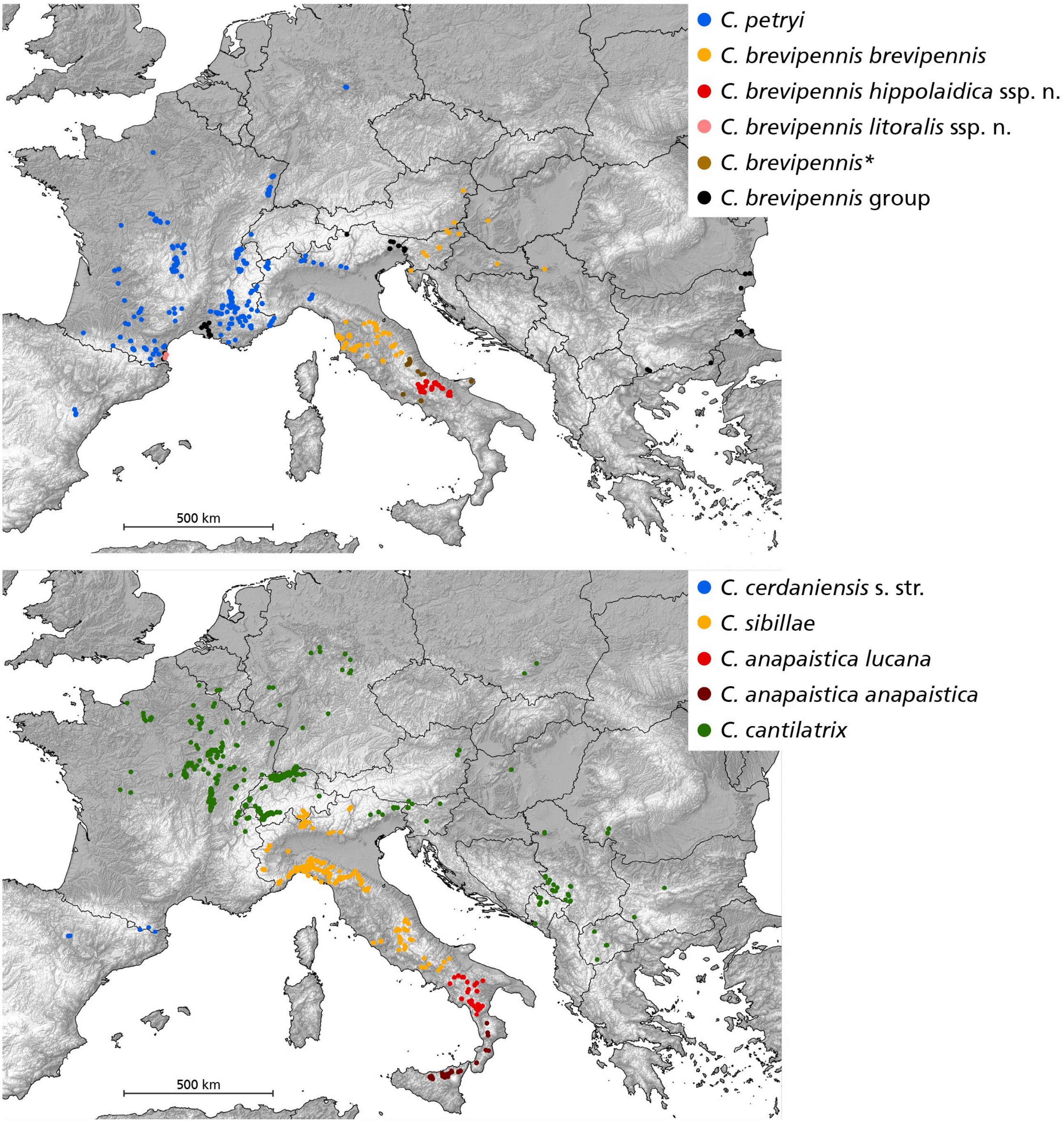

Fig 4.10. Distribution maps of *Cicadetta brevipennis* song-group taxa (top) and *Cicadetta cerdaniensis* song-group taxa (below). Many records in our database were sampled prior to our recent studies and do not contain sufficient audio information. In these cases taxon assignment to the various *Cicadetta brevipennis* group taxa is based on biogeography and must be verified especially for black dotted populations. Some of the records were previously published, essentially records of the *cerdaniensis* group (Trilar & Holzinger, 2004; Boitier & Brugel, 2006; Puissant, 2006; Trilar *et al.*, 2006; Hertach, 2007; Sueur & Puissant, 2007; Hugel *et al.*, 2008; Trilar & Hertach, 2008; Meineke, 2012; Trilar & Gogala, 2012; Chapter 6 = Hertach & Nagel, 2013; Delorme *et al.*, 2015; Gurcel, 2015; Chapter 2 = Hertach *et al.*, 2015; Klasa & Bokłak, 2015; Pfeifer, 2015). *Brown dots are observations of *Cicadetta brevipennis* without assignment to a subspecies in central Italy. Some of these populations mark hybrids between *C. b. brevipennis* and *C. b. hippolaidica* ssp. n.

Our system illustrates that it is not wise to rely on single or even two standard sources of taxonomic information (mtDNA, colouration) when delimiting species. Many difficult taxa can only be resolved when data from multiple data sources are combined. Songs often evolve faster than morphology and are considered to be the best markers of the early stages of reproductive isolation (e.g. Alexander & Moore, 1962; Dugdale & Fleming, 1978; Marshall *et al.*, 2009; 2011). Cicada complexes with morphologically similar but acoustically different species have repeatedly been shown to exhibit irregular mtDNA phylogenies (Buckley *et al.*, 2006; Marshall *et al.*, 2011; Popple, 2013; Nunes *et al.*, 2014).

In empirical studies most often morphological differences are combined with mitochondrial DNA, either the first are validated with the second or vice versa (Schlick-Steiner *et al.*, 2010; Yeates *et al.*, 2011). When these two data sources contradict each other, many authors evaluate results against a nuclear background and (if divergent enough) can demonstrate incomplete lineage sorting or introgression among lineages (e.g. Berthier *et al.*, 2006; Barbanera *et al.*, 2009; Warren *et al.*, 2012; Gvoždík *et al.*, 2015). Our study taxa have the disadvantage, that the nuclear genes examined to date (*elongation factor 1-alpha* and *period*) are not informative for recent cladogenic events, such as those we are studying here, when subjected to a variety of species delimitation methods (Chapter 3 = Wade *et al.*, 2015). The closest model organism to cicadas with a complete genome sequenced is an aphid (Sternorhyncha) and next-generation sequencing is needed to target fast evolving protein-coding genes for cicada taxonomy (Owen *et al.*, 2015). Luckily, cicada songs provide an applicable, nuclear-encoded, powerful discrimination tool more relevant to cicada biology than the average nuclear gene.

Different authors recently criticised the statistical rigour and objectivity of species delimitation (e.g. Tobias *et al.*, 2010; Hey & Pinho, 2012). However, it is equally problematic to rely exclusively on generalised species delimitation models that are imperfect imitations of the biological reality, most of them for example not allowing divergence with gene flow or the inclusion of non-molecular data (e.g. Yeates *et al.*, 2011; Carstens *et al.*, 2013; Solís-Lemus *et al.*, 2015). Our simple but practical guide is to use a combination of song, morphology, mtDNA and distributional data to recognise lines of evidence for “separately evolving metapopulation lineages” (De Queiroz, 2007). We emphasise however that “separately evolving” does not imply a lack of gene flow between species. De Queiroz (2007) qualified his concept by saying that, lineages do not have to be “phenetically distinguishable, diagnosable, monophyletic or intrinsically reproductively isolated”. We suggest that De Queiroz’ view of “separately evolving metapopulations”, just as Stebbins (1950) idea of species occupying “separate evolutionary trajectories”, are not in conflict with Mallet’s (2008) restatement of the Darwinian view that species are one step in a continuum from varieties to full species and that, “the existence of this continuum provides good evidence for gradual evolution of species from ecological races and biotypes, to hybridizing species and, ultimately, to species that no longer cross.”

Our data analysis, species definitions, and evolutionary scenarios are all consistent with these ideas. Our results suggest that hybridisation can take place between local populations of the *Cicadetta* species studied here within the range suggested by Mallet (2008; < 0.1% per generation). We designate as 'species', taxa that appear to have little to no gene flow among them as measured by our suite of characters. We designate as 'subspecies' taxa that appear to be exchanging genes to a degree that blurs the differences.

We analyse here data from the wide distribution area of *C. brevipennis* s. l. where song differences were initially not obvious but became clearer when quantitative song characteristics were measured. Combining small acoustic, morphological and ecological traits with the molecular data we noticed unexpected congruence and spatial coherence among datasets and among OTUs. Phenotypic differences corresponded to genotypic clades, even at localities where biogeographical patterns are unusual. This is especially true for the subspecies *C. b. litoralis* ssp. n. in southern France and for the Apenninian populations of *C. petryi* (Brallo di Pregola 'BR'), but also for the disjunct *C. b. brevipennis* distribution. The detection of the patterns in these three cases was the breakthrough in our study, and we infer two valid species. We can reject the alternative hypothesis that the distinct mtDNA clades are solely caused by genetic contacts of some metapopulations with the closely related *cerdaniensis* song group for *C. brevipennis* and *C. petryi*.

In contrast to the *C. brevipennis*/*C. petryi* example, the data do not indicate that *C. b. brevipennis* and *C. b. hippolaidica* ssp. n. are evolving separately. We describe them as subspecies. They may be an example of geographically allopatric clades (see Butlin *et al.*, 2008) that were separately evolving but later came back into secondary contact before reproductive isolation was complete. At some point in time (or at multiple time points), these two lineages met in central Italy and likely mixed over large areas (**Figs 4.3B and 4.4B**). As with many subspecies, it is not easy to draw the spatial limits of the two taxa. It could be argued that *C. b. hippolaidica* ssp. n. is endemic to the Monti della Daunia ('MD'), but our distribution data suggests that such a limit would not reflect the most natural system, at least not in the present day. The region between the two National Parks Monti Sibillini and Majella acts as a barrier where subspecific exchange is hindered by the presence of *C. sibillae* from the *cerdaniensis* group (i.e. competitive exclusion).

Additional contact zones may exist in the south-eastern Alps (*C. b. brevipennis* and *C. petryi*) and in southern France (*C. b. litoralis* and *C. petryi*) but not in the northern Apennine where *C. b. brevipennis* and *C. petryi* are clearly separated. These potential hybrid zones are small compared to the wider distributional area (**Fig 4.10**) and, therefore, are not influential in our taxonomic decisions. Only two populations (Torreilles 'TO', Travesio 'TR') among 31 showed intermediate signals in acoustics at the species level (**Figs 4.5 and 4.6**).

Cicadetta b. litoralis ssp. n. is certainly more than an ecotype with exceptional habitat requirements; it appears to have evolved unique attributes that could lead it to speciate along a separate trajectory. Our data supports a close relationship to *C. b. brevipennis* and *C. b. hippolaidica* despite the spatial separation. However, some elements are similar to *C. petryi* ($\mathrm{IED}_{\mathrm{S\_L}}$ minimum, colouration of basal junction of anal veins). The genetic distance to *C. b. brevipennis* implies that it is a very young taxon (**Fig 4.3A and Table 4.1**). The remarkable ecological and phenotypic adaptations were possibly favoured by drift in a small founder population (e.g. Grant & Grant, 1995; Podnar *et al.*, 2005).

The *brevipennis* basic song pattern is relatively simple and provides only a few variables in the time and carrier frequency domains. Therefore, we were looking for additional potential traits and experimented with the power succession until we found indicative characters (**Fig 4.2**), which are presumably being used for the first time in cicada taxonomy. Acoustic differences between the taxa are generally small, but the sum of specific traits is convincing (**Table 4.4**). The three best song characters in the data set have 0, 3 and 4 individuals in the overlap for *C. petryi* and *C. b. brevipennis* (**Fig 4.5**) and are probably reliable on similar levels when applied to randomly sampled individuals. Combining these characters results in a well-supported separation of the two taxa (**Fig 4.6**) and may contain relevant acoustic information when communicating with conspecific versus non-conspecific females. There are other examples within European cicadas, where inherited acoustic specific-mate recognition systems are fine-tuned (Quartau & Simões, 2006; Sueur & Aubin, 2003; Chapter 2 = Hertach *et al.*, 2015). With one colouration character (basal junction of the anal veins) we assign only 4.5% of the specimens to the wrong group of taxa (normally dark: *C. b. brevipennis* and *C. b. hippolaidica*, normally yellowish: *C. petryi* and *C. b. litoralis*).

Evolutionary hypotheses for the origin of the *C. cerdaniensis* and *C. brevipennis* group taxa

The complicated but regular evolutionary pattern between the *cerdaniensis* and the *brevipennis* song groups is unusual and intriguing. We suggest a possible explanation. This scenario combines the specific traits with traditional biogeographic models. Hereby, acoustic patterns serve as predictors of the most parsimonious evolution as well as indicators of recent hybridisation (Drosopoulos *et al.*, 2006; Marshall *et al.*, 2011; Chapter 2 = Hertach *et al.*, 2015). Three pieces of evidence form the basis of our scenario: 1) The molecular data are structured spatially, 2) the genetic distances (Brower, 1994; Papadopoulou *et al.*, 2010) between taxa of the three main clades (≈ mean 2.5%) suggest a radiation in the Pleistocene with its drastic climatic changes and severe impacts on populations (Taberlet *et al.*, 1998; Hewitt, 2000; 2001), and 3) the inter-group taxa pairs are likely to have been in contact.

Combining these three aspects, we hypothesise that widespread ancestors of the *cerdaniensis* and *brevipennis* groups were isolated from intra-group populations in different refugia during Pleistocene glaciation periods. European cicadas were likely displaced to the classic southern refugia (Taberlet *et al.*, 1998; Hewitt, 2000; 2001) since they are thermophilic and most of them are connected to temperate forests or their margins. We suggest an Iberian refuge for *C. petryi* and *C. cerdaniensis* s. str., a central Apenninian for *Cicadetta b. brevipennis* and *C. sibillae*, a southern Apenninian for *C. b. hippolaidica* ssp. n., *C. a. anapaistica* and *C. a. lucana* and a Balkan for *C. cantilatrix* and possibly bulg (**Figs 4.9 and 4.11A**). During these long cold periods, genetic exchange with other intra-group metapopulations was at least hindered (within Apenninian peninsula) or interrupted. Isolated metapopulations began to evolve specific traits. However, we speculate that they lived and reproduced in parapatry or even sympatry with populations belonging to the other song group. In these isolated populations, non-conspecific matings with sporadic gene flow could have taken place. This transfer led to mtDNA haplotypes (and presumably at least some nuclear genes) being shared among species and the mtDNA clades became diagnostic for refugia rather than species (see Nicholls *et al.*, 2012). Even rare hybridisation is enough to allow extensive introgression of mtDNA across species boundaries (Funk & Omland, 2003; Chan & Levin, 2005; Sullivan *et al.*, 2014). Mitochondrial capture in the absence of song introgression has been seen in other cicada taxa (Marshall *et al.*, 2011). Incomplete lineage sorting as an alternative for non-monophyly predicts no consistent patterns with respect to geography (Funk & Omland, 2003; Buckley *et al.*, 2006; Nicholls *et al.*, 2012) and is improbable for the main clades of our data set. We also suggest that the observed pattern is unlikely a result of a phenotypic convergence, even when taking into account, that astonishing cases have been documented for other insect groups but for morphological adaptations (Buckley *et al.*, 2009; Moore & Robertson, 2014; Bennik *et al.*, 2016). Phenotypic convergence is not able to explain why *brevipennis* and *cerdaniensis* song groups not only form taxa pairs but in many cases also share identical haplotypes. Additionally, the allopatric intra-group distributions are too perfect and the evolutionary costs of switching complex song patterns several times appear to be too high.

A similar explanation could be devised for the distribution of colouration characters, in particular the basal junction of the anal veins of the fore wing (**Fig 4.9**). Yellowish and dark phenotypes were displaced to the different refugia by glacial cycles. Probably, phenotypes – at least of one taxon – coming to the Apenninian peninsula were often dark and rarely yellowish and the individuals from the Iberian peninsula had a reciprocal frequency scale. We suggest that selection eliminated the rare phenotypes over thousands of generations and sporadic inter-group hybridisation spread the dominant phenotypes to cohabiting taxa. In a southern Apenninian refuge, *C. a. lucana* or *C. b. hippolaidica* ssp. n. evolved lighter morphs, and obviously began to share them after episodic heterospecific reproduction.

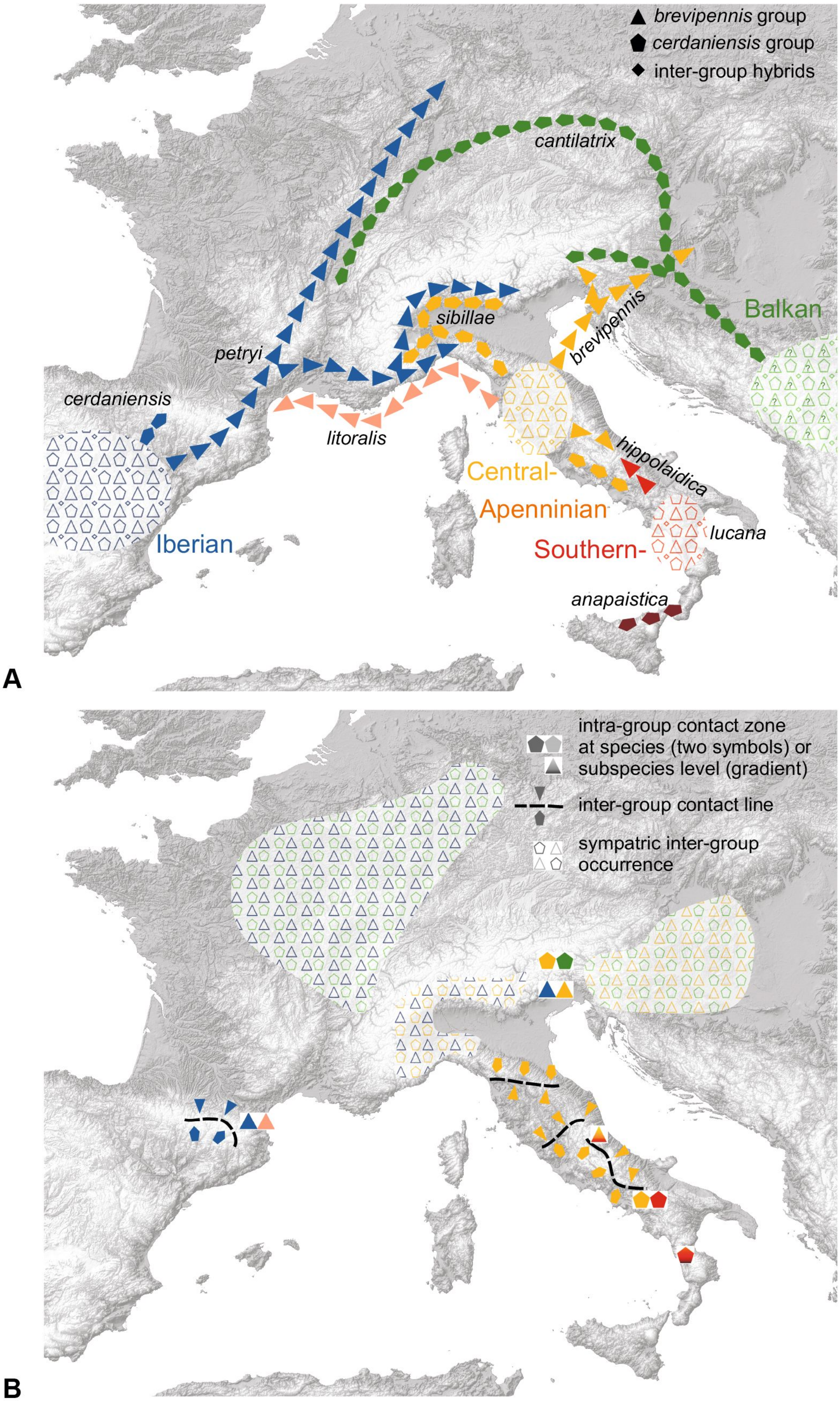

Fig 4.11. Biogeographical aspects within the *Cicadetta brevipennis* and *Cicadetta cerdaniensis* song groups. (A) Potential recolonisation routes from supposed common inter-group Pleistocene refuges at the Iberian, Apenninian and Balkan peninsulas. Glacial refuges are visualised schematically by coloured-outline hollow symbols. (B) Sympatric occurrences and contact zones of current populations. Blue = Iberian clade, red shades (pink – orange – red – brown red) = Apenninian clade, green = Balkan clade; triangles = *brevipennis* group, pentagons = *cerdaniensis* group.

Surprising is the fact, that the refugia scenario discussed above simultaneously took place in three to four different regions. The closely related ecological niches of *cerdaniensis* and *brevipennis* groups serve as a possible explanation, which inevitably leads to spatial contacts. Our data are paradoxical in the sense that on the one hand, gene trees and genetic distances were helpful to find cryptic species such as *C. sibillae* (Chapter 2 = Hertach *et al.*, 2015) and *C. petryi* and to roughly calibrate main clades. On the other hand, introgression affects our system so thoroughly that genetic distances are difficult to interpret in the whole *cerdaniensis-brevipennis* group. We cannot assess in which directions gene flow occurred or whether it occurs bidirectionally or unidirectionally. Thus, we also do not know which haplotypes are more original for the one or the other song group (see Funk & Omland, 2003). We do not even have evidence that *cerdaniensis* and *brevipennis* group ancestors are sister taxa.

All elements of our scenario are documented in other animal groups but as far as we know not in combination. Most similar to our scenario we found the gall wasp system (genus *Andricus*) described by Nicholls *et al.* (2012) in which the mtDNA haplotypes of four hybridizing species cluster in multiple clades indicative of three common Pleistocene refugia. However, in this case, speciation during isolation in separate refugia did not involve reproductively important traits and the prediction of refugial membership was not as perfect as in the *cerdaniensis-brevipennis* groups, especially not for specimens collected far from the core refuge; e.g. our *C. petryi* from central Germany still have haplotypes closely related to endemic *C. cerdaniensis* from the Pyrenees (**Fig 4.9**). Similarly, north Iberian hare species possess the mtDNA of *Lepus timidus* Linnaeus, 1758, a species that retreated from this region to the Alps and northern latitudes at the end of the last ice age (Melo-Ferreira *et al.*, 2005). Postglacial mtDNA replacement was seen in the New Zealand cicada *Kikihia muta* (Fabricius, 1775) after it crossed from North Island to South Island, invaded the territory of a non-sister species and hybridised with it (Marshall *et al.*, 2011). In North American chipmunks, Sullivan *et al.* (2014) found two pairs of non-sister species where the mtDNA of one member of each pair had completely replaced that of the other member prior to the Pleistocene with the taxa in question showing no current evidence of gene flow.

Postglacial biogeography

From the current geographic distribution (**Fig 4.10**) and the scenario above we can hypothesise potential postglacial recolonisation routes (**Fig 4.11A**; compare Taberlet *et al.*, 1998; Hewitt, 1999; 2000; 2001). *Cicadetta petryi* and *C. cantilatrix* were obviously successful dispersers. Apenninian lineages of both groups have conquered similar areas, and the presence of the Alpine barrier prohibited them from colonising more northern areas (Chapter 2 = Hertach *et al.*, 2015; Taberlet *et al.*, 1998; Hewitt, 1999; 2000; 2001). We propose that *Cicadetta b. brevipennis* and *C. b. litoralis* ssp. n. profited by temporary migration routes when coastlines moved seaward due to the enormous amount of water stored in the continental ice sheets in the Late Pleistocene (Shackleton *et al.*, 1984; Correggiari *et al.*, 1996; Zunino & Zullini, 2004) as known for other organisms (e.g. Fritz *et al.*, 2005; Podnar *et al.*, 2005; Gogala, 2008; Lecocq *et al.*, 2013). Later these coastal plains were inundated again and populations were isolated and went locally extinct.

Postglacial expansions led to secondary contacts with intra-song group taxa (**Fig 4.11B**). When reproductive isolation was incomplete, lineages hybridised (e.g. Hewitt, 2001). Such contact zones can be found in central and southern Italy today. These Apenninian taxa share mtDNA haplotypes which are likely a result of intra-group introgression (compare Barbanera *et al.*, 2009): *C. sibillae* completely asymmetrically captured the mtDNA of *C. a. lucana* (Chapter 2 = Hertach *et al.*, 2015), while *C. b. brevipennis* drove the distinct haplotype of *C. b. hippolaidica* ssp. n. almost to extinction (**Fig 4.9**; red symbols). Therefore, in both song groups the central Apenninian haplogroups became dominant. Another zone, where lineages having supposedly different origins meet, is found in the south-eastern Alps. Interestingly, the Trentino-Veneto region serves as a suture zone for both song groups (compare Baroni Urbani *et al.*, 1977; Hellrigl, 1996; Hewitt, 1999; Minelli *et al.*, 2006). The *brevipennis* group is notable since Apenninian *C. b. brevipennis* approached *C. petryi* from the east.

Where *brevipennis* and *cerdaniensis* song groups met postglacially they exhibit an unusual and puzzling distribution: Inter-group taxa pairs for which we predict common glacial refugia and origins are nowadays not distributed sympatrically but parapatrically or allopatrically (**Figs 4.10 and 4.11B**). Only a few isolated individuals contradict this rule. Parapatric inter-group distribution areas end sharply within a few kilometres in the Apennine Mountains, and we can define contact lines. When populations of the two groups occur in sympatry or even in syntopy they stem from different mtDNA geographical clades. Three known regions show such patterns of associations (**Fig 4.11B**). We currently do not have an explanation for this phenomenon.

Current distribution, ecology and threat

Fig 4.10 shows probable assignments of all records in our database to the new *brevipennis* group taxa (approximately 500 records, 50% from France, 33% from Italy, **Appendix S4.6**). Interestingly, the southern extents of the *brevipennis* group distribution in the Balkan, Apenninian and Iberian peninsulas are at similar latitudes. For the Apennine Mountains, the ridge decreases here to lower altitudes and classically divides the fauna into a central and a southern sector with changing species communities (Baroni Urbani *et al.*, 1977; Minelli *et al.*, 2006; Chapter 2 = Hertach *et al.*, 2015).

Like many other species of the *C. montana* complex, *brevipennis* song group taxa prefer ecotone habitats, e.g. between extensively cut or grazed open land and sparse woodlands. Often *brevipennis* group taxa sing in lower substrates (small bushes, herb layer) than is usually known for *C. montana* s. str. or for the *cerdaniensis* song group (Hertach, 2007; Chapter 6 = Hertach & Nagel, 2013; Delorme *et al.*, 2015). *Cicadetta brevipennis* group species tend to live in drier habitats than *cerdaniensis* group species. However, the ecological differences between the *cerdaniensis* and the *brevipennis* groups are generally small and, as mentioned above, syntopic populations do occur. Indeed, the habitat preferences among the *C. brevipennis* subspecies are remarkable and an additional diagnostic trait (**Table 4.4**).

Our discovery of four different taxa within the former *C. brevipennis* species provides evidence useful for the re-evaluation of endangerment potential (Puissant, 2006; Hertach, 2007; Chapter 6 = Hertach & Nagel, 2013; Delorme *et al.*, 2015). All newly recognised taxa are restricted to rather small or dissected distributional ranges, even *C. petryi*. The highest priority taxon is *C. b. litoralis* ssp. n., a subspecies that seems critically endangered. One of the two known localities (Alénya) has already been destroyed due to land use intensification. We hope that this subspecies is present in coastal Spanish habitats and that the undetermined populations in the French Rhone delta also belong to *C. b. litoralis* ssp. n. (**Fig 4.10**, black dots). The habitat in Torreilles should be protected immediately. Second in priority would be the *C. b. hippolaidica* ssp. n. population from the Monti della Daunia. It exhibits the purest and most extreme characters of this lineage. At third priority are the completely fragmented local populations of *C. petryi* remote from the core populations in southern France. It is rare with regionally restricted habitat requirements in Switzerland, Germany and Italy as well as in northern France (Hertach, 2007; Hugel *et al.*, 2008; Meineke, 2012; Chapter 6 = Hertach & Nagel, 2013; Delorme *et al.*, 2015). Many of these isolated populations appear as relicts of a postglacial warmer period with tens of kilometres between them. *Cicadetta b. brevipennis* needs to be investigated more deeply in the Balkans and in Eastern Europe to judge its distributional area and the potential threats. It seems not to be endangered in central Italy and Slovenia. Last but not least, the Black Sea lineage (bulg) should be investigated in more detail, checking to see if it forms another endemic and possibly endangered species or, for example, a peripheral *C. b. brevipennis* metapopulation with some individuals possessing the mitochondrial genome of *C. cantilatrix*.

CONCLUSIONS

We resolve an intricate pattern of mtDNA haplotype distributions by searching for congruent traits from different data sources in order to draw taxonomic conclusions and present a plausible biogeographic history. The system is easiest to understand when we attach the highest importance to the reproductively most relevant traits such as distinct song patterns. The former polyphyly is divided into the *brevipennis* and *cerdaniensis* song groups, and subtaxa of both groups are now much easier to comprehend geographically and taxonomically. We chose a system-adapted hierarchic approach, continually and consequently searching for lines of evidence of separately evolving metapopulation lineages versus partially isolated geographic varieties. The taxonomic result was a paradox: Introgression helped to find cryptic species. Our approach required the availability of good distributional data and illustrates the importance of more integrated species delimitation procedures.

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REFERENCES CHAPTER 4

Abascal F, Zardoya R, Telford MJ. 2010. TranslatorX: multiple alignment of nucleotide sequences guided by amino acid translations. *Nucleic Acids Research* 38: W7-W13.

Alexander RD, Moore TE. 1962. The evolutionary relationships of 17-year and 13-year cicadas, and three new species (Homoptera, Cicadidae, *Magicicada*). *Miscellaneous Publications of the Museum of Zoology, University of Michigan* 121: 1-59.

Barbanera F, Zuffi MAL, Guerrini M, Gentilli A, Tofanelli S, Fasola M, Dini F. 2009. Molecular phylogeography of the asp viper *Vipera aspis* (Linnaeus, 1758) in Italy: Evidence for introgressive hybridization and mitochondrial DNA capture. *Molecular Phylogenetics and Evolution* 52: 103-114.

Baroni Urbani C, Ruffo S, Vigna Taglianti A. 1977. Materiali per una biogeografia italiana fondata su alcuni generi di coleotteri Cicindelidi, Carabidi e Crisomelidi. *Memorie della Società Entomologica Italiana* 56: 35-92.

Bennik RM, Buckley TR, Hoare RJB, Holwell GI. 2016. Molecular phylogeny reveals the repeated evolution of complex male genital traits in the New Zealand moth genus *Izatha* (Lepidoptera: Xyloryctidae). *Systematic Entomology* 41: 309-322.

Berthier P, Excoffier L, Ruedi M. 2006. Recurrent replacement of mtDNA and cryptic hybridization between two sibling bat species *Myotis myotis* and *Myotis blythii*. *Proceedings of the Royal Society B: Biological Sciences* 273: 3101-3109.

Boitier E, Brugel E. 2006. Les Cigales en Auvergne: un essai de synthèse des connaissances (Hemiptera: Cicadidae). *Arvernsis* 37-38: 7-12.

Boulard M. 1995. Postures de cymbalisation, cymbalisations et cartes d’identité acoustique des cigales. Généralités et espèces méditerranéennes (Homoptera, Cicadoidea). *Ecole pratique des hautes Etudes, Biologie et Evolution des Insectes* 7-8: 1-72.

Boulard M, Mondon B. 1995. *Vie et Mémoires de Cigales. Provence, Languedoc, Méditerranée*. Barbentane: Édition de l’Équinoxe, 159 pp. + CD.

Brower AVZ. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences* 91: 6491-6495.

Buckley TR, Attanayake D, Bradler S. 2009. Extreme convergence in stick insect evolution: phylogenetic placement of the Lord Howe Island tree lobster. *Proceedings of the Royal Society B: Biological Sciences* 276: 1055-1062.

Buckley TR, Cordeiro M, Marshall DC, Simon C. 2006. Differentiating between hypotheses of lineage sorting and introgression in New Zealand alpine cicadas (*Maoricicada* Dugdale). *Systematic Biology* 55(3): 411-425.

Butlin RK, Galindo J, Grahame JW. 2008. Sympatric, parapatric or allopatric: the most important way to classify speciation? *Philosophical Transactions of the Royal Society B: Biological Sciences* 363: 2997-3007.

Carstens BC, Knowles LL. 2007. Estimating species phylogeny from gene-tree probabilities despite incomplete lineage sorting: an example from *Melanoplus* grasshoppers. *Systematic Biology* 56: 400-411.

Carstens BC, Pelletier TA, Reid NM, Satler JD. 2013. How to fail at species delimitation. *Molecular Ecology* 22: 4369-4383.

Chan KMA, Levin SA. 2005. Leaky prezygotic isolation and porous genomes: rapid introgression of maternally inherited DNA. *Evolution* 59: 720-729.

Correggiari A, Roveri M, Trincardi F. 1996. Late-Pleistocene and Holocene evolution of the North Adriatic Sea. *Il Quaternario, Italian Journal of Quaternary Sciences* 9: 697-704.

De Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* 56: 879-886.

Delorme Q, Cury D, Bernier C. 2015. *Tettigetalna argentata* (Olivier, 1790) et les Cigales du groupe *Cicadetta* cf. *montana* (Scopoli, 1772) dans la moitié nord de la France: nouveaux éléments de répartition et d'écologie impliquant la conservation des espèces (Hemiptera Cicadidae). *L'Entomologiste* 71: 31-40.

Den Hollander J. 1995. Acoustic signals as specific mate recognition signals in leafhoppers (Cicadellidae) and planthoppers (Delphacidae) (Homoptera: Auchenorrhyncha). In: Lambert DM, Spencer HG, eds. *Speciation and the recognition concept, theory and application*. Baltimore: Johns Hopkins University Press, 440-463.

Drosopolos S, Eliopoulos E, Tsakalou P. 2006. Is migration responsible for the peculiar geographical distribution and speciation based on acoustic divergence of two cicadas in the Aegean archipelago? In: Drosopoulos S, Claridge M, eds. *Insect sounds and communication: Physiology, behaviour, ecology and evolution.* Boca Raton: CRC Press Taylor & Francis Group, 219-226.

Dugdale JS, Fleming CA. 1978. New Zealand cicadas of the genus *Maoricicada* (Homoptera: Tibicinidae). *New Zealand Journal of Zoology* 5: 295-340.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792-1797.

Feder JL, Flaxman SM, Egan SP, Nosil P. 2013. Hybridization and the build-up of genomic divergence during speciation. *Journal of Evolutionary Biology* 26: 261-266.

Fieber FX. 1876. Les Cicadines d'Europe d'après les originaux et les publications les plus récentes. Deuxième partie: Descriptions des espèces. *Revue et Magazin de Zoologie* 4: 11-268.

Fonseca PJ, Revez MA. 2002. Temperature dependence of cicada songs (Homoptera, Cicadoidea). *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology* 187: 971-976.

Fritz U, Fattizzo T, Guicking D, Tripepi S, Pennisi MG, Lenk P, Joger U, Wink M. 2005. A new cryptic species of pond turtle from southern Italy, the hottest spot in the range of the genus *Emys* (Reptilia, Testudines, Emydidae). *Zoologica Scripta* 34: 351-371.

Funk DJ, Omland KE. 2003. Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics* 34: 397-423.

Gogala A. 2008. Survival of the endemic Hemiptera species in Slovenia during the Holocene. In: Grozeva S, Simov N, eds. *Advances in Heteroptera Research, Pensoft Series Faunistica*. Sofia-Moscow: Pensoft Publishers, 121-128.

Gogala M, Trilar T. 1999. The song structure of *Cicadetta montana macedonica* Schedl with remarks on songs of related singing cicadas (Hemiptera: Auchenorrhyncha: Cicadomorpha: Tibicinidae). *Reichenbachia, Dresden* 33: 91-97.

Gogala M, Trilar T. 2004. Bioacoustic investigations and taxonomic considerations on the *Cicadetta montana* species complex (Homoptera: Cicadoidea: Tibicinidae). *Anais da Academia Brasileira de Ciências* 76: 316-324.

Gogala M, Trilar T, Krpač V. 2014. New subspecies *Cicadetta concinna arachnocepta* (Hemiptera: Cicadidae) from Macedonia. *Acta entomologica slovenica* 22: 101-114.

Gottsberger B, Mayer F. 2007. Behavioral sterility of hybrid males in acoustically communicating grasshoppers (Acrididae, Gomphocerinae). *Journal of Comparative Physiology A* 193: 703-714.

Grant PR, Grant BR. 1995. The founding of a new population of Darwin's Finches. *Evolution* 49: 229-240.

Gurcel K. 2015. Contribution à la connaissance des Cigales de France: première synthèse des observations et répartition des espèces pour le département de la Haute-Savoie (Hemiptera Cicadidae). *L'Entomologiste* 71: 245-260.

Gvoždík V, Canestrelli D, García-París M, Moravec J, Nascetti G, Recuero E, Teixeira J, Kotlík P. 2015. Speciation history and widespread introgression in the European short-call tree frogs (*Hyla arborea* sensu lato, *H. intermedia* and *H. sarda*). *Molecular Phylogenetics and Evolution* 83: 143-155.

Hanski I, Gilpin M. 1991. Metapopulation dynamics: brief history and conceptual domain. *Biological journal of the Linnean Society* 42: 3-16.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B: Biological Sciences* 270: 313-321.

Hellrigl K. 1996. *Die Tierwelt Südtirols.* Bozen: Naturmuseum Südtirol, 831 pp.

Hertach T. 2007. Three species instead of only one: Distribution and ecology of the *Cicadetta montana* species complex (Hemiptera: Cicadoidea) in Switzerland. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 80: 37-61.

Hertach T. 2011. Spectacular song pattern from the Sicilian Mountains: The new cicada species *Cicadetta anapaistica* sp. n. (Hemiptera: Cicadidae). *Zootaxa* 2771: 25-40.

Hertach T, Nagel P. 2013. Cicadas in Switzerland: a scientific overview of the historic and current knowledge of a popular taxon (Hemiptera: Cicadidae). *Revue suisse de Zoologie* 120: 229-269.

Hertach T, Trilar T, Wade EJ, Simon C, Nagel P. 2015. Songs, genetics, and morphology: revealing the taxonomic units in the European *Cicadetta cerdaniensis* cicada group, with a description of new taxa (Hemiptera: Cicadidae). *Zoological Journal of the Linnean Society* 173: 320-351.

Hewitt GM. 1999. Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* 68: 87-112.

Hewitt GM. 2000. The genetic legacy of the Quaternary ice ages. *Nature* 405: 907-913.

Hewitt GM. 2001. Speciation, hybrid zones and phylogeography – or seeing genes in space and time. *Molecular Ecology* 10: 537-549.

Hey J. 2006. On the failure of modern species concepts. *Trends in Ecology & Evolution* 21: 447-450.

Hey J, Pinho C. 2012. Population genetics and objectivity in species diagnosis. *Evolution; International Journal of Organic Evolution* 66: 1413-1429.

Hugel S, Matt F, Callot H, Feldtrauer JJ, Brua C. 2008. Présence de *Cicadetta brevipennis* Fieber, 1876 en Alsace (Hemiptera, Cicadidae). *Bulletin de la Société Entomologique de Mulhouse* 64: 5-10.

ICZN (International Commission on Zoological Nomenclature). 1999. *International Code of Zoological Nomenclature.* 4th edition. Available from: http://iczn.org (accessed 16 June 2015).

Klasa A, Bokłak E. 2015. Cykada *Cicadetta cantilatrix* w Ojcowskim Parku Narodowym – Cicada *Cicadetta cantilatrix* in the Ojców National Park. *Chrońmy Przyrodę Ojczystą* 71: 108-115.

Lanfear R, Calcott B, Ho SYW, Guindon S. 2012. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695-1701.

Lecocq T, Dellicour S, Michez D, Lhomme P, Vanderplanck M, Valterová I, Rasplus JY, Rasmont P. 2013. Scent of a break-up: phylogeography and reproductive trait divergences in the red-tailed bumblebee (*Bombus lapidarius*). *BMC Evolutionary Biology* 13: 263.

Mallet J. 2008. Hybridization, ecological races and the nature of species: empirical evidence for the ease of speciation. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 363: 2971-2986.

Marshall DC, Hill KBR, Cooley JR, Simon C. 2011. Hybridization, mitochondrial DNA phylogeography, and prediction of the early stages of reproductive isolation: Lessons from New Zealand cicadas (Genus *Kikihia*). *Systematic Biology* 60: 482-502.

Marshall DC, Hill KBR, Fontaine KM, Buckley TR, Simon C. 2009. Glacial refugia in a maritime temperate climate: Cicada (*Kikihia subalpina*) mtDNA phylogeography in New Zealand. *Molecular Ecology* 18: 1995-2009.

Martin SH, Dasmahapatra KK, Nadeau NJ, Salazar C, Walters JR, Simpson F, Blaxter M, Manica A, Mallet J, Jiggins CD. 2013. Genome-wide evidence for speciation with gene flow in *Heliconius* butterflies. *Genome Research* 23: 1817-1828.

Meineke T. 2012. Bergsingzikaden *Cicadetta cantilatrix* Sueur & Puissant, 2007, *Cicadetta brevipennis* Fieber, 1876 und *Cicadetta montana* s. str. (Scopoli, 1772) im mittleren Deutschland (Auchenorrhyncha, Cicadidae, Cicadettinae). *Entomologische Nachrichten und Berichte* 56: 133-142.

Melo-Ferreira J, Boursot P, Suchentrunk F, Ferrand N, Alves PC. 2005. Invasion from the cold past: extensive introgression of mountain hare (*Lepus timidus*) mitochondrial DNA into three other hare species in northern Iberia. *Molecular Ecology* 14: 2459-2464.

Minelli A, Ruffo S, Vigna Taglianti A. 2006. The Italian faunal provinces. In: Ruffo S, Stoch F, eds. *Checklist and distribution of the Italian fauna – 10,000 terrestrial and inland water species.* Memorie del Museo Civico di Storia Naturale di Verona, 2. Serie, Sezione Scienza della Vita 17, 37-39.

Moore W, Robertson JA. 2014. Explosive adaptive radiation and extreme phenotypic diversity within ant-nest beetles. *Current biology* 24: 2435-2439.

Moulds MS. 2005. An appraisal of the higher classification of cicadas (Hemiptera: Cicadoidea) with special reference to the Australian fauna. *Records of the Australian Museum* 57: 375-446.

Nicholls JA, Challis RJ, Mutun S, Stone GN. 2012. Mitochondrial barcodes are diagnostic of shared refugia but not species in hybridizing oak gallwasps. *Molecular Ecology* 21: 4051-4062.

Nunes VL, Mendes R, Marabuto E, Novais BM, Hertach T, Quartau JA, Seabra SG, Paulo OS, Simões PC. 2014. Conflicting patterns of DNA barcoding and taxonomy in the cicada genus *Tettigettalna* from southern Europe (Hemiptera: Cicadidae). *Molecular Ecology Resources* 14: 27-38.

Owen CL, Marshall DC, Hill KBR, Simon C. 2015. The phylogenetic utility of acetyltransferase (ARD1) and glutaminyl tRNA synthetase (QtRNA) for reconstructing Cenozoic relationships as exemplified by the large Australian cicada *Pauropsalta* generic complex. *Molecular Phylogenetics and Evolution* 83: 258-277.

Papadopoulou A, Anastasiou I, Vogler AP. 2010. Revisiting the insect mitochondrial molecular clock: The mid-Aegean trench calibration. *Molecular Biology and Evolution* 27: 1659-1672.

Paterson HEH. 1985. The recognition concept of species. In: Vrba ES, ed. *Species and Speciation.* Pretoria: Transvaal Museum, Monograph No. 4, 21-29.

Pfeifer MA. 2015. Die Berg-Singzikaden-Art *Cicadetta cantilatrix* Sueur & Puissant 2007 (Auchenorrhyncha: Cicadidae) in Rheinland-Pfalz. *Fauna und Flora in Rheinland-Pfalz* 13: 121-124.

Pinho C, Hey J. 2010. Divergence with gene flow: models and data. *Annual Review of Ecology, Evolution, and Systematics* 41: 215-230.

Podnar M, Mayer W, Tvrtković N. 2005. Phylogeography of the Italian wall lizard, *Podarcis sicula*, as revealed by mitochondrial DNA sequences. *Molecular Ecology* 14: 575-588.

Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* 55: 595-609.

Popov AV, Beganović A, Gogala M. 1997. Bioacoustics of singing cicadas of the Western Palearctic: *Tettigetta brullei* (Fieber 1876) (Cicadoidea: Tibicinidae). *Acta entomologica slovenica* 5: 89-101.

Popple LW. 2013. A revision of the *Pauropsalta annulata* Goding & Froggatt species group (Hemiptera: Cicadidae) based on morphology, calling songs and ecology, with investigations into calling song structure, molecular phylogenetic relationships and a case of hybridisation between two subspecies. *Zootaxa* 3730: 1-102.

Puissant S. 2001. Eco-Ethologie de *Cicadetta montana* (Scopoli, 1772) en France (Auchénorhyncha, Cicadidae, Cicadettini). *Ecole pratique des hautes Etudes, Biologie et Evolution des Insectes* 14: 141-155.

Puissant S. 2006. *Contribution à la connaissance des cigales de France: Géonémie et écologie des populations (Hemiptera, Cicadidae).* Bédeilhac/Aynat: Association pour la Caractérisation et l'Etude des Entomocénoses, 193 pp.

Puissant S, Boulard M. 2000. *Cicadetta cerdaniensis*, espèce jumelle de *Cicadetta montana* décryptée par l'acoustique (Auchenorhyncha, Cicadidae, Tibicinidae). *Ecole pratique des hautes Etudes, Biologie et Evolution des Insectes* 13: 111-117.

Puissant S, Sueur J. 2010. A hotspot for Mediterranean cicadas (Insecta: Hemiptera: Cicadidae): new genera, species and songs from southern Spain. *Systematics and Biodiversity* 8: 555-574.

Quartau JA, Simões PC. 2006. Acoustic evolutionary divergence in cicadas: the species of *Cicada* L. in Southern Europe. In: Drosopoulos S, Claridge M, eds. *Insect sounds and communication: Physiology, behaviour, ecology and evolution.* Boca Raton: CRC Press Taylor & Francis Group, 227-237.

R Development Core Team. 2008. *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

Rambaut A, Drummond A. 2007. *Tracer v1.5.* Available from: http://tree.bio.ed.ac.uk/software/tracer/ (accessed 15 January 2016).

Ronquist F, Teslenko M, Mark P van der, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539-542.

Schilthuizen M. 2002. *Frogs, flies and dandelions: The making of species*. New York, NY: Oxford University Press, USA, 256 pp.

Schlick-Steiner BC, Steiner FM, Seifert B, Stauffer C, Christian E, Crozier RH. 2010. Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology* 55: 421-438.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671-675.

Schumacher F. 1924. Auftreten der Bergzikaden in der Mark Brandenburg und Übersicht über die deutschen Formen (Hem. Homopt.). *Berliner Entomologische Zeitschrift* 4: 329-334.

Shackleton JC, van Andel TH, Runnels CN. 1984. Coastal paleogeography of the Central and Western Mediterranean during the last 125,000 years and its archaeological implications. *Journal of Field Archaeology* 11: 307-314.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Floors P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87: 651-701.

Smadja CM, Butlin RK. 2011. A framework for comparing processes of speciation in the presence of gene flow. *Molecular Ecology* 20: 5123-5140.

Solís-Lemus C, Knowles LL, Ané C. 2015. Bayesian species delimitation combining multiple genes and traits in a unified framework. *Evolution* 69: 492-507.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics (Oxford, England)* 30: 1312-1313.

Stebbins GL. 1950. *Variation and evolution in plants*. New York, NY: Columbia Univerity Press, 643 pp.

Sueur J, Aubin T. 2003. Specificity of cicada calling songs in the genus *Tibicina* (Hemiptera: Cicadidae). *Systematic Entomology* 28: 481-492.

Sueur J, Aubin T, Simonis-Sueur C. 2008. Seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics* 18(2): 213-226.

Sueur J, Puissant S. 2007. Similar look but different song: a new *Cicadetta* species in the *montana* complex (Insecta, Hemiptera, Cicadidae). *Zootaxa* 1442: 55-68.

Sullivan J, Demboski JR, Bell KC, Hird S, Sarver B, Reid N, Good JM. 2014. Divergence with gene flow within the recent chipmunk radiation (*Tamias*). *Heredity* 113: 185-194.

Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* 7: 453-464.

Tobias JA, Seddon N, Spottiswoode CN, Pilgrim JD, Fishpool LDC, Collar NJ. 2010. Quantitative criteria for species delimitation. *Ibis* 152: 724-746.

Trilar T, Gogala M. 2012. Three species of Mountain Cicadas (*Cicadetta montana* complex) found in Hungary with bioacoustic methods (Hemiptera: Cicadidae). *Folia Entomologica Hungarica* 73: 9-14.

Trilar T, Gogala M, Popa V. 2006. Contribution to the knowledge of the singing cicadas (Auchenorrhycha: Cicadoidea) of Romania. *Acta entomologica slovenica* 14: 175-182.

Trilar T, Hertach T. 2008. Three species of Mountain Cicadas *Cicadetta montana* (sensu lato) found in northern Italy. *Bulletin of Insectology* 61: 185-186.

Trilar T, Holzinger WE. 2004. Bioakustische Nachweise von drei Arten des *Cicadetta montana*-Komplexes aus Österreich (Insecta: Hemiptera: Cicadoidea). *Linzer biologische Beiträge* 36: 1383-1386.

Via S. 2012. Divergence hitchhiking and the spread of genomic isolation during ecological speciation-with-gene-flow. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 367: 451-460.

Villet M. 1995. Intraspecific variability in SMRS signal: some causes and implications in acoustic signalling systems. In: Lambert DM, Spencer HG, eds. *Speciation and the recognition concept, theory and application*. Baltimore: Johns Hopkins University Press, 422-439.

Wade EJ, Hertach T, Gogala M, Trilar T, Simon C. 2015. Molecular species delimitation methods recover most song-delimited cicada species in the European *Cicadetta montana* complex. *Journal of Evolutionary Biology* 28: 2318-2336.

Warren BH, Bermingham E, Bourgeois Y, Estep LK, Prys-Jones RP, Strasberg D, Thébaud C. 2012. Hybridization and barriers to gene flow in an island bird radiation. *Evolution* 66: 1490-1505.

Wickham H. 2009. *ggplot2: Elegant graphics for data analysis.* New York: Springer, VIII + 213 pp.

Wiens JJ. 2007. Species delimitation: new approaches for discovering diversity. *Systematic Biology* 56: 875-878.

Yang Z, Rannala B. 2010. Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences* 107: 9264-9269.

Yeates DK, Seago A, Nelson L, Cameron SL, Joseph L, Trueman JWH. 2011. Integrative taxonomy, or iterative taxonomy? *Systematic Entomology* 36: 209-217.

Zunino M, Zullini A. 2004. *Biogeografia. La dimensione spaziale dell'evoluzione*. 2nd ed. Milano: Casa Editrice Ambrosiana, 374 pp.

APPENDIXES

Appendix S4.1. Spatial origin of the specimens analysed acoustically, morphologically or molecularly.

Appendix S4.2. Maximum likelihood mtDNA phylogeny (*COI* and *COII*) with bootstrap values from RAxML analysis for the *Cicadetta brevipennis* group.

Appendix S4.3. Maximum likelihood mtDNA phylogeny (*COI* and *COII*) with bootstrap values from RAxML analysis for the *Cicadetta brevipennis* and *Cicadetta cerdaniensis* groups.

Appendix S4.4. Power differences between phrases 1 and 2 in a spatial context.

Appendix S4.5. Nomenclature of *Cicadetta brevipennis*.

Appendix S4.6. Database of the records in the *Cicadetta brevipennis* group.

Appendix S4.1. Spatial origin of the specimens analysed acoustically, morphologically or molecularly.

(ordered by taxon name → country → latitude; sheet 1)

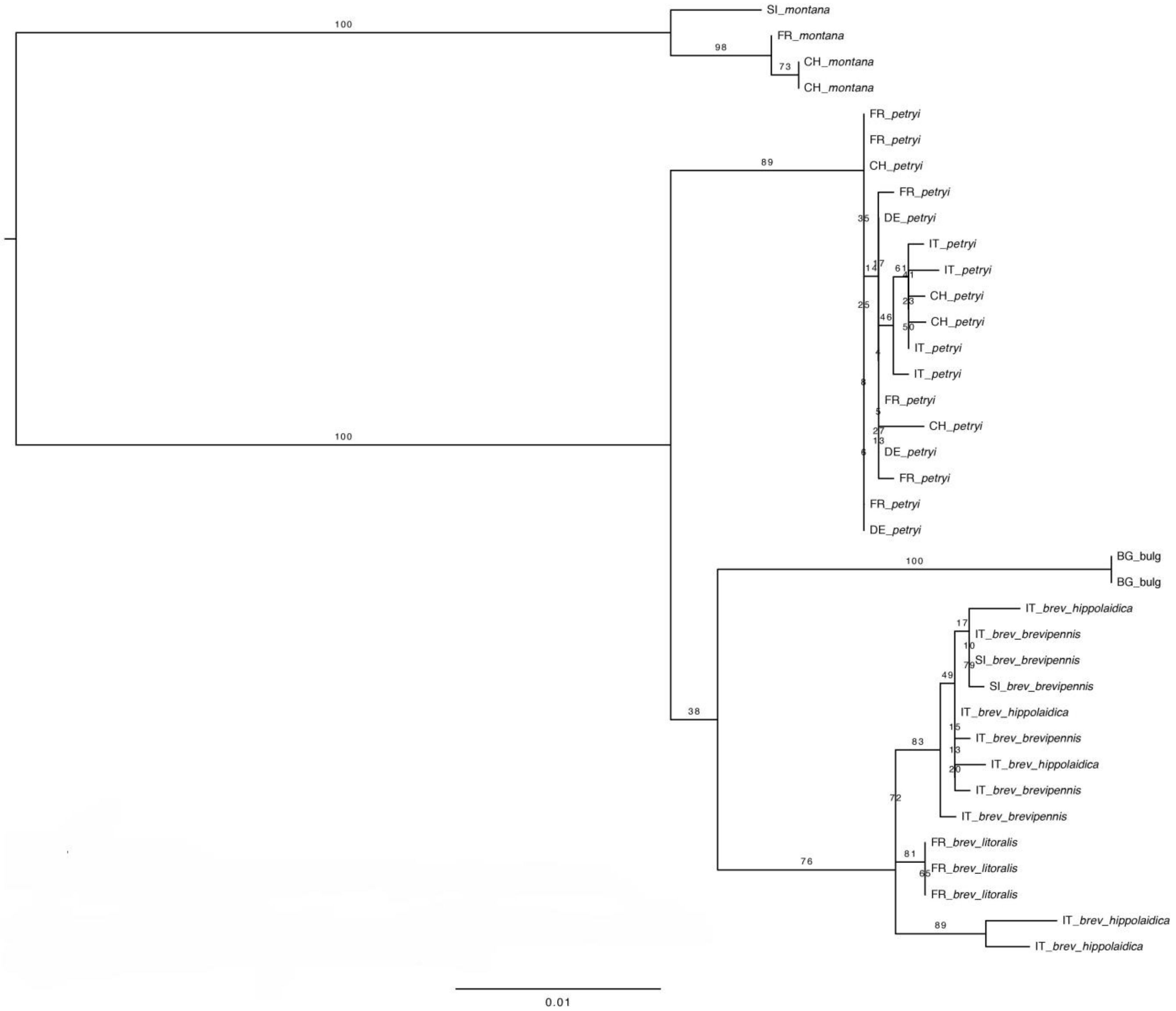
										Number of analysed specimens				
Taxon	**Location, region**	**Political region**	**Country**	**Altitude [m asl]**	**Lat.**	**Long.**	**Observer**	**Date**	**Collection**	**morphology**	**mtDNA**	**1. song phrase**	**2. song phrase**	**occurrence of the second phrase**
Cicadetta brevipennis brevipennis	Glanz an der Weinstrasse	Styria	Austria	400	46.6560	15.5220	Kunz/Gogala/Trilar	diverse	Kunz	2		3		6
Cicadetta brevipennis brevipennis	Velika, Papuk	Požeško slavonska	Croatia	380	45.4624	17.6400	Trilar/Gogala	30.05.2012						2
Cicadetta brevipennis brevipennis	NW Melezzole	Umbria	Italy	680	42.6762	12.3281	Thomas Hertach	13.07.2011						1
Cicadetta brevipennis brevipennis	Poggie le Perazzete, Monte Amiata	Tuscany	Italy	870	42.8235	11.6453	Thomas Hertach	15.07.2011				2		3
Cicadetta brevipennis brevipennis	Monte Ceci, Colline Metallifere	Tuscany	Italy	310	43.1110	10.6525	Thomas Hertach	13.07.2012				1		2
Cicadetta brevipennis brevipennis	Fontazzi, Murlo	Tuscany	Italy	200	43.1677	11.2955	Thomas Hertach	08.07.2012					0.5	3
Cicadetta brevipennis brevipennis	S Bivio Ercole, Fiuminata	Marches	Italy	550	43.1731	12.8867	Thomas Hertach	06.07.2012				2	0.5	3
Cicadetta brevipennis brevipennis	Crevole, Murlo	Tuscany	Italy	290	43.1839	11.3720	Thomas Hertach	08.07.2012				1		2
Cicadetta brevipennis brevipennis	Busco, Cortona	Tuscany	Italy	590	43.2466	12.0973	Thomas Hertach	07.07.2012						2
Cicadetta brevipennis brevipennis	Foresta di Monterufoli	Tuscany	Italy	500	43.2488	10.7485	Thomas Hertach	14.07.2012	Hertach	2	2	1	1	2
Cicadetta brevipennis brevipennis	N Castellina Marittima	Tuscany	Italy	400	43.4217	10.5833	Thomas Hertach	11.07.2008						2
Cicadetta brevipennis brevipennis	W San Gimignano	Tuscany	Italy	530	43.4561	10.9512	Thomas Hertach	14.07.2012						2
Cicadetta brevipennis brevipennis	Colline Livornesi, Livorno	Tuscany	Italy	350	43.5117	10.4267	Thomas Hertach	13.07.2008	Hertach	1	1			
Cicadetta brevipennis brevipennis	Monte Acuto, Cantiano	Marches	Italy	760	43.5126	12.6554	Thomas Hertach	05.07.2012				1		4
Cicadetta brevipennis brevipennis	Colombara - Serravalle di Carda	Marches	Italy	590	43.5587	12.4608	Thomas Hertach	05.07.2012				1		4
Cicadetta brevipennis brevipennis	Passo dell'Incisa, Alfero	Emilia-Romagna	Italy	820	43.8575	12.0451	Thomas Hertach	04.07.2012						1
Cicadetta brevipennis brevipennis	E Corniolo, Casentino-Falterona	Emilia-Romagna	Italy	470	43.9051	11.8154	Thomas Hertach	04.07.2012						2
Cicadetta brevipennis brevipennis	Fruška gora, Bešenovački	Vojvodina	Serbia	215	45.1145	19.7131	Gogala/Trilar	04.06.2015				(1)		
Cicadetta brevipennis brevipennis	Golo, Stražar	Osrednjeslovenska	Slovenia	760	45.9125	14.5151	Tomi Trilar	18.06.2013				1		1
Cicadetta brevipennis brevipennis	Log, Lukovica	Osrednjeslovenska	Slovenia	330	46.0090	14.3590	Matija Gogala	diverse	PMSL	10	2	2	2	3
Cicadetta brevipennis brevipennis	Dol pri Hrastniku	Zasavska	Slovenia	400	46.1450	15.1141	Gogala/Trilar/Kapla	06.06.1999	PMSL	3		1		2
Cicadetta brevipennis hippolaidica	N San Bartolomeo in Galdo	Campania	Italy	590	41.4533	15.0385	Thomas Hertach	20.07.2010					1	1
Cicadetta brevipennis hippolaidica	Monte Sambuco, Monti della Daunia	Apulia	Italy	790-920	41.5311	15.0825	Thomas Hertach	diverse	NHMB/NMBE/Hertach	6	3	6	12	27
Cicadetta brevipennis hippolaidica	N Cerasuolo, Le Mainarde	Lazio	Italy	780	41.5953	14.0225	Thomas Hertach	03.07.2011						3
Cicadetta brevipennis hippolaidica	Lago Selva, Cardito, Le Mainarde	Lazio	Italy	930	41.6071	13.9758	Thomas Hertach	03.07.2011	Hertach	1		2	1	3
Cicadetta brevipennis hippolaidica	E Picinisco, Le Mainarde	Lazio	Italy	1200	41.6527	13.9037	Thomas Hertach	03.07.2011	Hertach	1				
Cicadetta brevipennis hippolaidica	S Pietrabbondante	Molise	Italy	960	41.7158	14.3767	Thomas Hertach	02.07.2011	NMBE	1				
Cicadetta brevipennis hippolaidica	NW Civitacampomarano	Molise	Italy	850	41.7972	14.6506	Thomas Hertach	01.07.2011				2	0.5	4
Cicadetta brevipennis hippolaidica	N Pescasseroli	Abruzzo	Italy	1260	41.8564	13.7847	Thomas Hertach	04.07.2011	NMBE/Hertach	2		1	1	2
Cicadetta brevipennis hippolaidica	N Torrebruna	Abruzzo	Italy	750	41.8846	14.5297	Thomas Hertach	01.07.2011	Hertach	3	2	1	1.5	5
Cicadetta brevipennis litoralis	Alénya	Pyrénées Orientales (66)	France	4	42.65XX	2.99XX	Stéphane Puissant	31.05.1999	Puissant	2				
Cicadetta brevipennis litoralis	Torreilles	Pyrénées Orientales (66)	France	2	42.76XX	3.02XX	Stéphane Puissant	diverse	MNHN/PMSL/Puissant/Hertach	21	3	4	6	5
Cicadetta brevipennis s.l. ("bulg?")	SE Meden Buk	Haskovo Province	Bulgaria	110	41.3697	26.0522	Gogala/Gjonov	06.07.2012						2
Cicadetta brevipennis s.l. ("bulg?")	E Melnik	Blagoevgrad Province	Bulgaria	520	41.5247	23.4224	Gogala/Trilar/Gjonov	18.06.2009						2
Cicadetta brevipennis s.l. ("bulg?")	NE Kalovo, Strandzha	Burgas Province	Bulgaria	160	42.1307	27.4930	Gogala/Trilar/Gjonov	22.06.2009						2
Cicadetta brevipennis s.l. ("bulg?")	Mladezhko, Strandzha	Burgas Province	Bulgaria	210	42.1517	27.3618	Gogala/Trilar/Gjonov	24.06.2009						2
Cicadetta brevipennis s.l. ("bulg")	Batovo	Dobrich Province	Bulgaria	120	43.4161	27.9414	Ilia Gjonov	23.06.2014	Hertach	2	2			2
Cicadetta brevipennis s.l. ("bulg?")	Rezervatie Padurea Hagieni, Albeşti	Constanţa	Romania	20	43.8024	28.4501	Gogala/Trilar/Popa	26.06.2004	PMSL	2				

Appendix S4.1. Spatial origin of the specimens analysed acoustically, morphologically or molecularly.

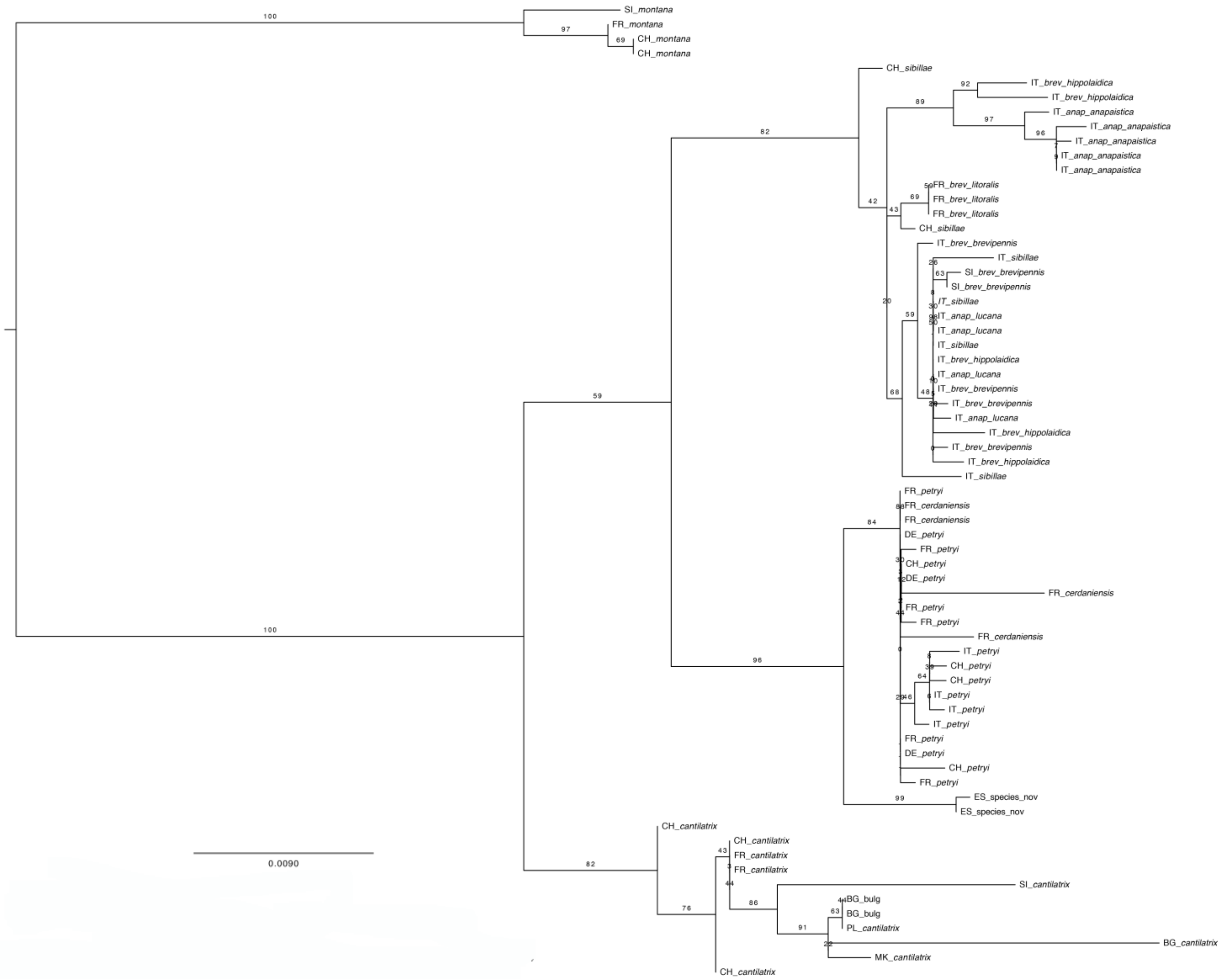
(ordered by taxon name → country → latitude; sheet 2)

										Number of analysed specimens				
Taxon	**Location, region**	**Political region**	**Country**	**Altitude [m asl]**	**Lat.**	**Long.**	**Observer**	**Date**	**Collection**	morphology	mtDNA	1. song phrase	2. song phrase	occurrence of the second phrase
Cicadetta brevipennis s.l. (Hybrid?)	Travesio	Friuli–Venezia Giulia	Italy	470	46.2058	12.8590	Thomas Hertach	12.06.2010				1		1
Cicadetta brevipennis s.l. (Hybrid?)	Avaglio near Villa Santina	Friuli–Venezia Giulia	Italy	680	46.4280	12.9155	Thomas Hertach	08.06.2010	Hertach	1	1			
Cicadetta brevipennis	Cona di Selvapiana, Monti Lepini	Lazio	Italy	570	41.5720	13.1351	Thomas Hertach	24.07.2010				2		3
Cicadetta brevipennis	Forca di Penne, Villa Santa Lucia degli Abruzzi	Abruzzo	Italy	920	42.2827	13.8221	Thomas Hertach	06.07.2011						2
Cicadetta brevipennis	SE Campotosto, Monti della Laga	Abruzzo	Italy	1370	42.5366	13.4013	Thomas Hertach	11.07.2011				1		3
Cicadetta petryi	Badebany, Fuilla	Pyrénées Orientales (66)	France	640	42.5749	2.3610	Thomas Hertach	15.06.2011	Hertach	1	1	3		3
Cicadetta petryi	Duilhac-sous-Peyrepertuse (castle)	Aude (11)	France	670	42.8700	2.5570	Stéphane Puissant	20.07.2001	Puissant	1				
Cicadetta petryi	Saint-Félix-de-l'Héras	Hérault (34)	France	630	43.8311	3.3065	Matthieu Aubert	11.07.2013	Hertach	1	1			
Cicadetta petryi	Châlet Reynard, Mt. Ventoux	Vaucluse (84)	France	1400	44.1510	5.3190	Stéphane Puissant	29./30.06.1998	Puissant	3				
Cicadetta petryi	Sérignan-du-Comtat	Vaucluse (84)	France	150	44.2033	4.8353	Puissant/Boulard/Yense	diverse	MNHN/Puissant	11				
Cicadetta petryi	Lagarde-Paréol	Vaucluse (84)	France		44.22XX	4.85XX	Michel Boulard	10.07.1978	MNHN	1				
Cicadetta petryi	Mostuéjouls, Gorges du Tarn	Aveyron (12)	France	410	44.2215	3.2225	Cédric Mroczko	07.07.2013	Hertach	1	1			
Cicadetta petryi	Col d'Anelle, Mercantour	Alpes-Maritimes (06)	France	1730	44.2730	6.8910	Hannes Baur	24.06.2012	NMBE	1	1			
Cicadetta petryi	Demafé, Aspres-sur-Buëch	Haut-Alpes (05)	France	785	44.5421	5.7784	Kevin Gurcel	30.06.2001	Gurcel	1				
Cicadetta petryi	Bouthoux, Clelles	Isère (38)	France	820	44.8131	5.6291	Kevin Gurcel	15.07.2011	Gurcel	1				
Cicadetta petryi	Les Calloudes, Montmélian	Savoie (73)	France	500	45.5087	6.0475	Kevin Gurcel	12.07.2013	Gurcel	1				
Cicadetta petryi	Combefolle, Saint-Jean-de-la-Porte	Savoie (73)	France	770	45.5601	6.1116	Kevin Gurcel	14.06.2009	Gurcel	1				
Cicadetta petryi	Le Rosay, Saint-Ferréol	Haut-Savoie (74)	France	530	45.7624	6.3206	Kevin Gurcel	09.06.2012	Gurcel	1				
Cicadetta petryi	Les Savioz, Marlens	Haut-Savoie (74)	France	830	45.7698	6.3321	Kevin Gurcel	diverse	Gurcel	2				
Cicadetta petryi	Montagne des Princes, Val de Fier	Haut-Savoie (74)	France	900	45.9442	5.8782	Kevin Gurcel	diverse	Gurcel	3				
Cicadetta petryi	Le Rucher-Les Vorziers, Vanzy	Haut-Savoie (74)	France	320	46.0214	5.8714	Kevin Gurcel	12.06.2013	Gurcel	1				
Cicadetta petryi	Le Mont, Chaumont	Haut-Savoie (74)	France	600	46.0378	5.9570	Kevin Gurcel	18.06.2007	MNHN/PMSL/Puissant/Hertach	1				
Cicadetta petryi	Pougny, Pays de Gex	Ain (01)	France	360	46.1406	5.9343	Thomas Hertach	25.05.2011				1		2
Cicadetta petryi	Grasberg, Bergheim	Haut-Rhin (68)	France	280	48.2125	7.3586	Thomas Hertach	23.05.2009	Hertach	1	1	1		4
Cicadetta petryi	La Roche-Guyon, Haut-Isle	Val d'Oise (95)	France	75	49.0850	1.6420	Jérôme Sueur	29.06.2006	Puissant	3	1			
Cicadetta petryi	Frankenhausen, Kyffhäuser	Thuringia	Germany	150-210	51.3638	11.0985	Hertach/Petry	diverse	ZMHB/Hertach	5	3	3		4
Cicadetta petryi	Capanne di Cosola, Monte Lesima	Emilia-Romagna	Italy	1420	44.6679	9.2052	Thomas Hertach	22.06.2012				1		1
Cicadetta petryi	Corbesassi, Brallo di Pregola	Lombardy	Italy	1000	44.7077	9.2653	Thomas Hertach	04.07.2013				1		3
Cicadetta petryi	Monte d'Alpe/Passo del Penice	Lombardy	Italy	1050	44.8022	9.3149	Thomas Hertach	03.07.2013	Hertach	4	3		1	3
Cicadetta petryi	SE Epinel, Cogne	Aosta	Italy	1510	45.6214	7.3334	Thomas Hertach	14.06.2012	Hertach	2	1	1		
Cicadetta petryi	Pont El, Cogne	Aosta	Italy	900	45.6783	7.2207	Thomas Hertach	13.06.2012	Hertach	1		2		2
Cicadetta petryi	S Doues, Valpelline	Aosta	Italy	1120	45.8083	7.3067	Trilar/Hertach	10.06.2007						2
Cicadetta petryi	Monte Barro, Lecco	Lombardy	Italy	720	45.8288	9.3800	Thomas Hertach	02.07.2013						2
Cicadetta petryi	NE Valpelline	Aosta	Italy	1150	45.8300	7.3317	Trilar/Hertach	10.06.2007						1
Cicadetta petryi	Ejulve-Villarluengo	Aragon	Spain	1480	40.7532	-0.6037	Stéphane Puissant	7./9.06.2015	Puissant	(7)		(2)		
Cicadetta petryi	Monte San Giorgio, Meride	Ticino	Switzerland	1040	45.9128	8.9491	Thomas Hertach	diverse	Hertach	8	2	3		7
Cicadetta petryi	Bois de Treulaz, Aire-la-Ville	Geneva	Switzerland	380	46.1880	6.0345	Thomas Hertach	20.05.2011	Hertach	1				3
Cicadetta petryi	Mauregard, Russin	Geneva	Switzerland	380	46.1969	6.0270	Hertach/Trilar/Gurcel	diverse	PMSL/Hertach/Gurcel	4	1	1	1	4
Cicadetta petryi	Bois de la Grille, Vernier	Geneva	Switzerland	410	46.2145	6.0974	Trilar/Hertach	diverse	PMSL/Hertach	5		1	2	2
Cicadetta petryi	Les Vaux, Arnex-sur-Orbe	Vaud	Switzerland	480	46.6843	6.5195	Thomas Hertach	diverse	Hertach	6	1	2		4

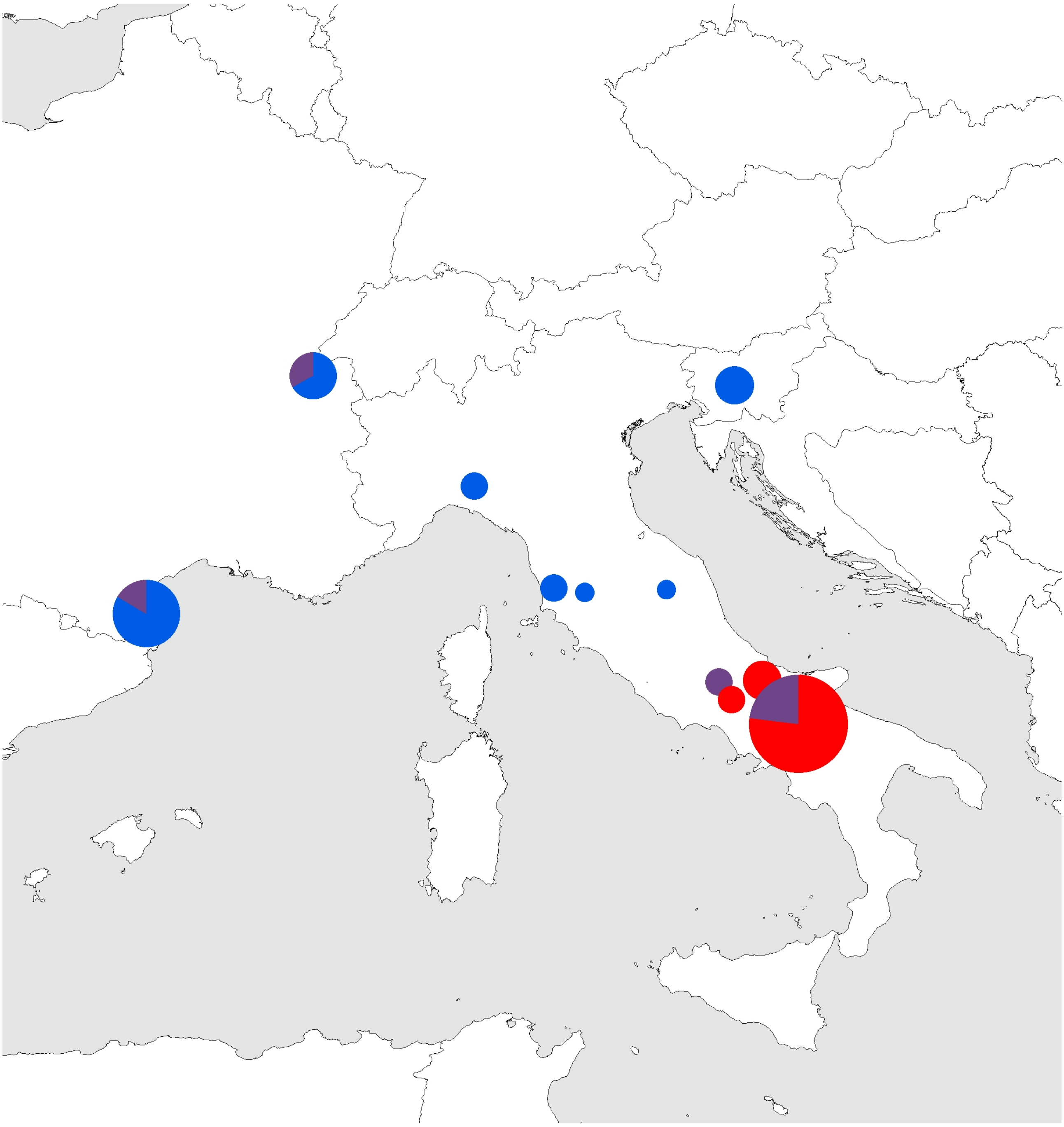
Appendix S4.2. Maximum likelihood mtDNA phylogeny (*COI* and *COII*) with bootstrap values from RAxML analysis for the *Cicadetta brevipennis* group.



Appendix S4.3. Maximum likelihood mtDNA phylogeny (*COI* and *COII*) with bootstrap values from RAxML analysis for the *Cicadetta brevipennis* and *Cicadetta cerdaniensis* groups.



Appendix S4.4. Power differences between phrases 1 and 2 in a spatial context.



Significant differences between *Cicadetta brevipennis hippolaidica* ssp. n. and other taxa. $EP_{L/5}$ - EP_{2L} blue > 12.3 dB, red < 11.7 dB, violet = overlap. Size of circles relative to the number of investigated individuals (n_{max} = 13).

Appendix S4.5. Nomenclature of *Cicadetta brevipennis.*

Fieber (1876) described *C. montana* var. *longipennis* and *C. montana* var. *brevipennis* in his comprehensive taxonomic work on European Auchenorrhyncha, which was posthumously printed in a French translation by Reiber. The names '*longipennis*' and '*brevipennis*' are available (Art. 10.2; ICZN, 1999). Both taxa were classified as synonyms of *Cicadetta montana* (s. l.) for several decades. Gogala & Trilar (2004) regarded '*brevipennis*' as a valid taxon of the binary long-short-echeme song pattern and raised the variety to species rank. They mainly referred to Slovenian populations (e.g. figure with oscillogram) where the OTU brev was found within our study. Since Fieber did not describe songs, we have learned after the publication by Gogala & Trilar (2004) that Fieber's detailed but complicated morphological descriptions of both, '*brevipennis*' and '*longipennis*', more or less apply to all seven acoustically defined species subsequently discovered in this complex. His intraspecific boundaries are within the interspecific variability. Thus, *C. montana* var. *brevipennis* sensu Fieber and *C. brevipennis* sensu Gogala & Trilar is perhaps not the same. It is even likely that in Fieber's original type series specimens of multiple song-defined taxa have been treated.

Fieber did not provide a precise type locality but described the distribution area as (together with *C. montana* var. *longipennis*): "Distributed in whole Europe, rarer in northern parts, additionally found on the Crimea peninsula, the Ural Mountains and Siberia". His unpublished colour plates preserved in the National Museum of Natural History Paris (Soulier-Perkins, pers. comm.) do not contribute important new morphological information; the basal junction of the anal veins is not visible. At least a notice indicates that one drawn specimen originates from the Graz region in Austria (Styria) where OTU brev in fact occurs (Trilar & Holzinger, 2004 and this work: population at Glanz an der Weinstrasse 'GL'; see **Fig 4.5C**). Two other species have been found (*C. montana* s. str.; Trilar & Holzinger, 2004) or can be expected (*C. cantilatrix*) in Styria with respect to biogeographical evidence. However, Fieber's description does not match our OTU brev specimens (see below) nor our *C. cantilatrix* from the whole distribution area. In both taxa, more or less the same characters are either predominantly appropriate or rarely true. *Cicadetta montana* s. str. seems to be congruent with Fieber's *Cicadetta megerlei* and can therefore be excluded from the debatable species.

The name *Cicadetta brevipennis* sensu Gogala & Trilar has been accepted and consequently used by subsequent entomologists during the last ten years (e.g. Trilar & Holzinger, 2004; Puissant, 2006; Boitier & Brugel, 2006; Hertach, 2007; Sueur & Puissant, 2007; Hugel *et al.*, 2008; Meineke, 2012; Delorme *et al.*, 2015; Gurcel, 2015). A type specimen has never been reported in literature. According to Melichar (1896), Handlirsch (1901) and Horn *et al.* (1990) the Fieber collection was given to Reiber, Puton, Lethierry and Noualhier and then came to the National Museum of Natural History Paris, the Museum of Natural History Vienna and the Royal Belgian Institute of Natural Sciences Brussels. We checked these collections ourselves and with the help of the curators and found two debatable specimens in Paris and Vienna.

The Paris specimen found in the Puton collection is labelled "montana/Smyrna" (= Izmir, Turkey). Asia Minor has never been mentioned by Fieber as part of the geographic range. We conclude that Fieber did not know this specimen and it was probably Puton himself who added a typed label listing all the synonyms of *Cicadetta montana*, and ending with "var. *brevipennis* Fieb.".

The Vienna specimen (**Fig 4.8B**) is without location but labelled with "*Cicadetta montana* var. *brevipenis*" (with one 'n'!) and "*brevipennis* det. Fieber" (together with a putative *Cicadetta montana* var. *longipennis* specimen from Mehadia in Romania). The former label could be written by Fieber, the latter by Lethierry (see copies of handwritings in Horn *et al.*, 1990).

We extracted around 15 morphological characters, which Fieber provided to distinguish between *C. montana* var. *longipennis* and var. *brevipennis* first from the Reiber translation and then controlled it with the original unpublished handwritten German text kept in the National Museum of Natural History Paris (Soulier-Perkins, pers. comm.). Since no constitutive characters exist or were provided by Fieber, we generally did not weight the characters. The concordance between the putative Vienna type and Fieber's description was low. We considered 36 to 47% of the characters as discordant, 8 to 43% as ambiguous and 29 to 54% as fitting the description. The range results from weak characters, which were independently evaluated by the first four authors of the study. Obvious characters do not fit such as 1) the lateral part of the pronotal collar straight instead of "rounded"; 2) in the fore wing, the outer rim of the costal vein with different colouration than the radial and subcostal veins instead of "monochrome"; 3) the apical part of the operculum with a larger instead of "marginal portion yellowish"; and 4) the median lobe of uncus rather long-oval instead of "short and almost semi-circular". These four characters are also easily visible on the unpublished colour drawings (National Museum of Natural History Paris; Soulier-Perkins, pers. comm.) and the Vienna specimen fits much better with the '*longipennis*' plate and description. Though, it is also unlikely that the Vienna '*longipennis*' and '*brevipennis*' specimens have been interchanged. The concordance between the '*longipennis*' specimen and the description would then decrease and in the '*brevipennis*' specimen other characters would no longer fit. Fieber also stressed (1876: p. 88, description of *C. montana*) that among the considered material of both varieties some have "aberrant *one-sided* fused" median and cubitus anterior veins, but the Vienna specimen has an exceptional long fusion even on both sides (**Fig 4.8B and F**). A specimen so poorly fitting the description can scarcely be regarded as a type.

Another uncertainty arises from the spelling of the names '*brevipennis*' (short-winged) and '*longipennis*' (long-winged). Fieber's description does not make any statement about the wing shape. It was only Schumacher (1924) who suggested that the wing ratio was a good character to use to distinguish between his '*brevipennis*' and '*longipennis*' groups. Fieber's (1872) published list and handwritten original works (labels, unpublished texts and illustrations) suggest that his intention was to distinguish between *C. montana* var. *brevipenis nomen nudum* (short genitalia, probably: short median lobe of uncus and/or pseudoparameres) and *C. montana* var. *longipenis nomen nudum* (long genitalia), but Reiber translated the names incorrectly. By the way, the pseudoparameres can protrude more or less depending on the position and preparation of the specimen and this character is rather delicate in the manner used by Fieber. The spelling with two 'n' has been consequently used after 1876 and subsequent authors focussed more on wing shapes than on Fieber's original characters; a vernacular French name has even established as "la Cigalette à ailes courtes" (≈ short-winged lesser cicada; Puissant, 2006; Deroussen *et al.*, 2014). '*Brevipenis*' and '*longipenis*' are both *nomina nuda* since Art. 12.1. (ICZN, 1999) is not satisfied in Fieber (1872) lacking a description, definition or indication.

Summarizing, in our opinion *Cicadetta montana* var. *brevipennis* sensu Fieber cannot be linked to a song defined species. For optimal stability and universality of the nomenclature, we favour petitioning the ICZN to set aside the doubtful Vienna specimen as a potential type and to designate a neotype of the OTU brev. For the neotype, we would like to choose a specimen from the population at Glanz an der Weinstrasse ('GL') which is only 40 km away from Graz (Art. 75.3.6; ICZN, 1999). This population belongs to the same metapopulation as Slovenian brev and we can bring together *Cicadetta montana* var. *brevipennis* (sensu Fieber) and *Cicadetta brevipennis* (sensu Gogala & Trilar) in the most suitable way.

References Appendix S4.5

Boitier E, Brugel E. 2006. Les cigales en Auvergne: un essai de synthèse des connaissances (Hemiptera: Cicadidae). *Arvernsis* 37-38: 7-12.

Delorme Q, Cury D, Bernier C. 2015. *Tettigetalna argentata* (Olivier, 1790) et les Cigales du groupe *Cicadetta* cf. *montana* (Scopoli, 1772) dans la moitié nord de la France: nouveaux éléments de répartition et d'écologie impliquant la conservation des espèces (Hemiptera Cicadidae). *L'Entomologiste* 71(1): 31-40.

Deroussen F, Sueur J, Puissant S. 2014. *Cigales de France. Cicadas of France.* Paris: Muséum national d'Histoire naturelle. La sonothèque du Muséum. CD album.

Fieber FX. 1872. *Katalog der europäischen Cicadinen nach Originalen mit Benützung der neuesten Literatur.* Vienna: Carl Gerold's Sohn, 19 pp.

Fieber FX. 1876. Les Cicadines d'Europe d'après les originaux et les publications les plus récentes. Deuxième partie: Descriptions des espèces. Traduit de l'allemand par Ferd. Reiber. *Revue et Magazin de Zoologie* 4: 11-268.

Gogala M, Trilar T. 2004. Bioacoustic investigations and taxonomic considerations on the *Cicadetta montana* species complex (Homoptera: Cicadoidea: Tibicinidae). *Anais da Academia Brasileira de Ciências* 76(2): 316-324.

Gurcel K. 2015. Contribution à la connaissance des Cigales de France: première synthèse des observations et répartition des espèces pour le département de la Haute-Savoie (Hemiptera Cicadidae). *L'Entomologiste* 71(4): 245-260.

Handlirsch A. 1901. Geschichte der Zoologie in Oesterreich von 1850-1900. III. Arthropoden, D. Insecten, Rhychoten. In: Hölder A, ed. *Botanik und Zoologie in Österreich in den Jahren 1850 bis 1900.* Vienna: Zoologisch-Botanische Gesellschaft, K. U. K. Hof- und Universitäts-Buchhändler, 302-313.

Hertach T. 2007. Three species instead of only one: Distribution and ecology of the *Cicadetta montana* species complex (Hemiptera: Cicadoidea) in Switzerland. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 80: 37-61.

Horn W, Kahle I, Friese G, Gaedike R. 1990. *Collectiones entomologicae. Ein Kompendium über den Verbleib entomologischer Sammlungen der Welt bis 1960.* Berlin: Akademie der Landwirtschaftswissenschaften der Deutschen Demokratischen Republik, 220 pp.

Hugel S, Matt F, Callot H, Feldtrauer J, Brua C. 2008. Présence de *Cicadetta petryi* Fieber, 1876 en Alsace (Hemiptera, Cicadidae). *Bulletin de la Société Entomologique de Mulhouse*, 64(1): 5-10.

ICZN (International Commission on Zoological Nomenclature). 1999. *International Code of Zoological Nomenclature.* 4th edition. Available from: http://iczn.org (accessed 16 June 2015).

Meineke T. 2012. Bergsingzikaden *Cicadetta cantilatrix* Sueur & Puissant, 2007, *Cicadetta petryi* Fieber, 1876 und *Cicadetta montana* s. str. (Scopoli, 1772) im mittleren Deutschland (Auchenorrhyncha, Cicadidae, Cicadettinae). *Entomologische Nachrichten und Berichte* 56(2): 133-142.

Melichar L. 1896. *Cicadinen (Hemiptera-Homoptera) von Mittel-Europa.* Berlin: Felix L. Dames, 364 pp.

Puissant S. 2006. *Contribution à la connaissance des cigales de France: Géonémie et écologie des populations (Hemiptera, Cicadidae).* Bédeilhac/Aynat: Association pour la Caractérisation et l'Etude des Entomocénoses, 193 pp.

Schumacher F. 1924. Auftreten der Bergzikaden in der Mark Brandenburg und Übersicht über die deutschen Formen (Hem. Homopt.). *Berliner Entomologische Zeitschrift* 4: 329-334.

Sueur J, Puissant S. 2007. Similar look but different song: a new *Cicadetta* species in the *montana* complex (Insecta, Hemiptera, Cicadidae). *Zootaxa* 1442: 55-68.

Trilar T, Holzinger WE. 2004. Bioakustische Nachweise von drei Arten des *Cicadetta montana*-Komplexes aus Österreich (Insecta: Hemiptera: Cicadoidea). *Linzer biologische Beiträge* 36: 1383-1386.

Appendix S4.6. Database of the records in the *Cicadetta brevipennis* group.

(ordered by taxon name → country → latitude; sheet 1)

Taxon	Location	Country	Altitude [m asl]	Lat.	Long.	Observer	Year	Month	Day
Cicadetta brevipennis brevipennis	Glanz an der Weinstrasse	AT	390	46.6558	15.5216	Kunz Gernot	2015	6	2
Cicadetta brevipennis brevipennis	Glanz an der Weinstrasse	AT	400	46.6560	15.5220	Gogala Matija, Trilar Tomi	2015	6	10
Cicadetta brevipennis brevipennis	Glanz an der Weinstrasse	AT	430	46.6567	15.5232	Kunz Gernot	2014	5	27
Cicadetta brevipennis brevipennis	Bad Gleichenberg, Steinbruch Klausen	AT	400	46.8943	15.8992	Tomi Trilar, Werner Holzinger	2004	7	9
Cicadetta brevipennis brevipennis	Steinbruch N Müllendorf	AT	310	47.8568	16.4515	Kunz Gernot	2015	6	8
Cicadetta brevipennis brevipennis	Velika, Papuk, Poljani	HR	385	45.4624	17.6400	Matija Gogala, Tomi Trilar	2012	5	30
Cicadetta brevipennis brevipennis	Tapolca, Szent György-hegy	HU	308	46.8406	17.4456	Matija Gogala, Tomi Trilar	2011	5	26
Cicadetta brevipennis brevipennis	E Santa Restituta	IT	540	42.6474	12.3550	Hertach Thomas	2011	7	13
Cicadetta brevipennis brevipennis	NW Melezzole	IT	680	42.6762	12.3281	Hertach Thomas	2011	7	13
Cicadetta brevipennis brevipennis	Poggie le Perazzete, Mte. Amiata	IT	870	42.8235	11.6453	Hertach Thomas	2011	7	15
Cicadetta brevipennis brevipennis	N Cerreto di Spoleto	IT	560	42.8332	12.9146	Hertach Thomas	2011	7	12
Cicadetta brevipennis brevipennis	W Piancastagnaio	IT	800	42.8479	11.6642	Hertach Thomas	2011	7	15
Cicadetta brevipennis brevipennis	E Sassofortino	IT	500	43.0299	11.1386	Hertach Thomas	2012	7	8
Cicadetta brevipennis brevipennis	NW Meleta	IT	420	43.0427	11.0486	Hertach Thomas	2012	7	8
Cicadetta brevipennis brevipennis	NE Massa Marittima	IT	460	43.0776	10.9458	Hertach Thomas	2012	7	8
Cicadetta brevipennis brevipennis	SW Monticiano	IT	370	43.0913	11.1289	Hertach Thomas	2012	7	8
Cicadetta brevipennis brevipennis	Montarone, Subasio	IT	750	43.1023	12.7304	Hertach Thomas	2012	7	6
Cicadetta brevipennis brevipennis	above Prata (Suvereto)	IT	290	43.1057	10.6462	Hertach Thomas	2012	7	13
Cicadetta brevipennis brevipennis	above Villa Postignano	IT	540	43.1097	12.7464	Hertach Thomas	2012	7	6
Cicadetta brevipennis brevipennis	Monte Ceci	IT	310	43.1110	10.6525	Hertach Thomas	2012	7	13
Cicadetta brevipennis brevipennis	Montarone, Subasio	IT	550	43.1113	12.7459	Hertach Thomas	2012	7	6
Cicadetta brevipennis brevipennis	Sassetta	IT	330	43.1146	10.6545	Hertach Thomas	2012	7	13
Cicadetta brevipennis brevipennis	S. Monticiano	IT	360	43.1201	11.1713	Hertach Thomas	2012	7	8
Cicadetta brevipennis brevipennis	Passo Cornello	IT	740	43.1334	12.8524	Hertach Thomas	2012	7	6
Cicadetta brevipennis brevipennis	E Monticiano	IT	260	43.1453	11.2076	Hertach Thomas	2012	7	8
Cicadetta brevipennis brevipennis	Fontazzi	IT	200	43.1677	11.2955	Hertach Thomas	2012	7	8
Cicadetta brevipennis brevipennis	Fiuminata	IT	550	43.1731	12.8867	Hertach Thomas	2012	7	6
Cicadetta brevipennis brevipennis	Crevole (Murlo)	IT	290	43.1839	11.3720	Hertach Thomas	2012	7	8
Cicadetta brevipennis brevipennis	SW Sinalunga	IT	390	43.2009	11.7017	Hertach Thomas	2012	7	7
Cicadetta brevipennis brevipennis	Foresta di Monterufoli-Caselli	IT	470	43.2241	10.7768	Hertach Thomas	2012	7	13
Cicadetta brevipennis brevipennis	Foresta di Monterufoli-Caselli	IT	470	43.2274	10.7750	Hertach Thomas	2012	7	13
Cicadetta brevipennis brevipennis	Foresta di Monterufoli-Caselli	IT	460	43.2409	10.7655	Hertach Thomas	2012	7	13
Cicadetta brevipennis brevipennis	Busco (Cortona)	IT	590	43.2466	12.0973	Hertach Thomas	2012	7	7
Cicadetta brevipennis brevipennis	Foresta di Monterufoli-Caselli	IT	500	43.2488	10.7485	Hertach Thomas	2012	7	13/14
Cicadetta brevipennis brevipennis	W Mercatale	IT	390	43.2553	12.1113	Hertach Thomas	2012	7	7
Cicadetta brevipennis brevipennis	SW Micciano	IT	450	43.2768	10.7748	Hertach Thomas	2012	7	14
Cicadetta brevipennis brevipennis	Castellina Marittima	IT	410	43.3917	10.5767	Hertach Thomas	2008	7	12
Cicadetta brevipennis brevipennis	Les Plaines, ferme du Merque	IT	400	43.4217	10.5833	Hertach Thomas	2008	7	11
Cicadetta brevipennis brevipennis	above Castellina Marittima	IT	460	43.4217	10.5933	Hertach Thomas	2008	7	11
Cicadetta brevipennis brevipennis	above Castellina Marittima	IT	460	43.4217	10.5933	Hertach Thomas	2008	7	12
Cicadetta brevipennis brevipennis	above Castellina Marittima	IT	490	43.4217	10.6017	Hertach Thomas	2008	7	11
Cicadetta brevipennis brevipennis	Monte Vaso	IT	500	43.4317	10.6100	Hertach Thomas	2008	7	11
Cicadetta brevipennis brevipennis	NW Monte Santa Maria Tiberina	IT	440	43.4489	12.1359	Hertach Thomas	2012	7	4
Cicadetta brevipennis brevipennis	W San Gimignano	IT	530	43.4561	10.9512	Hertach Thomas	2012	7	14
Cicadetta brevipennis brevipennis	Paltratico, Castelnuovo	IT	150	43.4567	10.4417	Hertach Thomas	2008	7	13
Cicadetta brevipennis brevipennis	S Gabbro	IT	120	43.4733	10.4367	Hertach Thomas	2008	7	13
Cicadetta brevipennis brevipennis	W Castagno	IT	320	43.4835	10.8887	Hertach Thomas	2012	7	14
Cicadetta brevipennis brevipennis	Gabbro	IT	240	43.4900	10.4383	Hertach Thomas	2008	7	13
Cicadetta brevipennis brevipennis	Malavolta, Gabbro	IT	300	43.4967	10.4317	Hertach Thomas	2008	7	13
Cicadetta brevipennis brevipennis	Monte Acuto	IT	920	43.5044	12.6565	Hertach Thomas	2012	7	5
Cicadetta brevipennis brevipennis	E Monte Maggiore, Valle Benedetta	IT	320	43.5050	10.4300	Hertach Thomas	2008	7	13
Cicadetta brevipennis brevipennis	NE Monte Maggiore, Valle Benedetta	IT	320	43.5083	10.4333	Hertach Thomas	2008	7	13
Cicadetta brevipennis brevipennis	N Monte Maggiore, Valle Benedetta	IT	350	43.5117	10.4267	Hertach Thomas	2008	7	13
Cicadetta brevipennis brevipennis	Monte Acuto	IT	760	43.5126	12.6554	Hertach Thomas	2012	7	5
Cicadetta brevipennis brevipennis	SE Valle Benedetta	IT	340	43.5133	10.4200	Hertach Thomas	2008	7	13
Cicadetta brevipennis brevipennis	NE Bocca Serriola	IT	660	43.5247	12.3633	Hertach Thomas	2012	7	5
Cicadetta brevipennis brevipennis	S Montaione	IT	460	43.5309	10.9247	Hertach Thomas	2012	7	14
Cicadetta brevipennis brevipennis	S Colombara	IT	590	43.5587	12.4608	Hertach Thomas	2012	7	5
Cicadetta brevipennis brevipennis	N Sansepolcro	IT	550	43.6338	12.1184	Hertach Thomas	2012	7	4
Cicadetta brevipennis brevipennis	S Caprile	IT	830	43.7053	12.1368	Hertach Thomas	2012	7	4
Cicadetta brevipennis brevipennis	N Caprile	IT	840	43.7212	12.1242	Hertach Thomas	2012	7	4
Cicadetta brevipennis brevipennis	Balze	IT	1150	43.7793	12.0868	Hertach Thomas	2012	7	4
Cicadetta brevipennis brevipennis	Riofreddo	IT	800	43.8384	12.0660	Hertach Thomas	2012	7	4
Cicadetta brevipennis brevipennis	Passo dell'Incisa	IT	820	43.8575	12.0451	Hertach Thomas	2012	7	4
Cicadetta brevipennis brevipennis	above Londa	IT	290	43.8599	11.5792	Hertach Thomas	2012	7	3
Cicadetta brevipennis brevipennis	Acquapartita	IT	630	43.8602	12.0067	Hertach Thomas	2012	7	4
Cicadetta brevipennis brevipennis	E Corniolo	IT	470	43.9051	11.8154	Hertach Thomas	2012	7	4
Cicadetta brevipennis brevipennis	E Marliana	IT	310	43.9367	10.7850	Hertach Thomas	2012	7	15
Cicadetta brevipennis brevipennis	Fruška gora, Bešenovački	RS	215	45.1145	19.7131	Matija Gogala, Tomi Trilar	2015	6	4
Cicadetta brevipennis brevipennis	Brič, Škrline, near river Dragonja	SI	81	45.4667	13.7630	Matija Gogala, Tomi Trilar	2003	6	19
Cicadetta brevipennis brevipennis	Golo, Stražar	SI	758	45.9125	14.5151	Matija Gogala, Tomi Trilar	multiple dates		
Cicadetta brevipennis brevipennis	Dragomer, Lukovica pri Brezovici	SI	335	46.0188	14.3883	Matija Gogala, Tomi Trilar	multiple dates		
Cicadetta brevipennis brevipennis	Krnice	SI	505	46.1227	15.1034	Matija Gogala, Tomi Trilar, Andrej Kapla	multiple dates		
Cicadetta brevipennis brevipennis	Dol pri Hrastniku	SI	401	46.1442	15.1125	Matija Gogala, Tomi Trilar, Andrej Kapla	multiple dates		
Cicadetta brevipennis brevipennis	Dol pri Hrastniku, Jelenca, Lužar	SI	629	46.1528	15.1063	Tomi Trilar	2013	6	12
Cicadetta brevipennis brevipennis	Mala Nedelja	SI	231	46.5155	16.0542	Matija Gogala	2011	5	31
Cicadetta brevipennis brevipennis	Kamnica, Nad elektrarno	SI	365	46.5719	15.6045	Tomi Trilar	multiple dates		
Cicadetta brevipennis hippolaidica	Montalbo Alberana	IT	900	41.4432	15.1142	Hertach Thomas	2011	6	27
Cicadetta brevipennis hippolaidica	N San Bartolomeo in Galdo	IT	590	41.4533	15.0385	Hertach Thomas	2010	7	20
Cicadetta brevipennis hippolaidica	Coppa San Pietro	IT	900	41.5158	15.0812	Hertach Thomas	2011	6	27
Cicadetta brevipennis hippolaidica	Monte Sambuco	IT	850	41.5180	15.0797	Hertach Thomas	2010	7	19
Cicadetta brevipennis hippolaidica	W Monte Sambuco	IT	860	41.5283	15.0571	Hertach Thomas	2011	6	30
Cicadetta brevipennis hippolaidica	Monte Sambuco	IT	920	41.5284	15.0838	Hertach Thomas	2011	6	27
Cicadetta brevipennis hippolaidica	Monte Sambuco	IT	920	41.5284	15.0838	Hertach Thomas	2011	6	28

Appendix S4.6. Database of the records in the *Cicadetta brevipennis* group.

(ordered by taxon name → country → latitude; sheet 2)

Taxon	Location	Country	Altitude [m asl]	Lat.	Long.	Observer	Year	Month	Day
Cicadetta brevipennis hippolaidica	Monte Sambuco	IT	890	41.5311	15.0825	Hertach Thomas	2011	6	27
Cicadetta brevipennis hippolaidica	Monte Sambuco	IT	890	41.5311	15.0825	Hertach Thomas	2011	6	28
Cicadetta brevipennis hippolaidica	Monte Sambuco	IT	860	41.5312	15.0830	Hertach Thomas	2010	7	19
Cicadetta brevipennis hippolaidica	Monte Sambuco	IT	770	41.5489	15.1063	Hertach Thomas	2011	6	29
Cicadetta brevipennis hippolaidica	Monte Sambuco	IT	790	41.5490	15.1036	Hertach Thomas	2011	6	29
Cicadetta brevipennis hippolaidica	Monte Sambuco	IT	720	41.5528	15.1061	Hertach Thomas	2011	6	29
Cicadetta brevipennis hippolaidica	S Castelnuovo della Daunia	IT	660	41.5538	15.1068	Hertach Thomas	2010	7	19
Cicadetta brevipennis hippolaidica	N Cerasuolo	IT	780	41.5953	14.0225	Hertach Thomas	2011	7	3
Cicadetta brevipennis hippolaidica	N Cerasuolo	IT	750	41.6005	14.0217	Hertach Thomas	2011	7	3
Cicadetta brevipennis hippolaidica	Lago Selva	IT	930	41.6071	13.9758	Hertach Thomas	2011	7	3
Cicadetta brevipennis hippolaidica	W San Biagio Saracinisco	IT	750	41.6108	13.9142	Hertach Thomas	2011	7	3
Cicadetta brevipennis hippolaidica	W San Biagio Saracinisco	IT	800	41.6124	13.9228	Hertach Thomas	2011	7	3
Cicadetta brevipennis hippolaidica	W San Biagio Saracinisco	IT	700	41.6135	13.9123	Hertach Thomas	2011	7	3
Cicadetta brevipennis hippolaidica	W San Biagio Saracinisco	IT	620	41.6194	13.8961	Hertach Thomas	2011	7	3
Cicadetta brevipennis hippolaidica	Picinisco	IT	700	41.6385	13.8753	Hertach Thomas	2011	7	3
Cicadetta brevipennis hippolaidica	above Picinisco	IT	1070	41.6499	13.9011	Hertach Thomas	2011	7	3
Cicadetta brevipennis hippolaidica	Picinisco-Mainarde	IT	1200	41.6527	13.9037	Hertach Thomas	2011	7	3
Cicadetta brevipennis hippolaidica	S Pescolanciano	IT	840	41.6684	14.3433	Hertach Thomas	2011	7	2
Cicadetta brevipennis hippolaidica	S Ripabottoni	IT	630	41.6837	14.8184	Hertach Thomas	2011	6	30
Cicadetta brevipennis hippolaidica	S Ripabottoni	IT	630	41.6849	14.8166	Hertach Thomas	2011	6	30
Cicadetta brevipennis hippolaidica	W Ripabottoni	IT	370	41.6886	14.7649	Hertach Thomas	2011	6	30
Cicadetta brevipennis hippolaidica	W Ripabottoni	IT	400	41.6895	14.7719	Hertach Thomas	2011	6	30
Cicadetta brevipennis hippolaidica	Monte Lupone, Pescolanciano	IT	840	41.7000	14.3424	Hertach Thomas	2011	7	2
Cicadetta brevipennis hippolaidica	Collemelluccio	IT	890	41.7139	14.3578	Hertach Thomas	2011	7	2
Cicadetta brevipennis hippolaidica	Il Monte, Pietrabbondante	IT	960	41.7158	14.3767	Hertach Thomas	2011	7	2
Cicadetta brevipennis hippolaidica	Colle della Croce, Barrea	IT	1130	41.7435	13.9860	Hertach Thomas	2011	7	4
Cicadetta brevipennis hippolaidica	W Pietrabbondante	IT	1100	41.7478	14.3627	Hertach Thomas	2011	7	2
Cicadetta brevipennis hippolaidica	Forca d'Acero	IT	1480	41.7600	13.8290	Hertach Thomas	2011	7	4
Cicadetta brevipennis hippolaidica	S Opi	IT	1290	41.7685	13.8386	Hertach Thomas	2011	7	4
Cicadetta brevipennis hippolaidica	E Opi	IT	1070	41.7803	13.8551	Hertach Thomas	2011	7	4
Cicadetta brevipennis hippolaidica	W Villetta Barrea	IT	1010	41.7821	13.9103	Hertach Thomas	2011	7	4
Cicadetta brevipennis hippolaidica	San Giovanni, Civitacampomarano	IT	850	41.7964	14.6527	Hertach Thomas	2011	7	1
Cicadetta brevipennis hippolaidica	San Giovanni, Civitacampomarano	IT	850	41.7972	14.6506	Hertach Thomas	2011	7	1
Cicadetta brevipennis hippolaidica	N Castel di Sangro	IT	820	41.8010	14.1059	Hertach Thomas	2011	7	4
Cicadetta brevipennis hippolaidica	San Giovanni, Civitacampomarano	IT	790	41.8105	14.6184	Hertach Thomas	2011	7	1
Cicadetta brevipennis hippolaidica	Valloni, Torrebruna	IT	950	41.8489	14.4968	Hertach Thomas	2011	7	1
Cicadetta brevipennis hippolaidica	Fosse di Paolo, Pescasseroli	IT	1220	41.8526	13.7812	Hertach Thomas	2011	7	4
Cicadetta brevipennis hippolaidica	Les Plaines, ferme du Merque	IT	1260	41.8564	13.7847	Hertach Thomas	2011	7	4
Cicadetta brevipennis hippolaidica	E Castiglione Messer Marino	IT	990	41.8735	14.4701	Hertach Thomas	2011	7	1
Cicadetta brevipennis hippolaidica	N Torrebruna	IT	800	41.8772	14.5343	Hertach Thomas	2011	7	1
Cicadetta brevipennis hippolaidica	N Torrebruna	IT	750	41.8846	14.5297	Hertach Thomas	2011	7	1
Cicadetta brevipennis hippolaidica	between Gamberale and Palena	IT	1490	41.9201	14.1705	Hertach Thomas	2011	7	5
Cicadetta brevipennis hippolaidica	between Gamberale and Palena	IT	1370	41.9252	14.1625	Hertach Thomas	2011	7	5
Cicadetta brevipennis hippolaidica	S Palena	IT	1120	41.9330	14.1222	Hertach Thomas	2011	7	5
Cicadetta brevipennis litoralis	Alénya	FR	4	42.65XX	2.99XX	Puissant Stéphane	1999	5	31
Cicadetta brevipennis litoralis	Torreilles	FR	2	42.76XX	3.02XX	Puissant Stéphane	multiple dates		
Cicadetta brevipennis s.l.	Dolno Lukovo, Meden Buk	BG	115	41.3697	26.0522	Matija Gogala, Ilia Gjonov	2012	7	6
Cicadetta brevipennis s.l.	Kalimantsi, Kalimanska reka	BG	390	41.4656	23.5138	Matija Gogala, Ilia Gjonov	2010	7	23
Cicadetta brevipennis s.l.	Melnik, dolina pod Rozhen Monastery	BG	520	41.5247	23.4224	Matija Gogala, Tomi Trilar, Ilia Gjonov	2009	6	18
Cicadetta brevipennis s.l.	Strandzha, Stoilovo, reka Mechi Dol	BG	228	42.0309	27.5132	Matija Gogala, Tomi Trilar, Ilia Gjonov	2009	6	22
Cicadetta brevipennis s.l.	Sinemorets, ustje Veleka reka	BG	5	42.0643	27.9704	Matija Gogala, Tomi Trilar, Ilia Gjonov	2009	6	23
Cicadetta brevipennis s.l.	Strandzha, Kalovo, Mladezhka reka	BG	162	42.1307	27.4930	Matija Gogala, Tomi Trilar, Ilia Gjonov	2009	6	22
Cicadetta brevipennis s.l.	Strandzha, Mladezhko, izvir Mladeshka reka	BG	212	42.1517	27.3618	Matija Gogala, Tomi Trilar, Ilia Gjonov	2009	6	24
Cicadetta brevipennis s.l.	Batovo, Dobrichka	BG	120	43.4161	27.9414	Gjonov Ilia	2014	6	23
Cicadetta brevipennis s.l.	Mas Bellric	FR		42.6177	2.9232	Puissant Stéphane	1999	6	20
Cicadetta brevipennis s.l.	Au nord-ouest du village sur la D11	FR		42.7967	2.9581	Puissant Stéphane	1999	6	19
Cicadetta brevipennis s.l.	Au nord-ouest du village sur la D11	FR		42.7967	2.9581	Puissant Stéphane	2001	5	19
Cicadetta brevipennis s.l.	Mas de l'Audience	FR	1	43.4611	4.8902	Braud Yoan	2009	6	4
Cicadetta brevipennis s.l.	Lac du Réaltor, rive Sud-Est	FR	160	43.4624	5.3359	Braud Yoan	2004	6	14
Cicadetta brevipennis s.l.	Marais du Coucou	FR	1	43.4839	4.8469	Braud Yoan	2004	6	11
Cicadetta brevipennis s.l.	Etang des Aulnes	FR	18	43.6004	4.7907	Braud Yoan	2007	7	17
Cicadetta brevipennis s.l.	Digue du Petit Rhône	FR		43.6691	4.4721	Braud Yoan	2009	6	18
Cicadetta brevipennis s.l.	Mas du Castellet	FR	13	43.7135	4.6878	Braud Yoan	2009	5	28
Cicadetta brevipennis s.l.	Les Prêcheurs	FR		43.7343	4.6421	Braud Yoan	2004	6	25
Cicadetta brevipennis s.l.	Sud du Mas de Roustan, le long du canal	FR		43.7889	4.5949	Aubert M.	2012	6	10
Cicadetta brevipennis s.l.	La Sarinette	FR	8	43.8420	4.7907	Braud Yoan	2009	7	6
Cicadetta brevipennis s.l.	Terme di Suio	IT	20	41.3231	13.8833	Hertach Thomas	2010	7	23
Cicadetta brevipennis s.l.	Fiume Garigliano, SW Santa Maria di Mortola	IT	20	41.3317	13.8779	Hertach Thomas	2010	7	23
Cicadetta brevipennis s.l.	Fruška gora, Bešenovački	IT	50	41.3394	13.8708	Hertach Thomas	2010	7	23
Cicadetta brevipennis s.l.	Cona di Selvapiana	IT	590	41.5706	13.1384	Hertach Thomas	2010	7	24
Cicadetta brevipennis s.l.	Cona di Selvapiana	IT	570	41.5720	13.1351	Hertach Thomas	2010	7	24
Cicadetta brevipennis s.l.	Val del Tesoro, Foresta Umbra	IT	450	41.8400	16.0451	Hertach Thomas	2010	7	17
Cicadetta brevipennis s.l.	Val del Tesoro, Foresta Umbra	IT	370	41.8535	16.0438	Hertach Thomas	2010	7	17
Cicadetta brevipennis s.l.	Salle Vecchia	IT	530	42.1686	13.9543	Hertach Thomas	2011	7	6
Cicadetta brevipennis s.l.	Colle della Civita, Majelletta	IT	1110	42.1818	14.0711	Hertach Thomas	2011	7	6
Cicadetta brevipennis s.l.	W Forca di Penne	IT	920	42.2827	13.8221	Hertach Thomas	2011	7	6
Cicadetta brevipennis s.l.	S Lago di Campotosto	IT	1300	42.5071	13.3590	Hertach Thomas	2011	7	11
Cicadetta brevipennis s.l.	E Ortolano	IT	1050	42.5206	13.4319	Hertach Thomas	2011	7	11
Cicadetta brevipennis s.l.	SE Campotosto	IT	1390	42.5309	13.4126	Hertach Thomas	2011	7	11
Cicadetta brevipennis s.l.	SE Campotosto	IT	1370	42.5366	13.4013	Hertach Thomas	2011	7	11
Cicadetta brevipennis s.l.	Nerito	IT	790	42.5533	13.4805	Hertach Thomas	2011	7	11
Cicadetta brevipennis s.l.	N Nerito	IT	860	42.5603	13.4740	Hertach Thomas	2011	7	11
Cicadetta brevipennis s.l.	S Cervaro	IT	870	42.5727	13.4741	Hertach Thomas	2011	7	11

Appendix S4.6. Database of the records in the *Cicadetta brevipennis* group.

(ordered by taxon name → country → latitude; sheet 3)

Taxon	Location	Country	Altitude [m asl]	Lat.	Long.	Observer	Year	Month	Day
Cicadetta brevipennis s.l.	Crognaleto	IT	1130	42.5879	13.4857	Hertach Thomas	2011	7	11
Cicadetta brevipennis s.l.	between Crognaleto and Cortino	IT	1180	42.6144	13.4976	Hertach Thomas	2011	7	11
Cicadetta brevipennis s.l.	NE Ceppo	IT	1230	42.6828	13.4794	Hertach Thomas	2011	7	11
Cicadetta brevipennis s.l.	between Gualdo/Castelsantangelo sul Nera	IT	920	42.8876	13.1593	Hertach Thomas	2011	7	12
Cicadetta brevipennis s.l.	NW. Castelsantangelo sul Nera	IT	740	42.9066	13.1340	Hertach Thomas	2011	7	12
Cicadetta brevipennis s.l.	Scrutto	IT	170	46.1219	13.5223	Hertach Thomas	2010	6	11
Cicadetta brevipennis s.l.	SW Savogna	IT	200	46.1520	13.5194	Hertach Thomas, Gogala Matija, Trilar Tomi	2010	6	11
Cicadetta brevipennis s.l.	Perchinie	IT	680	46.1800	13.5283	Hertach Thomas, Trilar Tomi	2010	6	11
Cicadetta brevipennis s.l.	Montemaggiore	IT	730	46.1877	13.5291	Hertach Thomas	2010	6	11
Cicadetta brevipennis s.l.	above Travesio	IT	370	46.2032	12.8606	Hertach Thomas	2010	6	12
Cicadetta brevipennis s.l.	above Travesio	IT	470	46.2058	12.8590	Hertach Thomas	2010	6	12
Cicadetta brevipennis s.l.	above Travesio	IT	530	46.2076	12.8590	Hertach Thomas	2010	6	12
Cicadetta brevipennis s.l.	Micottis	IT	530	46.2797	13.2812	Hertach Thomas	2010	6	10
Cicadetta brevipennis s.l.	Pradielis	IT	360	46.2829	13.2627	Hertach Thomas	2010	6	10
Cicadetta brevipennis s.l.	Tolmezzo - Illegio	IT	460	46.4118	13.0438	Hertach Thomas	2010	6	9
Cicadetta brevipennis s.l.	below Avaglio	IT	680	46.4280	12.9155	Hertach Thomas	2010	6	8
Cicadetta brevipennis s.l.	E Karthaus, Schnalstal	IT	1270	46.7065	10.9173	Hertach Thomas	2010	6	3
Cicadetta brevipennis s.l.	Albeşti, Rezervatie Padurea Hagieni	RO	20	43.8024	28.4501	Matija Gogala, Tomi Trilar, Valentin Popa	2004	6	26
Cicadetta brevipennis s.l.	Negru Vodă, Rezervatie Padurea Negru Vodă	RO	127	43.8143	28.2561	Matija Gogala, Tomi Trilar, Valentin Popa	2004	6	26
Cicadetta petryi	Poncione d'Arzo, Costa di Prabello	CH	705	45.8858	8.9432	Hertach Thomas	2006	5	28
Cicadetta petryi	Monte San Giorgio, S Forello	CH	960	45.9100	8.9503	Hertach Thomas	2013	6	3
Cicadetta petryi	Monte San Giorgio, western slope	CH	980	45.9100	8.9516	Hertach Thomas	2013	6	3
Cicadetta petryi	Monte San Giorgio, Forello-Cugnoli	CH	960	45.9109	8.9516	Hertach Thomas	2005	6	5
Cicadetta petryi	Monte San Giorgio	CH	1040	45.9119	8.9491	Hertach Thomas	2006	5	27
Cicadetta petryi	Monte San Giorgio, W Forello	CH	1040	45.9128	8.9491	Hertach Thomas	2005	6	6
Cicadetta petryi	Monte San Giorgio, W Forello	CH	1040	45.9128	8.9491	Hertach Thomas	2013	6	3
Cicadetta petryi	Monte San Giorgio, W Forello	CH	1040	45.9128	8.9491	Hertach Thomas	2013	6	1
Cicadetta petryi	Denti della Vecchia	CH	1320	46.0459	9.0161	Hertach Thomas	2009	6	12
Cicadetta petryi	Denti della Vecchia	CH	1280	46.0486	9.0149	Hertach Thomas	2009	6	12
Cicadetta petryi	Nant des Crues, Cartigny	CH	410	46.1730	6.0136	Hertach Thomas	2013	6	26
Cicadetta petryi	Nant des Crues, Cartigny	CH	380	46.1757	6.0116	Hertach Thomas	2012	6	28
Cicadetta petryi	Nant des Crues, Cartigny	CH	380	46.1761	6.0122	Hertach Thomas	2011	6	20
Cicadetta petryi	Nant des Crues, Cartigny	CH	380	46.1761	6.0122	Hertach Thomas	2011	6	21
Cicadetta petryi	Russin	CH	380	46.1819	6.0116	Hertach Thomas	2011	6	21
Cicadetta petryi	Bois de Treulaz	CH	380	46.1880	6.0345	Hertach Thomas	2011	5	20
Cicadetta petryi	La Fin, Aire-la-Ville	CH	400	46.1888	6.0506	Hertach Thomas	2012	6	30
Cicadetta petryi	Bois de Treulaz	CH	350	46.1893	6.0337	Hertach Thomas	2011	6	20
Cicadetta petryi	Les Plaines, ferme du Merque	CH	400	46.1936	6.0668	Hertach Thomas	2012	6	30
Cicadetta petryi	Nant de Lagnon	CH	400	46.1936	6.0665	Hertach Thomas	2013	6	26
Cicadetta petryi	Mauregard, Russin	CH	400	46.1971	6.0269	Hertach Thomas	2011	5	26
Cicadetta petryi	Mauregard, Russin	CH	400	46.1971	6.0269	Hertach Thomas	2011	6	21
Cicadetta petryi	Mauregard, Russin	CH	380	46.1974	6.0265	Hertach Thomas, Trilar Tomi	2007	6	6
Cicadetta petryi	Vallon d'Allondon, Dardagny	CH	400	46.1989	5.9986	Hertach Thomas	2011	5	26
Cicadetta petryi	Vallon d'Allondon	CH	380	46.1990	6.0045	Hertach Thomas	2006	6	24
Cicadetta petryi	Le Châtelet, Peney-Dessus	CH	405	46.2011	6.0290	Hertach Thomas	2006	6	24
Cicadetta petryi	Vallon d'Allondon	CH	390	46.2016	5.9979	Hertach Thomas	2006	6	24
Cicadetta petryi	Les Rippes, Essertines	CH	440	46.2022	5.9854	Hertach Thomas	2011	5	26
Cicadetta petryi	Bois de Merdisel, Peney	CH	410	46.2050	6.0548	Hertach Thomas	2006	6	24
Cicadetta petryi	Bois de Merdisel, Satigny	CH	420	46.2054	6.0538	Hertach Thomas	2014	6	26
Cicadetta petryi	Bois de la Grille, Vernier	CH	405	46.2118	6.0948	Hertach Thomas	2006	6	24
Cicadetta petryi	Bois de la Grille, Vernier	CH	405	46.2118	6.0948	Hertach Thomas	2013	6	27
Cicadetta petryi	Bois de la Grille, Vernier	CH	370	46.2122	6.0947	Hertach Thomas	2011	5	20
Cicadetta petryi	Bois de la Grille, Vernier	CH	410	46.2126	6.0935	Hertach Thomas, Trilar Tomi	2007	6	6
Cicadetta petryi	Bois de la Grille, Vernier	CH	360	46.2128	6.0940	Hertach Thomas	2013	6	27
Cicadetta petryi	Bois de la Grille, Vernier	CH	380	46.2140	6.0974	Hertach Thomas	2011	5	20
Cicadetta petryi	Bois de la Grille, Vernier	CH	410	46.2145	6.0974	Hertach Thomas	2006	6	24
Cicadetta petryi	Vallon d'Allondon, Les Granges	CH	420	46.2185	5.9962	Hertach Thomas	2011	5	26
Cicadetta petryi	Les Vaux, Arnex	CH	480	46.6847	6.5197	Hertach Thomas	2011	5	9
Cicadetta petryi	Les Vaux, Arnex	CH	480	46.6847	6.5197	Hertach Thomas	2012	5	28
Cicadetta petryi	Schlachtberg, Kyffhäuser	DE	210	51.3638	11.0985	Hertach Thomas	2014	6	21
Cicadetta petryi	Schlachtberg, Kyffhäuser	DE	210	51.3638	11.0985	Hertach Thomas	2014	6	22
Cicadetta petryi	Schlachtberg, Kyffhäuser	DE	210	51.3646	11.1002	Hertach Thomas	2014	6	22
Cicadetta petryi	Kosackenberg, Kyffhäuser	DE	150	51.3667	11.0817	Hertach Thomas	2014	6	23
Cicadetta petryi	W Kattenburg, Kyffhäuser	DE	170	51.3716	11.0656	Hertach Thomas	2014	6	23
Cicadetta petryi	Habichtstal, Kyffhäuser	DE	230	51.3863	11.0427	Hertach Thomas	2014	6	20
Cicadetta petryi	Canada de Benatanduz	ES	1375	40.5962	-0.5407	Puissant Stéphane	2015	6	9
Cicadetta petryi	Villarluengo	ES	1110	40.6523	-0.5352	Puissant Stéphane	2015	6	9
Cicadetta petryi	Ejulve	ES	1530	40.7531	-0.6110	Puissant Stéphane	2015	6	7
Cicadetta petryi	Fruška gora, Bešenovački	ES	1485	40.7532	-0.6037	Puissant Stéphane	2015	6	7
Cicadetta petryi	Coussères (Sud-Est)	FR	332	42.4104	2.4549	Braud Yoan	2010	6	29
Cicadetta petryi	Badebany, Fuilla	FR	650	42.5741	2.3616	Hertach Thomas	2011	6	15
Cicadetta petryi	nördlich Veïnat de Baix, Fuilla	FR	470	42.5744	2.3584	Hertach Thomas	2011	6	13
Cicadetta petryi	Badebany, Fuilla	FR	640	42.5749	2.3610	Hertach Thomas	2011	6	15
Cicadetta petryi	Sur la D7	FR		42.7452	2.4594	Sueur Jérôme	2001	7	23
Cicadetta petryi	Soulas del Llinas	FR	164	42.7576	2.7024	Braud Yoan	2010	6	30
Cicadetta petryi		FR	650	42.8272	1.6353	Defaut Bernard	1999	6	18
Cicadetta petryi	Sur la D14	FR		42.8549	2.5707	Puissant Stéphane, Sueur Jérôme	2001	7	20
Cicadetta petryi	Soudour	FR	650	42.8619	1.5800	Defaut Bernard, Puissant Stéphane	2011	7	10
Cicadetta petryi	Sur la D14	FR		42.8700	2.5570	Puissant Stéphane, Sueur Jérôme	2001	7	20
Cicadetta petryi	D117, Les Lauses	FR	650	42.8746	2.1526	Puissant Stéphane	2011	7	9
Cicadetta petryi	Le long de la D117 à l'ouest de Quillan	FR		42.8841	2.1342	Puissant Stéphane	2011	7	9
Cicadetta petryi		FR	700	42.8900	0.7186	Menand Mathieu	2012	6	
Cicadetta petryi		FR	420	42.9004	2.5697	Bousquet J.-M., Puissant Stéphane	2007	6	27

Appendix S4.6. Database of the records in the *Cicadetta brevipennis* group.

(ordered by taxon name → country → latitude; sheet 4)

Taxon	Location	Country	Altitude [m asl]	Lat.	Long.	Observer	Year	Month	Day
Cicadetta petryi	D117	FR	500	42.9192	2.0605	Puissant Stéphane	2011	7	9
Cicadetta petryi		FR	310	42.9895	2.8844	Puissant Stéphane	2013	6	3
Cicadetta petryi		FR		43.0395	2.9359	Puissant Stéphane	2011	5	22
Cicadetta petryi		FR	320	43.0990	1.4496	Defaut Bernard, Puissant Stéphane	2011	7	10
Cicadetta petryi	Forêt domaniale de Barbaira	FR		43.1671	2.4799	Soldati L.	1999	7	4
Cicadetta petryi	Carrière des Ciments Lafarge par la D 62	FR		43.1809	0.9952	Puissant Stéphane	2005	6	30
Cicadetta petryi	Oloron-Sainte-Marie	FR	230	43.1947	-0.6055	Février Jérémie	2015	6	17
Cicadetta petryi	250 m à l'est de Font Blanche	FR	316	43.2523	5.6757	Braud Yoan	2004	6	27
Cicadetta petryi	la Barrière - les Neufs Fonts	FR	394	43.3414	5.9639	Gurcel Kevin	2013	7	5
Cicadetta petryi		FR	180	43.6175	1.2856	Menand Mathieu	2011	6	30
Cicadetta petryi		FR	180	43.6286	1.1572	Menand Mathieu	2011	6	30
Cicadetta petryi	Bord de la Durance	FR	245	43.6889	5.6580	Puissant Stéphane	2013	6	19
Cicadetta petryi	Combe de la Roque	FR	990	43.7218	6.6438	Braud Yoan	2010	7	22
Cicadetta petryi	Vers La Font du Buis	FR	1020	43.7850	6.5996	Braud Yoan	2007	7	5
Cicadetta petryi	Saint-Maurin	FR	600	43.7967	6.2620	Braud Yoan	2009	6	28
Cicadetta petryi	Le Mousteiret	FR	1066	43.7996	6.6376	Braud Yoan	2007	7	5
Cicadetta petryi		FR	190	43.7997	1.8328	Menand Mathieu	2011	7	8
Cicadetta petryi	Foret de Menton	FR	414	43.8215	7.4757	Braud Yoan	2010	7	6
Cicadetta petryi	Lujerneta, vers col de verroux	FR	937	43.8216	7.4468	Braud Yoan	2010	6	7
Cicadetta petryi	Les mallans	FR	232	43.8286	5.2812	Braud Yoan	2002	5	17
Cicadetta petryi	Saint-Félix-de-l'Héras	FR	630	43.8311	3.3065	Aubert Matthieu	2013	7	11
Cicadetta petryi	Moulin	FR	670	43.8385	5.6935	Gurcel Kevin	2010	7	7
Cicadetta petryi	Sainte Roustagne	FR	433	43.8481	5.7942	Braud Yoan	2009	7	14
Cicadetta petryi	Erc sur la D2566a	FR		43.8493	7.4516	Puissant Stéphane, Sueur Jérôme	1999	7	1
Cicadetta petryi		FR	140	43.8664	1.5058	Menand Mathieu	2012	6	
Cicadetta petryi	La Bégude-Fontaine Ss Pareille	FR	635	43.9043	6.1387	Gurcel Kevin	2012	7	6
Cicadetta petryi	Tour de la Cruella	FR	479	43.9389	7.5188	Braud Yoan	2010	7	7
Cicadetta petryi	Repentance	FR	770	43.9524	6.3950	Braud Yoan	2004	6	17
Cicadetta petryi	Arbitelle	FR	800	43.9625	6.3729	Braud Yoan	2004	6	17
Cicadetta petryi	Pont de Castou (aval)	FR	426	43.9769	7.5765	Braud J., Braud Yoan	2010	7	7
Cicadetta petryi		FR	200	44.0042	1.6908	Menand Mathieu	2012	6	
Cicadetta petryi	Jas de la Roche	FR	849	44.0049	5.7820	Braud Yoan	2010	7	15
Cicadetta petryi	La Partie	FR		44.0241	6.0550	Braud Yoan	2002	7	8
Cicadetta petryi	Forêt de Grésigne	FR		44.0544	1.7436	Puissant Stéphane, Valladarès L.	2002	7	6
Cicadetta petryi	Bois du Pinet	FR	1196	44.0585	7.6382	Braud Yoan	2010	7	29
Cicadetta petryi	Les Dourbes	FR	1070	44.0673	6.3120	Gurcel Kevin	2010	7	2
Cicadetta petryi	Saint-Sauveur	FR	700	44.0746	3.2708	Février Jérémie	2013	7	21
Cicadetta petryi	Les Plaines, ferme du Merque	FR		44.0860	6.1911	Braud Yoan	2010	7	3
Cicadetta petryi	Le Purgatoire	FR	640	44.0882	6.2649	Braud Yoan, Gurcel Kevin	2010	7	3
Cicadetta petryi	Amon du barrage	FR	1171	44.0885	6.5337	Braud Yoan	2007	7	4
Cicadetta petryi	Mouiroués SW	FR	650	44.0902	6.2669	Gurcel Kevin, Bence Stéphane	2010	7	2
Cicadetta petryi	Aire de repos du Viaduc de Millau	FR		44.0925	3.0226	Puissant Stéphane	2011	5	17
Cicadetta petryi	Route des crêtes au sud de la ville	FR		44.1376	1.7861	Puissant Stéphane, Valladarès L.	2003	6	29
Cicadetta petryi		FR	400	44.1383	5.1495	Puissant Stéphane	1998	6	26
Cicadetta petryi		FR	1410	44.1505	5.3170	Puissant Stéphane	1998	6	28
Cicadetta petryi	Le long de la D974	FR	800	44.1677	5.1772	Boulard Michel, Puissant Stéphane	1999	6	15
Cicadetta petryi	Vers St-Amant	FR		44.1787	5.0875	Puissant Stéphane	1998	6	25
Cicadetta petryi	Entre Champ-Brancou et Le Virail	FR	500	44.2018	5.9060	Braud Yoan	2006	6	29
Cicadetta petryi		FR		44.2033	4.8353	Boulard Michel, Puissant Stéphane	1998	6	13
Cicadetta petryi		FR		44.2040	4.8492	Puissant Stéphane	1998	6	12
Cicadetta petryi	Grosse Grange	FR	760	44.2181	5.5626	Braud Yoan	2010	8	1
Cicadetta petryi	Mostuéjouls, Gorges du Tarn	FR	410	44.2215	3.2225	Mroczko Cédric	2013	7	7
Cicadetta petryi	La Labrède	FR	920	44.2427	3.5555	Barataud J.	2010	6	26
Cicadetta petryi	Col d'Anelle, Mercantour	FR		44.2730	6.8910	Baur Hannes	2012	6	24
Cicadetta petryi	Rivière Durance	FR		44.3138	5.9156	Braud Yoan	2008	7	19
Cicadetta petryi	Village	FR	822	44.3190	5.9582	Braud Yoan	2008	6	22
Cicadetta petryi	10 km avant Col de la Cayolle	FR	1400	44.3194	6.6273	Braud Yoan	2006	7	24
Cicadetta petryi		FR		44.3211	5.3258	Sueur Jérôme	2000	7	7
Cicadetta petryi		FR		44.3411	1.0189	Sueur Jérôme	2005	7	10
Cicadetta petryi	Vallon au nord du village	FR		44.3459	6.0240	Braud Yoan	2008	8	3
Cicadetta petryi	Vers le village	FR	695	44.3544	5.7074	Braud Yoan	2006	5	29
Cicadetta petryi	Village	FR		44.3547	5.7092	Braud Yoan	2007	6	24
Cicadetta petryi	Vers col de Faye	FR	898	44.3883	5.8653	Braud Yoan	2008	6	18
Cicadetta petryi	La Perrière	FR	600	44.3954	5.3446	Braud Yoan	2002	6	24
Cicadetta petryi	Torrent de Channes	FR	633	44.3955	5.7439	Braud Yoan	2008	6	23
Cicadetta petryi	Bergerie de Monrond	FR	850	44.3996	5.3738	Braud Yoan, Sardet E.	2010	6	15
Cicadetta petryi	Ravin de Bataric	FR	648	44.4019	5.7481	Braud Yoan	2008	6	23
Cicadetta petryi	Rivière Blème	FR	815	44.4144	5.6115	Braud Yoan	2007	6	6
Cicadetta petryi	Fruška gora, Bešenovački	FR	1350	44.4487	6.4287	Gurcel Kevin	2010	7	10
Cicadetta petryi	Torrent du Maraise	FR	730	44.4575	5.7670	Braud Yoan	2008	6	23
Cicadetta petryi	Rivière Buech	FR	737	44.4993	5.7668	Braud Yoan	2008	6	23
Cicadetta petryi	Babaillon SE	FR	728	44.5007	5.7325	Gurcel Kevin	2012	6	30
Cicadetta petryi	Les Glacières	FR	763	44.5220	5.7616	Braud Yoan, Maurel N.	2007	7	27
Cicadetta petryi	Demafé E	FR	785	44.5410	5.7792	Gurcel Kevin	2011	7	15
Cicadetta petryi	L'Isère, Est	FR	800	44.5658	6.4961	Braud Yoan	2010	6	17
Cicadetta petryi	La Rochette	FR	862	44.5874	5.7203	Braud Yoan	2008	6	18
Cicadetta petryi	Rivière Buech, Neuvillard	FR	946	44.6341	5.6939	Braud Yoan	2008	6	18
Cicadetta petryi	La Caisse	FR	1000	44.6619	5.6957	Braud Yoan	2009	6	24
Cicadetta petryi	La jarjatte, station de ski	FR	1226	44.6743	5.7622	Braud Yoan	2001	7	8
Cicadetta petryi	Les Fauries	FR	1060	44.6859	5.6871	Braud Yoan	2003	6	8
Cicadetta petryi	Ruisseau de la Croix Haute	FR	790	44.7574	5.6827	Braud Yoan	2008	7	2
Cicadetta petryi	N 44,76788 - E 5,33724	FR	385	44.7679	5.6957	Braud Yoan	2006	6	24
Cicadetta petryi	Bord de route nationale	FR	845	44.7970	5.6376	Braud Yoan	2007	6	15

Appendix S4.6. Database of the records in the *Cicadetta brevipennis* group.

(ordered by taxon name → country → latitude; sheet 5)

Taxon	Location	Country	Altitude [m asl]	Lat.	Long.	Observer	Year	Month	Day
Cicadetta petryi	Centre Terre Vivante	FR	700	44.8041	5.7031	Braud Yoan	2007	7	17
Cicadetta petryi	Bouthoux SW	FR	821	44.8132	5.6292	Gurcel Kevin	2011	7	15
Cicadetta petryi	RD4, La Casse de France	FR	1219	44.8433	6.5769	Braud Yoan	2008	8	8
Cicadetta petryi		FR		44.8617	0.6629	Duhazé Benoit	2014	7	2
Cicadetta petryi	Ravin de Grosse-Eau	FR	747	44.8655	5.6010	Braud Yoan	2008	7	2
Cicadetta petryi	Amont du pont de Cros	FR	1250	44.8987	6.6283	Braud Yoan	2003	7	10
Cicadetta petryi		FR		45.2605	0.4561	Duhazé Benoit	2014	7	1
Cicadetta petryi	Le Breuilh	FR		45.2606	0.4554	Duhazé Benoit	2013	7	24
Cicadetta petryi		FR		45.2636	3.4547	Boitier Emmanuel	2004		
Cicadetta petryi	La Chauterie	FR		45.3083	0.6412	Duhazé Benoit	2011	8	11
Cicadetta petryi	La Chauterie	FR		45.3105	0.6375	Duhazé Benoit	2011	8	11
Cicadetta petryi	Les Chaupres	FR		45.3177	0.6284	Duhazé Benoit	2014	7	1
Cicadetta petryi		FR	800	45.3903	3.1467	Boitier Emmanuel, Brugel Eric	2004	7	8
Cicadetta petryi		FR	900	45.3956	3.0444	Boitier Emmanuel	2004	7	21
Cicadetta petryi		FR		45.4125	3.1828	Boitier Emmanuel	2002	6	11
Cicadetta petryi		FR		45.4158	3.2169	Boitier Emmanuel	2002	7	18
Cicadetta petryi		FR	1000	45.4697	2.9881	Boitier Emmanuel	2002	6	15
Cicadetta petryi	Les Calloudes W	FR	500	45.5091	6.0535	Gurcel Kevin	2013	8	14
Cicadetta petryi	Les Calloudes W	FR	500	45.5091	6.0535	Gurcel Kevin	2013	6	14
Cicadetta petryi	Les Calloudes W	FR	500	45.5091	6.0535	Gurcel Kevin	2013	7	12
Cicadetta petryi	Les Calloudes	FR	430	45.5093	6.0562	Gurcel Kevin	2013	6	14
Cicadetta petryi	Les Calloudes	FR	430	45.5093	6.0562	Gurcel Kevin	2013	7	12
Cicadetta petryi	Cruet-Ferroux	FR	500	45.5435	6.1037	Gurcel Kevin	2013	8	14
Cicadetta petryi	Cruet-Ferroux	FR	500	45.5435	6.1037	Gurcel Kevin	2013	7	12
Cicadetta petryi	Cruet-Ferroux	FR	560	45.5448	6.1049	Gurcel Kevin	2013	6	14
Cicadetta petryi	Cruet-Ferroux	FR	560	45.5448	6.1049	Gurcel Kevin	2013	7	12
Cicadetta petryi		FR		45.5519	3.1078	Brugel Eric	2002	6	
Cicadetta petryi	Combefolle-Le Féal NW	FR	770	45.5603	6.1118	Gurcel Kevin	2013	6	19
Cicadetta petryi	Combefolle-Le Féal NW	FR	770	45.5603	6.1118	Gurcel Kevin	2013	7	13
Cicadetta petryi	Combefolle-Le Féal NE	FR	735	45.5606	6.1132	Gurcel Kevin	2013	6	19
Cicadetta petryi		FR		45.5636	3.1053	Boitier Emmanuel	2002	6	
Cicadetta petryi	Combefolle-Pierre Bénat Rnes	FR	750	45.5638	6.1126	Gurcel Kevin	2013	6	19
Cicadetta petryi	Las Chaussis	FR	520	45.5831	6.0075	Gurcel Kevin	2013	8	15
Cicadetta petryi	Les Chaussis	FR	520	45.5831	6.0075	Gurcel Kevin	2013	7	13
Cicadetta petryi	Les Chaussis	FR	520	45.5831	6.0075	Gurcel Kevin	2013	6	19
Cicadetta petryi	En proximité du Bourg	FR		45.5869	3.0906	Boitier Emmanuel	2002	6	
Cicadetta petryi		FR		45.5878	3.1800	Boitier Emmanuel	2002	6	20
Cicadetta petryi	Les Plaines, ferme du Merque	FR	460	45.5894	3.1297	Boitier Emmanuel	2001	6	
Cicadetta petryi	La Pierre Plate S	FR	700	45.5902	5.9946	Gurcel Kevin	2013	7	13
Cicadetta petryi		FR		45.6717	3.2369	Boitier Emmanuel	2003	6	17
Cicadetta petryi		FR		45.6850	3.1906	Brugel Eric	2004	8	17
Cicadetta petryi		FR		45.7422	3.1972	Boitier Emmanuel	2002	7	22
Cicadetta petryi	La Balmette	FR	700	45.7610	6.2846	Gurcel Kevin	2006	6	30
Cicadetta petryi	La Balmette	FR	700	45.7610	6.2846	Gurcel Kevin	2007	6	24
Cicadetta petryi	Le Rosay	FR	530	45.7625	6.3206	Gurcel Kevin	2012	6	9
Cicadetta petryi	Le Chenay	FR	1050	45.7671	6.2775	Gurcel Kevin	2006	6	30
Cicadetta petryi	Les Torches NO	FR	537	45.7706	6.3585	Gurcel Kevin	2007	5	19
Cicadetta petryi	Les Savioz	FR	827	45.7711	6.3321	Gurcel Kevin	2009	6	14
Cicadetta petryi	Les Savioz	FR	827	45.7711	6.3321	Gurcel Kevin	2013	6	22
Cicadetta petryi	Vesonne	FR	600	45.7719	6.2577	Gurcel Kevin	2007	6	24
Cicadetta petryi		FR		45.7831	3.0824	Boitier Emmanuel	2002	7	22
Cicadetta petryi		FR		45.8267	3.1456	Brugel Eric	2003	7	
Cicadetta petryi	Château de Menthon	FR	550	45.8620	6.2027	Gurcel Kevin	2009	7	14
Cicadetta petryi	Montagne des Princes	FR	900	45.9456	5.8767	Bal Bernard, Kevin Gurcel, Christophe Bernier	2007	5	26
Cicadetta petryi	Montagne des Princes	FR	900	45.9456	5.8767	Gurcel Kevin	2006	6	17
Cicadetta petryi	Montagne des Princes	FR	900	45.9456	5.8767	Gurcel Kevin	2011	5	8
Cicadetta petryi	Montagne des Princes	FR	900	45.9456	5.8767	Gurcel Kevin	2013	6	30
Cicadetta petryi		FR		45.9997	3.5067	Brugel Eric	2004	6	28
Cicadetta petryi	Le Rucher - Les Vorziers	FR	320	46.0212	5.8717	Gurcel Kevin	2013	6	12
Cicadetta petryi	Le Rucher - Les Vorziers	FR	320	46.0212	5.8717	Gurcel Kevin	2013	6	7
Cicadetta petryi	Le Mont	FR	600	46.0382	5.9568	Gurcel Kevin	2007	6	18
Cicadetta petryi	Le Mont	FR	600	46.0382	5.9568	Gurcel Kevin	2012	6	23
Cicadetta petryi	Vuache	FR	870	46.0449	5.9514	Gurcel Kevin	2007	6	18
Cicadetta petryi	Vuache	FR	870	46.0449	5.9514	Gurcel Kevin	2012	6	23
Cicadetta petryi		FR		46.1269	2.9917	Brugel Eric	2001	6	25
Cicadetta petryi	Jonnay (est), Pougny	FR	360	46.1406	5.9343	Hertach Thomas	2011	5	25
Cicadetta petryi	Bois du Coutard, Pougny	FR	400	46.1449	5.9345	Hertach Thomas	2011	5	25
Cicadetta petryi	Ferruaz Rnes, Asserans	FR	400	46.1601	5.9401	Hertach Thomas	2011	5	25
Cicadetta petryi	Fruška gora, Bešenovački	FR	440	46.1608	5.9253	Hertach Thomas	2011	5	25
Cicadetta petryi		FR		46.1622	3.3939	Brugel Eric	2001	6	21
Cicadetta petryi	oberhalb Airans-Farges	FR	550	46.1660	5.8980	Hertach Thomas	2011	5	25
Cicadetta petryi		FR		46.1853	2.9353	Brugel Eric	2004	6	10
Cicadetta petryi		FR		46.2083	3.1906	Brugel Eric	2004	7	27
Cicadetta petryi		FR		46.2561	3.4758	Brugel Eric	2004	6	2
Cicadetta petryi	Réserve naturelle du Pinail	FR		46.6973	0.5234	Veneau F.	1992	5	
Cicadetta petryi	Wassergarten	FR		47.9455	7.2802	André Antoine	2014	6	23
Cicadetta petryi	Sternehmuehle	FR		47.9491	7.2746	André Antoine	2014	6	23
Cicadetta petryi	Clos Saint-Landelin	FR		47.9497	7.2766	André Antoine	2014	6	23
Cicadetta petryi	Haulen	FR		47.9526	7.2745	André Antoine	2014	6	23
Cicadetta petryi	Lutzelthal	FR		47.9532	7.2717	André Antoine	2014	6	23
Cicadetta petryi		FR		47.9546	7.2641	André Antoine	2010	6	27
Cicadetta petryi		FR		47.9546	7.2641	André Antoine	2010	7	3
Cicadetta petryi		FR		47.9546	7.2641	André Antoine	2011	5	21

Appendix S4.6. Database of the records in the *Cicadetta brevipennis* group.

(ordered by taxon name → country → latitude; sheet 6)

Taxon	Location	Country	Altitude [m asl]	Lat.	Long.	Observer	Year	Month	Day
Cicadetta petryi		FR		47.9546	7.2641	André Antoine	2012	6	17
Cicadetta petryi	Schlossberg	FR		47.9552	7.2644	André Antoine	2014	6	26
Cicadetta petryi	Strangenberg, Rouffach	FR	300	47.9553	7.2694	Hertach Thomas	2009	5	24
Cicadetta petryi	Schlossberg	FR		47.9564	7.2644	André Antoine	2011	7	9
Cicadetta petryi		FR		47.9570	7.2498	André Antoine	2014	7	16
Cicadetta petryi		FR		47.9578	7.2594	Blanchet S.	2007	6	23
Cicadetta petryi	Strangenberg, Rouffach	FR	360	47.9592	7.2681	Hertach Thomas	2009	5	24
Cicadetta petryi		FR		47.9593	7.2679	Blanchet S.	2007	6	23
Cicadetta petryi		FR		47.9603	7.2438	André Antoine	2011	7	9
Cicadetta petryi	Carrière du Strangenberg	FR		47.9629	7.2769	Audinot S.	2014	6	24
Cicadetta petryi	Sultzelberg	FR		47.9630	7.2477	André Antoine	2011	7	9
Cicadetta petryi	Zinnkoepfle	FR		47.9684	7.2539	André Antoine	2011	5	29
Cicadetta petryi	Schlossrain	FR		48.0038	7.2165	André Antoine	2014	6	10
Cicadetta petryi		FR		48.0445	7.2974	André Antoine	2010	7	8
Cicadetta petryi	Chapelle des Bois	FR		48.0615	7.2832	André Antoine	2014	6	17
Cicadetta petryi	Hengst	FR		48.0646	7.2909	André Antoine	2014	6	17
Cicadetta petryi	Letzenberg	FR		48.0912	7.2917	André Antoine	2014	6	1
Cicadetta petryi	Letzenberg	FR		48.0919	7.2909	André Antoine	2013	7	9
Cicadetta petryi	Weingarten	FR		48.0921	7.2850	André Antoine	2014	6	8
Cicadetta petryi	Letzenberg	FR		48.0924	7.2976	André Antoine	2014	6	1
Cicadetta petryi	Letzenberg	FR		48.0927	7.2871	André Antoine	2014	6	7
Cicadetta petryi	Weingarten	FR		48.0927	7.2829	André Antoine	2014	6	8
Cicadetta petryi	Weingarten	FR		48.0933	7.2857	André Antoine	2014	6	1
Cicadetta petryi	Boland	FR		48.0959	7.2919	André Antoine	2014	6	1
Cicadetta petryi	Boland	FR		48.0975	7.2985	André Antoine	2014	6	1
Cicadetta petryi	Florimont	FR		48.0995	7.2941	André Antoine	2013	7	23
Cicadetta petryi	Dorfbourg	FR		48.1002	7.2923	André Antoine	2014	6	8
Cicadetta petryi	Dorfbourg	FR		48.1007	7.2939	André Antoine	2014	6	8
Cicadetta petryi	Dorfbourg	FR		48.1007	7.2911	André Antoine	2014	6	8
Cicadetta petryi	Dorfbourg	FR		48.1015	7.2902	André Antoine	2014	6	8
Cicadetta petryi	Schorr	FR		48.1020	7.2874	André Antoine	2014	6	8
Cicadetta petryi	Schorr	FR		48.1022	7.2893	André Antoine	2014	6	8
Cicadetta petryi	Dorfbourg	FR		48.1024	7.2905	André Antoine	2014	6	8
Cicadetta petryi	Florimont	FR		48.1029	7.2923	André Antoine	2012	6	24
Cicadetta petryi	Dorfbourg	FR		48.1034	7.2908	André Antoine	2014	6	8
Cicadetta petryi	Nécropole militaire	FR		48.1406	7.3105	André Antoine	2014	6	9
Cicadetta petryi	Mont de Sigolsheim	FR		48.1411	7.3061	André Antoine	2011	5	13
Cicadetta petryi	Les Plaines, ferme du Merque	FR		48.1416	7.3037	André Antoine	2014	6	9
Cicadetta petryi	Nécropole militaire	FR		48.1418	7.3075	André Antoine	2014	6	9
Cicadetta petryi	Nécropole militaire	FR		48.1431	7.3054	André Antoine	2014	6	9
Cicadetta petryi	Mont de Sigolsheim	FR		48.1445	7.3034	André Antoine	2014	6	9
Cicadetta petryi	Mont de Sigolsheim	FR		48.1447	7.3016	André Antoine	2014	6	9
Cicadetta petryi	Mont de Sigolsheim	FR		48.1460	7.3014	André Antoine	2014	6	9
Cicadetta petryi	Mont de Sigolsheim	FR		48.1467	7.2991	André Antoine	2014	6	9
Cicadetta petryi	Mont de Sigolsheim	FR		48.1471	7.2964	André Antoine	2014	6	9
Cicadetta petryi	Mont de Sigolsheim	FR		48.1477	7.2932	André Antoine	2014	6	9
Cicadetta petryi	Grasberg, Bergheim	FR	320	48.2114	7.3519	Hertach Thomas	2009	5	23
Cicadetta petryi	Grasberg	FR		48.2119	7.3592	André Antoine	2014	5	25
Cicadetta petryi	Grasberg, Bergheim	FR	280	48.2125	7.3586	Hertach Thomas	2009	5	23
Cicadetta petryi		FR	335	48.2133	7.3575	Hugel Sylvain	2006	6	18
Cicadetta petryi	Krapenhummel, Dinsheim	FR	265	48.5458	7.4200	Brua Christophe	2007	6	18
Cicadetta petryi	Krapenhummel, Dinsheim	FR	270	48.5458	7.4200	Hertach Thomas	2009	5	22
Cicadetta petryi		FR	265	48.5636	7.4931	Hugel Sylvain	2003	6	14
Cicadetta petryi		FR	245	48.5783	7.4931	Hugel Sylvain	2006	6	11
Cicadetta petryi	Schutterbronn	FR		48.5827	7.4957	André Antoine	2011	5	15
Cicadetta petryi		FR		49.0847	1.6636	Gadoum S., Sueur Jérôme	2006	6	16
Cicadetta petryi	oberhalb S. Martino, Maira	IT	1020	44.5070	7.0952	Hertach Thomas	2012	6	18
Cicadetta petryi	oberhalb S. Martino, Maira	IT	1150	44.5214	7.0871	Hertach Thomas	2012	6	18
Cicadetta petryi	Capanne di Cosola	IT	1420	44.6679	9.2052	Hertach Thomas	2012	6	22
Cicadetta petryi	Capanne di Cosola	IT	1410	44.6759	9.2031	Hertach Thomas	2012	6	22
Cicadetta petryi	Passo del Giova	IT	1270	44.6827	9.2215	Hertach Thomas	2012	6	22
Cicadetta petryi	oberhalb Corbesassi	IT	1000	44.7077	9.2653	Hertach Thomas	2013	7	4
Cicadetta petryi	Cima di Colletta	IT	1310	44.7094	9.2598	Hertach Thomas	2012	6	22
Cicadetta petryi	nördl. Corbesassi	IT	940	44.7179	9.2722	Hertach Thomas	2013	7	4
Cicadetta petryi	südwestl. Brallo di Pregola	IT	970	44.7341	9.2759	Hertach Thomas	2013	7	4
Cicadetta petryi	Brallo di Pregola	IT	900	44.7401	9.2931	Hertach Thomas	2012	6	22
Cicadetta petryi	Monte d'Alpe	IT	1050	44.8022	9.3149	Hertach Thomas	2013	7	3
Cicadetta petryi	Monte d'Alpe	IT	1010	44.8087	9.3186	Hertach Thomas	2013	7	3
Cicadetta petryi	Fruška gora, Bešenovački	IT	1340	44.9464	6.7972	Hertach Thomas	2012	6	16
Cicadetta petryi	südwest. Salbertrand	IT	1020	45.0551	6.8577	Hertach Thomas	2012	6	16
Cicadetta petryi	südöstl. Epinel, Cogne	IT	1490	45.6214	7.3334	Hertach Thomas	2012	6	14
Cicadetta petryi	Epinel, Cogne	IT	1520	45.6301	7.3281	Hertach Thomas	2012	6	13
Cicadetta petryi	nördlich Spiazzi, Monte Baldo	IT	900	45.6570	10.8550	Hertach Thomas	2010	6	17
Cicadetta petryi	Pont El, Cogne	IT	900	45.6783	7.2207	Hertach Thomas	2012	6	13
Cicadetta petryi	Valle Toscolano	IT	470	45.6956	10.6117	Hertach Thomas	2010	6	17
Cicadetta petryi	unterhalb Selvino	IT	770	45.7690	9.7424	Hertach Thomas	2010	6	18
Cicadetta petryi	zw. Roisan-Porossan	IT	770	45.7746	7.3161	Hertach Thomas	2011	6	24
Cicadetta petryi	südl. Doues, östl. Meylan	IT	950	45.7950	7.3017	Hertach Thomas, Trilar Tomi	2007	6	10
Cicadetta petryi	südl. Doues, östl. Dialley	IT	1120	45.8083	7.3067	Hertach Thomas, Trilar Tomi	2007	6	10
Cicadetta petryi	Monte Barro, Lecco	IT	720	45.8288	9.3800	Hertach Thomas	2013	7	2
Cicadetta petryi	oberhalb Valpelline	IT	1150	45.8300	7.3317	Hertach Thomas, Trilar Tomi	2007	6	10
Cicadetta petryi	Monte Chiusarella, Rasa	IT	900	45.8727	8.8142	Hertach Thomas	2013	6	2
Cicadetta petryi	Monte Chiusarella, Rasa	IT	900	45.8732	8.8155	Hertach Thomas	2013	6	2

Chapter 5: Sing me your name: Additional *Cicadetta montana* complex species in Italy

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ABSTRACT

The *Cicadetta montana* species complex was shown to be diverse in Italy (see previous Chapters 1 to 4). However, we only discussed the taxa belonging to the *C. cerdaniensis* and *C. brevipennis* song groups. *Cicadetta montana* s. str. was the only additional species found in Italy. The homogeneity of the observations throughout Europe in general and Italy in particular suggests that it is a species without complicated taxonomy. *Cicadetta montana* s. str. is widely distributed but only abundant locally in Italy.

MATERIALS AND METHODS

See Chapters 1 to 4

RESULTS AND DISCUSSION

Taxonomy

The extensive data collected in Italy revealed only one additional species namely *Cicadetta montana* s. str. (Scopoli, 1772) which belongs neither to the *Cicadetta cerdaniensis* nor to the *Cicadetta brevipennis* song groups. This species can easily be recognised by the swelling long-lasting single echeme which is interrupted afterwards by several seconds or even minutes of acoustic inactivity (**Fig 5.1**). In the entire Italian distribution range the song patterns did not show any important variations. Therefore, we conclude the presence of a single taxon. Song-based delimitation is supported by the molecular result from the mitochondrial DNA (Chapter 3 = Wade *et al.*, 2015), where the three specimens from distant Italian localities (Calabria, Piedmont, Emilia-Romagna) cluster with all the other European *C. montana* s. str. in a monophyletic clade with rather low intraspecific genetic distances.

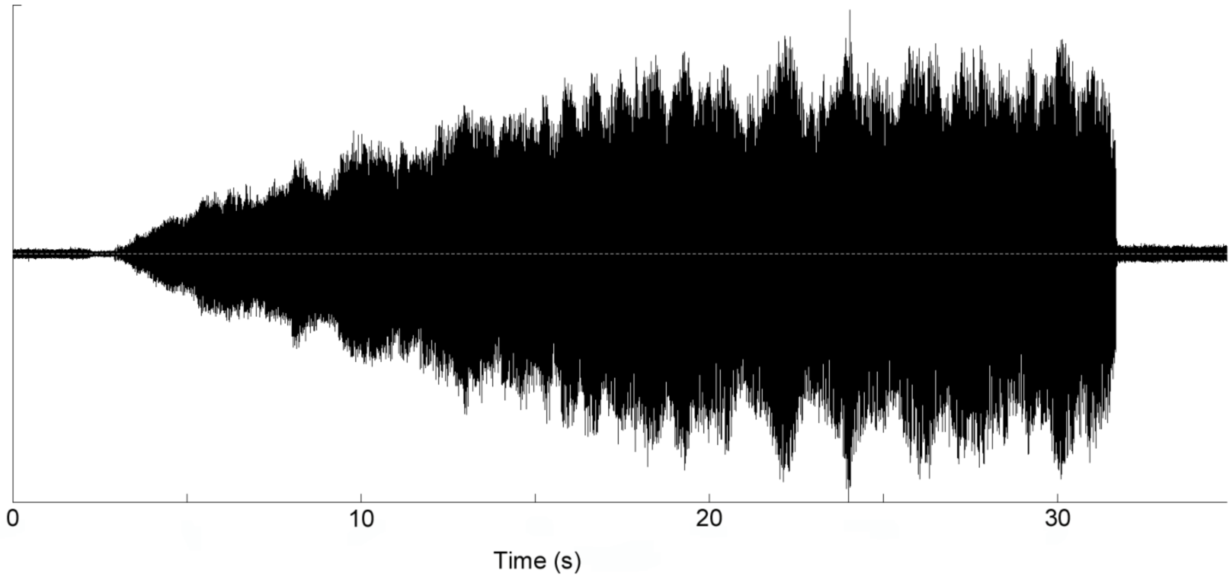

Fig 5.1. Oscillogram (time versus amplitude) of calling song of *Cicadetta montana* s. str. from Mount Etna (Sicily). A second specimen is singing in the background.

Distribution

Cicadetta montana s. str. is one of the widest distributed cicada species in Italy (**Fig 5.2**). It was recorded from Brixen (South Tyrol) in the north to Mount Etna in Sicily in the south and from Aosta in the west to the Slovenian border in the east. However, its distribution is patchy and it generally does not seem to be an abundant species as it has only 103 records [by comparison: *Cicadetta sibillae* Hertach & Trilar, 2015 237 records, *C. anapaistica* Hertach, 2011 (both subspecies) 140 records, *C. brevipennis* Fieber, 1876 (both subspecies) 127 records]. Numerous populations have only been found in two distant places namely the eastern Alps (**Fig 5.3A**) and Calabria (**Fig 5.3B**). However, no populations were found for almost 200 km in the western Alps and in parts of the central Apennine Mountains (Toscana-Umbria). We suspect methodological problems to be the reason for the absence from the western Alps. *Cicadetta montana* s. str. occurs and reproduces in many different habitats. Some habitats are not obvious at first glance such as European Beech forests (*Fagus sylvatica*), others have small dimensions like rocky ridges or slopes, and others are at elevated altitudes which have not been searched intensively. The absence recorded in the Apennines is probably accurate since the mountains are lower and therefore provide ideal conditions for *Cicadetta brevipennis brevipennis* Fieber, 1876 (Chapter 4). *Cicadetta sibillae* has also not been recorded in this Apenninian range (Chapter 2 = Hertach *et al.*, 2015).

Cicadetta montana s. str. was found with high probability in very small habitats such as rocky clearcuttings along roads in the beech forest zone in the Apennine Mountains (**Fig 5.3C**). This is interesting since there are vast gaps of seemingly no occurrence for tens of kilometres. However, we expect a local network of similar small habitats spread over the landscape which are difficult and very time consuming to find.

No other cicada species prefers habitats as elevated as *Cicadetta montana* s. str. The Italian finds are located at a mean altitude of 1160 ± 350 m asl. Thereby, the Alpine records are situated clearly below (880 ± 280 m asl) and the Apenninian ones above (1310 ± 290 m asl) this mean altitude. A well-supported trend from south to north exists in the Apennine Mountains: the altitudes of the habitats are negatively correlated with geographical latitude (linear regression: $R^2 = 0.38$). Its culmination point is at Sierra di Crispo (Pollino National Park) at 1960 m asl, syntopically occurring with *Cicadetta anapaistica lucana* Hertach, 2015 (Chapter 2 = Hertach *et al.*, 2015).

Fig 5.2. Distribution map of *Cicadetta montana* s. str. in Italy.

Cicadetta montana s. str. is by far the widest distributed cicada in Europe. Its north to south expansion is remarkable, reaching from southern Scandinavia (Endrestøl, pers. comm.) to Mount Etna in Sicily. The origin of the species is not as obvious as in the *C. cerdaniensis* and *C. brevipennis* song group taxa (Chapter 2 = Hertach *et al.*, 2015; Chapter 4). From the mtDNA genetic distances (Chapter 3 = Wade *et al.*, 2015; compare Brower, 1994; Papadopoulou *et al.*, 2010), it can be inferred that *C. montana* s. str. supposedly diverged before the Pleistocene ice ages. It is well possible that multiple distant regions served as refuges in cold periods.

Since *Cicadetta fangoana* Boulard, 1976 is the most abundant species on Corsica (Puissant & Sueur, 2001), the taxon was expected to occur in Sardinia as well. We visited Sardinia once at the end of June/beginning of July and once in late July and did not find any species of the *Cicadetta montana* complex. *Cicadetta fangoana* could only have remained undiscovered if it formes ecotypes with a clearly earlier adult seasonal pattern than is known for Corsica. The Elba Island also completely lacks *Cicadetta* species. Thus, it seems that *C. fangoana* is a strictly endemic species of Corsica and the 12 km wide sea between Corsica and Sardinia (Bocche di Bonifacio) is an effective barrier hindering the dispersal. This has been observed for many other organisms such as amphibians (Grill *et al.*, 2007). Cicadas are excellent flyers, but for short distances only (Simões & Quartau, 2007), as is underlined by this finding.

Fig 5.3. Typical habitats of *Cicadetta montana* s. str. from Italy. (A) Near Pontebba (Val Canale, Friuli-Venezia) in Scots Pine wood *(Pinus sylvestris)* on limestone, (B) at Montalto (Aspromonte, Calabria) in the European Beech forest zone *(Fagus sylvatica)*, (C) at Mount Maggiorasca (Aveto, Emilia-Romagna) in a rocky clearcutting along the road, (D) at Mount Etna (Sicily) in endemic *Betula aetnensis* and other sparse woods.

Threat and conservation

Many habitats of *Cicadetta montana* s. str. are natural (e.g. steep rocky woods) and not endangered. The most serious threat arises from grazing. Species of the *Cicadetta montana* complex have been shown to react sensitively to intense or early pasturing, probably because the last larval instar lives close to the ground surface is trampled upon (Joint Nature Conversation Committee, 2007; Hertach, 2007; Chapter 1 = Hertach, 2011; Chapter 2 = Hertach *et al.*, 2015). The preferred altitudinal belt of *Cicadetta montana* s. str. overlaps to a large extent with traditional pastures in the Apennine Mountains. Many of the pastures have large populations of cattle and are usually overgrazed even in the case of protected areas such as National or Natural Parks. Local cicada populations probably suffer from the intensive pasturing. However, *C. montana* s. str. is not in need of special conservation measures, with one exception namely the three known Sicilian populations. The first priority should be to protect the large Mount Etna population (30-50 males singing). The semi-open structure of trees including endemic *Betula aetnensis* in combination with the extensively grazed meadows must be maintained (**Fig 5.3D**).

REFERENCES CHAPTER 5

Brower AVZ. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences USA* 91: 6491-6495.

Grill A, Casula P, Lecis R, Menken S. 2007. Endemism in Sardinia. In: Weiss S, Ferrand N, eds. *Phylogeography of Southern European Refugia.* Dodrecht: Springer, 273-296.

Hertach T. 2007. Three species instead of only one: Distribution and ecology of the *Cicadetta montana* species complex (Hemiptera: Cicadoidea) in Switzerland. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 80: 37-61.

Hertach T. 2011. Spectacular song pattern from the Sicilian Mountains: The new cicada species *Cicadetta anapaistica* sp. n. (Hemiptera: Cicadidae). *Zootaxa* 2771: 25-40.

Hertach T, Trilar T, Wade EJ, Simon C, Nagel P. 2015. Songs, genetics, and morphology: revealing the taxonomic units in the European *Cicadetta cerdaniensis* cicada group, with a description of new taxa (Hemiptera: Cicadidae). *Zoological Journal of the Linnean Society* 173(2): 320-351.

Joint Nature Conversation Committee. 2007. *UK action biodiversity plan – action plan for Cicadetta montana.* Available from: http://www.ukbap.org.uk/ukplans.aspx?ID=216 (accessed 5 March 2010).

Papadopoulou A, Anastasiou I, Vogler AP. 2010. Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. *Molecular Biology and Evolution* 27: 1659-1672.

Puissant S, Sueur J. 2001. Contribution à l'étude des Cigales de Corse (Hemiptera, Cicadidae). *Bulletin de la Société entomologique de France* 106(5): 429-436.

Simões PC, Quartau JA. 2007. On the dispersal of males of *Cicada orni* in Portugal (Hemiptera: Cicadidae). *Entomologia Generalis* 30(3): 245-252.

Wade EJ, Hertach T, Gogala M, Trilar T, Simon C. 2015. Molecular species-delimitation methods recover most song-delimited cicada species in the European *Cicadetta montana* complex. *Journal of Evolutionary Biology* 28(12): 2318-2336.

Part III:

Faunistics, distribution and conservation of the Swiss cicadas

Chapter 6: Cicadas in Switzerland: A scientific overview of the historic and current knowledge of a popular taxon (Hemiptera: Cicadidae)

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ABSTRACT

Cicadas are charismatic and are widely appreciated, even by the general public, but knowledge of species diversity and distribution is patchy, incomplete and sometimes misleading. This study presents an overview on the historic and current knowledge on the cicadas of Switzerland. For the first time, data retrieved from historic and recent literature, review of public and private collections and detailed recent field-work including up-to-date recording techniques have been combined. Our work during the last decade has resulted in the doubling of the number of known species. We now report the existence of ten native species in Switzerland: *Cicada orni, Lyristes plebejus, Tibicina quadrisignata, T. steveni, T. haematodes, Cicadetta montana* s. str.*, C. cantilatrix, C. sibillae, C. petryi* and *Tettigettalna argentata*. All species are presented with detailed distribution maps, data on habitat and conservation status. Centres of cicada diversity are the southern Swiss Cantons of Valais, Ticino and Geneva. Nine species have restricted habitat requirements, seven species are rare and three species are of high national conservation importance in Switzerland.

Front page: *Tibicina steveni* (top left), cicada habitats in Switzerland: above Gampel in the Valais (top right) and near Chancy, Canton of Geneva (bottom left), *Tettigettalna argentata* (bottom right).

INTRODUCTION

Cicadas (Cicadidae) form an attractive group of potential flagship species that are recognised and liked by many non-scientists in Switzerland. Some Swiss species attract attention by their loud calling songs, but many species are rare and very difficult to see or capture. Furthermore, recent studies have shown Central European cicadas to be taxonomically interesting. Acoustic investigations have demonstrated that *Cicadetta montana* s. l. consists of several species with clearly different calling song patterns (e.g. Gogala & Trilar, 2004; Sueur & Puissant, 2007a). The consideration of song patterns as species-specific characters has permitted a reassessment of the Swiss cicada fauna (Hertach, 2004; 2007; Chapter 2 = Hertach *et al.*, 2015; Chapter 4).

Very few publications are available on Swiss cicadas, although the country includes Mediterranean regions in Ticino and Grisons and xerothermic valleys in the Valais that potentially favours high diversity in cicadas. Before 2004, only four references in scientific journals referred to Swiss cicadas in their titles (Artmann, 1987; Pillet, 1993; Vernier, 1996; Sueur *et al.*, 2003). In contrast more than twenty publications on local occurrences of *Cicadetta montana* s. l. (e.g. Hüeber, 1904; Schwoerbel, 1957; Müller 1969; Eitschberger, 1972; Niehuis & Simon, 1994; Dorda, 2012; Meineke, 2012) and *Tibicina haematodes* (e.g. Geisenheyner, 1887; Vogel, 1935; 1937; Schwoerbel, 1957; Niehuis & Simon, 1994) in Germany have been published, even though the cicada fauna of Germany is less rich (Nickel & Remane, 2003; Biedermann & Niedringhaus, 2004; Kunz *et al.*, 2011; Meineke, 2012).

The poor understanding of Swiss cicada distribution and diversity is a big hurdle to plan and implement conservation strategies for their protection. A summary of our basic knowledge of this group has been lacking completely for Switzerland. Our work intends to update the knowledge on the species diversity, distribution, and habitat and conservation requirements of cicadas in Switzerland. The presented data are based on field studies conducted during the last decade, studies of entomological collections and published data from the 18th century onwards.

MATERIALS AND METHODS

Over the last years systematic field work has been conducted in regional projects across the country: eastern part of the Jura Mountains (Cantons of Aargau, Solothurn, Basel-Stadt and Basel-Land), Canton of Ticino, Canton of Graubünden (Grisons), Lake Geneva Basin (Canton of Geneva and parts of Canton of Vaud). Other regions have been visited occasionally. Regions and subregions mentioned several times within this work are illustrated in **Fig 6.1**.

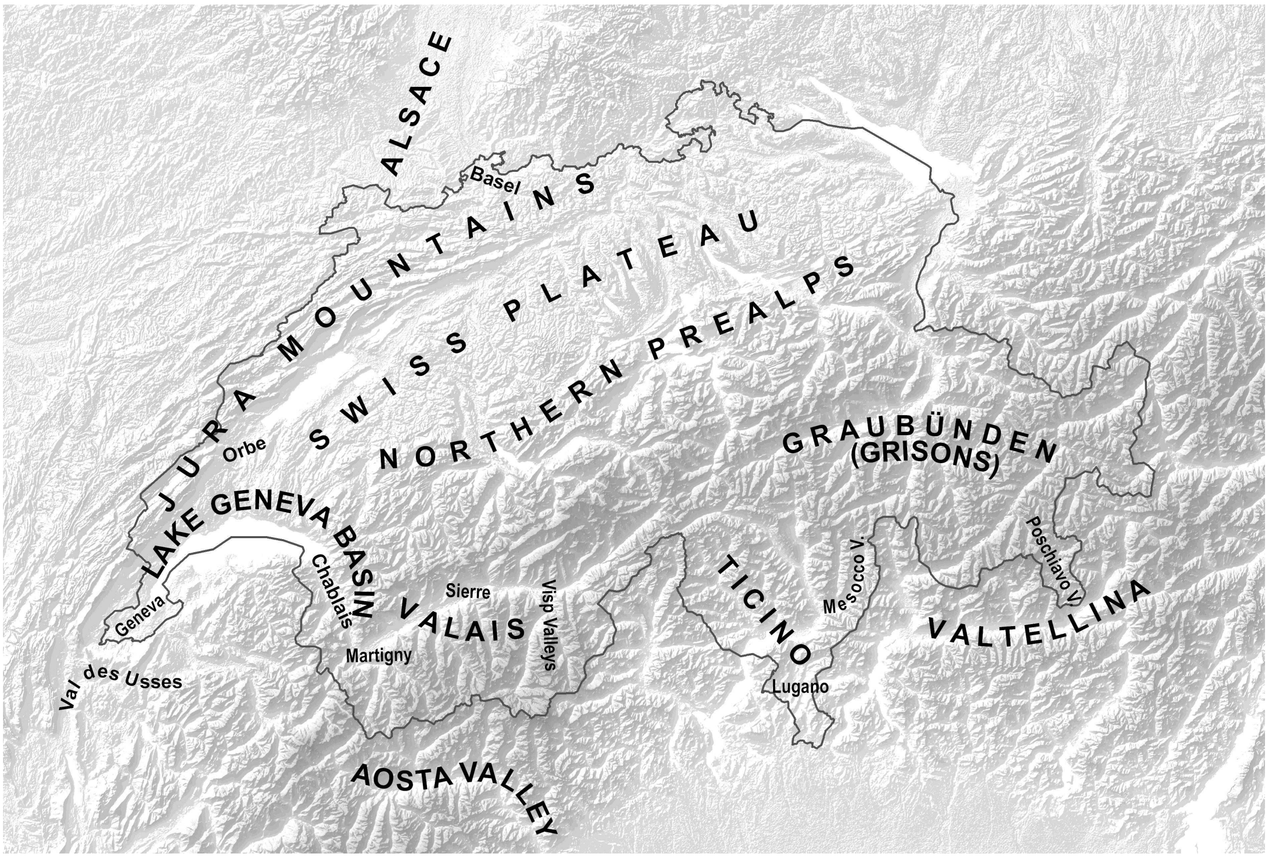

Fig 6.1. Regions (capital letters) and subregions (normal letters) used for cicada recording within this study.

For recent records, the presence of a species at a locality was normally detected acoustically. Recordings were made with a Marantz PMD 660 (sampling frequency at 48 kHz) and mainly analysed using Raven Pro 1.3 (Cornell Lab of Ornithology). The number of singing individuals was counted or estimated. For detecting and recording the species in the *Cicadetta montana* complex, which sing in a high carrier frequency range (10-20 kHz), an ultrasonic detector (Pettersson D 200) was used whose microphone was fitted into a Telinga parabola or a smaller parabola with a 15 cm diameter (adapted from Popov *et al.*, 1997).

Records of cicadas from the public collections of the Swiss natural history museums (Aarau, Basel, Bern, Chur, Frauenfeld, Fribourg, Geneva, Lausanne, Liestal, Lucerne, Lugano, Neuchâtel, Olten, Schaffhausen, Sion, Solothurn, St. Gallen, Winterthur), of the Eidgenössische Technische Hochschule Zürich and of the Agroscope Changins Nyon have been reviewed and records compiled. A few museums outside Switzerland (Paris, Vienna, Ljubljana) and private collections (Pillet, Marchesi, Chittaro, Vernier, Keim, Artmann) have been checked for usable data. We attempted to review all potentially relevant regional, national and international zoological journals or series for references on cicadas from Switzerland, with particular attention to local publications for the Cantons of Ticino, Valais, Grisons, Vaud and Geneva. Digital literature databases have been searched using relevant key words. The data from the 'Swiss Central Fauna Data Bank' was included (CSCF, Neuchâtel). Unexpected or questionable records from all these sources have been verified by personal contacts with other entomologists or naturalists where possible. Several requests have been published in newspapers and magazines to stimulate reports on observations of the few remarkable species in the Basel region, Ticino and the Lake of Geneva Basin.

According to the Swiss graticule, chorological data is visualised in a grid of 5 x 5 km^2 cells with ArcGIS (map source: http://srtm.csi.cgiar.org and http://www.diva-gis.org). Distribution maps are based on the two parameters 'abundance' and 'time period of the observation' each of them divided into three categories (**Table 6.1**). Note that 'time period' is the latest record and 'abundance' is the highest density and these are presented in the maps for each cell. Observations made within one time period but from different locations are pooled for each cell. Although we only include reliable records, the level of certainty is different when comparing literature references, voucher specimens or data gathered during field-work. For example, voucher specimens are not helpful in distinguishing species morphologically in the *Cicadetta montana* complex. Only verified acoustic records can be included. In contrast, acoustic field observations of some species belonging to the genus *Tibicina* Amyot, 1847 are uncertain, because the songs are very similar (Sueur & Aubin, 2003). Within this genus we have normally integrated records based on either voucher specimens or digital recordings of the calling song analysed on the computer. The distribution maps show some unpublished records from adjoining countries of Switzerland that could be helpful for the understanding of the chorological pattern.

Based on historical data, the current distribution and habitat requirements, the level of extinction threat of the species is estimated for Switzerland. These data will provide information for Red List assessment of each species. Furthermore, we discuss the abundance of species found in Switzerland in an international context. We highlight those important populations that occur within the borders of Switzerland and are therefore of high national conservation importance for the country.

Nomenclature follows Puissant (2006), Gogala (2015; www.cicadasongs.eu) for European species, Boulard (1981), Hayashi & Saisho (2011) for exotic taxa and Moulds (2005) for higher classification. We provide German common names in addition to previous works. We have chosen the most established and accurate term in cases with several common names.

Table 6.1. Parameters and their categories for the production of the distribution maps.

Parameter	Category	Description
Abundance	High	more than 30 specimens in *Cicadetta, Tettigettalna* and *Cicada*, more than 20 specimens in *Lyristes* and *Tibicina*
	Low	less than 30 specimens in *Cicadetta, Tettigettalna* and *Cicada*, less than 20 specimens in *Lyristes* and *Tibicina*, but reproductive population expected or confirmed
	Single specimen	only one specimen reported within one time period, reproductive population not expected
Time period	2000-2012	recent observations
	1950-1999	elderly observations
	before 1950	historic occurrences
Data source	Field observation	observations unpublished, stored in the Swiss Central Fauna Data Bank or/and made by the authors (discrimination normally according to acoustic characters)
	Collection	specimens found in public and private collections (discrimination according to morphology)
	Literature	references published by other scientists or naturalists

Acronyms

(see Evenhuis, 2014)

BNM	Bündner Naturmuseum Chur
ETHZ	Eidgenössische Technische Hochschule Zürich
MCSN	Museo Cantonale di Storia Naturale Lugano
MHNG	Muséum d'Histoire Naturelle de la Ville de Genève
MHNN	Muséum d'Histoire Naturelle Neuchâtel
MNHN	Muséum National d'Histoire Naturelle Paris
MZLS	Musée de Zoologie Lausanne
NAAG	Naturama Aarau
NHMB	Naturhistorisches Museum Basel
NMBE	Naturhistorisches Museum der Burgergemeinde Bern
NMLS	Natur-Museum Luzern
NMS	Naturmuseum Solothurn
NMT	Naturmuseum Thurgau Frauenfeld
NMWI	Naturmuseum Winterthur

RESULTS

General overview

1288 field records from Switzerland have been included in the final analysis, most of them gathered within the last 10 years and 824 made by the first author. The study is supported by 128 sufficiently labelled voucher specimens from public and private collections and by 194 reliable and spatially precise literature references, 78% referring to Pillet (1993) (**Table 6.2**). Literature references have been found in general works on insects, descriptions of Swiss nature and the popular science literature, rather than in specific scientific entomological publications.

Table 6.2. Number of records per species integrated to the maps. Field records, literature references and voucher specimens and grid cells occupied (without records from the neighboured foreign countries). *Field records which have already been published in own studies and vouchers in the own private collection are only counted in this second column.

	Field*	Literature	Vouchers	total	Grid cells
Cicada orni	309	98	57	464	68
Lyristes plebejus	65	8	15	88	15
Tibicina quadrisignata	35	47	27	109	18
Tibicina steveni	26	36	21	83	16
Tibicina haematodes	5	0	10	15	3
Cicadetta montana	532	1	0	533	182
Cicadetta cantilatrix	233	4	0	237	66
Cicadetta sibillae	39	0	0	39	11
Cicadetta petryi	31	0	0	31	8
Tettigettalna argentata	13	0	0	13	2
total	1288	194	128	1610	

Moffet *et al.* (1634) looked intensively for cicadas in “Helvetia” but without any success, although it is not clear if they visited Ticino and the Valais. They were convinced that other contemporary naturalists had confused cicadas with grasshoppers and other insects. In the 18th and early 19th century a general confusion existed in the taxonomy and nomenclature of European cicadas. Even common and currently well-known species have been mixed up due to inexact descriptions or illustrations, missing type specimens and scarce chorological data. For example, Dietrich (1872) was, according to our knowledge, the first Swiss author to use the name *Cicadetta montana* (Scopoli, 1772), exactly one century after the description. Previously this species was erroneously called *Tibicina haematodes*, *Cicada orni* or *Cicadetta concinna* by different authors (e.g. Sulzer, 1761; Fuesslin, 1775; Heer & Blumer, 1846; Bremi, 1849). These confusions sometimes lead to difficulties in the interpretation of historical data.

Fuesslin (1775, p. 24) mentions the four cicadas "*Cicada orni"*, "*Cicada plebeja"*, "*Cicada haematodes,* der Blutring" and "*Cicada violacea*, die Blaue" in his list of Swiss insects. "*Cicada orni"* and "*Cicada plebeja"* are obviously identical to the species currently known as *Cicada orni* Linnaeus, 1758 and *Lyristes plebejus* (Scopoli, 1763). "*Cicada haematodes*" is referenced to Sulzer's illustration (1761, plate 10, fig. 65) which, however, shows *Cicadetta montana* s. l. (see e.g. Hagen, 1856b; Fieber, 1876). The fourth species, "*Cicada violacea*", remains mysterious (see section "Comments on other species" below). Sulzer (1761) and Von Salis (1807) did not mention any cicada records from Switzerland.

Bremi (1849) recorded five cicada species from Switzerland, but with the exception of "*Cicada concinna*" which can reliably be referred to *C. montana* s. l., does not name the remaining four species. Some species must have been locally abundant in the Valais and Ticino, as Bremi stated (1849, p. 333) "für feine Ohren in belästigender Menge" (= in irritating density for sensitive ears). Hagen (1855; 1856a; 1856b) refers to the local knowledge of Bremi. The information on Swiss cicadas that these early studies contributed was substantially greater than was to be published in the next hundred years.

Stoll (1901) listed the three species *Cicada orni*, *Tibicina haematodes* (Valais) and *Cicadetta montana*. Robert (1937) knew five species sporadically entering Central Europe, but only provides one record for a more precisely defined area (*C. montana* s. l.). Other authors mentioned cicadas casually and only generally as remarkable representatives of the insects (e.g. Stabile & Stabile, 1856; Bettelini, 1919; Zschokke, 1928; Segantini, 1937), completely omit this group despite promising general study titles (e.g. Pavesi, 1873; Carlini, 1887), or do not provide any information for Switzerland (Melichar, 1896; Oshanin, 1912). Nast (1972) listed seven species in his catalogue on Palaearctic Auchenorrhyncha for Switzerland, but three of them are obviously erroneous as will be demonstrated below. The author corrected some of them himself when listing only five species in his second catalogue (Nast, 1987).

The absence of publications on the cicadas from the southern part of the Alps (Ticino and some valleys in Grisons) is particularly notable. Neither the first 99 volumes of the *Bollettino della Società ticinese di scienze naturali* nor the 300 issues of the more popular *Il nostro paese* include a single reference to cicadas at the local or national scale. In contrast many non-scientists from these regions perceive and know well the song of 'le cicale', which normally refers to *Cicada orni*. Perty (1879, p. 315) writes about two species occurring in Ticino, one singing by day and one by night, but the latter observation must refer to an Orthoptera species. A detailed study of the cicadas of Ticino has just been published (Hertach & Pollini Paltrinieri, 2012).

Cicadas have been treated in popular, well illustrated literature on nature in the Valais (Bille, 1980; Bille & Werner, 1986; Delarze, 1988; Oggier, 1995). Pillet (1993) published the first modern and comprehensive work on cicadas focussing on the Valais region. He reported five species, each of them presented with detailed distribution maps and ecological data: *Cicada orni, Lyristes plebejus, Cicadetta montana* (Scopoli, 1772)*, Tibicina quadrisignata* (Hagen, 1855) and *T. haematodes* (Scopoli, 1763). He concludes that these five species are the only ones in Switzerland. Cicadas from the Valais have been observed by different local naturalists since the study by Pillet, with some notable records published in the annual reports of the *Bulletin de la Murithienne* (Sierro & Keim, 1999; Sierro *et al.*, 2000; Marchesi *et al.*, 2002).

In more recent literature on a national scale, Günthart & Mühlethaler (2002) and Holzinger *et al.* (2003) listed the six species *C. orni, L. plebejus, T. quadrisignata, T. haematodes, C. montana* and *Cicadatra atra* (Olivier, 1790) mainly referring to Schedl (2000). Almost the same species spectrum is available from the Fauna Europaea project, solely missing *T. quadrisignata* (Hoch, 2011). In 2007, the small cicada *Tettigettalna argentata* (Olivier, 1790) was found for the first time in Switzerland (Hertach, 2008). Additionally, the presence of *Cicada orni* and *Lyristes plebejus* has been confirmed within the current study, besides the genera *Cicadetta* and *Tibicina* which have to be discussed in more detail.

Cicadetta montana species complex

In the 18th and 19th centuries many species and subspecies similar to *Cicadetta montana* (Scopoli, 1772) were described using morphological characters. Most authors later assigned them to the two taxa, *C. montana* and *C. concinna* (Germar, 1821 = *C. podolica* Eichwald, 1830) for Central Europe, since the characters showed large and overlapping variability (e.g. Duffels & Van der Laan, 1985; Nast, 1987; Schedl, 2000). The introduction of acoustic methods has revealed sibling species even in Central Europe, which is probably one of the most astonishing taxonomic findings in entomology for this region in recent decades (Gogala & Trilar, 2004; Puissant & Boulard, 2000; Sueur & Puissant, 2007a). Currently, 14 European species, many of them from Greece, have been described within the complex (Gogala *et al.*, 2011; Chapter 1 = Hertach, 2011; Chapter 2 = Hertach *et al.*, 2015; Chapter 4).

Acoustic differences among cicada species have been noted earlier and it would not do justice to the 19th century's entomologists from Germany and Switzerland to assume they did not consider discriminating species on this basis. Bremi (1849, p. 333) wrote 150 years before the elucidation of the *Cicadetta montana* species complex: "Ich vermuthe, dass sich neben jener gewöhnlichen Art noch eine oder zwei in unsern warmgelegenen Laubwäldern vorfinden. Sie zu entdecken, wird man auf den Gesang derselben lauschen müssen, der unter den Arten sehr verschieden ist, wie Hr. Professor Von Siebold an zwei bei Freiburg im Breisgau entdeckten Arten beobachtet hat." [= I suppose that one or two species will be detected in our thermophilous deciduous forests in addition to the common species. You have to listen to their songs which are clearly different between species, what has been observed by Professor Von Siebold in two detected species near Freiburg (Germany)]. It seems that Siebold (1847, p. 10) had still misinterpreted the songs in his own work two years before. He described a song that repeated echemes 10 to 12 times in a fast rate. This description fits well to the song of *Cicadetta cantilatrix* Sueur & Puissant, 2007a. And he concluded: "… geben ... ziemlich laute Töne von sich, welche, wenn diese Sänger in grossen Gesellschaften auf Bäumen und Buschwerk umher vertheilt ihre Stimme hören lassen, so in einander schmelzen, dass nur noch ein einziger ununterbrochener Ton durch die Luft getragen wird" (non-literal translation: If calling males occur in dense populations, the tones will merge resulting in one single steady sound). In fact, Siebold probably observed a syntopic population of *Cicadetta cantilatrix* and *C. montana* s. str. whose song fits well with the steady song pattern. Neither Bremi nor Siebold published later descriptions of a new species and their findings were not commented on by subsequent authors.

Artmann (1987) described the song of *C. cantilatrix* from the Swiss Jura Mountains in words, but he concluded to have found a regional dialect of *C. montana* s. str. It took another 15 years until these acoustic differences were investigated more deeply (Puissant & Boulard, 2000; Gogala & Trilar, 2004; Hertach, 2004). Currently we know that in Switzerland four sibling species of the complex exist (Hertach, 2007; Chapter 1 = Hertach, 2011; Chapter 2 = Hertach *et al.*, 2015; Chapter 4).

Genus *Tibicina* Amyot, 1847

The genus *Tibicina* consists of larger sized species (up to 85 mm wingspan), singing loudly and with fairly similar continuous song patterns. The species can be separated relatively easily by the specific colouration of the thorax and the wing venation, but are difficult to catch as solitary singers. Bugnion (1890) listed "*Cicada haematodes*" and "*Cicada viridinervis*" for the Valais, the latter currently known as a variety of *Tibicina haematodes* described by Fieber (1876). Bugnion had already realised that two different taxa occur in the Valais. Other authors solely mention *Tibicina haematodes*, the most widely distributed European species, as the single Swiss species of this genus occurring in the Valais (Meyer-Dür, 1860; Stoll, 1901; Kuntze, 1931). Pillet (1993) was the first to publish that *Tibicina quadrisignata* occurs in the Valais. Ten years later, Sueur *et al.* (2003) published that the second species is the eastern species *Tibicina steveni* (Krynicki, 1837) and that *T. haematodes* does not occur in the Valais. The surprising presence of *T. steveni* in the Valais had actually been reported indirectly long previously. Hagen (1855) assigned a specimen received from Bremi to his variety "*Cicada sanguinea*" which he supposed to be identical to Krynicki's "*C. steveni*". He also wrote that none of the Swiss specimens belonged to *T. haematodes*.

Nevertheless, a single reference by Vernier (1996) indicated that *T. haematodes* could belong to the Swiss fauna. He listed voucher specimens deposited in the MHNG (see also Hollier, 2012). They were collected in the Canton of Geneva in the first half of the 20th century. We studied these vouchers and confirm Vernier's comment. *Tibicina haematodes* is, therefore, the tenth, but extremely rare indigenous Swiss species, the third of the genus *Tibicina* (**Fig 6.2**).

Annotated list of Swiss cicadas

Cicada orni Linnaeus, 1758

German common name: Mannazikade (e.g. Remane & Wachmann, 1993; Schedl, 2000)
French common name: La Cigale de l'orne (Pillet, 1993)

Distribution (Fig 6.3)

Cicada orni is the most popular European cicada due to its remarkable chorus song and its wide distribution area. If Swiss non-scientists speak about cicadas they normally refer to this species.

Fuesslin (1775) knew *C. orni* from the Valais as "very abundant". Saussure (1780) wrote that *C. orni* is completely missing in the Geneva region and provides herewith an interesting absence record in very old literature. Labram & Imhoff (1845) reported it from the most southern parts of Switzerland where it was locally abundant. Curti (1846) thought *C. orni* to be restricted to Ticino, some parts of Grisons and found occasionally in the Valais. Seiler (1847) listed the species as uncommon for the Canton of Schaffhausen, but we doubt its validity since he does not report *Cicadetta montana* s. l. Hagen (1856a) cites Bremi who has found it as the most common species in Ticino. Tschudi (1872) listed *C. orni* as abundant in both Ticino and Valais with a record altitude of "3800 feet" (≈ 1150 m asl) for the upper Valais. Rougemont (1880, p. 33) mentioned "un charivari vraiment méridional" at Fully (VS) (= a true Mediterranean pandemonium).

Fig 6.2. Three species of the genus *Tibicina* deposited in Swiss Museum collections. *Tibicina quadrisignata* (♂ top, Valais, 1928, leg. Cerutti, MZLS), *Tibicina steveni* [♀ centre, Sierre (VS), leg. Maerky, MHNG] and *Tibicina haematodes* [♂ bottom, “Pt. Butin” (GE), 1943, leg. Schauenberg, MHNG]. Bar = 1 cm.

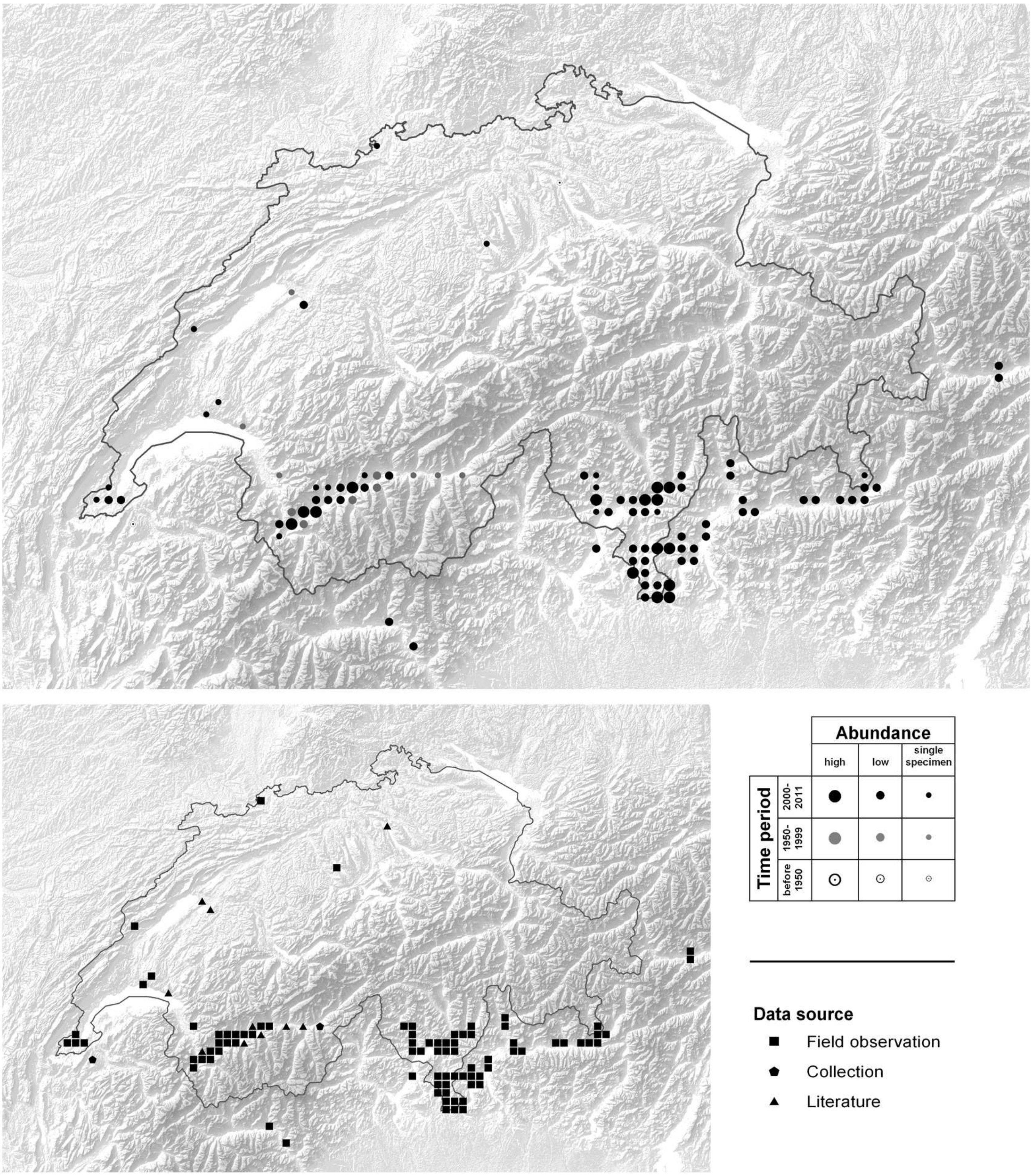

Fig 6.3. Distribution map of *Cicada orni* (top) including the data source for the records presented (bottom).

Bugnion (1890) described the species as very common from Martigny (VS) to Sierre (VS), but missing at Montreux (VD) and Lausanne (VD). Stoll (1901) repeated the occurrence in the Valais and Ticino and added a remarkable observation by Dr. Ris on a Manna Ash (*Fraxinus ornus*) from the city of Zurich

(“Kantonsschule”), which seems reliable. Another two records far away from the known range have been published from Mont Vully (FR) (Musée d’histoire naturelle Fribourg, 2004) registered in the 1960’s (Beaud, pers. comm.) and from Vevey (VD) (1976; Barbey, 2010). Bille (1982) probably refers to *C. orni* when writing that the fabulous concert can sporadically be heard in the Lake Geneva Basin and at the southern base of the Jura Mountains. Many of the older records are not clearly localised but are normally overlapping with regions with many recent observations. The distribution map for the Valais provided by Pillet (1993) is very detailed and even presents two abundance classes. Among 104 sufficiently labelled specimens identified in collections during the present study, 90 originate from the Valais, 8 from the Mesocco Valley (GR) and only 6 from Ticino. Eight of them are remarkable with respect to the current distribution: Grono (GR) (1883, four specimens, NHMB), Roveredo (GR) (1922, NHMB), Brig (VS) (1929 and 1980, NHMB), Valle Morobbia (TI) (1937, ETHZ). We consider a voucher labelled solely “Aarau” (AG, NAAG) doubtful.

The current distribution area is well known and almost congruent with the historical data. *Cicada orni* populations are restricted to the lower Valais from Martigny to Sierre, the most southern parts of Ticino (south of Lugano) and to a belt extending from the lowest Centovalli (TI) along the northern slope of the Piano di Magadino (TI) to the Mesocco Valley (GR). The species is locally common.

Local observers indicate for example for Grono and Roveredo (Mesocco Valley) that the species has appeared only the last few years. According to voucher specimens collected at the end of the 19th and at the beginning of the 20th centuries, it is therefore more accurate to speak about a supposedly re-colonisation.

Similar to older observations, isolated singers sporadically appear outside the continuous distribution area as far as south-western Germany (Nickel, 2003), often in urban areas and gain remarkable publicity: Murten (FR) (25.6.2003; Musée d’histoire naturelle Fribourg, 2004), Lausanne (VD) (13. and 19.7.2010; Ducret, 2010; Bertaudon/Barros, pers. comm.), Epalinges (VD) (July 2010; Alexander, pers. comm.), Binningen (BL) (2.8.2010; Blattner, pers. comm.), Sursee (LU) (9.7.2011; Birrer, pers. comm.) and Ste-Croix above Yverdon (VD) (August 2011; Burnier, pers. comm.). In the Geneva agglomeration, the records have accumulated in a manner that suggests the establishment of a small new population (see also Hollier, 2012). We assembled eleven reliable observations between 2003 and 2011 for this region, eight communicated by naturalists (Prosenc, Hollier, Dandliker, pers. comm.), two printed in newspapers (Grosjean, 2008; Nikolic, 2010) and one even broadcasted on national television (Radio Télévision Suisse Romande, 2010). Some of these isolated singers have probably been transported as larvae in the root balls of plants, as is the case for the Murten (FR) specimens, which were introduced with olive trees for an exhibition. Others have reached their singing site on their own, which probably applies to all observations in the Lake of Geneva Basin.

Populations are normally restricted to altitudes below 600 m asl in Ticino and Grisons and 800 m in the Valais, but solitary singers can exceptionally rise up to 1530 m asl (La Forclaz, VS; Marchesi, pers. comm.).

Ecology and threat

Cicada orni is the most eurytopic species of the Swiss cicadas, colonizing man-made habitats such as vineyards along forest edges, woods, hedges and solitary trees and even parks and gardens in urban areas. Nevertheless, some of the most important populations are found in natural habitats of generally high conservation value such as forests dominated by Downy (*Quercus pubescens*) and Sessile Oak (*Quercus petraea*) (= *Quercion pubescenti-petraeae*; see Delarze & Gonseth, 2008) for the Valais and a plant association characterised by Downy Oak, Turkey Oak (*Quercus cerris*), Hop Hornbeam (*Ostrya carpinifolia*) and Manna Ash (*Fraxinus ornus*) for Ticino (= *Orno-Ostryon*; see Delarze & Gonseth, 2008). A long exposure to the sun is very important for this species, therefore most habitats have south-facing slopes. The distribution area is limited by the less favourable climate in other parts of Switzerland.

Given its habitat associations we assume that *C. orni* will extend its distribution area if a predicted rise in temperatures will occur. Currently this can probably be observed in the Lake Geneva Basin, where the species obviously did not occur in the 18th and 19th century (Saussure, 1780; Bugnion, 1890). The species is not endangered but we consider it to be important for public awareness and education with respect to bioacoustic experience and climate change.

Lyristes plebejus (Scopoli, 1763)

German common name: Grosse Zikade (Gogala, 2002)
French common name: La grande Cigale commune (e.g. Boulard, 1995)

Distribution (Fig 6.4)

Lyristes plebejus is the second largest European cicada after the south-eastern European species *Lyristes gemellus* Boulard, 1988, and has a very loud and distinctive song pattern. The great Swiss savant Albrecht Von Haller (1765, p. 77) knew “die echte Cicade der Alten” (= the true cicada of the ancients) from Aigle (Chablais, VD) that we assume probably refers to *Lyristes plebejus*. This is the first record found for cicadas in Switzerland. Fuesslin (1775) reports the species from the so-called “Italienische Vogteien” (= Italian Bailiwicks; at that period comprising the Swiss Ticino as well as the Italian Valtelline Valley and Chiavenna region) and from Roche (Chablais, VD). Schnetzler (1877) mentioned *L. plebejus* from Bex (VD) one century later, located like Roche in the neighbourhood of Aigle. Pillet (1993) concluded that the remarkable species must have been quite frequent in the Valais in the 19th century referring to a notice and several specimens found in the MHNG. Milde (1866), Huber (1916) and later Schedl (2000) mentioned the species only for the most southern parts. Nevertheless, concrete historic records from Ticino are missing in the literature and in the museum collections. Vernier (1996) could not find *L. plebejus* in Ticino, in contrast to the local abundant *C. orni* during his journey in summer 1994. Schertenleib observed the species several times at Lake Neuchâtel during the first half of the 20th century (Vernier, 1996). We could not prove this historic occurrence, and with the recent death of Schertenleib and without information from his colleagues, the Lake Neuchâtel records remain uncertain. Vernier also mentioned a nymphal skin from the Canton of Geneva found in the same period (1945) and preserved in the MHNG, but this exuvia belongs to the genus *Tibicina* (T.H. vidit 2010). Pillet (1993) observed the species only three times for the Valais between 1985 and 1993. Scherdlin (1910), Döderlein (1913) and Huber (1916) also reported few records from the French Alsace region which seem to be reliable.

Lyristes plebejus is currently a very rare species in Switzerland and with an unusual distribution pattern. It is mainly restricted to two established, dense populations situated in the Lugano region (TI). The first includes the slope between Bré-Gandria-Castagnola, the second located in the San Salvatore Mountain, both with a maximum of 25 calling males. The two populations are described for the first time within this study. A third, new population seems to be established in the surrounding of Verchiez (Chablais, VD). Keim (pers. comm.) registered between two and eight singing specimens in the years 2004, 2005, 2007, 2009 and 2010. Our survey of this site revealed the presence of 10 to 12 males in 2012, but no other specimens in the whole region. Interestingly, Verchiez is situated in the municipality of Aigle, where Von Haller (1765) had indicated the species 250 years before.

The species can sporadically be found far away from established populations with isolated singers especially in the Lake Geneva Basin. Vernier (1996) discussed in detail one record from the Vallon d'Allondon (Russin, GE) in 1995. Further observations have been made in 1999 in Gland (VD) (♀ drowned in a pool, leg. Breitenmoser), in 2004 at La Tour-de-Peilz (VD) (det. Martin/Göldin; Muhieddine, 2004), and potentially even in Allschwil (BL) in 2006 (Currat, pers. comm.). Established populations can be found less than two kilometres behind the Swiss border near the Poschiavo Valley (GR) in the Valtellina (Italy). Juillerat (pers. comm.) heard one specimen singing at the Swiss side of the border in 2009 (Campocologno). *Lyristes plebejus* is also currently known from the Val des Usses in Haute-Savoie (France; Vernier, 1996; Gurcel, 2015).

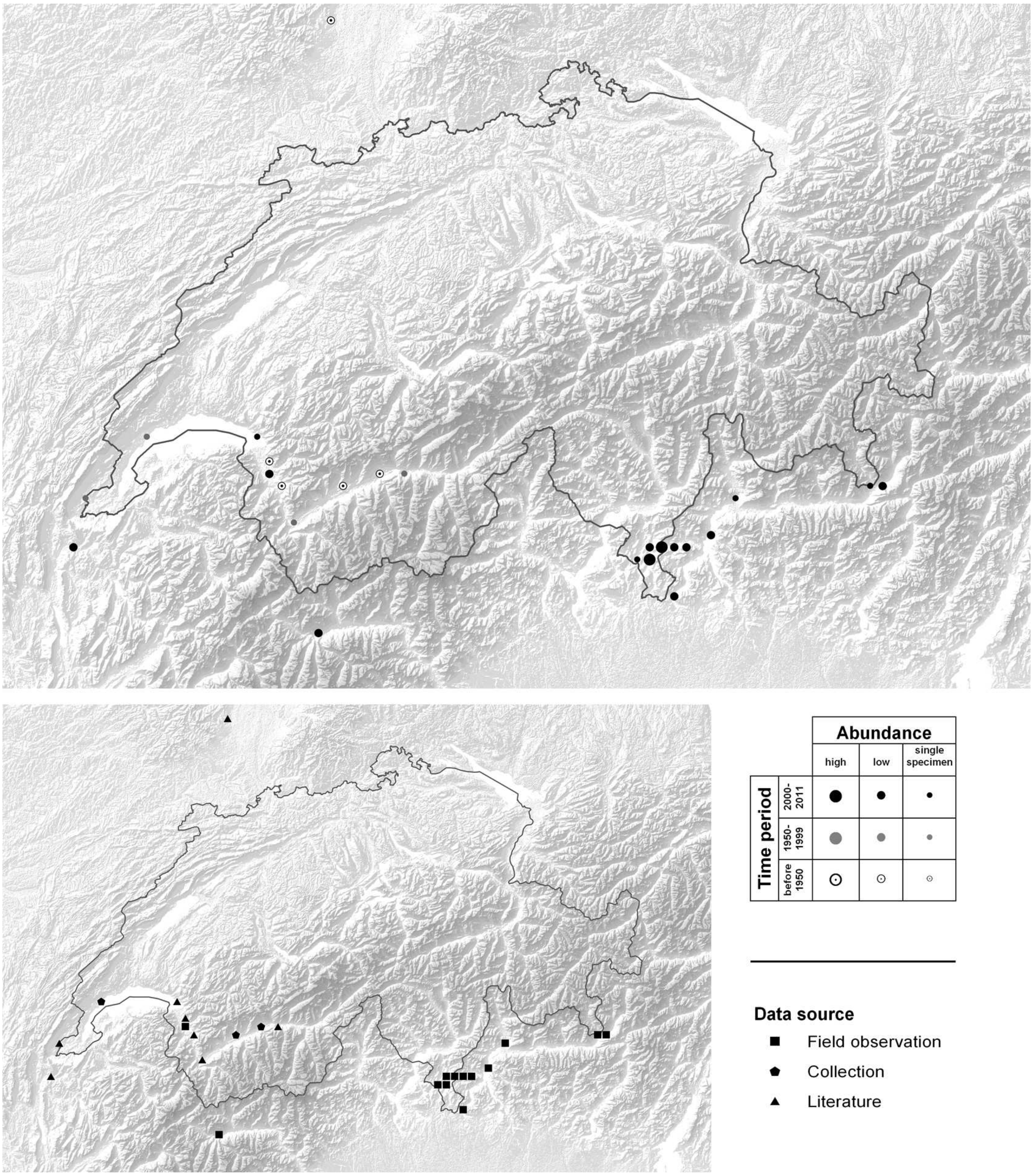

Fig 6.4. Distribution map of *Lyristes plebejus* (top) including the data source for the records presented (bottom).

Ecology and threat

The two habitats in Ticino consist of almost natural, rocky and steep slopes (*Orno-Ostryon*, see previous paragraph). Here, at the northern limit of its range, *L. plebejus* is restricted to this mostly undisturbed, Mediterranean landscape, while in its core area some populations even colonise city parks. In spite of the rareness, the species does not seem to be threatened in Ticino by habitat loss. The habitats are scarcely influenced by human activities and classified as protected 'dry habitats of national importance' by Swiss authorities.

In contrast, *L. plebejus* has become extinct in the Valais (Keim, 1999). Pillet (1993) could not find a cogent reason for the dramatic decline. The population in the Chablais is restricted to the close surrounding of three cliffs with sparse forests dominated by oaks (*Quercus* sp.). This site is almost natural as in Ticino but in vicinity of intensively cultivated vineyards. The recent observations may indicate a re-colonisation in the south-western part of Switzerland which could be promoted by climate change. The Chablais (VD) could play an important role for this species with its steep rocky slopes and the mild climate.

Tibicina quadrisignata **(Hagen, 1855)**

German common name: Schwarzer Scherenschleifer
French common name: La Cigale quadrisignée (e.g. Pillet, 1993)

We could not find a common German name for this species. “Scheereschliffer” (= scissor grinder) is an old popular and local name used for *T. haematodes* from southern Germany (Geisenheyner, 1887; Jacobs & Renner, 1988) and representing the sound of all species of the genus in a suitable and inventive manner. The black colouration of the wing venation is diagnostic within the genus for Central European species.

Distribution (Fig 6.5)

Tibicina quadrisignata is distributed in the western Mediterranean region and has been confirmed recently for the Mediterranean regions of France (Puissant, 2006) and for Portugal (Sueur *et al.*, 2004). The species is also reported from the Aosta and Susa Valleys in Italy (Schedl, 2000 and pers. data). Pillet (1993) was the first to report this species for Switzerland and presented a detailed distribution map with occurrences in the upper Rhone Valley (VS) from Grengiols to Salgesch and in parts of the very dry Visp Valleys. He concludes a strict vicariance with *T. haematodes* (later corrected to *T. steveni*), the latter colonizing the lower, western parts of the Rhone Valley and some side valleys west of Salgesch, including the Finges Forest (VS, Sierre region).

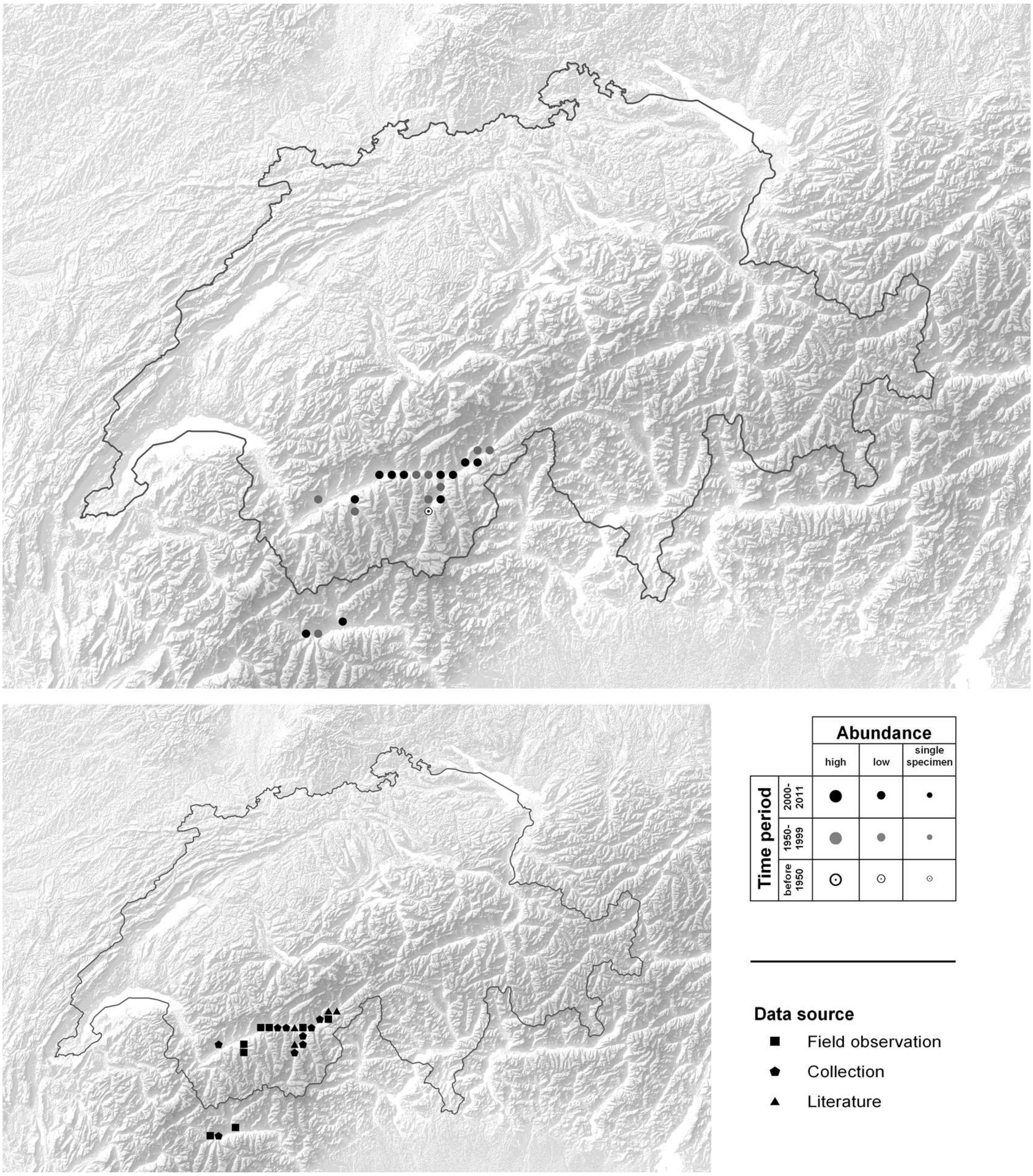

Fig 6.5. Distribution map of *Tibicina quadrisignata* (top) including the data source for the records presented (bottom).

One voucher specimen (1977, NMWI) and our acoustic recordings suggest that the Finges forest population belongs to *T. quadrisignata*, five further specimens are labelled with “Sierre” (NMHN and MNHG). The limit of the known distribution range is therefore slightly shifted westward for *T. quadrisignata* when comparing to Pillet (1993). Moreover the allopatry seems not to be that strict. *Tibicina quadrisignata* has also been found clearly west of Sierre but only very few observations can be validated: Chamoson (Sierro & Keim, 1999; 2 ♂ leg. Carron, MHNN and private collections of Keim), Bramois (Sierro *et al.*, 2000; Keim, pers. comm.; ♀ seen) and Euseigne (Praz, pers. comm.). Vernier (pers. comm.) knows one site where *T. quadrisignata* and *T. steveni* occur in complete syntopy in the Val d'Hérens. This range overlap makes the delimitation of the distribution areas of *T. quadrisignata* and *T. steveni* more complicated. The songs are very difficult to discriminate in the field and it is probable that Pillet was not able to verify all of his observations, neither by analysing fine song differences on computer (Sueur & Aubin, 2003) nor by checking colouration characters. There is no evidence that *T. steveni* occurs to the east of Sierre, and all other 19 voucher specimens of *T. quadrisignata* deposited in the private and public collections with reliable labels are from within the distribution area published by Pillet. Interestingly one voucher from Stalden (Visp Valleys) was determined correctly in the 19th century (1883, det. Lethierry, NHBE). According to museum collections many specimens have been captured in this region. Our distribution map reports all voucher specimens, recent acoustic recordings and visual observations. Furthermore, Pillet's records were assessed as being valid if they originate from spatial unities where the suspicion on an occurrence of *T. steveni* is very low.

Ecology and threat

Tibicina quadrisignata is a specialised species in Switzerland. It lives in very xerothermic habitats with a mosaic of scattered Scots Pine (*Pinus sylvestris*) and Savin Juniper (*Juniperus sabina*), sometimes entering dry pine forests. Singing has been observed on ten different woody plants (Pillet, 1993). Comparable habitats are occupied in the Aosta Valley. In contrast, southern French populations are most abundant in woodland ("lande haute fermée", "bois"; Puissant, 2006). Therefore, the Swiss and adjacent Italian populations possibly form a specialised ecotype.

The national threat to *T. quadrisignata* results from the following facts: the small distribution area within Switzerland, the low population densities typical of the genus, the spatial isolation at the north-eastern border of the distribution area, the stenoecious habitat requirements and habitat loss by artificial irrigation and expansion of vineyards or settlements. Moreover, the general international rareness of both the species and the specific ecotypes increase the necessity for the conservation of the species at the Swiss level. *Tibicina quadrisignata* is an attractive flagship species for the driest habitats of the country and could be highlighted in nature conservation programmes.

Tibicina steveni (Krynicki, 1837)

German common name: Gelber Scherenschleifer
French common name: La Cigale de Steven (Puissant, 2006)

The German name has been formed similarly to *T. quadrisignata*. It is the only species of the genus with yellow marks on the mesonotum, a broad yellow stripe at the posterior margin of the pronotum and with a yellow colouration of the basal wing venation in Central Europe.

Distribution (Fig 6.6)

Tibicina steveni was originally known as an eastern Mediterranean cicada. It was described from the Taurus Mountains (Turkey) and is distributed in Azerbaijan, Armenia, Georgia, Ukraine (Crimea) (Sueur *et al.*, 2003) and Bulgaria (Gogala *et al.*, 2005). The occurrence in Switzerland and in one place in France was unexpected (Sueur *et al.*, 2003). It has recently also been found in a wider but fragmented range in the northern and southern Apennine Mountains in Italy (pers. data).

Swiss populations of *T. steveni* have actually been known as "*Cicada sanguinea*" for more than 150 years (Bremi in Hagen, 1855). He estimated that the species was not rare in the Valais, although less abundant than *C. orni*, but he had probably included observations of *T. quadrisignata* in his assessment. Judging by nine specimens caught between 1891 and 1899 and preserved in different public collections (NMBE, NMLS and NMS), the species must have occurred fairly frequently in the Martigny region (VS) at that time. Farquet's (1940) "*C. haematodes*" at Mont Ravoire near Martigny must be *T. steveni* and is the only detailed chorological information published before Pillet (1993).

Tibicina steveni only occurs in the western parts of the Rhone Valley (VS) and a few of its side valleys (Pillet, 1993). Some of the records made by Pillet are doubtful since *T. quadrisignata* has been reported from the same site subsequently (see previous section). For that reason the exact distribution area has to be reinvestigated for the western part of the Valais. Specimens from collections labelled with "Aïre" (GE, MHNG), "Peney" (GE, MHNG) and "Clarens" (VD, NMBE), all of them far away from the known distribution area, we suppose to be erroneous. The first two (Aïre and Peney) were reported by Maerky, who is known to have mixed up localities when labelling specimens. Moreover, the Peney label originates from Tournier, who was also an insect dealer (Hollier, pers. comm.). The third (Clarens) is without any further information, but there is also a specimen of *Tibicina quadrisignata* preserved in the same collection and with the same handwriting from this site and it is probable that there have been labelling errors.

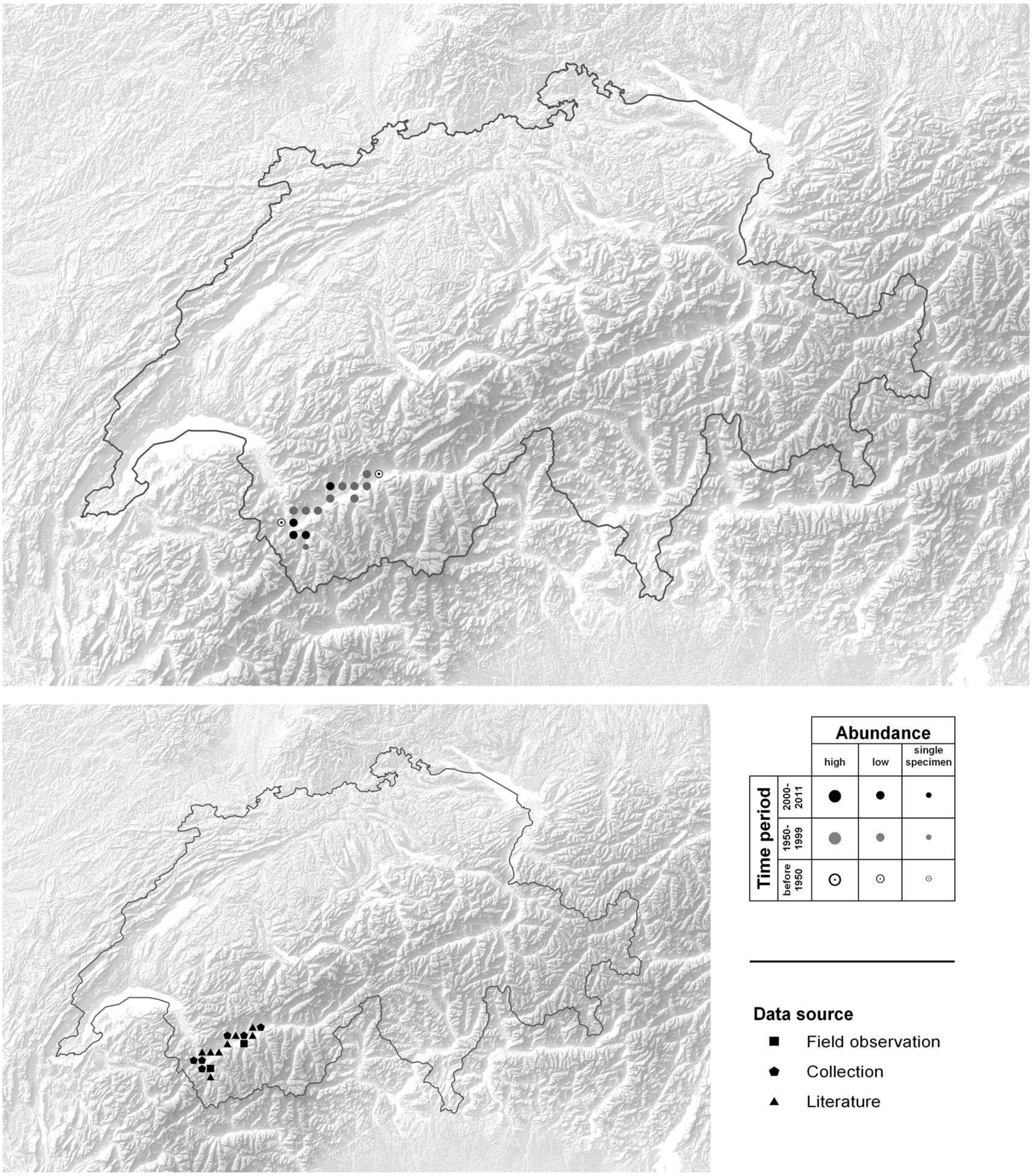

Fig 6.6. Distribution map of *Tibicina steveni* (top) including the data source for the records presented (bottom).

Sueur *et al.* (2003) hypothesised that the Swiss and French populations must be a relict of a formerly wider distribution of the species across Europe. We support this opinion based on the following considerations: The hypothesis of a relictual occurrence in the western part of the distribution is verified by the recent observations in Italy, isolated populations are common for the Valais with its characteristic dry climate, and the biogeographical pattern with the main distribution area in the east (Europe and/or Asia minor) is not unique for species occurring in the Valais. Delarze (1987) has published a list of 21 beetles, two grasshoppers and one spider occurring in the Valais that are at least more abundant in Eastern Europe than in the Rhone Basin. He discussed the origins of the xerothermic flora and fauna and concluded that a colonisation of the Valais along the Jura Mountains and north of the Alps could not be excluded for some oriental insects since the late glacial period.

Ecology and threat

Tibicina steveni is a stenoecious species preferring dry Scots Pine tree forests (= *Ononido-Pinion*; see Delarze & Gonseth, 2008) and oak woods (*Quercion pubescenti-petraeae*). The species inhabits closed habitats (Sueur *et al.*, 2003). Singing males are often observed sitting on Scots Pine (*Pinus sylvestris*) or on Downy Oak (*Quercus pubescens*) (Pillet, 1993). Sometimes they also sing in neighboured vineyards.

A good knowledge of the distribution and ecology of this attractive species is essential, because Switzerland is exclusively responsible for the conservation of the Central European populations. The population densities are low, the occurrence isolated and restricted to a very small area. Pillet (1993) already pointed out the severe threat especially due to the continuous expansion of the vineyards. Similar to *T. quadrisignata*, this species is a perfect model for flagship species concepts in nature conservation.

Tibicina haematodes (Scopoli, 1763)

German common name: Weinzwirner (e.g. Schedl, 2000)
French common name: La Cigale rouge (e.g. Boulard, 1995)

Distribution (Fig 6.7)

All studies reporting *Tibicina haematodes* from the Valais are considered to be incorrect (Fuesslin, 1775; Stoll, 1901; Kuntze, 1931; Schedl, 2000) as well as two voucher specimens labelled with “Wallis” (NMT) and “Martigny” (VS, MNHN). Nevertheless, it is possible that the species could have reached Switzerland from three different directions historically, from the north, the south or the west, since it has been published for all neighbouring countries except Liechtenstein: Italy [up to Bozen (Schedl, 2002) and around Torino (Griffini, 1896; Della Beffa, 1925)], France [including central parts (e.g. Hagen, 1855; Puissant, 2006)], Austria [Vienna region (e.g. Hagen, 1855; Wagner & Franz, 1961; Schedl, 2002)] and Germany [Rhine-Main region (e.g. Hagen, 1855; Vogel, 1935; 1937; Schwoerbel, 1957)].

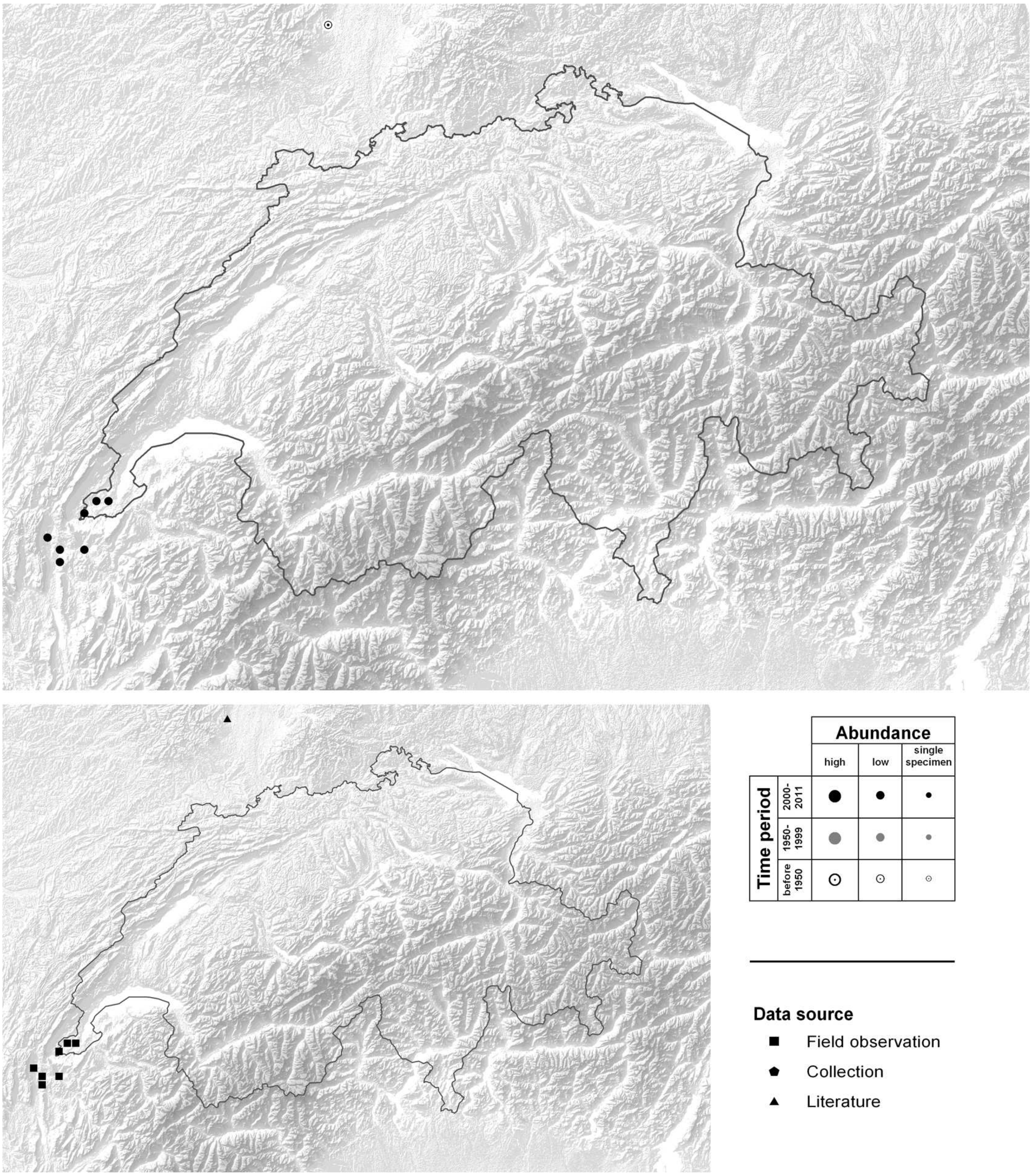

Fig 6.7. Distribution map of *Tibicina haematodes* (top) including the data source for the records presented (bottom).

With respect to the north, Labram & Imhoff (1845) mentioned *T. haematodes* as a very rare species in the Basel region. One historic population is mentioned from the vineyards in Rouffach (Alsace, France) situated only 50 km north of Basel (Döderlein, 1913; Huber, 1916). 16 specimens of this time are deposited in the collection of the Zoological Museum of Strasbourg labelled with the nearby village of Westhalten (Hugel *et* *al.*, 2008). The German population nearest to Switzerland was described from Tübingen (Baden-Württemberg) with a population of 80 to 100 specimens (Schwoerbel, 1957). Fischer (1847) cited an occurrence from Tuttlingen and Immendingen (Baden-Württemberg) that is only situated 15 km north of the Swiss border, but had doubts about the identification. To summarise, it is possible that Labram & Imhoff (1845) are correct and *T. haematodes* had sporadically occurred in the Basel region, but nevertheless it is more likely that the authors confused it with *C. montana* s. l. A specimen labelled "Winterthur" (ZH) (1900-1910, leg. Huber, NMWI) is considered as doubtful, since it was near a handwritten label with "cicada from the Mediterranean for comparison". The location probably refers to the domicile of the collector.

With respect to the south, no reliable historic references could be found that *T. haematodes* once occurred in Ticino. Two unlabelled specimens are deposited in the MCSN collected by Scherrer and Maestri who were active mainly in southern Ticino and especially in the surroundings of Morcote and Melide at the beginning of the 20th century (Pollini Paltrinieri, pers. comm.).

Vernier (1996) mentioned a historical occurrence of the species for the Geneva region, the most western part of Switzerland. In fact, six adult specimens (1 ♀, 5 ♂) and four nymphal skins of *T. haematodes* from the Canton of Geneva are preserved in the collection of the MHNG. They are labelled from the two localities "Pont Butin/Aïre" (western city limit of Geneva) and "Nant de Lagnon" (east of Aire-la-Ville, municipality of Bernex) (leg. Simonet, Schauenberg, Maerky or anonymous). We therefore conclude that a reproductive population must have existed along a four kilometre length of the Rhone River from 1914 to 1947.

Despite recent intense field work, *T. haematodes* was found neither in southern Ticino nor in the Basel region. When investigating the slopes of the Rhone River downstream of Geneva two small populations of 3 to 4 singing males each were discovered at the historically known localities Pont Butin and Nant de Lagnon in 2012 and a single singing specimen was discovered at Cartigny (GE) in 2011 and in 2012. These are the first known observations of the species for more than 60 years in Switzerland.

Ecology and threat

The historic and recent Swiss habitats in the Geneva Region are oak dominated deciduous woodland on steep slopes. At the Pont Butin locality two specimens entered a small orchard in 2012. An occurrence in vineyards is not documented for Switzerland. The site Pont Butin/Aïre seems to be adversely affected by settlements. The Rouffach population (Alsace, France) was endangered through the collecting of this attractive species by dealers (Döderlein, 1913).

Tibicina haematodes is an indigenous, but extremely rare Swiss species. The current population size is about 10 calling males. It is interesting that we have found the species 60 years after the last record at exactly the same localities. Either the two small but striking populations have been continuously present without any public recognition or the species has re-immigrated to its former habitats. The population in the Val des Usses (Haute-Savoie, France) mentioned by Vernier (1996) is still present according to regular recent observations (Gurcel, 2015). Given its short distance of only 15 km from the Swiss border, it might serve as a pool for immigration. With the rise of more ecologically sensitive approaches to viticulture, supported by a milder climate, *T. haematodes* possibly establishes more numerous populations in Switzerland. Nevertheless, the species is currently critically endangered.

Cicadetta montana s. str. (Scopoli, 1772)

German common name: Pechader-Bergzikade (Hertach, 2010)

It seems obvious to keep the well-established name ‘Bergzikade’ (= Mountain Cicada) and add a prefix for all species of the complex in order to demonstrate their close relationship. *Cicadetta montana* s. str. often can be distinguished from all other Central European species of the clade by its black or very dark coloured basal wing venation (‘Pechader’ = pitch coloured venation).

Distribution (Fig 6.8)

It is difficult to present a historical overview of the distribution of *C. montana* s. str., because until 2004 only records referring to the whole species complex have been published, with the exception of one record by Artmann (1987). Nevertheless, we report here some historical data for *C. montana* s. l. with special focus on records originating from regions where only *C. montana* s. str. is expected although these are not presented on the map.

In a coloured figure Sulzer (1761) drew an accurately shaped *C. montana* s. l. but named it *C. orni*. He did not provide any Swiss localities. Referring to this drawing, Fuesslin listed the taxon for Leuk (VS, Sierre region). Heer & Blumer (1846, p. 210) published a sporadic occurrence up to the “Wart” for the Canton of Glarus at “2000 feet” (≈ 600 m asl, probably near Schwanden in the Sernf Valley). They call the species erroneously “Blutcicade (*Cicada haematodes*)”, but the presence of *T. haematodes* can be reliably excluded from this region because of its harsh climatic conditions. Bremi (1849; Bremi in Hagen, 1856b) gives the most complete overview on the occurrence in the middle of the 19th century stating that the species is widespread but not common in warm lowland areas including the Zurich region, and that it was found at high altitude locations near a glacier in the Valais. The following further sites have been published: Glarus and Bern (Milde, 1866), a few exactly described places in the Canton of Zurich (Dietrich, 1872; Stoll, 1901), in the lower parts of the Engadine Valley (GR) (Hofmänner, 1924; Günthart, 1987) and in the Finges Forest (VS) (Bille, 1980; Artmann, 1987). Pillet (1993) published a detailed map of the Valais populations. But since a second species of the complex occurs in this region, conclusions are difficult to reach and his data could unfortunately not been integrated into our map. Notable older voucher specimens for regions which are not well represented in the map, but probably only with this single species, originate in chronological order from Weissenburg (BE) (1849, NAAG), Frauenfeld (TG) (1892 and 1904, NMT), Gerzensee (BE) (1915, NHMB), Erstfeld (UR) (1916, NAAG), Davos Monstein (GR) (1935, ETHZ), Röthenbach i. E. (BE) (1961, NMBE), Hagneck (BE) (1979, NMBE), Isenthal (UR) (1986, NMLS) and Warth-Weiningen (TG) (1997, NMT).

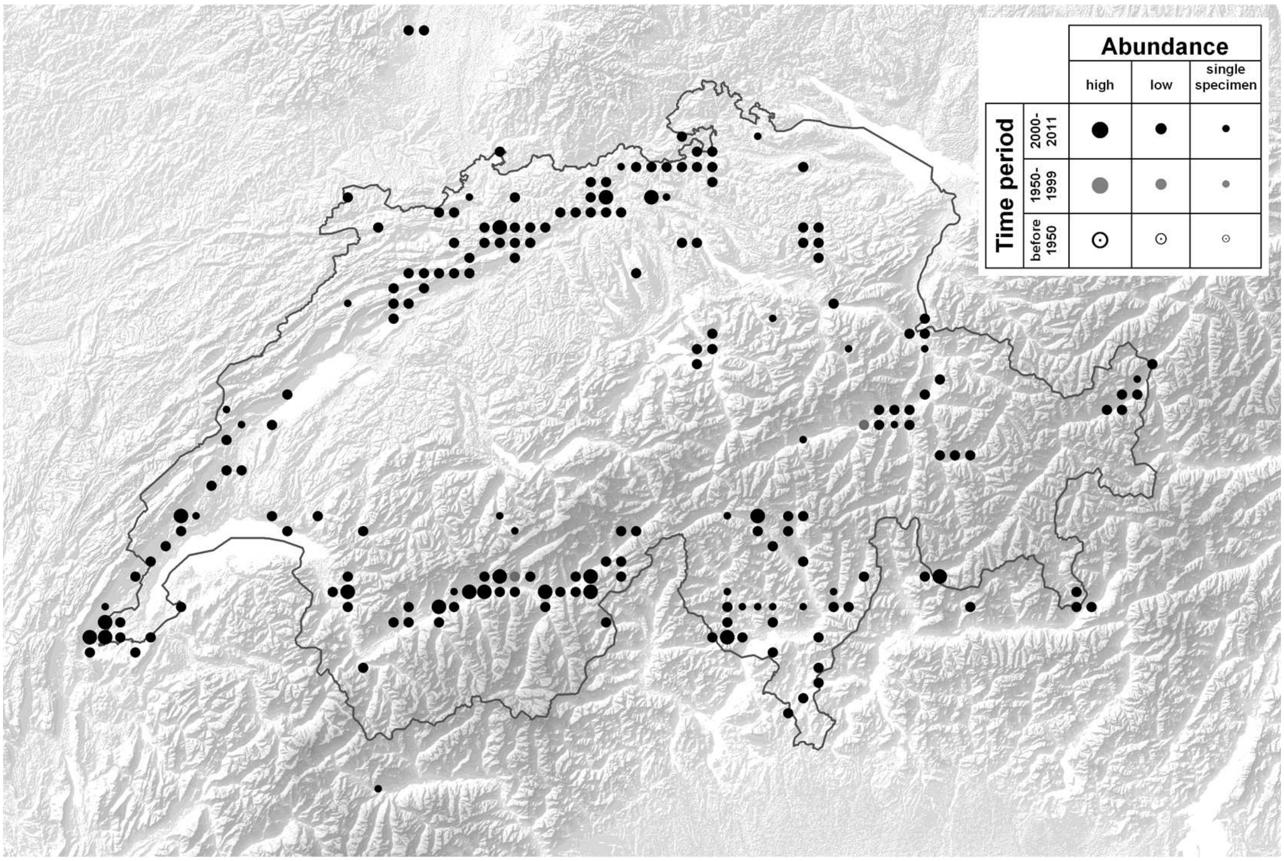

Fig 6.8. Distribution map of *Cicadetta montana* s. str. including solely acoustically checked records.

In Switzerland, *C. montana* s. str. is by far the most widely distributed species, although scarcely observed even by many entomologists. The previously published map (Hertach, 2007) has now been complemented with new records mainly from northern Ticino, Grisons and the Lake Geneva Basin. *Cicadetta montana* s. str. occurs in almost all regions of Switzerland but is very rare in the hilly Swiss Plateau. In the entire Grisons except for the Mesocco Valley and for the Poschiavo Valley, in the northern Prealps and in northern Ticino it is the only representative of the family Cicadidae. In the Valais, the most elevated population is established up to 2010 m asl (Rosswald; Hertach, 2007), which as far as we know has not been surpassed by any other Central European Cicadidae, except for the slight surpassing by observations in Haute-Savoie (Gurcel, 2015).

Ecology and threat

The habitat requirements of this and the other three species in the *C. montana* complex have been described in detail (Hertach, 2007). The rather broad habitat requirements have been confirmed within the last years, with a clear preference of sparse Scots Pine (*Pinus sylvestris*) woodlands and dry to mesophilous ecotone habitats. This species is the most difficult to observe due to its preference for singing in the treetops in combination with its rather faint almost ultrasonic song.

The species has probably suffered due to habitat loss in the hilly Swiss Plateau judging by the description of Bremi (in Hagen, 1856b) and some of the sites given by Dietrich (1872). In Ticino, suitable habitats have been lost by the abandonment of traditional land use. Nevertheless, *C. montana* s. str. is widely distributed and currently only locally endangered. The European distribution is large but we do not know of other regions with comparable population densities. It is rare in the Italian Alps. Therefore, we assume that Switzerland has a responsibility for the conservation and maintenance of large populations of this species. *Cicadetta montana* s. str. benefits at warm and sunny sites by thinning out forests on poor soils (especially pine forests) and by improving the structure of forest edges near dry and semidry meadows. Such measures have been conducted by regional authorities over the last decades.

Cicadetta cantilatrix Sueur & Puissant, 2007

German common name: Honigader-Bergzikade (Hertach, 2010)

Cicadetta cantilatrix can often be distinguished from *C. montana* s. str. by a yellow to brown colouration of the basal fore wing venation (‘Honigader’ = honey coloured venation).

Distribution (Fig 6.9)

Cicadetta cantilatrix was described recently and separated from *C. cerdaniensis* by acoustic characters (Sueur & Puissant, 2007a). For the acoustic description Sueur & Puissant integrated recordings from Switzerland (Martigny-Combe, VS), but the holotype and paratype were collected in France.

It is possible that Bremi (1849) and his German colleague Siebold already knew about this species. There is an older reference to a population in Diegten (BL, Jura Mountains; Artmann, 1987) where the species is still present and frequent. *Cicadetta cantilatrix* is not rare in Switzerland and it is surprising that it was not detected earlier (Hertach, 2007). It is well distributed in the Jura Mountains including the Randen massif in the east and entering to the eastern part of the hilly Swiss Plateau. We have found 17 local populations solely in the Canton of Aargau, some of them abundant. It also occurs quite regularly in the lower Valais and in the Chablais (VD). The main distribution is split into two big disjunct metapopulations in Switzerland (Jura and Chablais/Valais). Additionally, there are three supposedly isolated local populations: Immenberg (TG, discovered by Keist), Uetliberg (ZH, discovered by Rey) and Epesses (VD).

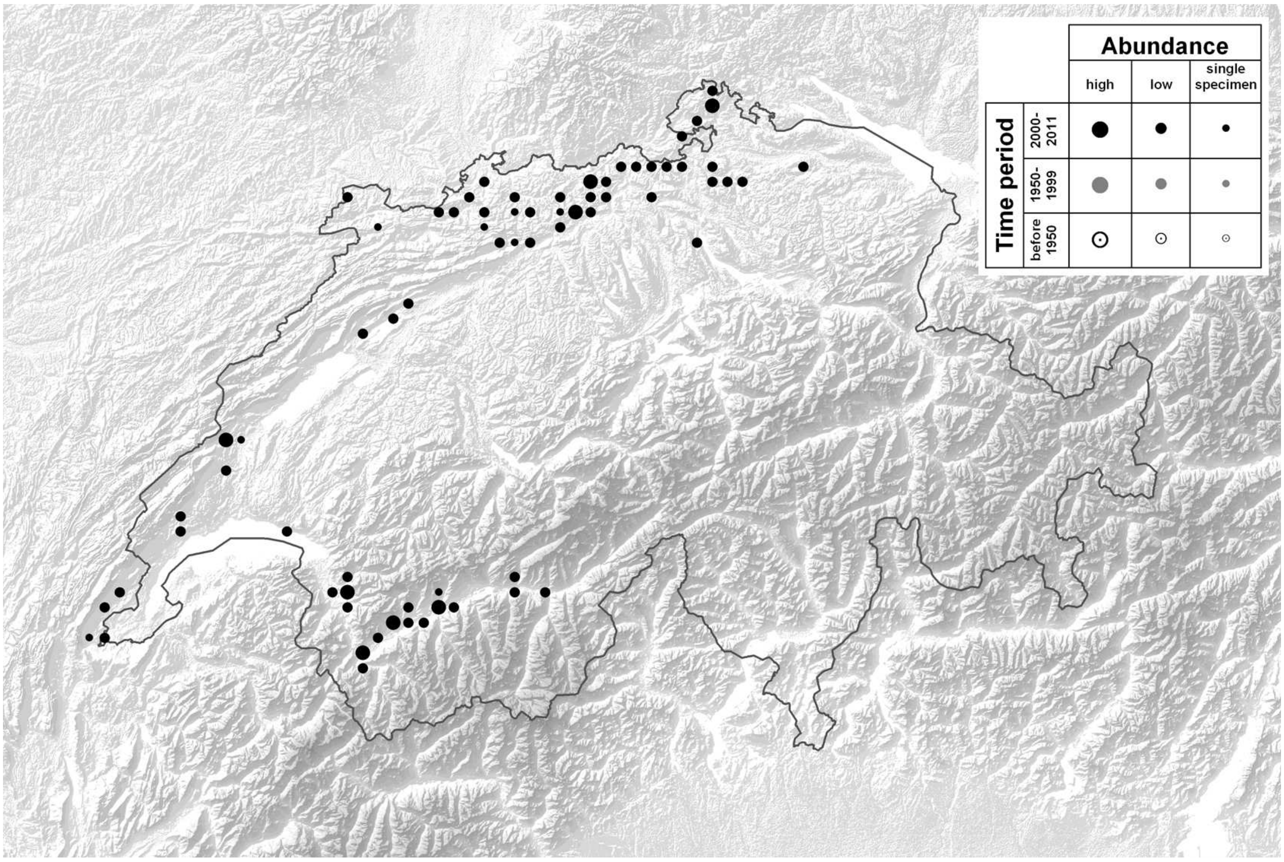

Fig 6.9. Distribution map of *Cicadetta cantilatrix* including solely acoustically checked records.

Ecology and threat

Cicadetta cantilatrix is specialised on sparse Scots Pine forests with a grass-rich understorey on marl (= *Molinio-Pinion*; see Delarze & Gonseth, 2008) and variable edges of the forest along extensively used semidry meadows normally with Upright Brome (*Bromus erectus*) (= *Mesobromion*; see Delarze & Gonseth, 2008) in the Jura Mountains and in the Chablais, and on sparse woods with Downy Oak (*Quercus pubescens*) in the Valais (Hertach, 2007). In contrast to *C. montana*, it scarcely occurs on rocky cliffs and ridges. It is a stenoecious species whose abundance is often correlated with nature conservation measures and traditional land use.

Beyond Switzerland, the distribution area is large, but a comparable abundance is not known. It therefore seems that the Swiss responsibility for its conservation is high. As in *C. montana* s. str., it benefits from recent improvements of suitable habitats (Hertach, 2007). The very active role of cantonal authorities in thinning out pine forests might create an important positive factor for the current frequency in the Cantons of Aargau and Basel-Land.

Cicadetta sibillae Hertach & Trilar, 2015

German common name: Italienische Bergzikade

The geographical attribute (‘italienisch’) is justified insofar that more than 90% of the distribution area is located in Italy. Moreover, *C. sibillae* is by far the most abundant species belonging to the *C. montana* species complex on the Apenninian peninsula.

Distribution (Fig 6.10)

The Swiss distribution of *C. sibillae* is well known. In the Lugano region, the species is the most common representative of the *C. montana* complex but there are only nine local populations. The Monte San Giorgio (TI) population is one of the most numerous of the Alps (up to 50 singers). Additionally the species was found north of Monte Ceneri (TI) three times with isolated singers (Castaneda, GR, Juillerat, pers. comm.; Gudo, TI; Tegna, TI). The location in Castaneda (Mesocco Valley, GR) might be a reproductively viable population.

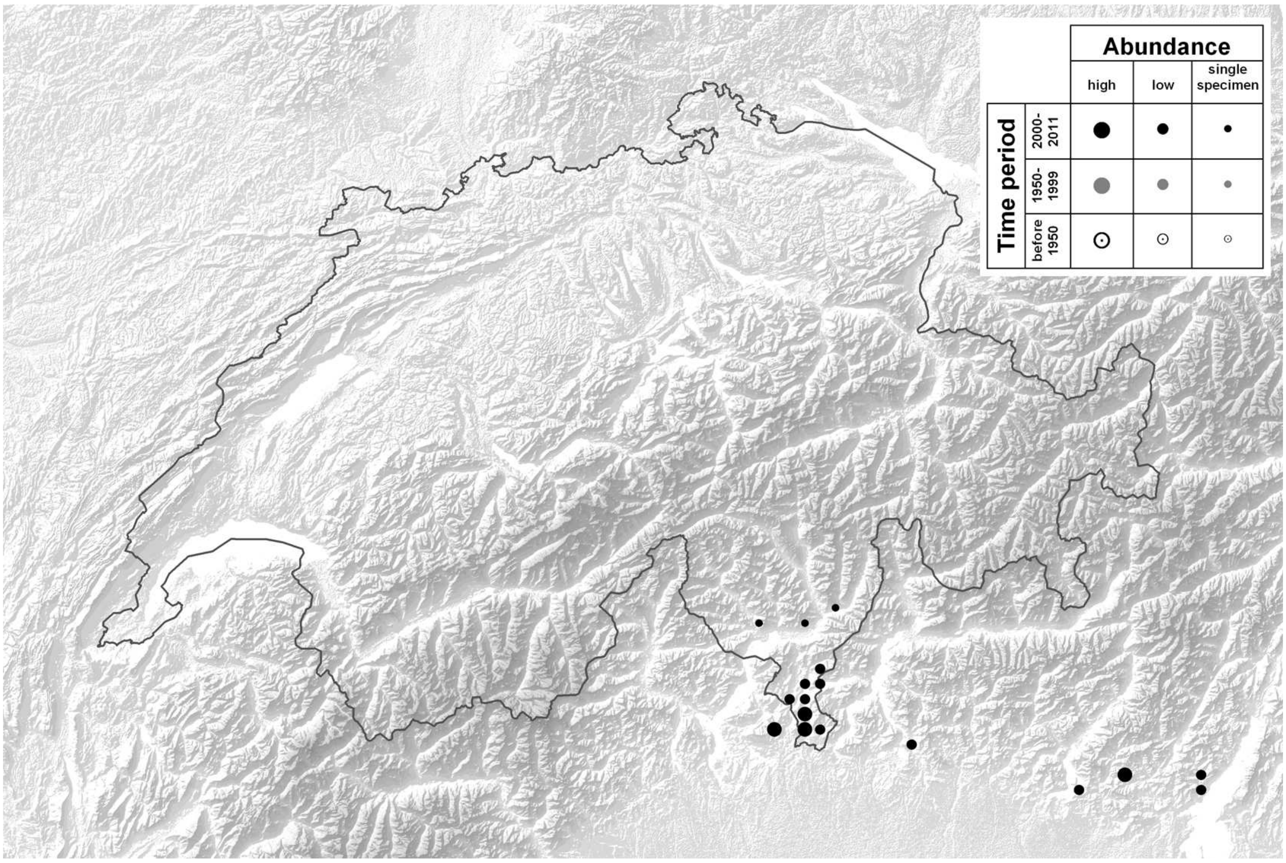

Fig 6.10. Distribution map of *Cicadetta sibillae* including solely acoustically checked records.

Ecology and threat

Cicadetta sibillae is specialised on sunny and sparsely vegetated woodland (*Orno-Ostryon*) and semidry meadows with shrubs or forest edges in Switzerland. Similar to *C. cantilatrix*, this species prefers low trees and bushes when singing. Almost all habitats are situated on calcareous soils.

The species probably already suffered in the Celto-Latin period, when early settlers transformed open deciduous woodland into forests of European Chestnut (*Castanea sativa*) (e.g. Hegg *et al.*, 1993). A serious decline may have taken place after the abandonment of semidry meadows and the expansion of settlement areas in the last century. These last two adverse processes have been mitigated but not stopped by nature conservation measures and stricter land use planning regulations. *Cicadetta sibillae* is endangered in Switzerland.

Cicadetta petryi Schumacher, 1924

German common name: Gras-Bergzikade
French common name: La Cigalette à ailes courtes (Puissant, 2006)

The common German name is derived from an unusual behaviour within Central European cicadas: *Cicadetta petryi* is regularly singing in the herb layer (‘Gras’ = grass).

Distribution (Fig 6.11)

Historic records of this fourth member of the *C. montana* species complex cannot be reported. It is the rarest species within the complex in Switzerland, but the most frequent in the Mediterranean parts of France (Puissant, 2006; Chapter 4). The sister species *Cicadetta brevipennis* Fieber, 1876 is not present in Switzerland and is a more south-eastern species (Chapter 4).

The distribution area reaches Switzerland in the Geneva region and southern Ticino. Not more than two populations and an isolated calling male are known from Ticino, but the population on Monte San Giorgio is very numerous with more than 100 calling males. The Ticino populations are not well interconnected with Italian ones. *Cicadetta petryi* has its most important Swiss populations along slopes of the river Rhone in the Canton of Geneva. Furthermore, an isolated population was discovered in the Orbe region in 2011. The Alsace populations (Hugel *et al.*, 2008 and pers. data) do not reach the Swiss border from the north.

Ecology and threat

Cicadetta petryi resembles *C. sibillae* in habitat selection in Ticino and it can occur syntopically with the latter. The composition of the woody plants is similar, but *C. petryi* prefers more open ecotone habitats with a tendency to semidry meadows. In the Canton of Geneva, *C. petryi* more or less replaces *C. cantilatrix* in sparse Scots Pine forests (*Pinus sylvestris*) and ecotone habitats with a slight preference for drier soils. For more ecological data see Hertach (2007).

The species is stenoecious and endangered by the abandonment of traditional land use and by grazing or intensification of semidry or dry meadows, especially in Ticino and at the location near Orbe. Swiss populations do not seem to be connected with large populations, at least not in Italy.

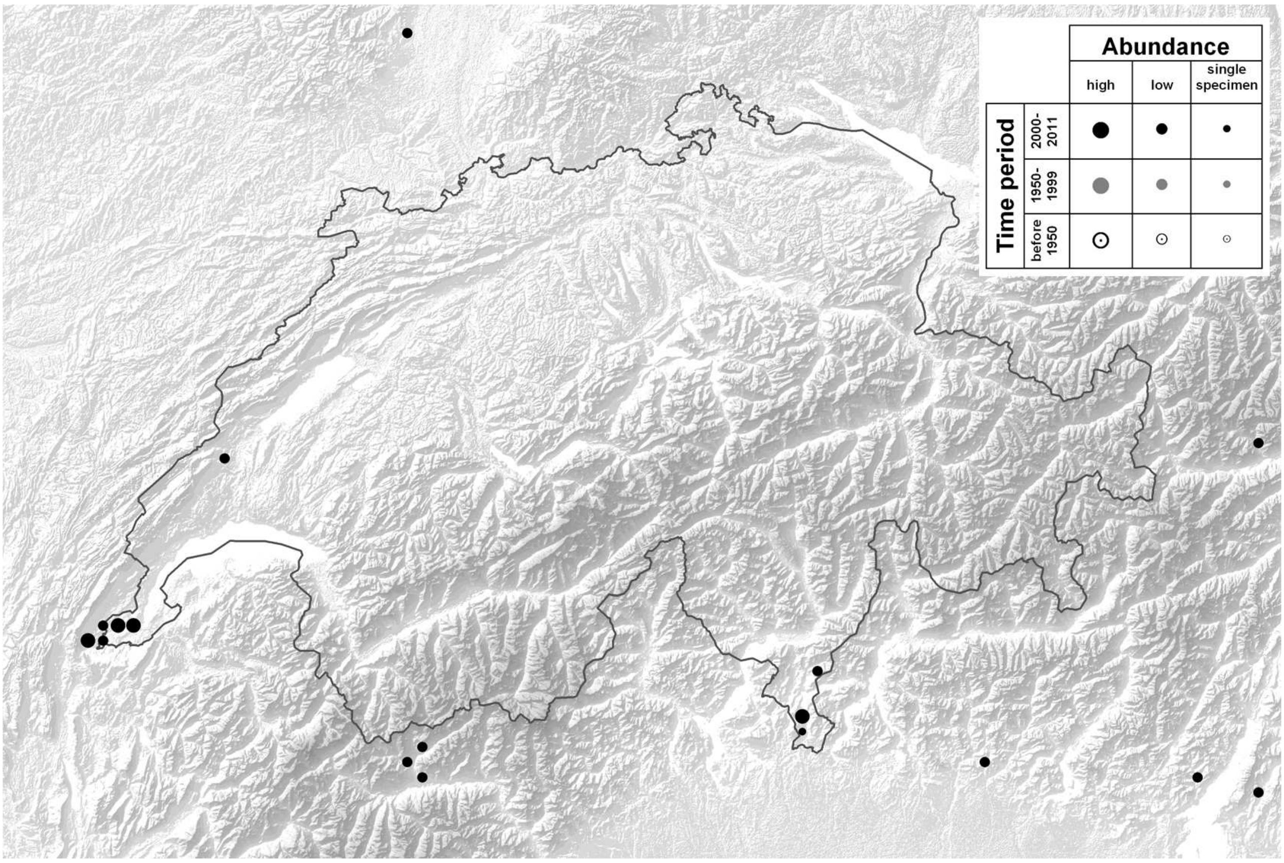

Fig 6.11. Distribution map of *Cicadetta petryi* including solely acoustically checked records.

Tettigettalna argentata (Olivier, 1790)

German common name: Silbrige Zikade (Gogala, 2002)
French common name: La Cigalette argentée (e.g. Boulard, 1995)

Distribution (Fig 6.12)

Tettigettalna argentata (Olivier, 1790) is a small western Mediterranean cicada species that has been recorded in Spain and Portugal (Sueur *et al.*, 2004), France (Puissant, 2006), northern Italy (Ferrari, 1892; Schedl, 1973; 2000) and western Slovenia (Gogala & Gogala, 1999). Some of the records of Schedl (1973) are situated not far from the Swiss border. The occurrence near Meran in the Italian South Tyrol had already been detected and described in the middle of the 19th century (Milde, 1865).

Tettigettalna argentata was discovered for the first time in Switzerland at Monte Caslano (TI, Lugano region) in 2007 (Hertach, 2008). The species is abundant there with more than 30 males singing in 2008.

No other populations have been found in Switzerland, but a single and supposedly immigrant specimen was observed and recorded near San Vittore (GR) in the Mesocco Valley on 29 July 2011. Other current populations nearly reach the Swiss border in different regions: 1.7 km from the Poschiavo Valley (GR) (Tirano, 2010 and 2011), 10.4 km from the Valais (Doues, Aosta Valley, 2007) and 29.5 km from the Val Mustair (GR) (Kastelbell-Latsch, Vinschgau, 2009). Immigration into the Poschiavo Valley might be expected in the near future.

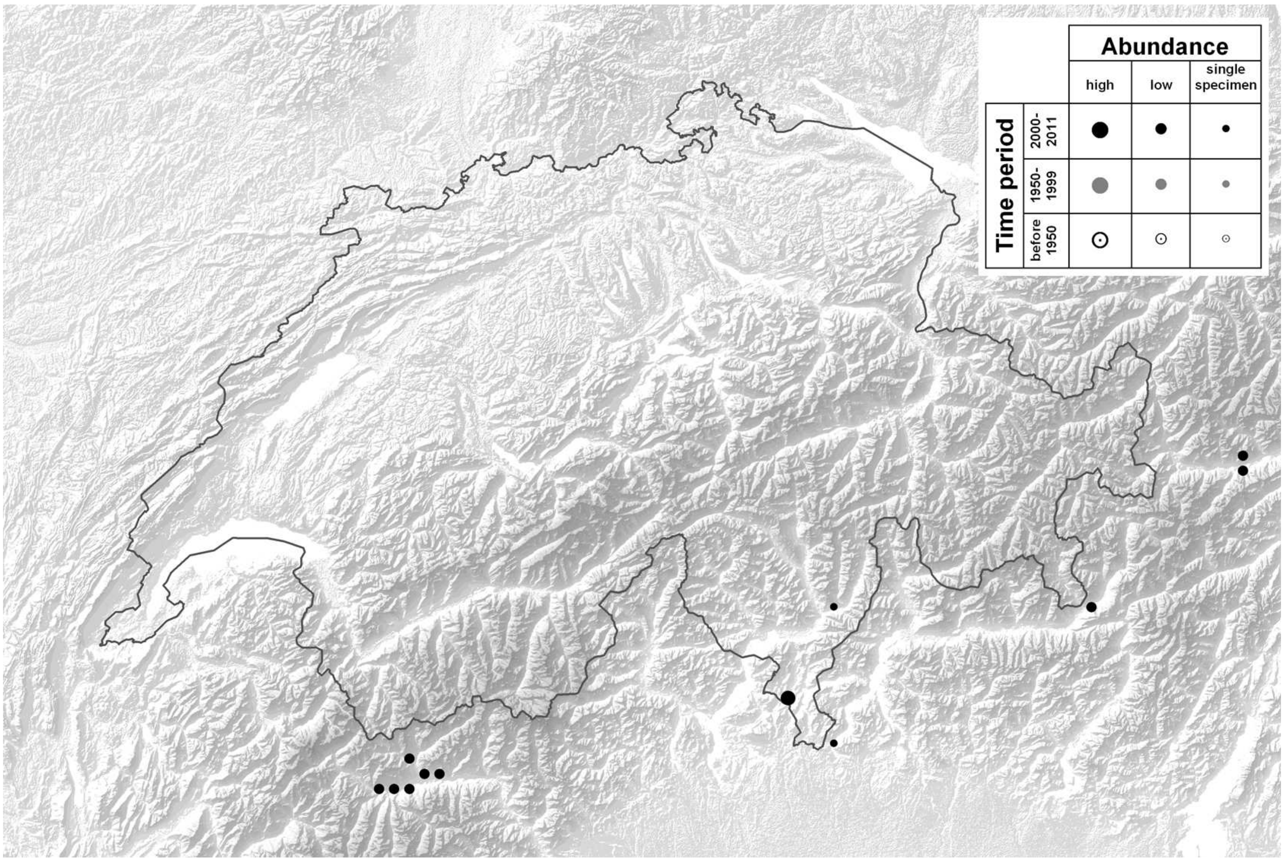

Fig 6.12. Distribution map of *Tettigettalna argentata*.

Ecology and threat

The habitat at Monte Caslano consists of a very steep and rocky slope on limestone (*Orno-Ostryon*). Some areas of Monte Caslano are cultivated as dry meadows with bushes and small woods for habitat conservation by regional authorities (Hertach, 2008). Maintenance or slight extension of these activities seems to be crucial for the conservation of this population of *T. argentata*. The Mesocco Valley specimen sang in intensively cultivated land with a garden, hedges and pastures, which is not typical for the species.

With only one established population the occurrence of this species in Switzerland is endangered. Nevertheless, the responsibility for the species is not high in the international context.

Comments on other species

Cicadatra atra **(Olivier, 1790)**

Bremi (1849) named the most frequent Swiss species "*Cicada concinna*", which, according to this description, must be assigned to *Cicadetta montana* s. l. Some authors classify *Cicada concinna* Germar, 1821 as a synonym of *Cicadatra atra* (e.g. Hagen, 1855; Fieber, 1876; Metcalf, 1963). Bremi was therefore probably misinterpreted as his record of "*Cicada concinna*" seems to have lead several authors to the conclusion that *C. atra* is a Swiss species: Haupt [1935, "reaching the southern parts of Switzerland (Valais)"], Robert (1937, "more abundant than *C. orni*, *L. plebejus* and *T. haematodes* in Central Europe"), Metcalf (1963), Nast (1972; 1987), Schedl (2000; "southern Switzerland", the symbol in the corresponding distribution map is located in the Valais), Holzinger *et al.* (2003) and Hoch (2011). Neither historic nor recent evidence could be found in collections or in the field. It is rather unlikely that *C. atra* has occurred in Switzerland. The nearest recent observations are situated in the Mediterranean parts of France (Puissant, 2006) and in the most northern Apennine Mountains (pers. data). Servadei (1967) did not mention of Italian records north of the Emilia Romagna.

Tibicina nigronervosa **Fieber, 1876**

Puton (1899) listed *T. nigronervosa* for Switzerland, Spain and Corsica. Nast (1972) followed him and added Portugal (and Russia). Both most probably mistake *T. nigronervosa* for *T. quadrisignata* in Switzerland. Incorrect labels reflecting the same confusion have been found in collections (e.g. Salgesch, 1889, BNM). Nast (1987), obviously aware of his mistake, eliminated *T. nigronervosa* from the second list for Switzerland. *Tibicina nigronervosa* is currently known to occur as an endemic species in Corsica and Sardinia (Puissant, 2006). It is considered to have never been native to Switzerland.

Saticula coriaria **Stål, 1866**

Saticula coriaria is a northern African species (Boulard, 1981), which has been listed for Switzerland tentatively but erroneously by Fieber (1876) and later by Nast (1972). The confusion started with "*Cicada violacea*, die Blaue" reported from Geneva by Fuesslin (1775) and referring to *Cicada violacea* Linné 1758 which is actually not a European species. Boulard (1981) demonstrated in detail that several authors concluded erroneously the two taxa mentioned to be in synonymy. We are unable to speculate to which species Fuesslin was referring to.

Cicadivetta tibialis (Panzer, 1798)

This very small cicada is one of two species that could expand their distribution areas towards Switzerland supported by global warming in the future. The species has been described and later listed by Wagner & Franz (1961) and by Schedl (2002) for the Vienna region, which is climatically comparable to some parts in Switzerland. The nearest, spatial occurrences are reported from the Lake Garda region (Schedl, 2002 and pers. data) and from the Piedmont (Novi Ligure; Ferrari, 1892).

Tettigettula pygmea (Olivier, 1790)

Tettigettula pygmea is very widespread and frequent in central and southern Italy, reaching elevated locations in the mountains. Because of its nearly ultrasonic song it is probably often missed. Servadei (1967) reports this species from Lombardy, unfortunately without specifying a more precise locality. Schedl (2004) mentions a single voucher specimen found at the Lake Neusiedl region in 1973. He has never listed the species for the south-eastern Alps (Schedl, 1973; 2000), but we have found it for example at Monte Collalto (Bassano di Grappa, Veneto). This is currently the nearest location to the Swiss border and an expansion towards the Ticino region would not be surprising.

Platypleura kaempferi (Fabricius, 1794)

This common Japanese species (Hayashi & Saisho, 2011) was captured in a garden in Gland (VD, Lake Geneva Basin) on 20 August 2011 after having already sung the previous evening (leg. Delapierre, det. Duffels/Boulard/Hayashi). *Platypleura kaempferi* is certainly not a species native to Switzerland and must have been introduced by human activity. There is a garden centre selling exotic trees within 200 metres of Gland locality, and we suppose the specimen to have been introduced with root balls in a larval stage. Furthermore, we assign song recordings made in a settlement in Jegenstorf (23.8.2003; Cordillot, pers. comm.) to another exotic but currently undeterminable cicada.

DISCUSSION

Due to detailed fieldwork and the use of bioacoustic methods, the number of cicada species in Switzerland has been doubled within the last few years. Pillet (1993) included five species; now we are able to present ten species as certainly indigenous. It is likely that none of these species has immigrated recently. The ten species recorded from Switzerland demonstrate the transition between the northern regions harbouring only few species (e.g. Germany: 5 species; Nickel & Remane, 2003; Meineke, 2012) and the southern regions rich in species (e.g. France: 21 species; Puissant, 2006; Puissant & Sueur, 2011; Chapter 4; Macedonia: 15 species; Gogala *et al.*, 2005; Portugal: 13 species; Sueur *et al.*, 2004). Similar species numbers have been published for well studied countries Austria (8 species; Schedl, 2002; 2004; Trilar & Holzinger, 2004) and Slovenia (11 species; Gogala & Gogala, 1999; Gogala & Trilar, 2004). Four of the five largest European species are present in Switzerland, and five of the ten native species have impressive songs. The *Cicadetta montana* species complex is well represented with four species. For comparison, three species have been published for Austria (Trilar & Holzinger, 2004) and Germany (Meineke, 2012) and six for France (Sueur & Puissant, 2007a; 2007b; Chapter 4). Many species reach Switzerland only marginally, *Tettigettalna argentata* and *Cicadetta sibillae* from the south, *Tibicina haematodes* from the west and *Cicadetta petryi* and *Lyristes plebejus* from the south and west. Swiss national responsibility for the conservation of these species is not that high in an international context. In contrast, the isolated populations of *Tibicina quadrisignata* and *T. steveni* are potentially good flagship species with a high international responsibility for their conservation. The abundant local populations of *C. cantilatrix* are important and, at least until comparable data from elsewhere is available, must be assumed to result in a high national responsibility for its conservation.

We demonstrate that some species which have been published for Switzerland in previous works should be omitted from the species list (*Cicadatra atra*, *Tibicina nigronervosa*, *Saticula coriaria*). We also publish records of an exotic Asian cicada species (*Platypleura kaempferi*) found in Central Europe for the first time. Such incidents are rare since adult specimens appear to die within a few days without suitable nutrition.

Three parts of the country are clearly important with respect to the regional diversity of species: The Valais (e.g. Pillet, 1993), Ticino and more surprisingly the Lake Geneva Basin (**Figs 6.13 and 6.14**). The grid cell “Sierre” is the only one with five species occurring. 15 cells with four species detected are situated in the Valais (9), Ticino (3), the Canton of Geneva (2) and Grisons (1). Up to four species are associated syntopically in the Lugano region (TI) and parts of the Rhone Valley (VS). The Jura Mountains and especially the eastern parts in the Cantons of Aargau and Basel-Land are recognised as of international importance for their numerous *Cicadetta cantilatrix* populations. Populations of three species in the *C. montana* group occur within a distance of a few kilometres in the Canton of Geneva as well as in the Lugano (TI) and Orbe (VD) regions. An almost deafening concert of dozens of calling males for example of *L. plebejus* and *C. orni* between Gandria and Castagnola (TI, Lugano region) possess an additional value. At these locations natural acoustic spectacles can be found that may only be surpassed in power by a chorus of European Tree Frog (*Hyla arborea*) or Natterjack Toad (*Bufo calamita*) in Switzerland. The potential of this experience should be used for nature education in a country more and more dominated by artificial sounds and noises.

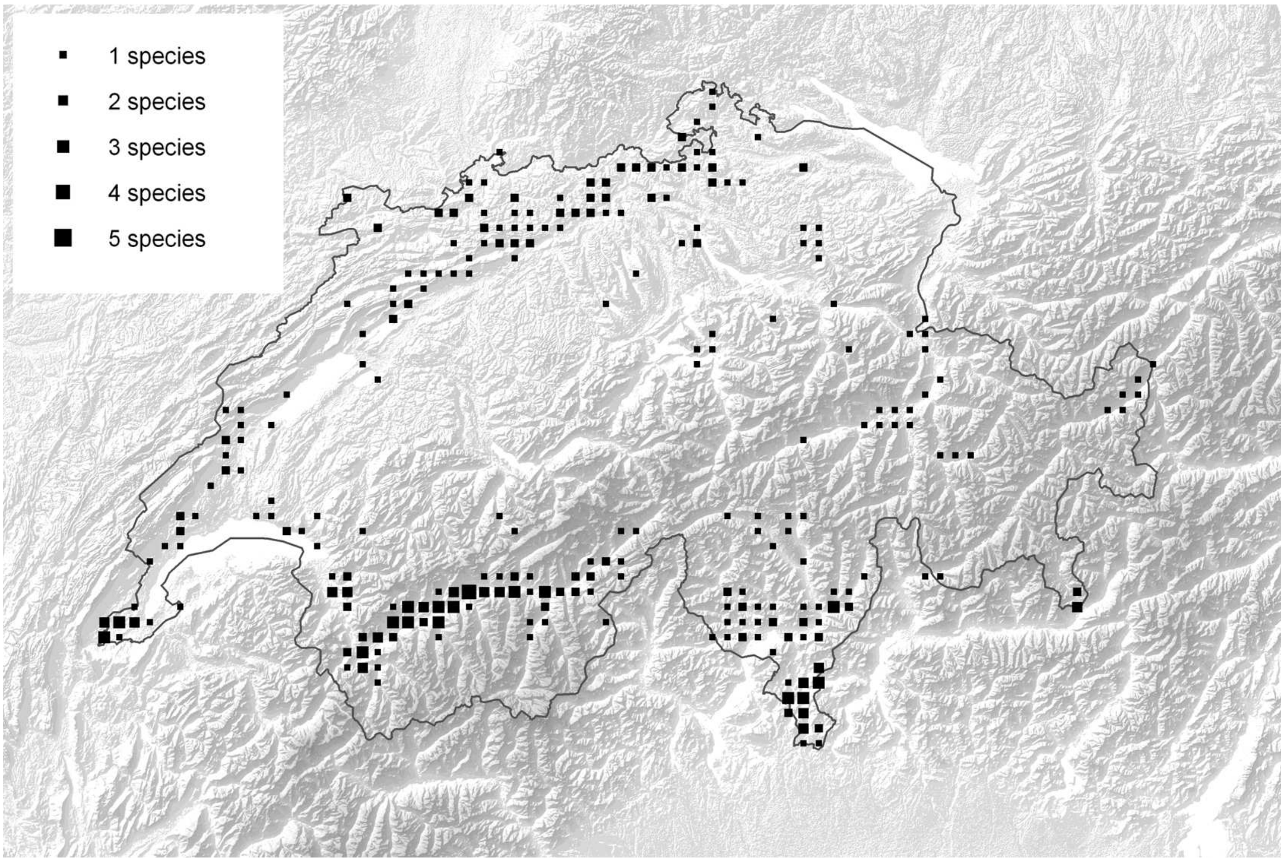

Fig 6.13. Species number for each grid cell showing the centres of species diversity.

Nine of the ten species are critical with respect to the ecological quality of their habitats (**Fig 6.15**). *Cicada orni* is the only species that has been recorded several times in man-made habitats such as parks or gardens. Favourite habitats for Swiss cicadas consist of different oak and pine forests and traditionally used semidry and dry meadows with bushes and trees. They are often restricted to scattered locations on the driest, poorest soils and the sunniest exposures. The plant association (*Orno-Ostryon*) characterised by Downy (*Quercus pubescens*) and Turkey Oak (*Quercus cerris*), Hop Hornbeam (*Ostrya carpinifolia*) and Manna Ash (*Fraxinus ornus*) in Ticino and xerothermic habitats with Scots Pine (*Pinus sylvestris*), Savin

Juniper (*Juniperus sabina*) and/or Downy Oak in the Valais form very important habitats. Apart from local climatic conditions, habitat structure is more important for species of the *C. montana* group than for any other species. These species prefer ecotone habitats or open woodlands with a well-developed herb layer.

Fig 6.14. Centres of cicada diversity. Four syntopically occurring species at Lintellière (central Valais, top left), three species of the *C. montana* group within a few square kilometres at Denti della Vecchia mountains (Lugano region, TI, top right) and up to five regularly and irregularly observed species at slopes of the Rhone river in the Canton of Geneva (bottom).

A clear decline in population numbers and distribution area can be documented for *L. plebejus*, which has become extinct in the Valais (Pillet, 1993; Keim, 1999). Other species have probably suffered locally due to habitat loss. Considering Bremi (in Hagen, 1856b) and Dietrich (1872), *C. montana* s. str. and *C. cantilatrix* supposedly disappeared locally in the Swiss Plateau with the intensification of agriculture [loss of traditionally managed semidry meadows (*Mesobromion*), elimination of hedges and other ligneous structures, replacement of meadows by pastures] and the strict separation between forests and agricultural land (loss of open wood and ecotone habitats). A decline in all *Cicadetta* species probably took place after the abandonment of traditional land use and the expansion of settlements in Ticino. Both *Tibicina* species from the Valais have been eliminated locally by the expansion of vineyards (Pillet, 1993) and artificial irrigation. These two species are the most important to focus on in nature conservation programmes because of their spatial isolation, their small distribution areas and their low densities. Overall, the most important conservation measure is to stop the habitat loss in the Valais. *Cicada orni*, *L. plebejus*, *T. haematodes* and *T. argentata* may extend their distribution area in the future supported by a milder climate.

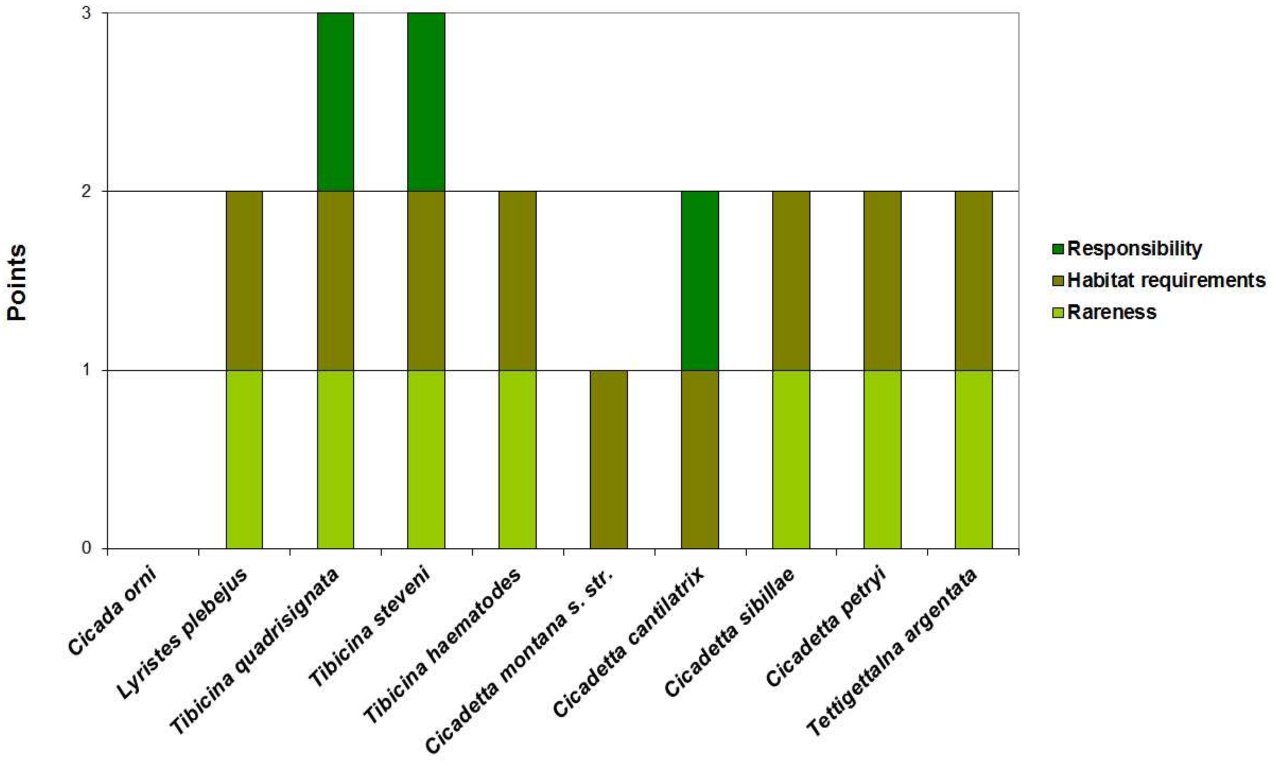

Fig 6.15. Conservation issues estimated for each species in Switzerland. High national responsibility for the conservation of the species, critical habitat requirements and rareness (less than 20 grid cells occupied).

Species of the *C. montana* complex can be assisted on sites with a warm and dry microclimate by thinning out forests on poor soil and by improving the structure of forest edges near dry and semidry meadows (Hertach, 2007). For example, well-adapted measures have been conducted in the Jura Mountains and in the Canton of Geneva within the last decades. We hope that the detailed knowledge on this insect group in combination with the planned Red List and the list of priority species will lead to more concrete conservation measures and an increasing political importance of these flagship species. The references found in non-scientific articles and the remarkable media coverage given to solitary calling males of *C. orni* or *L. plebejus* underline the popularity of this insect group in Switzerland.

The gathered knowledge is an excellent base for additional field-work. Gaps in the dataset can be found in the central Jura Mountains and in the northern Prealps. It is likely that no additional species will be found, because of climatic and biogeographic patterns. The completeness of the inventory work is important for an assessment of the threat according to the criteria established in the IUCN. Furthermore, it forms the baseline to refine conservation strategies from the local to the national scale. Detailed field-work has to be redone in the central Valais in order to provide more accurate data on the overlapping distribution areas of the two *Tibicina* species. Furthermore, sites with old references or vouchers of *C. montana* s. l. should be visited again to get an impression on the long term development and threat of the populations.

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REFERENCES CHAPTER 6

Artmann G. 1987. Die fremdsprachige Bergzikade (*Cicadetta montana peregrina*). *Mitteilungen der Entomologischen Gesellschaft Basel* 37(4): 190-192.

Barbey P. 2010. Un événement pas si inhabituel que ça! A propos de l'article intitulé «Une cigale chante en ville de Lausanne». *24heures (newspaper, online)*, issue of 13 August 2010. Available from: http://www.24heures.ch (accessed 23 August 2010).

Bettelini A. 1919. Discorso inaugurale del Congresso. *Verhandlungen der Schweizerischen Naturforschenden Gesellschaft* 100(2): 3-14.

Biedermann R, Niedringhaus R. 2004. *Die Zikaden Deutschlands – Bestimmungstafeln für alle Arten.* Scheessel: Fründ, 409 pp.

Bille RP. 1980. *Le Bois de Finges et son Rhône. L'une des plus belles pinèdes d'Europe.* Sierre: Edition W. Schoechli, 96 pp.

Bille RP. 1982. Les cigales de Romandie. *Diana* 99: 327-328.

Bille RP, Werner P. 1986. *Natur entdecken im Pfynwald.* Liestal: Verlag des Schweizerischen Vereins für Handarbeit und Schulreform, 144 pp.

Boulard M. 1981. Matériaux pour une révision de la faune cicadéenne de l'Ouest paléarctique. *Bulletin de la Société Entomologique de France* 86: 41-53.

Boulard M. 1995. Postures de cymbalisation, cymbalisations et cartes d'identité acoustique des cigales. Généralités et espèces méditerranéennes (Homoptera, Cicadoidea). *Ecole pratique des hautes Etudes, Biologie et Evolution des Insectes* 7/8: 1-72.

Bremi H. 1849. Uebersicht der schweizerischen Rhynchoten. *Mittheilungen der Naturforschenden Gesellschaft in Zürich* 34: 325-339.

Bugnion E. 1890. Introduction à la faune entomologique du Valais. In: Favre E, ed. *Faune des Coléoptères du Valais et des régions limitrophes.* Neue Denkschriften der allgemeinen schweizerischen Gesellschaft für die gesamten Naturwissenschaften, Band 31. Zürich: Druck Zürcher und Furrer, IX-XLIV.

Carlini A. 1887. Rincoti del Sottoceneri. *Bullettino de la Società Entomologica Italiana, Firenze* 19: 258-277.

Curti G. 1846. *Storia naturale disposta con ordine scientifico e adattata alla comune intelligenza*. Lucerna: Tipografia Meyer, 528 pp.

Delarze R. 1987. L'origine des pelouses steppiques valaisannes à la lumière de leurs liens de parenté avec les régions limitrophes. *Bulletin de la Murithienne* 105: 41-70.

Delarze R. 1988. *Die Follatères naturgetreu. Die Reichtümer der Natur im Wallis.* Departement für Umwelt des Kantons Wallis. Martigny: Editions Pillet, 63 pp.

Delarze R, Gonseth Y. 2008. *Lebensräume der Schweiz: Ökologie – Gefährdung – Kennarten*. 2. Auflage. Bern: Hep Verlag AG, 424 pp.

Della Beffa G. 1925. Contributo alla conoscenza della fauna entomologica del Piemonte: Omotteri (Emitteri). *Bollettino della Società Entomologica Italiana* 57: 141-147.

Dietrich K. 1872. Beiträge zur Kenntniss der im Kanton Zürich heimischen Insekten. *Entomologische Blätter aus der Schweiz* 2: 1-32.

Döderlein L. 1913. Beobachtungen über elsässische Tiere. *Mitteilungen der Philomathischen Gesellschaft in Elsass-Lothringen, Strassbourg* 5(1): 163-174.

Dorda D. 2012. *Die Tierwelt im Saarland – eine Auswahl typischer Arten.* Saarland-Hefte 5. Schiffweiler: Institut für Landeskunde im Saarland, 127 pp.

Ducret J. 2010. Une cigale chante en ville de Lausanne. *24heures (newspaper, online)*, issue of 26 July 2010. Available from: http://www.24heures.ch (accessed 23 August 2010).

Duffels JP, Van der Laan PA. 1985. *Catalogue of the Cicadoidea (Homoptera, Auchenorrhyncha) 1956–1980.* Series entomologica. Dordrecht, Boston, Lancaster: Dr. W. Junk Publishers, 414 pp.

Eitschberger U. 1972. Zur Verbreitung von *Cicadetta montana* und *Tibicen haematodes* in Mainfranken (Hom., Cicadidae). *Entomologische Zeitschrift, Frankfurt* 82(18): 210-213.

Evenhuis NL. 2014. *Abbreviations for insect and spider collections of the world.* Available at: http://hbs.bishopmuseum.org/codens/codens-inst.html (accessed 20 May 2015).

Farquet P. 1940. Le Mont Ravoire s/Martigny. *Bulletin de la Murithienne* 57: 34-89.

Ferrari PM. 1892. Res Ligusticae XIX. Elenco dei Rincoti Ligustici (Emitteri e Cicadarii) fin'ora osservati. *Annali del Museo Civico di Storia Naturale di Genova* Serie 2, Vol. XII: 549-576.

Fieber FX. 1876. Les Cicadines d'Europe d'après les originaux et les publications les plus récentes. Deuxième partie: Descriptions des espèces. *Revue et Magazin de Zoologie* 4: 11-268.

Fischer LH. 1847. Ueber Sing-Cicaden. *Stettiner entomologische Zeitung* 8: 237-243.

Fuesslin JC. 1775. *Verzeichnis der ihm bekannten Schweitzerischen Inseckten.* Zürich/Winterthur: Bey dem Verfasser und in Commission bey Heinrich Steiner und Compagnie, xii + 62 pp.

Geisenheyner L. 1887. Nicht eine, sondern zwei Singzikaden in der Rheinprovinz. *Verhandlungen des naturhistorischen Vereines der preussischen Rheinlande, Westfalens und des Reg.-Bezirkes Osnabrück* 44: 116-117.

Gogala M. 2002. Gesänge der Singzikaden aus Südost- und Mittel-Europa. *Denisia* 04: 241-248.

Gogala M. 2015, ed. *Songs of European singing cicadas.* Available from: www.cicadasong.eu (accessed 25 January 2016).

Gogala M, Gogala A. 1999. A checklist and provisional atlas of the Cicadoidea fauna of Slovenia (Homoptera: Auchenorrhyncha). *Acta entomologica slovenica* 7(2): 119-128.

Gogala M, Trilar T. 2004. Bioacoustic investigations and taxonomic considerations on the *Cicadetta montana* species complex (Homoptera: Cicadoidea: Tibicinidae). *Anais da Academia Brasileira de Ciências* 76(2): 316-324.

Gogala M, Trilar T, Drosopoulos S. 2011. Two new species and a new genus of Cicadettini (Hemiptera, Cicadidae) from the Greek island of Evia. *Deutsche Entomologische Zeitschrift* 58(1): 105-117.

Gogala M, Trilar T, Krpač VT. 2005. Fauna of singing Cicadas (Auchenorrhycha: Cicadoidea) of Macedonia – a bioacoustic survey. *Acta entomologica slovenica* 13(2): 103-126.

Griffini A. 1896. Notes sur la faune entomologique piémontaise. X. Cicadidae, Membracidae et Scaridae. *Miscellanea Entomologica. Revue Entomologique Internationale* 4(11-12): 143-144.

Grosjean A. 2008. Une cigale a pris ses quartiers à Plainpalais. *Tribune de Genève (newspaper)*, issue of 31 July/1 August 2008.

Günthart H. 1987. Zikaden (Auchenorrhyncha). In: Bündner Natur-Museum. *Ökologische Untersuchungen im Unterengadin.* Band 12. Chur: Bündner Natur-Museum, D203-D299.

Günthart H, Mühlethaler R. 2002. Provisorische Checkliste der Zikaden der Schweiz (Insecta: Hemiptera, Auchenorrhyncha). *Denisia* 04: 329-338.

Gurcel K. 2015. Contribution à la connaissance des Cigales de France: première synthèse des observations et répartition des espèces pour le département de la Haute-Savoie (Hemiptera Cicadidae). *L'Entomologiste* 71(4): 245-260.

Hagen H. 1855. Die Sing-Cicaden Europa's. *Stettiner entomologische Zeitung* 16: 340-358.

Hagen H. 1856a. Die Sing-Cicaden Europa's. *Stettiner entomologische Zeitung* 17: 27-37.

Hagen H. 1856b. Die Sing-Cicaden Europa's. *Stettiner entomologische Zeitung* 17: 66-91.

Haller Von A. 1765. *Kurzer Auszug einer Beschreibung der Salzwerke in dem Amte Aelen*. Bern: Dan. Brunner und Albr. Haller, 170 pp.

Haupt H. 1935. 2. Unterordnung: Gleichfülger, Homoptera. In: Brohmer P, Ehrmann P, Ulmer G, eds. *Die Tierwelt Mitteleuropas*, Band IV, Insekten I. Teil. Leipzig: Quelle & Meyer, 115-262.

Hayashi M, Saisho Y. 2011. *The Cicadidae of Japan.* Tokyo: Seibundo-shinkosha, 224 pp.

Heer O, Blumer JJ. 1846. *Der Kanton Glarus, historisch-geographisch-statistisch geschildert von den älteren Zeiten bis auf die Gegenwart.* St. Gallen/Bern: Huber und Compagnie, XIV + 665 pp.

Hegg O, Béguin C, Zoller H. 1993. *Atlas schutzwürdiger Vegetationstypen der Schweiz.* Bern: Bundesamt für Umwelt, Wald und Landschaft, 160 pp.

Hertach T. 2004. Beitrag zur Klärung des Artkomplexes *Cicadetta montana* – Bergzikade (Hemiptera, Cicadidae): Entdeckung einer Singzikadenart mit ungewissem taxonomischem Status in der Nordschweiz. *Mitteilungen der Entomologischen Gesellschaft Basel* 54(2): 58-66.

Hertach T. 2007. Three species instead of only one: Distribution and ecology of the *Cicadetta montana* species complex (Hemiptera: Cicadoidea) in Switzerland. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 80: 37-61.

Hertach T. 2008. A new cicada species for Switzerland: *Tettigetta argentata* (Olivier, 1790) (Hemiptera: Cicadoidea). *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 81: 209-214.

Hertach T. 2010. Pechader-Bergzikade (*Cicadetta montana*). In: Ineichen S, Ruckstuhl M, eds. *Stadtfauna – 600 Tierarten der Stadt Zürich.* Bern/Stuttgart/Wien: Haupt Verlag, 183.

Hertach T. 2011. Spectacular song pattern from the Sicilian Mountains: The new cicada species *Cicadetta anapaistica* sp. n. (Hemiptera: Cicadidae). *Zootaxa* 2771: 25-40.

Hertach T, Trilar T, Wade EJ, Simon C, Nagel P. 2015. Songs, genetics, and morphology: revealing the taxonomic units in the European *Cicadetta cerdaniensis* cicada group, with a description of new taxa (Hemiptera: Cicadidae). *Zoological Journal of the Linnean Society* 173(2): 320-351.

Hertach T, Pollini Paltrinieri L. 2012. Le cicale (Hemiptera: Cicadidae) del Cantone Ticino (Svizzera). *Bollettino della Società ticinese di scienze naturali* 100: 43-51.

Hoch H. 2011. *Cicadidae and Tibicinidae.* Fauna Europaea Version 2.4. Available from: www.faunaeur.org (accessed 7 June 2012).

Hofmänner B. 1924. Die Hemipterenfauna des Schweizerischen Nationalparkes (Heteroptera und Cicadinen). *Denkschrift der Schweizerischen Naturforschenden Gesellschaft* 60: 1-88.

Hollier J. 2012. Ordre Auchenorrhyncha. In: Merz B, ed. *Liste annotée des insectes (Insecta) du canton de Genève.* Instrumenta Biodiversitatis 8. Geneva: Muséum d'histoire naturelle, 59-62.

Holzinger WE, Kammerlander I, Nickel H. 2003. *The Auchenorrhyncha of Central Europe. Volume 1: Fulgoromorpha, Cicadomorpha excl. Cicadellidae.* Leiden: Brill Academic Publishers, 673 pp.

Huber A. 1916. Die wärmeliebende Tierwelt der weitern Umgebung Basels. *Archiv für Naturgeschichte, Berlin, Abteilung A* 82(7): 1-120.

Hüeber T. 1904. Systematisches Verzeichnis der deutschen Zikadinen. *Jahreshefte des Vereins für vaterländische Naturkunde in Württemberg* 60: 253-277.

Hugel S, Matt F, Callot H, Feldtrauer J, Brua C. 2008. Présence de *Cicadetta petryi* Fieber, 1876 en Alsace (Hemiptera, Cicadidae). *Bulletin de la Société Entomologique de Mulhouse*, 64(1): 5-10.

Jacobs W, Renner M. 1988. *Biologie und Ökologie der Insekten.* 2. Auflage. Stuttgart: Gustav Fischer Verlag, 609 pp.

Keim C. 1999. Les cigales musiciennes accomplis. *Diana, Chasse/Nature* 12: 13-15.

Kuntze R. 1931. Vergleichende Beobachtungen und Betrachtungen über die xerotherme Fauna in Podolien, Brandenburg, Österreich und der Schweiz. *Zeitschrift für Morphologie und Ökologie der Tiere* 21: 629-690.

Kunz G, Nickel H, Niedringhaus R. 2011. *Fotoatlas der Zikaden Deutschlands – Photographic Atlas of the Planthoppers and Leafhoppers of Germany.* Scheessel: Fründ, 293 pp.

Labram JD, Imhoff L. 1845. *Insekten der Schweiz. Die vorzüglichsten Gattungen je durch eine Art bildlich dargestellt.* Viertes Bändchen. Basel: Dahnmaier's Buchhandlung, 61-81.

Marchesi P, Sierro A, Fournier J. 2002. Chronique entomologique valaisanne pour 2001 et 2002. *Bulletin de la Murithienne* 120: 25-32.

Meineke T. 2012. Bergsingzikaden *Cicadetta cantilatrix* Sueur & Puissant, 2007, *Cicadetta petryi* Fieber, 1876 und *Cicadetta montana* s. str. (Scopoli, 1772) im mittleren Deutschland (Auchenorrhyncha, Cicadidae, Cicadettinae). *Entomologische Nachrichten und Berichte* 56(2): 133-142.

Melichar L. 1896. *Cicadinen (Hemiptera-Homoptera) von Mittel-Europa.* Berlin: Felix L. Dames, 364 pp.

Metcalf ZP. 1963. *General Catalogue of the Homoptera, Fascicle VIII, Cicadoidea. Part 1: Cicadidae, Section 1 Tibicinidae.* Raleigh: University of North Carolina State College, 585 pp.

Meyer-Dür R. 1860. *Ein Blick über die schweizerische Orthoptern-Fauna.* Neue Denkschriften der Allgemeinen schweizerischen Gesellschaft für die gesammten Naturwissenschaften, Band 17. Zürich: Allgemeine schweizerische Gesellschaft für die gesammten Naturwissenschaften, 32 pp.

Milde J. 1865. Zoologische Mitteilungen aus Meran. *Verhandlungen der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft in Wien* 15: 961-962.

Milde J. 1866. *Naturgeschichtliche Mittheilungen über Meran. Erste Mittheilung: Die Sing-Cicaden.* Breslau: Realschule zum heiligen Geist, 49 pp.

Moffet T, Wotton E, Gesner K, Penny T. 1634. *Insectorum sive minimorum animalium theatrum.* London: Ex officinâ typographicâ Thomas Cotes, 326 pp.

Moulds MS. 2005. An appraisal of the higher classification of cicadas (Hemiptera: Cicadoidea) with special reference to the Australian fauna. *Records of the Australian Museum* 57: 375-446.

Müller P. 1969. *Cicadetta montana* Scop. (Homoptera-Cicadidae) im Saarland. *Faunistisch-floristische Notizen aus dem Saarland* 2(3): 24-25.

Muhieddine F. 2004. Une cigale chante à la Tour-de-Peilz. *24heures (newspaper)*, issue of 17 August 2004.

Musée d'histoire naturelle Fribourg, Naturhistorisches Museum Freiburg. 2004. Rapport d'activité – Jahresbericht 2003, 83 pp.

Nast J. 1972. *Plaearctic Auchenorrhyncha (Homoptera). An annotated check list.* Warszawa: Polish scientific publishers, 550 pp.

Nast J. 1987. The Auchenorrhyncha (Homoptera) of Europe. *Annales Zoologici Warszawa* 40(15): 355-661.

Nickel H. 2003. *The leafhoppers and planthoppers of Germany (Hemiptera, Auchenorrhyncha): patterns and strategies in a highly diverse group of phytophagous insects.* Sofia/Moscow: Pensoft, 460 pp.

Nickel H, Remane R. 2003. Verzeichnis der Zikaden (Auchenorrhyncha) der Bundesländer Deutschlands. In: Klausnitzer B, ed. *Entomofauna Germanica 6.* Beiheft 8. Dresden: Entomologische Nachrichten und Berichte, 344 pp.

Niehuis M, Simon L. 1994. Zum Vorkommen der Blutaderzikade – *Tibicina haematodes* (Scop.) – und Bergzikade – *Cicadetta montana* (Scop.) – in Rheinland-Pfalz (Homoptera: Cicadidae). *Fauna Flora Rheinland Pfalz* 7(2): 253-264.

Nikolic D. 2010. La cigale est de retour à Plainpalais. *Tribune de Genève (newspaper, online)*, issue of 28 June 2010. Available from: http://www.tdg.ch (accessed 29 June 2010).

Oggier PA. 1995. *Erkenne die Natur im Wallis – die Fauna.* Deutsche Ausgabe. Martigny: Edition Pillet, 279 pp.

Oshanin B. 1912. *Katalog der paläarktischen Hemipteren (Heteroptera, Homoptera-Auchenorhyncha und Psylloideae).* Berlin: Friedländer & Sohn, 187 pp.

Pavesi P. 1873. *Materiali per una fauna del cantone Ticino.* Atti della Società Italiana di Scienze Naturali, Vol XVI. Fasc. I. Milano: Società Italiana di Scienze Naturali, 33 pp.

Perty M. 1879. *Erinnerungen aus dem Leben eines Natur- und Seelenforschers des neunzehnten Jahrhunderts.* Leipzig/Heidelberg: C. F. Winter'sche Verlagshandlung, 486 pp.

Pillet JM. 1993. Les Cigales du Valais. *Bulletin de la Murithienne* 111: 95-113.

Popov A, Beganovic A, Gogala M. 1997. Bioacoustics of singing cicadas on the western palaearctic: *Tettigetta brullei* (Fieber 1876) (Cicadoidea: Tibicinidae). *Acta entomologica slovenica* 5: 89-101.

Puissant S. 2006. *Contribution à la connaissance des cigales de France: Géonémie et écologie des populations (Hemiptera, Cicadidae).* Bédeilhac/Aynat: Association pour la Caractérisation et l'Etude des Entomocénoses, 193 pp.

Puissant S, Boulard M. 2000. *Cicadetta cerdaniensis,* espèce jumelle de *Cicadetta montana* décryptée par l'acoustique (Auchenorrhyncha, Cicadidae, Tibicinidae). *Ecole pratique des hautes Etudes, Biologie et Evolution des Insectes* 13: 111-117.

Puissant S, Sueur J. 2010. A hotspot for Mediterranean cicadas (Insecta: Hemiptera: Cicadidae): new genera, species and songs from southern Spain. *Systematics and Biodiversity* 8(4): 555-574.

Puissant S, Sueur J. 2011. *Dimissalna*, a cicada genus that remained unnoticed in France (Insecta: Hemiptera: Cicadidae). *Annales de la Société entomologique de France* 47(3-4): 519-523.

Puton A. 1899. *Catalogue des Hémiptères (Hétéroptères, Cicadines et Psyllides) de la faune Paléarctique.* Quatrième édition. Caen: Société française d'entomologie, 121 pp.

Radio Télévision Suisse Romande (RTSR). 2010. Le Journal. Broadcasted on 16 July 2010.

Remane R, Wachmann E. 1993. *Zikaden kennenlernen – beobachten.* Augsburg: Naturbuch Verlag, 288 pp.

Robert PA. 1937. *Les Insectes: Hyménoptères, Lépidoptères, Rhynchotes, Diptères.* Volume 2. Neuchâtel/Paris: Editions Delachaux & Niestlé S.A., 270 pp.

Rougemont P. 1880. Note sur l'helicopsyche sperata. *Bulletin de la Société des Sciences Naturelles de Neuchâtel* 12: 29-38.

Saussure HB. 1780. *Voyages dans les Alpes, précédés d'un Essai sur l'Histoire Naturelle des Environs de Genève.* Tome Premier. Neuchâtel: Samuel Fauche, Imprimeur & Libraire du Roi, 540 pp.

Schedl W. 1973. Zur Verbreitung, Bionomie und Ökologie der Singzikaden (Homoptera: Auchenorrhyncha, Cicadidae) der Ostalpen und ihrer benachbarten Gebiete. *Berichte des naturwissenschaftlich-medizinischen Vereins Innsbruck* 60: 79-94.

Schedl W. 2000. Taxonomie, Biologie und Verbreitung der Singzikaden Mitteleuropas (Insecta: Homoptera: Cicadidae et Tibicinidae). *Berichte des naturwissenschaftlich-medizinischen Vereins Innsbruck* 87: 257-271.

Schedl W. 2002. Die Verbreitung der fünf Singzikaden-Arten in Österreich (Hemiptera: Cicadoidea). *Denisia* 04: 231-239.

Schedl W. 2004. Die Singzikaden des Burgenlands (Österreich) (Insecta: Homoptera, Cicadoidea). *Linzer Biologische Beiträge* 36: 913-917.

Scherdlin P. 1910. Eine für die deutsche Fauna neue grosse Cicade, *Cicada plebeja* Scop. *Annales de la Société entomologique de Belgique* 54: 142-143.

Schnetzler JB. 1877. Discours d'ouverture de la 60^{e} session de la Société Helvétique des Sciences Naturelles à Bex. *Verhandlungen der Schweizerischen Naturforschenden Gesellschaft* 60: 3-30.

Schwoerbel W. 1957. Die Wanzen und Zikaden des Spitzberges bei Tübingen, eine faunistisch-ökologische Untersuchung (Hemipteroidea: Heteroptera und Cicadina = Homoptera Auchenorrhyncha). *Zeitschrift für Morphologie und Ökologie der Tiere, Berlin* 45: 462-560.

Segantini G. 1937. La Bregaglia. *Quaderni grigionitaliani* 6(1): 3-17.

Seiler A. 1847. Uebersicht der im Canton Schaffhausen vorkommenden Thiere. *Verhandlungen der Schweizerischen Naturforschenden Gesellschaft* 32: 160-175.

Servadei A. 1967. *Fauna d'Italia. Rhynchota (Heteroptera, Homoptera, Auchenorrhyncha). Catalogo topografico e sinonimico.* Bologna: Edizione Calderini, 851 pp.

Siebold CT. 1847. Ueber die Verbreitung der singenden Cicaden in Deutschland. *Stettiner entomologische Zeitung* 8: 6-18.

Sierro A, Keim C. 1999. Activité entomologique valaisanne pour 1997 et 1998. *Bulletin de la Murithienne* 117: 61-71.

Sierro A, Keim C, Marchesi P. 2000. Activité entomologique valaisanne pour 1999 et 2000. *Bulletin de la Murithienne* 118: 93-103.

Stabile AG, Stabile F. 1856. Degli Insetti del Cantone Ticino. *L'educatore della Svizzera italiana* Anno 1: 230-240.

Stoll O. 1901. Über xerotherme Relikte in der Schweizer Fauna der Wirbellosen. *Jahresberichte der Geographisch-Ethnographischen Gesellschaft in Zürich* 2: 145-208.

Sueur J, Aubin T. 2003. Specifity of cicada calling songs in the genus *Tibicina* (Hemiptera, Cicadidae). *Systematic Entomology* 28: 481-492.

Sueur J, Puissant S. 2007a. Similar look but different song: a new *Cicadetta* species in the *montana* complex (Insecta, Hemiptera, Cicadidae). *Zootaxa* 1442: 55-68.

Sueur J, Puissant S. 2007b. Biodiversity eavesdropping: bioacoustics confirms the presence of *Cicadetta montana* (Insecta: Hemiptera: Cicadidae) in France. *Annales de la Société entomologique de France* 43(1): 126-128.

Sueur J, Puissant S, Pillet JM. 2003. An Eastern mediterranean cicada in the West: first record of *Tibicina steveni* (Krynicki, 1837) in Switzerland and France (Hemiptera, Cicadidae). *Revue française d'Entomologie* 25: 105-111.

Sueur J, Puissant S, Simões PS, Seabra S, Boulard M, Quartau JA. 2004. Cicadas from Portugal: revised list of species with eco-ethological data (Hemiptera: Cicadidae). *Insect Systematics and Evolution* 35: 177-187.

Sulzer JH. 1761. *Die Kenntzeichen der Insekten.* Zürich/Winterthur: Steiner'sche Buchhandlung, xxviii + 203 pp.

Trilar T, Holzinger WE. 2004. Bioakustische Nachweise von drei Arten des *Cicadetta montana*-Komplexes aus Österreich (Insecta: Hemiptera: Cicadoidea). *Linzer biologische Beiträge* 36(2): 1383-1386.

Tschudi F. 1872. *Das Thierleben der Alpenwelt. Naturansichten und Thierzeichnungen aus dem schweizerischen Gebirge.* Neunte, vielfach vermehrte und verbesserte Auflage. Leipzig: Verlagsbuchhandlung J. J. Weber, XV + 593 pp.

Vernier R. 1996. Un chanteur isolé de *Lyristes plebejus* (Scop.) (Hemiptera, Cicadidae) à l'Allondon. *Bulletin romand d'entomologie* 14: 147-152.

Vogel R. 1935. Die blutrote Singzikade (*Tibicen haematodes* Scop.) in Württemberg und Deutschland. *Jahreshefte des Vereins für vaterländische Naturkunde in Württemberg* 91: 146-154.

Vogel R. 1937. Weiteres über Verbreitung und Lebensweise der Blutroten Singzikade (*Tibicen haematodes* Scop.). *Jahreshefte des Vereins für vaterländische Naturkunde in Württemberg* 93: 116-123.

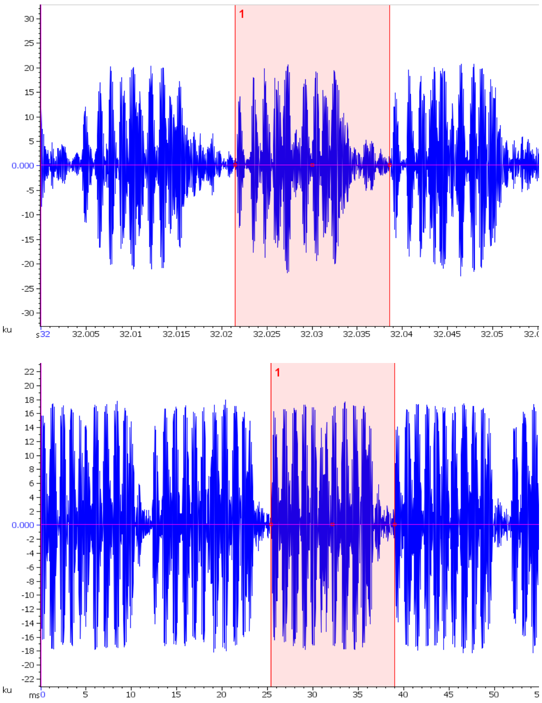
Von Salis CU. 1807. Fragmente zur Entomologie der Alpen. *Alpina – eine Zeitschrift der genauern Kenntnis der Alpen gewiedmet,* Winterthur 2: 64-104.

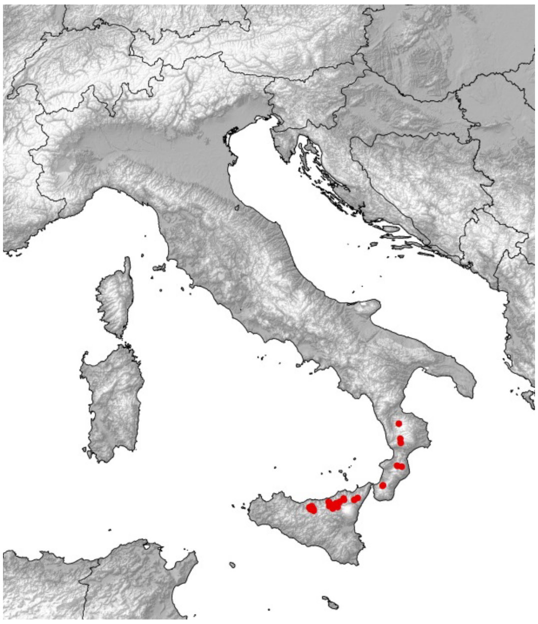
Wagner W, Franz H. 1961. Auchenorrhyncha (Zikaden). In: Franz H, ed. *Die Nordostalpen im Spiegel ihrer Landtierwelt.* Band 2. Innsbruck: Universitätsverlag Wagner, 74-158.

Zschokke F. 1928. *Die Tierwelt des Kantons Tessin. Ein Beitrag zur Kenntnis der Südschweiz.* Basel: Frobenius Verlag, 145 pp.

Part IV:

General discussion and conclusions





Introduction

In Part IV of this thesis the previous chapters are discussed in a more general context. The paragraphs are connected to the seven questions of the thesis listed at the end of the introduction (Part I). Again, we focus on the model group *Cicadetta montana* s. l. but also include the genus *Tibicina* for comparison, which formed a second field for in-depth investigations. In fact, all species found in Switzerland and many in Italy are part of cryptic species complexes. Thus, the genera *Tettigettalna* (Puissant & Sueur, 2010), *Lyristes* (Boulard, 1988; Drosopoulos *et al.*, 2006) and *Cicada* (Quartau & Simões, 2006) are represented by only one and the most widespread species and do not cause determination problems. Speciation hotspots of these genera are located far away on the Iberian peninsula (*Tettigettalna*), respectively Greek Islands and Asia Minor (*Lyristes, Cicada*). Repeating former discussions was avoided as far as possible as new aspects should be reported here. Hereby, we also deliver insight into ongoing studies.

Impact on the knowledge of the cicada biodiversity

(Questions 1 and 2)

The primary goal of taxonomic work has not changed in the last 250 years. To have a better understanding and knowledge of the biodiversity of our globe is still of major interest. The number of cryptic species described increased exponentially with the evolution of molecular approaches (Bickford *et al.*, 2007; Pfenninger & Schwenk, 2007). In tropical regions, a high number of species remain undescribed; many of them become extinct owing to habitat destructions before they are ever discovered (e.g. Reid, 1992). Nevertheless, it is remarkable that new cicada species can be found in Central Europe and Italy. Cicadas belong to the largest European insects and form a rather species-poor group.

This thesis is of considerable importance for the knowledge of the Italian and Swiss cicada diversity. For Switzerland, ten native species are reported now; the species number is doubled in comparison to Pillet (1993; Chapter 6 = Hertach & Nagel, 2013). One new species (*Cicadetta sibillae* Hertach & Trilar, 2015) was described (Chapter 2 = Hertach *et al.*, 2015), which provoked a real media hype; a second one was recognised to be different from the south-eastern populations and hence was renamed (Chapter 4; *Cicadetta petryi* Schumacher, 1924).

Front page: *Cicadetta brevipennis hippolaidica* ssp. n. female (top left), fine temporal acoustic distinction of *Tibicina steveni* (top) and *T. quadrisignata* (below) (55 ms sections; after Sueur & Aubin, 2003; top right), distribution of endemic *Cicadetta a. anapaistica* (bottom left), *Tibicina quadrisignata* male (bottom right).

The same discoveries are also valid for the cicadas of Italy. Here, 15 song-determined records of the *C. montana* complex species at the beginning of this thesis (Trilar & Hertach, 2008) multiplied to 664 records. We now report six species as well as two subspecies instead of only three. Two species (*Cicadetta sibillae*, *Cicadetta anapaistica* Hertach, 2011) and two subspecies (*C. anapaistica lucana* Hertach, 2015 and *C. brevipennis hippolaidica* Hertach ssp. n.*) are new to science (Chapters 1, 2 and 4). The studies additionally influence the nomenclature of the Mountain Cicadas in Germany (*C. petryi*) as well as nomenclature and diversity in France (*C. petryi*, *C. brevipennis litoralis* Puissant & Hertach ssp. n.*). They impact conservation assessment in a wide European range including France, Spain, Slovenia, Austria and adjoining countries, since the distribution areas of *C. brevipennis brevipennis* Fieber, 1876 and *Cicadetta cerdaniensis* Puissant & Boulard, 2000 are more limited than previously expected.

Taxonomy and species delimitation: An integrative approach as a key (Question 3)

Song patterns often provide the first evidence of hidden diversity in cicadas (e.g. Dugdale & Fleming, 1978; Gogala & Trilar, 2004; Quartau & Simões, 2006; Puissant & Sueur, 2010). However, they were not sufficient to explain the diversity of the taxa discussed here. We had to combine data sets from multiple sources towards an ‘integrative taxonomy’ (e.g. Schlick-Steiner *et al.*, 2010; Yeates *et al.*, 2011) in order to resolve these taxonomically challenging groups.

In the genus *Tibicina* (three species in Switzerland, Chapter 6 = Hertach & Nagel, 2013) identification difficulties are unusual for cicadas, contrary to the *C. montana* complex. These species are difficult to identify in the field because of very similar song patterns (and they are rarely seen). Indeed, well preserved vouchers in collections are easily recognised according to their specific colouration pattern. The application of recently published methods to distinguish songs from digital recordings (Sueur & Aubin, 2003; see front page of Part IV) established a powerful tool for the identification of the Swiss species. Combining vouchers and song analyses resulted in consistent allopatric to parapatric distribution patterns and demonstrated systematic failures in species identification of previous works (e.g. Pillet, 1993; see Chapter 6 = Hertach & Nagel, 2013; **Figs 6.5-6.7 and DI.1**). The *Tibicina* species in Switzerland have a straightforward taxonomy, but only an integrative approach for the determination was efficient and successful for the verification of their abundances.

*The new subspecies names are not yet available at the submission date of the present thesis.

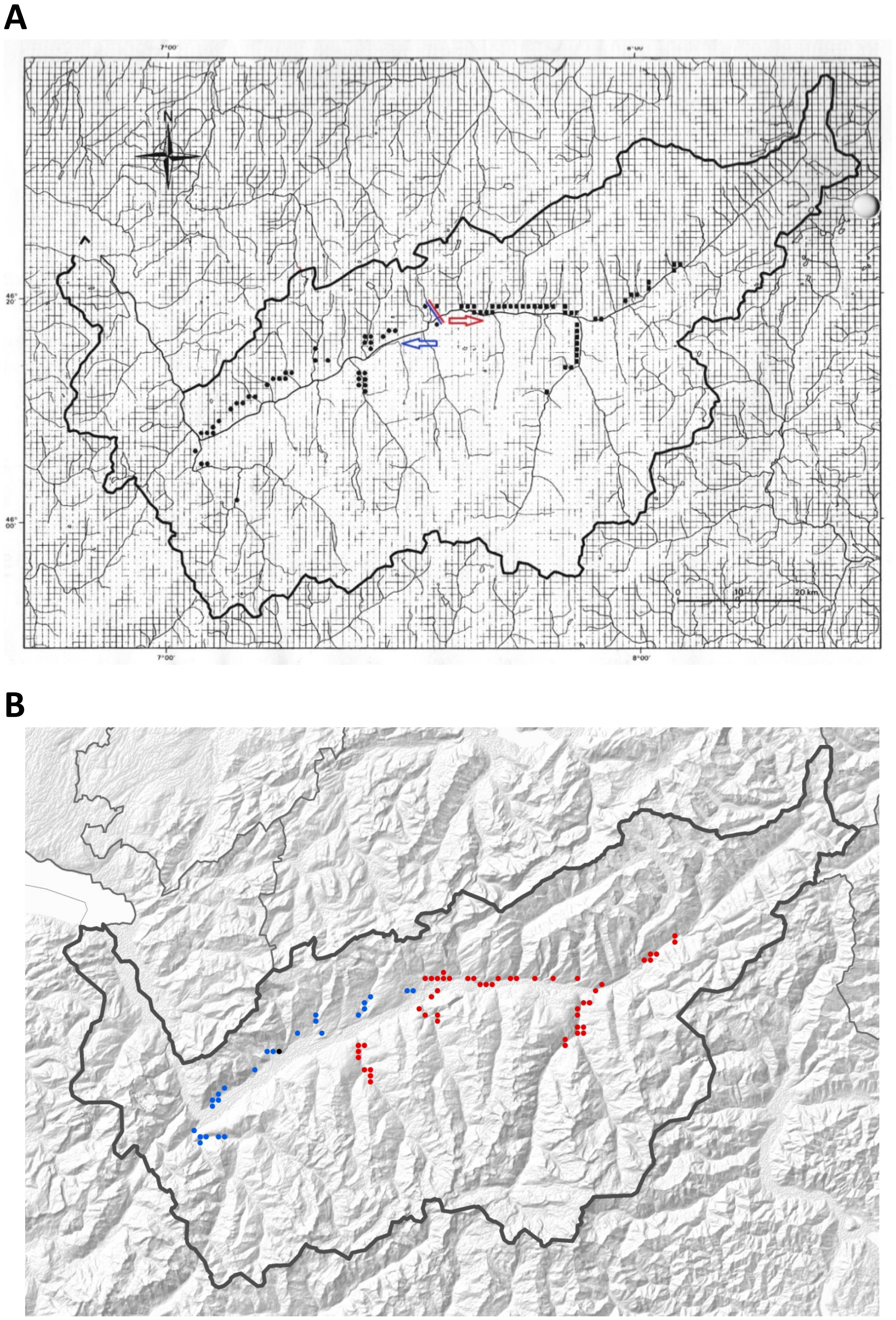


Fig DI.1. Distribution of *Tibicina quadrisignata* (squares resp. red) and *Tibicina steveni* (dots resp. blue) in the Swiss Valais. (A) After Pillet (1993), modified with marked contact line and arrows of parapatric taxa. (B) Current state of knowledge including field work of the years 2014 and 2015. Visualised in a 1 km^2 grid. Black dot = single syntopic occurrence of both species.

The *C. montana* complex in Switzerland and especially in Italy is intricate and has an unusual evolution. Almost all aspects of speciation can be demonstrated with these species and made the resolution of the problem challenging, including the nomenclature (*C. brevipennis*). Fundamental questions arose in multiple situations: How much difference is necessary that two allopactrically occurring taxa should be regarded as species? To which spatial extent are parapatric species allowed to hybridise? Or, back to the basics, what is a species?

De Queiroz' (2007) 'Unified Species Concept' was very helpful for our understanding of species in combination with Butlin *et al.*'s (2008) and Mallet's ideas (2008). "Separately evolving metapopulation lineages" can be inferred by combining different data sources; some of them may be contradictory (Chapter 2 = Hertach *et al.*, 2015 and Chapter 4). We used at least two alternative congruent data sets or several traits supporting the evidence of different lines. Additionally, we searched for reasons which could explain aberrant results.

Various authors have lately criticised the statistical rigour and objectivity of traditional species delimitation (e.g. Tobias *et al.*, 2010; Hey & Pinho, 2012). However, two trends on species delimitation have been observed in recent publications. On the one hand, applied molecular studies and other new methods demonstrate that speciation can happen in a complexity not suspected before. For example, hybridisation is widespread (Mallet, 2005) or phenotypic convergences are extreme in different groups (Moore & Robertson, 2014; Bennik *et al.*, 2016). On the other hand, trends to model species delimitation are upcoming and among the most frequently cited taxonomic works (review in Carstens *et al.*, 2013). These two trends are conflicting, and it is questionable whether the species delimitation models really contribute to a better taxonomy. Models are imperfect imitations of the biological reality, most of them for example not allowing divergence with gene flow (Carstens *et al.*, 2013; Solís-Lemus *et al.*, 2015). They may contribute to a more efficient taxonomy in poorly known species-rich groups (Pons *et al.*, 2006). However, in our complicated system they will fail. Or, a sophisticated model that fits our dataset, will in return suffer from objectivity: The results will only be convincing when the operator is allowed to manipulate the weight of input data. We agree with Sites & Marshall (2004) that all methods will sometimes fail to delimit species boundaries properly and virtually require researchers to make qualitative judgements. There seems to be one, time-consuming, approach to elevate the objectivity of the taxonomic decisions in our model group *C. montana* s. l.: We would have to investigate in-depth gene flow between taxa on spatial and temporal levels. Thus, we could estimate rates of gene flow typical for species, subspecies and populations (see Mallet, 2008).

Hybridisation among taxa of the *cerdaniensis* and *brevipennis* song groups is obviously present to different degrees. We found at least three intra-group hybridisation zones between taxa, inferred from obviously intermediate song patterns (**Figs 2.11 and 4.4**): Twice populations mixed to a degree that blurred the differences over vast regions (subspecific level: *C. a. anapaistica* versus *C. a. lucana*; Chapter 2 = Hertach *et al.*, 2015 and *C. b. brevipennis* versus *C. b. hippolaidica* ssp. n.; Chapter 4), once the hybridisation zone only affected a few peripheral populations (species level: *C. sibillae* versus *C. a. lucana*; Chapter 2 = Hertach *et al.*, 2015). Gene flow is obviously also present between the *cerdaniensis* and the *brevipennis* groups themselves; this can be concluded from the identical haplotypes (**Fig 4.9**). These basic song patterns are clearly different and the occurring gene flow surprises. However, the song patterns of **Fig DI.2A-B** were probably emitted by two hybrids between *C. sibillae* and *C. petryi* among hundreds of normal singing individuals in syntopic populations.

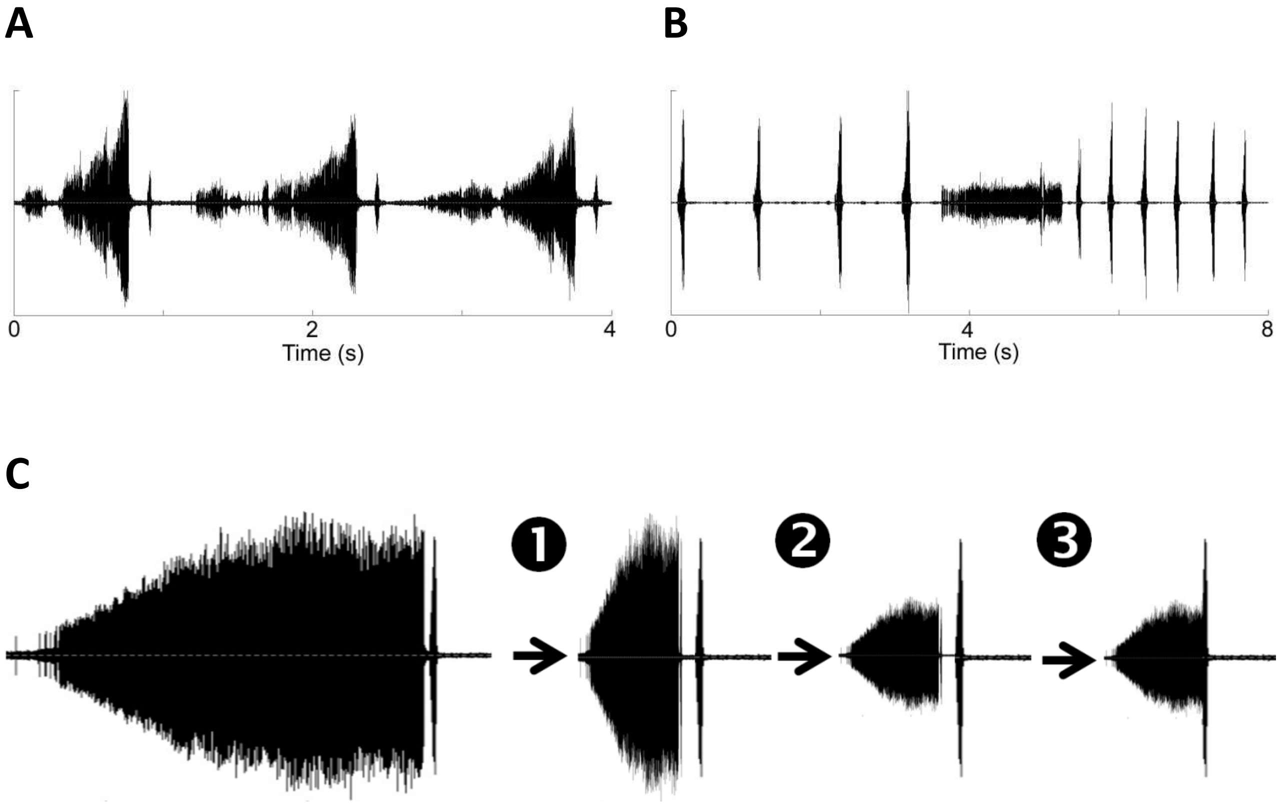


Fig DI.2. Oscillograms (time versus amplitude) of *brevipennis-cerdaniensis* group intermediates. (A) Weird song fragment of a supposed inter-group hybrid in syntopic *Cicadetta petryi* and *Cicadetta sibillae* populations from Brallo di Pregola (Apenninian Lombardy). (B) *Cicadetta sibillae* emitting (several times) an unusual long echeme, again in a population syntopically with *Cicadetta petryi* at Monte Barro (Lecco, Lombardy). (C) Schematic illustration of the evolutionary steps theoretically necessary to switch from the basic *brevipennis* song group pattern (left) to a basic *cerdaniensis* song group pattern (right).

Sueur (2006) postulated to make use of as many species-specific acoustic markers as possible, as long as it can be assumed that they are hereditary. We profit from the remarkable complexity of the songs and rayed them in a way which has probably never been done before for taxonomic purposes in cicadas. Thus, we partly compensated the lack of well-developed nuclear markers suitable for recent cladogenic events (Owen *et al.*, 2015). Songs are inherited and nuclear-encoded, and they can serve as predictors of the most parsimonious evolution as well as indicators of recent hybridisation (Drosopoulos *et al.*, 2006; Marshall *et al.*, 2011).

Classically, taxonomic studies on cicadas dealing with acoustics investigate the time and carrier frequency domains (e.g. Quartau & Simões, 2005; Puissant & Sueur, 2010; Gogala *et al.*, 2011). We enlarged the number of variables measuring 'relative power differences'. With this new approach, we could demonstrate: 1) the astonishing complexity of power modulations in *C. anapaistica* (Chapter 1 = Hertach, 2011; e.g. **Fig 1.3**), 2) natural selection towards stronger power levels, useful for communication over long distances (*C. brevipennis hippolaidica* ssp. n.; Chapter 4; **Appendix S4.4**), and 3) found an indicative trait for the distinction of *C. petryi* and *C. brevipennis* (power succession of a long lasting echeme; Chapter 4; **Figs 4.2 and 4.5A-C**). Thus, power levels are as fine-tuned as variables from the time domain. They probably contain information which is part of the specific mate recognition system (SMRS; Paterson, 1985). Independently from Popple (2013), we implemented the number of syllables forming an echeme to the cicada taxonomy and demonstrated this character to be species-specific (*C. cerdaniensis* versus *C. sibillae*; Chapter 2 = Hertach *et al.*, 2015; **Fig 2.8B**). Moreover, we disclosed that some time variables are temperature-dependent in the way that species' identifications from recordings without measured perch temperatures are very uncertain. In the present case, *Cicadetta cerdaniensis* clearly sings faster than *C. sibillae*, but recordings of the first taken at 20 °C and of the second taken at 30 °C are almost identical (Chapter 2 = Hertach *et al.*, 2015; **Fig 2.8**). In many other examples the ambient temperature is not that important, neither for temporal parameters, nor for the carrier frequencies. This robustness makes sense with respect to the SMRS (e.g. *Tibicina*, *C. brevipennis* group).

One Swiss-Italian species (*C. montana* s. str.) evolved independently from the rest of the species for a longer period and causes no systematic challenges (Chapter 5). However, single sources of data are in several other cases ambiguous or misleading. We were able to understand the *cerdaniensis* and the *brevipennis* song groups only step-by-step (**Fig DI.3**). At the beginning, we inferred contamination during the molecular analyses or mixing up of specimens, when interpreting the mtDNA results. However, we could rule out artefacts by resequencing and adding many new specimens. These taxa are morphologically, at first glance, indistinguishable, characterised by two clearly different basic song patterns and three well

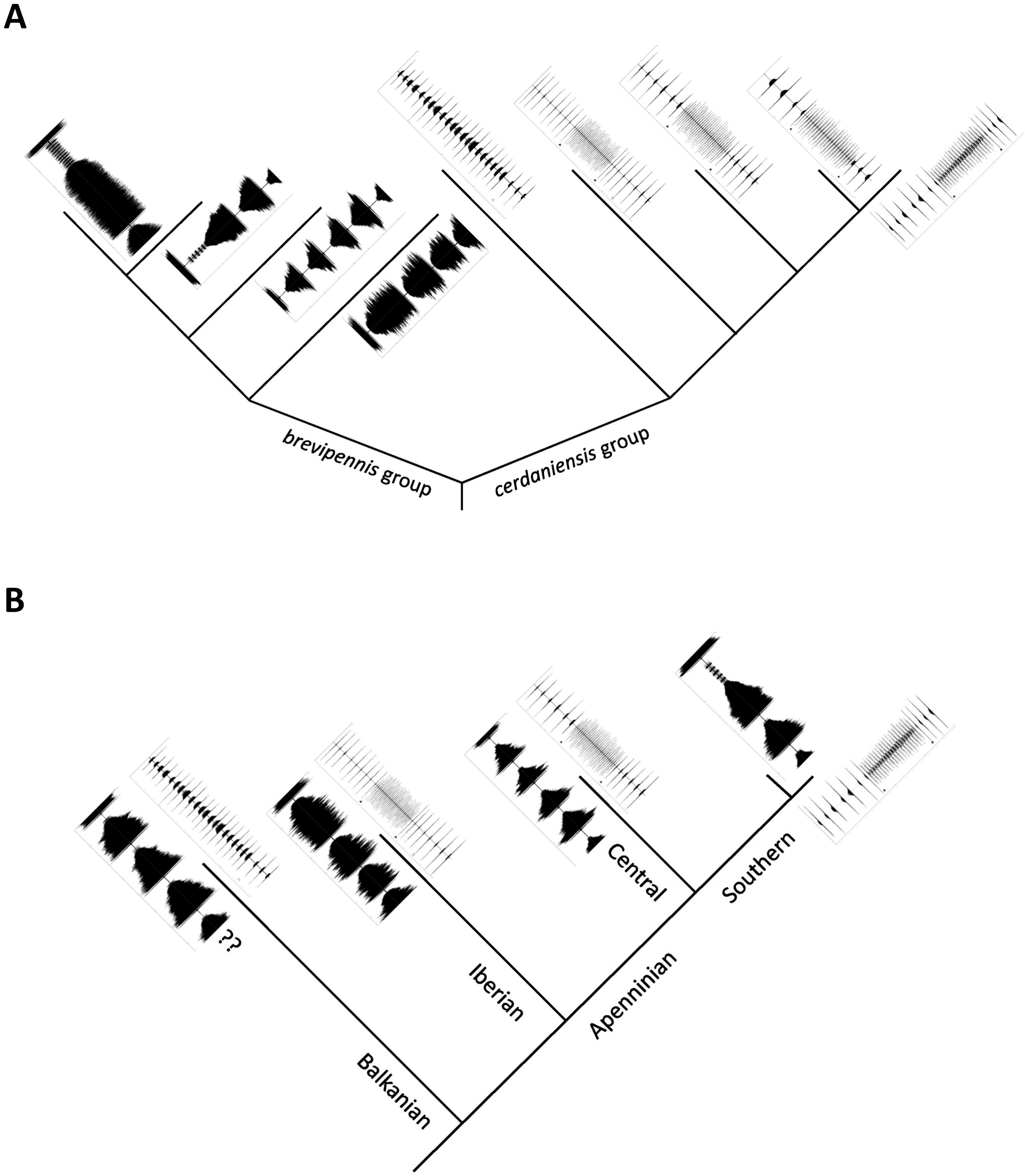


Fig DI.3. Genealogy of *Cicadetta cerdaniensis* and *Cicadetta brevipennis* song groups demonstrated with oscillograms of the calling songs. (A) Expected genealogy including the supposition that complex song patterns are generally more derived, (B) resulting genealogy from mtDNA with geographical structure.

supported, but interdigitated mtDNA clades. Acoustic song patterns are similar in some taxa with importantly different haplotypes, but clearly different in some taxa with closely related or identical haplotypes (**Fig 4.9**). A colouration character supports the molecular differences and opposes the acoustics. Only by making use of an integrative approach we were able to find surprising results: Mitochondrial molecular clades are most probably diagnostic for refugia and as a result of inter-group introgression not for species (compare Nicholls *et al.*, 2012). Moreover, the duration of the species isolation was obviously so long, that taxa speciated in these refugia to several species. Therefore, we have a paradox: Introgression helped find cryptic species in both song groups.

Finally, comprehensive morphological (276 males, 96 females) and morphometric investigations (273 males) on eleven taxa demonstrated three things (Hertach & Baur, in prep.): 1) Many species remained undistinguishable from others with a 100% certainty. However, qualitative characters often expose a taxa-typical appearance and combination of best characters (**Fig DI.4**). Some species are more variable (e.g. *C. cantilatrix*) and therefore cause serious problems when trying to establish a dichotomous key, others have outliers making difficulties (**Fig DI.5**). These outliers are probably more a result from missing selection pressure promoting morphological stasis (Bickford *et al.*, 2007) than from sporadic hybridisation. Songs are so important for mating that the morphological appearance is of minor significance in the specific mate recognition system (SMRS; Paterson, 1985). 2) **Fig DI.4** (last two rows) and **Fig DI.5** demonstrate the amazing fact that inter-group taxa in the *brevipennis* and *cerdaniensis* groups, for which we assume common Pleistocene refuges, have strikingly similar rates of character appearances: *C. petryi* and *C. cerdaniensis, C. b. brevipennis* and *C. sibillae* as well as *C. b. hippolaidica* ssp. n. and *C. a. lucana*. They do not only share mtDNA but also morphologic properties, which again supports our hypothesis of sporadic hybridisation between these taxa. 3) One song pair of closely related sister taxa (*C. cerdaniensis* contra *C. sibillae*) has characteristic (inherited) ratios in the fore wing and can be distinguished to about 85% in our morphometric study (**Fig DI.6**). Thus, these shapes establish additional traits supporting the taxonomic decisions that the mentioned taxa are valid species. Contrary, morphometrics do not help separate *C. b. brevipennis* from *C. petryi*.

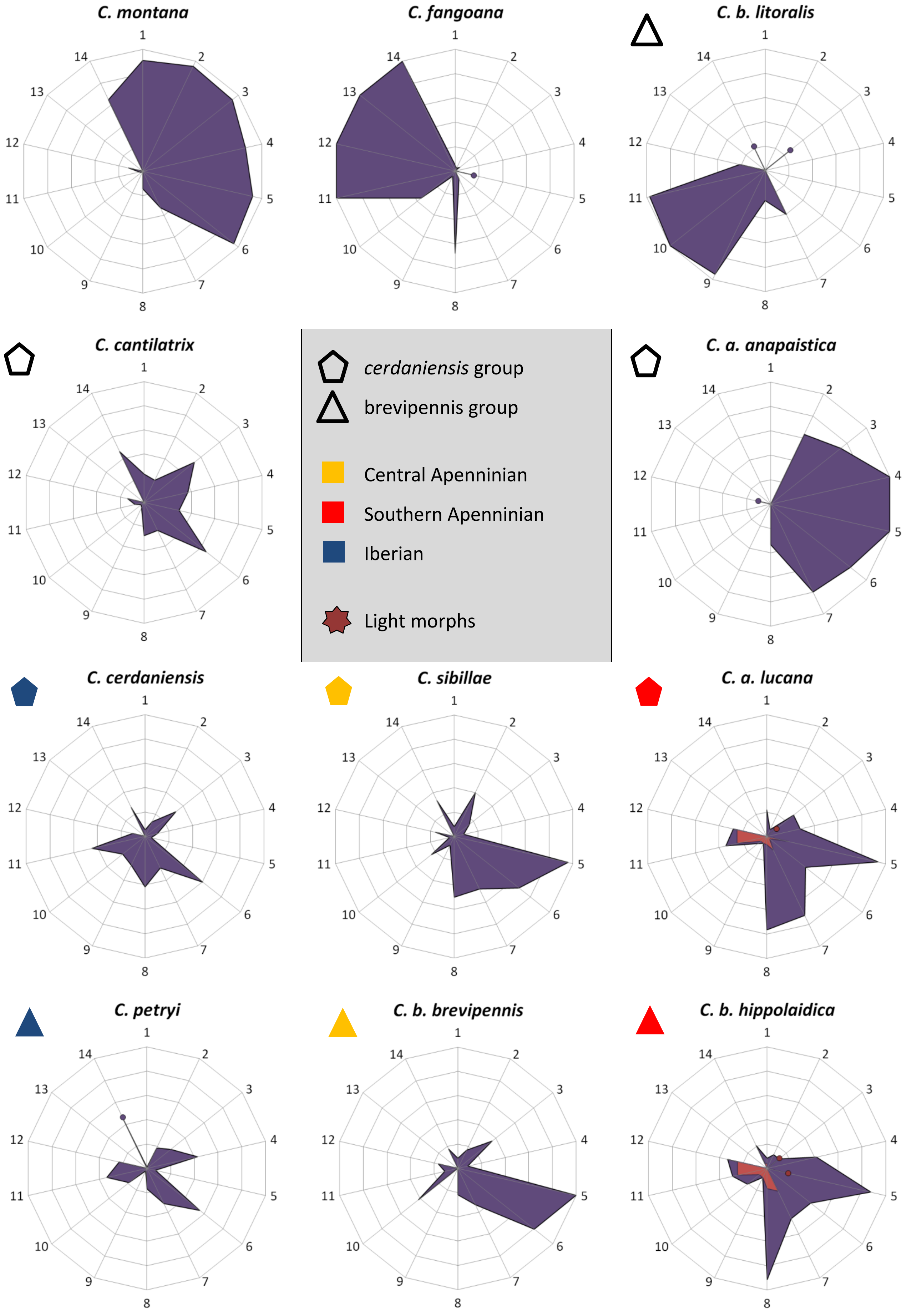
Evolution: Look similarly, sing differently
(Question 4)

Song patterns often evolve faster than morphology (Marshall *et al.*, 2011). This is certainly true for the cryptic *C. montana* species. As opposed to this, the genus *Tibicina* is characterised by monotonous long lasting songs, which are very similar. Swiss *Tibicina* species speciated latest in the Pliocene with more than 8% mtDNA differences (see Brower, 1994; Papadopoulou *et al.*, 2010; Genbank accession numbers

AY997107 – AY997125) and are older than the *C. montana* radiation. Sueur *et al.* (2007) infer a rapid radiation of the genus and hypothesise that this event was responsible for the low divergence in the acoustic communication. However, in this time frame the species developed clearly different colourations. Similar songs, but different colourations, are the exception rather than the rule in closely related cicadas (but see for example also the Australian genus *Tamasa*) and supposedly the result of an unusual selection pressure.

The *Cicadetta montana* complex species are a good example that selection took place in the song patterns and not in morphology. Song apparatus and neurological systems obviously provide a high plasticity for the evolution of very different and highly complex song patterns (**Fig 3.1**). The most diverse cicada complexes known worldwide from Australia (*Pauropsalta annulata* Goding & Froggatt, 1904; Popple, 2013) and New Zealand (*Kikihia* Dugdale, 1972; Marshall *et al.*, 2011) are probably slightly richer in species but less variable in song patterns. A particular nice example of song evolution is the *C. cerdaniensis* song group. The patterns within this group show stepwise increases in complexity from *C. cantilatrix* to *C. anapaistica anapaistica* (Chapter 2 = Hertach *et al.*, 2015; see **Fig 2.6**). None of the elements of this song evolution seem to have been lost until the present. Another interesting, newly discovered, case is the ability that song elements can evolve to different functions. *Cicadetta sibillae* and *C. cerdaniensis* emit a phrase in the calling song that is known only as part of the courtship behaviour in *C. cantilatrix* (Chapter 2 = Hertach *et al.*, 2015). The same development was observed for the subspecies *C. brevipennis hippolaidica* ssp. n. and *C. b. litoralis* ssp. n. in contrast to *C. b. brevipennis* and *C. petryi* (Chapter 4; **Fig 4.1**).

Fig DI.4. Qualitative morphological differences in *Cicadetta montana* complex taxa. See next page. Spider graph of 14 distinct characters of males (matching percentage coloured): 1 = fore wing: basal part of radial/subcostal vein and costal vein: outer rim of costa not darkest, 2 = fore wing: median vein dark brown to black, 3 = hind wing: apical part of cubitus anterior vein not lighter than neighbouring part of median vein, 4 = central dark patch of metanotum almost as broad as hind arms of cruciform elevation or even broader, 5 = fore wing: basal junction of anal veins predominantly dark, 6 = fore wing: basal cell apical dark surrounded, 7 = lateral shape of pronotal collar in dorsal view: frontal broad and rounded and towards the lateral angles smaller, 8 = more than 25 short, light and more or less fitted hairs between the scutal depressions, 9 = postclypeus with yellowish spot on the top of the groove, 10 = hind wing: cubitus anterior veins very light, 11 = lateral depressions of cruciform elevation light, 12 = scutum not completely black, 13 = postclypeus with ventrally (and laterally) broad reddish margin, 14 = upper lobes of pygofer pointed or pronounced thumb-like. Portions of light morphs in *C. a. lucana* and *C. b. hippolaidica* ssp. n. coloured differently.


C. montana
C. fangoana
C. b. litoralis
C. cantilatrix
cerdaniensis group
brevipennis group
Central Apenninian
Southern Apenninian
Iberian
Light morphs
C. a. anapaistica
C. cerdaniensis
C. sibillae
C. a. lucana
C. petryi
C. b. brevipennis
C. b. hippolaidica
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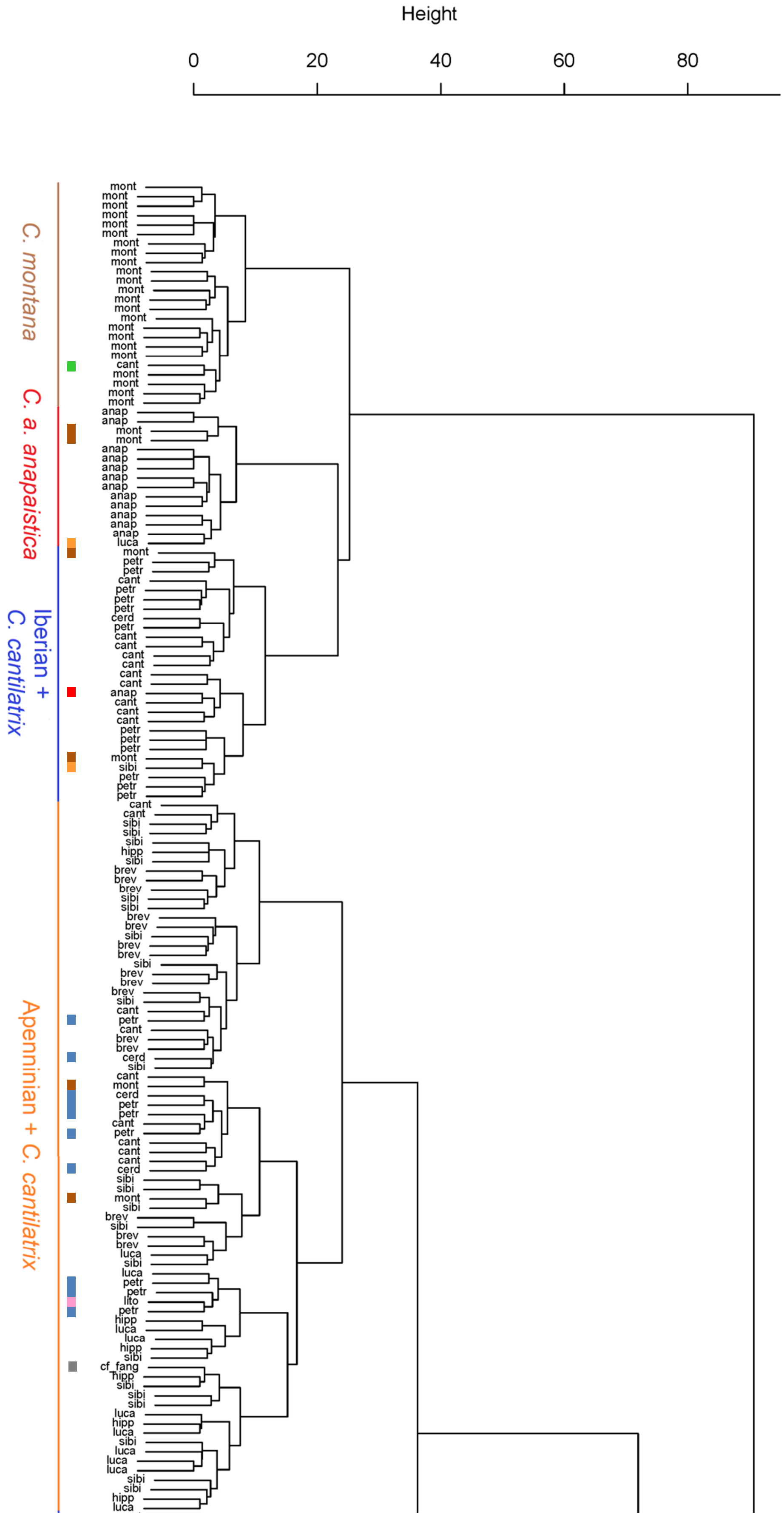

Fig DI.5. Dendrogram of the individual morphology of the *Cicadetta montana* complex males' from a cluster analysis. See next page.

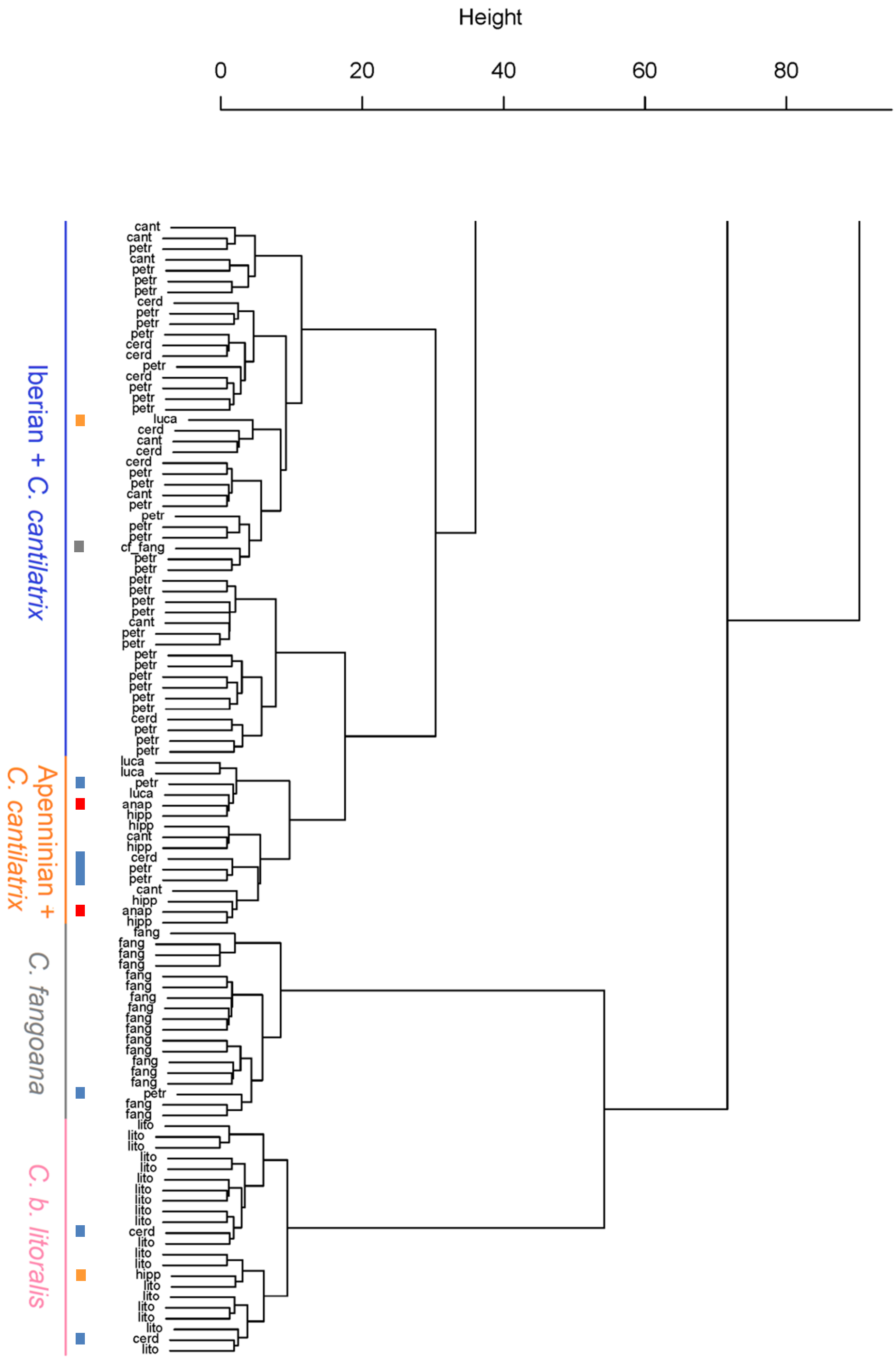

Fig DI.5. Dendrogram of the individual morphology of the *Cicadetta montana* complex males' from a cluster analysis. Continued. Hierarchical clustering of 16 qualitative morphological characters calculated under Euclidean distances in R. Character states are true (value 3), intermediate/ambiguous (2) and false (1). Outlying specimens not fitting named clusters marked with coloured small squares. anap = *Cicadetta a. anapaistica*, brev = *Cicadetta b. brevipennis*, cant = *Cicadetta cantilatrix*, cerd = *Cicadetta cerdaniensis*, fang = *Cicadetta fangoana*, hipp = *Cicadetta b. hippolaidica* ssp. n., lito = *Cicadetta b. litoralis* ssp. n., luca = *Cicadetta a. lucana*, mont = *Cicadetta montana* s. str., petr = *Cicadetta petryi*, sibi = *Cicadetta sibillae*.

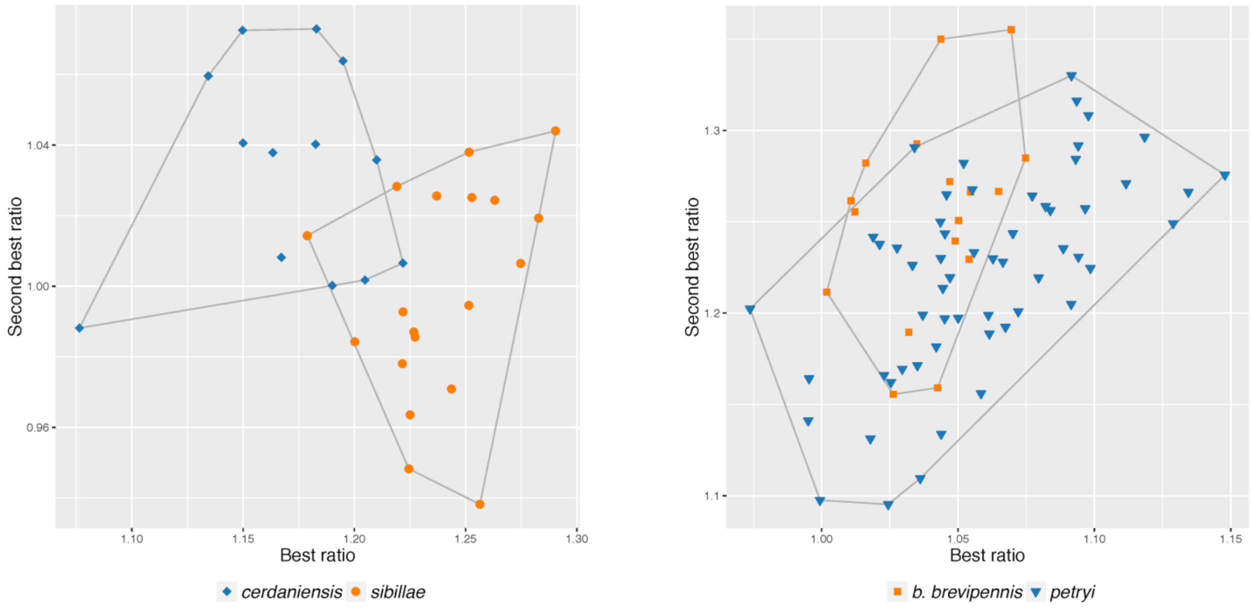

Fig DI.6. Morphometry of acoustic sister taxa. Best ratios selected by the LDA ratio extractor (sensu Baur & Leuenberger, 2011) using 15 landmarks of the left fore wing for *Cicadetta cerdaniensis*/*Cicadetta sibillae* (left) and *Cicadetta petryi*/*Cicadetta brevipennis brevipennis* (right).

Interestingly, the time and power, but not the frequency domains, contain important species-specific information in our *Cicadetta montana* model group (for example in contrast to the genus *Tibicina;* Sueur & Aubin, 2003). This does not mean that the highly variable carrier frequencies are without importance for conspecific females. Males singing at lower frequencies are expected to have a larger body size (Bennet-Clark & Young, 1994) and perhaps higher fitness than smaller individuals. This would make them more attractive for females. However, in choruses consisting of many different cicada species, the frequency domains of cicadas singing at lower frequencies overlap more relevantly with those of larger species and are, therefore, more difficult to cull for a female. This way, cicadas singing at higher frequencies would be selected against low-frequency-singers.

Songs of low complexity have to be very stable, i.e. hardly vary, to be recognised as conspecific (e.g. *Cicadetta montana* s. str.; **Fig 5.1**). High complexity allows higher intraspecific variability. *Cicadetta anapaistica* songs are variable among males in a way that many of them within a population can supposedly be recognised individually (Chapter 1 = Hertach, 2011; **Fig 1.5**). This species would be a good model to investigate individual preferences in acoustic female attraction-systems; this has scarcely been studied in insects (Seabra *et al.*, 2008). The most exciting question in this context is the following one: Are complex song patterns selected disproportionately as a more attractive phenotype (Bailey, 2006)?

The song evolution between the *cerdaniensis* and the *brevipennis* groups remains an unresolved issue. There are many reasons that the two taxa are sister groups. However, introgression affects our system so thoroughly that we do not know which haplotypes are more original for the one or the other song group (**Fig 4.9**). The basic songs are clearly different, but both of them contain dual patterns. Theoretically, three evolutionary steps of song adaptations are necessary for the basic *cerdaniensis* pattern (*C. cantilatrix*) to emerge from the basic *brevipennis* pattern (e.g. *C. brevipennis brevipennis*; **Fig DI.2C**): 1) The long echemes must be shortened. 2) The power of the long echemes must be reduced. 3) The breaks between the long- and short echemes must be omitted. Interestingly, the Korean *Cicadetta abscondita* Lee, 2008 song pattern incorporates elements of both the *cerdaniensis* and the *brevipennis* song structure (Lee, 2008).

The intraspecific variability of morphology is high and overlapping among species (**Figs DI.4-DI.6**). A supposed threshold of minimally two million years of reproductive isolation in *C. montana* s. str. (Chapter 3 = Wade *et al.*, 2015; see Brower, 1994; Papadopoulou *et al.*, 2010) was not enough to evolve one morphological or morphometric character valid for the correct separation of all specimens from other species within the complex. Closely restricted endemics (*C. fangoana*, *C. brevipennis litoralis* ssp. n., light morphs of *C. anapaistica lucana* and *C. brevipennis hippolaidica* ssp. n.; **Figs 2.4B and G and 4.8H**) are among the most aberrant taxa. These mutations were possibly favoured by drift in small founder populations after migration (*C. b. litoralis* ssp. n. and *C. fangoana*; see Grant & Grant, 1995; Podnar *et al.*, 2005) or dramatically reduced population sizes in refugia after climatic shifts (*C. a. lucana* and *C. b. hippolaidica* ssp. n.; see Taberlet *et al.*, 1998; Hewitt, 1999). Large populations conserved high intraspecific morphological variability (e.g. *C. cantilatrix*).

Biogeography: The importance of Pleistocene glacial refugia
(Question 5)

Biogeographic models were very helpful to understand the complicated taxonomy of the Italian and Swiss *C. montana* complex species. The refugia theory in southern isolates (see e.g. Hewitt, 1999; Taberlet *et al.*, 1998) was the key to come up with a scenario for the speciation of the *cerdaniensis* and *brevipennis* groups (**Figs 4.9 and 4.11**). The parallel speciation of these taxa is unique, as it probably took place nearly synchronously in four different regions during the Pleistocene glacial cycles. Our scenario explaining the *brevipennis-cerdaniensis* group pattern (Chapter 4) contains many elements published in literature (Chan & Levin, 2005; Melo-Ferreira *et al.*, 2005; Berthier *et al.*, 2006; Nicholls *et al.*, 2012). However, we demonstrate it in a combination which is probably new and of interest not only for cicadologists but for all biologists dealing with taxonomy, phylogeny and biogeography.

The above described scenario is an attempted explanation, with several independently fitting traits, but not a proof. However, we would like to elaborate a few additional aspects in the following, which were not presented in Chapter 4, but are nevertheless interesting contributions and partially suitable for further investigations:

1) Southern richness versus northern purity (Hewitt, 1999): The diversity (species, subspecies, haplotypes) in the proximity of supposed refugia is higher than in more northern countries. This is essentially true for the Apennine Mountains in comparison to central Europe (Chapter 2 = Hertach *et al.*, 2015; Chapter 4; **Fig 4.10**). Hewitt explains this phenomenon mainly with shorter migration distances linked to climatic oscillations. Southern lineages moved up and down altitudinal ranges of mountain chains and diverged, while northern lineages often went extinct with repeatedly decreasing temperatures.

2) Long common evolution time frame with a further relative: It is obvious for bio-acousticians that the next relative of *C. petryi* is *C. brevipennis* and not *C. cerdaniensis*. However, the mtDNA (**Figs 4.9 and DI.3**) and, even more surprising, the morphology suggest the opposite (**Figs DI.4 and DI.5**). We explain this phenomenon with the presumption that speciation in common inter-group glacial refugia with sporadic hybridisation was shaping the morphology and mtDNA more relevantly (and more recently) than the time before (and the very short time afterwards). Both the *cerdaniensis* and *brevipennis* group ancestors were possibly very young taxa when they became isolated in Pleistocene refugia. Furthermore, these taxa were obviously capable of rapid adaptive radiation. In many other species, Pleistocene isolation created intraspecific divergence only (e.g. Taberlet *et al.*, 1998; Hewitt, 1999; Lecocq *et al.*, 2013).

3) Interglacials: The purity of the mtDNA geographical structure is perhaps one of the most surprising facts revealed (compare Nicholls *et al.*, 2012; **Fig 4.9**). Iberian, Apenninian and Balkan clades are separated in a manner suggesting that no gene flow took place among them for at least 1 million years (≈ 2.5% uncorrected genetic distances). However, this time frame contains four interglacials. During these warmer periods lineages migrated northwards and Iberian, Apenninian and Balkan taxa could have met (compare **Fig 4.11**). Here, we present four hypotheses explaining the lack of such signs of contacts in our data:
3.1) Northern hybridised lineages became extinct in the beginning of the next colder period (see Hewitt, 1999). 3.2) Inter-group taxa hindered the migration through competitive exclusion, and intra-group contacts were as a result very limited. One demonstrative case is that *C. petryi* (or its ancestor) is likely to have obstructed the spread of *C. cerdaniensis* and *C. sibillae* (or its ancestors) to the lowlands of southern France in the Holocene but eventually also in the interglacials (compare **Fig 4.10**). The latter two species remained geographically separated (by long distances) and their similar song patterns did not have an opportunity to intermix. *Cicadetta sibillae* acts, at least currently, as separator of *C. petryi* and *C. b.*

brevipennis in the northern Apennine. Its presence is expected to support the conservation of stable song patterns in the *brevipennis* group. 3.3) The gene flow is not reflected in the mtDNA but may be found in other molecular markers. 3.4) The purity is a result of undersampling.

4) 'Holocene purity': The purity of the mtDNA geographical structure is also surprising with respect to the postglacial migrations in the Holocene (see Chapter 4; **Fig 4.11**). An illustrative example is provided in the following: Apenninian *C. sibillae* obviously exchanged mtDNA with *C. brevipennis brevipennis* on a relevant scale during ice ages (and maybe later) since all specimens investigated are introgressed. Contrary, *C. sibillae* and Iberian *C. petryi* meeting postglacially in the southern Alps and the northern Apennine hold their refugia-typical mtDNA. It is likely that this purity is a result of undersampling. The few acoustic intermediates between the *cerdaniensis* and the *brevipennis* song groups (**Fig DI.2**) were observed here. Therefore, subsequent researchers should not be taken by surprise if they find specimens singing like *C. sibillae*, but displaying haplotypes more typical for Iberia, near populations of *C. petryi*. This should not affect the taxonomic decisions, but rather support the described scenario.

5) 'Hubbs principle' (Hubbs, 1955): When migrating individuals meet an established population of a closely related species, the invading species is in a minority. This provokes heterospecific mating (see also Berthier *et al.*, 2006). Hubbs' principle could explain why invading *C. sibillae* obviously influenced postglacially the genome and song of *C. anapaistica lucana* (Chapter 2 = Hertach *et al.*, 2015; **Figs 2.3 and 2.10**). In case the *C. brevipennis* s. l. population near the Black Sea (= operational taxonomic unit 'bulg'; Chapter 4) turns out to form a peripheral *C. b. brevipennis* metapopulation possessing the mitochondrial genome of *C. cantilatrix*, Hubbs' principle can again serve as a possible explanation: According to this theory and our scenario *C. cantilatrix* established populations in the central Balkans long before *C. brevipennis brevipennis* arrived from the Apenninian refuge.

6) Sympatric inter-group occurrences: From data of the Apenninian peninsula we know that *cerdaniensis* and *brevipennis* song groups are strictly parapatric and competitively exclude each other (Chapter 4). However, the groups can coexist far away from potential glacial refugia and they always have a different geographical origin (**Fig 4.11**). The formation of sympatric occurrence was obviously possible when both species met after long-distance postglacial migrations (compare Hewitt, 1999).

The ten autochthonous Swiss species probably have very different biogeographic histories. We can infer from the climatic and habitat requirements that all of them colonised or re-colonised Switzerland in the Late Pleistocene or Holocene after the glaciers started retreating. We can speculate about their origin: *Tibicina quadrisignata* and *C. petryi* probably immigrated from the south-west (Iberian peninsula), *C.*

cantilatrix and supposedly *Tibicina steveni* from the Balkans, and *C. sibillae* from the south (Italy). For all other species Pleistocene refugia are currently not apparent (e.g. *Lyristes*, *Tettigettalna*); or we know about different refugia (*Cicada orni*; Pinto-Juma *et al.*, 2009) but not about the genotypes colonizing Switzerland.

Tibicina steveni and *T. quadrisignata*, both completely isolated in the Valais, appear as relicts of a postglacial warmer period (compare Delarze, 1987). Typically Mediterranean species such as *Lyristes plebejus*, *Tibicina haematodes*, *Tettigettalna argentata*, *C. petryi* and *C. sibillae* conquered only climatically most advantageous locations of Switzerland resulting in very limited distributions along the country borders.

Ecology and threat: Towards Red Lists
(Questions 6 and 7)

European cicadas are thermophilous insects. They prefer dry (e.g. *T. quadrisignata*) or mesophilous (e.g. *C. sibillae* in the south) or, as an exception, periodically wet habitats (*C. brevipennis litoralis* ssp. n., Chapter 4; **Fig 4.7E**). Cicadas in Italy and Switzerland, as well as in many other European countries, occur predominantly in ecotone and woodland habitats. They inhabit the entire range of habitats, from dry meadows with a few scattered bushes (e.g. *C. petryi*) to tall woods, which almost completely lack herb layers (e.g. some habitats of *C. orni*). The habitat requirements often differ importantly among regions for single species (e.g. Hertach, 2007). *Cicadetta sibillae* was found in developed beech forest in the southern Apennine, and mainly in sparse oak woods or ecotone habitats in the northern Apennine. Species are generally more ubiquitous in the core of the distribution area and stenoecious at the periphery. A particular illustrative example supporting this theory is *C. petryi* when the highly diverse and unspecific southern French habitats are compared to the Central European ones (Chapter 4). Preferences for certain bushes or trees (e.g. for *Quercus* or *Pinus* species) are scarcely linked to specific feeding requirements, but are formed owing to the similar climatic conditions favoured by plants and cicadas. Closely related *C. montana* complex species prefer different vertical niches in the vegetation structure: *Cicadetta montana* s. str. tend to sing in the treetop or in the crown, *cerdaniensis* group species in a medium range of smaller trees and bushes and *C. petryi* on low bushes or in the herb layer (e.g. Hertach, 2007). Very few species are independent from ligneous plants and can be regarded as grassland species (e.g. *Cicadetta mediterranea*).

Swiss cicadas live in an altitudinal range from 220 to 2010 m asl, Italian ones from sea level to 1960 m asl. No Italian species exclusively occurs in the lowlands, for example *Cicadetta mediterranea* ascends maximally at 830 m asl, *Cicadivetta tibialis* at 1220 m asl and *Tibicina haematodes* at 870 m asl (pers. data). A medium segment between 200 to 800 m asl is thought to have the highest diversity on the Apenninian peninsula.

The dataset presented for Switzerland in Chapter 6 (= Hertach & Nagel, 2013; **Figs 6.3-6.12**) was continually amended with additional field work in the years 2013 to 2015 (examples **Fig DI.7**). A random sample was defined for the cantons which had not been studied systematically before (Hertach, 2015). In the Valais, the chorology of the two *Tibicina* species has been verified more in detail (**Fig DI.1**). The data set is now so good that Red List assessment in collaboration with the 'Swiss Central Fauna Data Bank' (CSCF) is carried out according to the 'International Union for Conservation of Nature' criteria B2, C and D (IUCN, 2012; Cordillot & Klaus, 2011). Combining inferred or projected declines of populations, limited population sizes, severely fragmented distributions and the area of occupancy (AOO; modelled after Fivaz & Gonseth, 2014) resulted in the categories presented in **Table DI.1**.

Eight of the ten Swiss cicada species are part of the Red List. Only *C. orni* and *C. montana* s. str. (see **Fig DI.7A**) are recognised as 'not endangered' species. The first is partly living in artificial habitats and profiting from the climate change, the second is still widely distributed in the Alps and the Jura Mountains. *Cicadetta cantilatrix* and *L. plebejus* are 'vulnerable', the first because of split distribution (see **Fig DI.7C**) and threatened habitats, the second because of its very limited population size. All other species are 'endangered', and again fragmentation of populations (*C. petryi*, *T. argentata*), habitat loss (*T. quadrisignata*, *T. steveni*, *C. petryi*, *C. sibillae*) and population sizes (all three *Tibicina* species, *C. sibillae*, *T. argentata*) are the decisive criteria (see **Fig DI.7B and D**).

IUCN criteria adapted to national Red Lists have the disadvantage that they judge the regional extinction risk, the risk that a species will disappear from a country. However, this is not necessarily equivalent to a need for conservation action or for the stipulation of national conservation priorities (Keller & Bollmann, 2004). Swiss cicadas are particularly suitable to illustrate these deficits. Very rare species like *L. plebejus* and *T. haematodes* are widespread in Southern Europe and probably already had a very limited distribution in Switzerland (compare **Figs 6.4 and 6.7**), before mankind impacted their habitats negatively. We will react to these deficits with a list of national conservation concern or priority species (see BAFU, 2011). Herein *T. quadrisignata* and *T. steveni* will receive highest conservation concern for their protection.

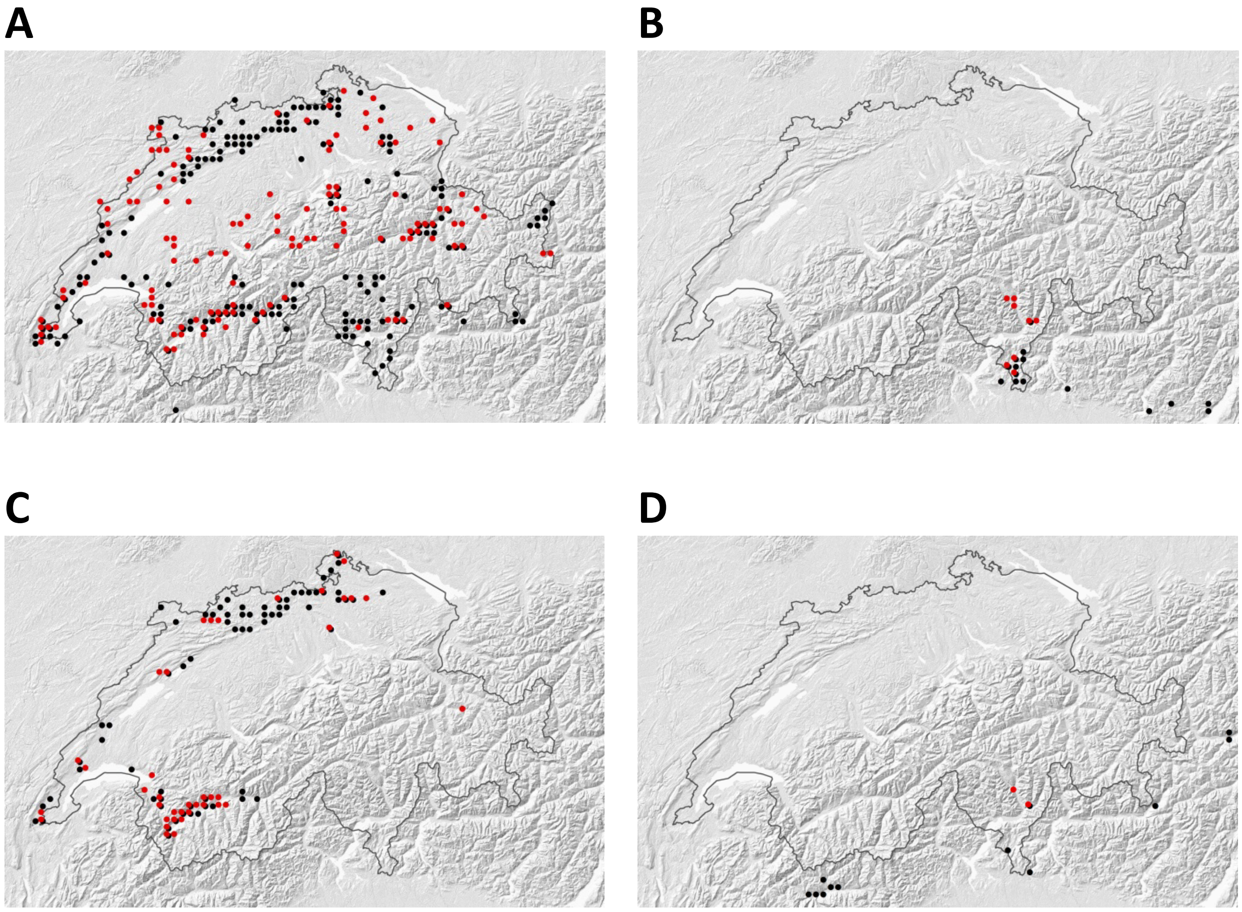

Fig DI.7. Complemented Swiss cicada distributions. The four species with the most important increase in the known populations between Chapter 6 (= Hertach & Nagel, 2013; black dots) and at the end of 2015 (additional red dots, slightly shifted for better visibility). (A) *Cicadetta montana* s. str., (B) *Cicadetta sibillae*, (C) *Cicadetta cantilatrix*, (D) *Tettigettalna argentata*.

Table DI.1. Red List assessment for Swiss cicadas according to the IUCN criteria (Hertach, in prep.) LC = least concern, VU = vulnerable, EN = endangered.

Species	Decisive criteria	Red List category
Cicada orni		LC
Lyristes plebejus	D	VU
Tibicina quadrisignata	C2a(i)	EN
Tibicina steveni	C2a(i)	EN
Tibicina haematodes	D	EN
Cicadetta montana		LC
Cicadetta cantilatrix	B2ab(iii)	VU
Cicadetta sibillae	C2a(i)	EN
Cicadetta petryi	B2ab(iii)	EN
Tettigettalna argentata	B2ab(iii) / C2a(i)	EN

The data collection in Italy was extensive and the data set is, as a result, finally comprehensive (almost 3,500 records). However, it is too soon for a Red List assessment. Additional taxonomic investigations need to be made for the genus *Tibicina*. Lower altitudinal ranges are underrepresented in the data set and few regions were not visited (e.g. the south of Sicily; compare **Fig IN.3**) or should be visited in a different season for data collection of late species (e.g. southern Alps). Here are some preliminary conclusions on the threat inferred from the travels between 2008 and 2013.

Italy has an excellent network of Apenninian mountain chains protected as Regional or National Parks. Although park authorities normally do not improve biodiversity directly – which is a pity – these parks guarantee large semi-natural conditions with important areas of forests. The anthropogenic impact on fragmentation in these mountain systems is currently pretty low. Transitions from dense forests to ecotone habitats (forest edges, abandoned or extensively used pastures) are represented in good quantities and qualities. If agriculture and forestry do not change dramatically, threat of species will be limited from this point of view. Cicadas generally suffer more from agriculture and settlements at lower altitudes. Species are particularly endangered when they prefer coastal habitats, as most of these are downgraded to small remains or completely destroyed for settlements and touristic infrastructures. Furthermore, grassland species appear more endangered than ecotone or woodland species.

Contrary, endemic taxa occur in higher altitudes and their spatial restrictions make them more prone to extinction (*C. anapaistica*, *C. brevipennis hippolaidica* ssp. n.; Chapter 1 = Hertach, 2011; Chapter 2 = Hertach *et al.*, 2015 and Chapter 4; see **Fig 4.10**). Mediterranean mountainous ecosystems are among the most vulnerable areas in the world towards climate change (Regato & Salman, 2008). Increasing temperatures and dryness could affect the populations by vegetation shift and changing species interactions (Chapter 1 = Hertach, 2011). Species of lower altitudes will be able to migrate to higher altitudes, i.e. to more suitable habitats. Mountainous cicada species currently do not inhabit mountain tops in Italy; they could still move up to these, at least theoretically, but fragmentation will increase and distribution areas decrease. However, in practice, higher altitudes are often grazed by cattle and it will be decisive whether the intensity of this utilisation allows or prevents the growth of woods (compare **Fig 2.10D**).

Potential levels of extinction threat are demonstrated in the following for four case species:

1) *Cicada orni* (**Fig DI.8A**): This famous species is very common throughout Italy and other Mediterranean countries. Its habitat requirements are unspecific, often it is a synanthrope. In the mountains and in northern parts of the country it is more limited by the climate. The species is not endangered in Italy.

2) *Cicadetta sibillae* (**Fig DI.8B**): Its distribution is well known and it is an abundant species, especially in the northern Apennine, occurring in medium altitudinal ranges (see Chapter 2 = Hertach *et al.*, 2015). However, the distribution area is fragmented and peripheral metapopulations in the Alps and in the central Apennine seem to be vulnerable. Anyway, more than 95% of the local populations known are situated in Italy and its responsibility for the conservation of *C. sibillae* is therefore very high. It is even higher for the two subspecies of *C. anapaistica*, completely endemic to southern Italy, as both have restricted distribution areas and endangered habitats (Chapter 1 = Hertach, 2011; Chapter 2 = Hertach *et al.*, 2015; **Fig 2.9** and front page of Part IV).

3) *Cicadetta mediterranea* (**Fig DI.8C**): This species does not belong to the *Cicadetta montana* complex and is in fact a species of a genus different to *Cicadetta*. It is restricted to coastal dunes and natural or semi-natural grassland habitats. *Cicadetta mediterranea* has been found very occasionally from Calabria to Emilia-Romagna. One would infer from this map a very low and patchy abundance and a high risk of regional extinction. This is probably true, but coastal habitats need to be investigated more systematically in order to get more evidence of the endangerment. We suspect that Italy has a high national responsibility for the conservation of this species.

4) *Tibicina quadrisignata* (**Fig DI.8D**): The species has a very limited distribution in Italy. Swiss and Italian populations have similar dimensions, a rather unusual situation for a typical Mediterranean cicada (compare **Figs 6.5 and DI.1**). Most important occurrences are reported from the Iberian peninsula and southern France (Sueur *et al.*, 2004; Puissant, 2006). Therefore, on a national scale *T. quadrisignata* is endangered, and the Italian populations contribute to the genetic diversity of this species. The populations are situated in two of the three driest valleys of the Italian Alps (Aosta and Susa); they occur in habitats similar to the Swiss Valais. Italian as well as Swiss populations are separated by high mountain chains from the other occurrences.

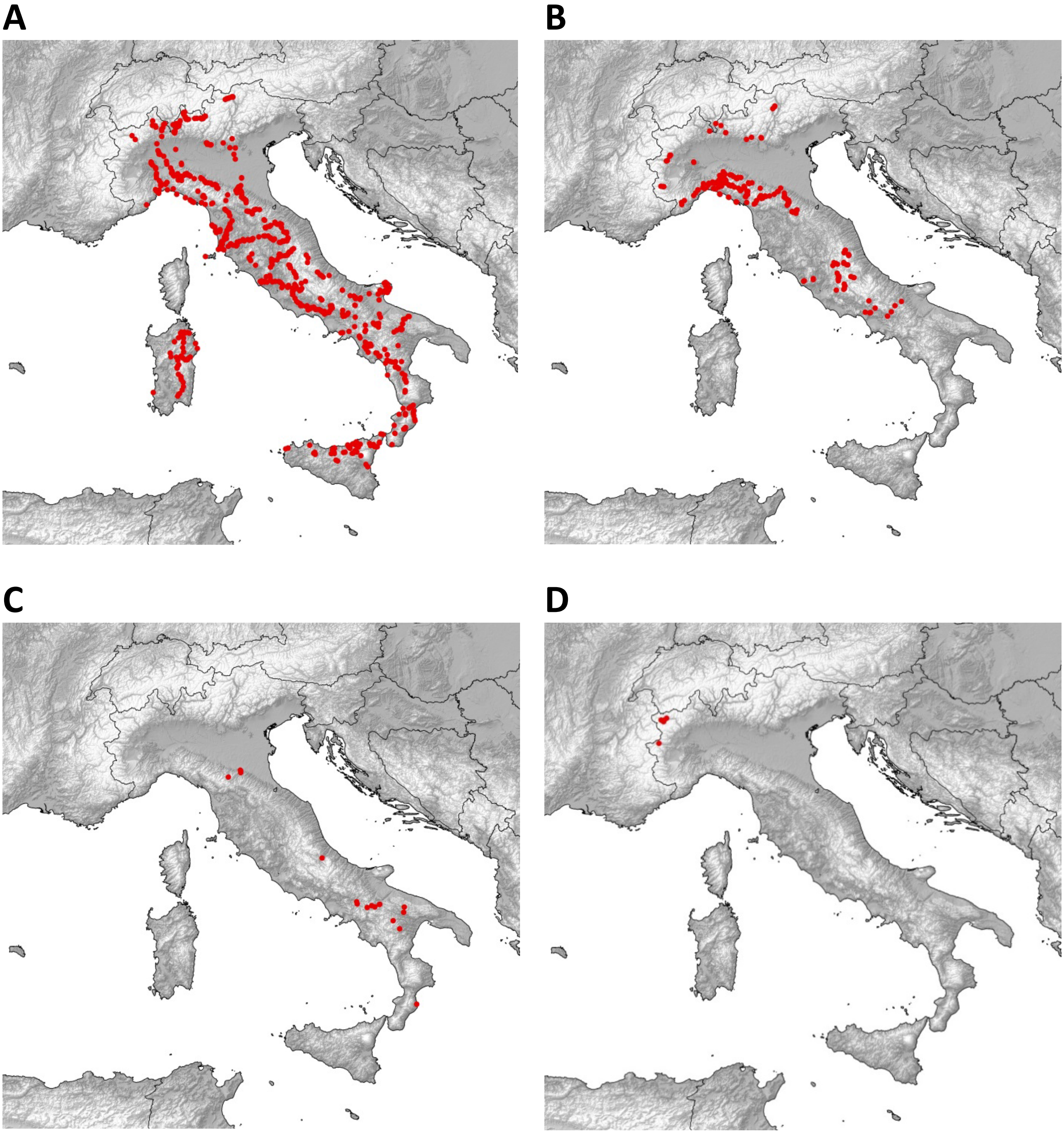

Fig DI.8. Locality data of four cicada species in Italy. (A) *Cicada orni*, (B) *Cicadetta sibillae*, (C) *Cicadetta mediterranea*, (D) *Tibicina quadrisignata*. 98% of the observations gathered during field work in the frame of this thesis.

Conclusions

This thesis importantly contributes to the knowledge of cicadas in Italy, Switzerland and the adjoining countries with respect to taxonomy, biodiversity, ecology and chorology. Two species and three subspecies are new to science. We demonstrate new methods for the investigation of song patterns. Integrative taxonomy and classic biogeography were the decisive research fields to understand the intricate speciation processes. The *cerdaniensis-brevipennis* group phylogenetic pattern nicely demonstrates the restrictions of species delimitation with single data sources or unified models. We found a plausible explanation – possibly assignable to other data sets not dealing with cicadas – why mitochondrial DNA is structured geographically and does not reflect species boundaries. In-depth analyses of acoustics enable us to infer interesting aspects of song evolution. The work is a solid basis for the conservation of Swiss and Italian cicadas on practical and political levels.

Last but not least, we should be amazed and pleased about the diversity and complexity of the song patterns which these insects have developed.

REFERENCES PART IV

Bailey WJ. 2006. Insect songs – The evolution of signal complexity. In: Drosopoulos S, Claridge M, eds. *Insect sounds and communication: Physiology, behaviour, ecology and evolution.* Boca Raton: CRC Press Taylor & Francis Group, 127-136.

Baur H, Leuenberger C. 2011. Analysis of ratios in multivariate morphometry. *Systematic Biology* 60(6): 813-825.

Bennet-Clark H, Young D. 1994. The scaling of song frequency in cicadas. *Journal of Experimental Biology* 191: 291-294.

Bennik RM, Buckley TR, Hoare RJB, Holwell GI. 2016. Molecular phylogeny reveals the repeated evolution of complex male genital traits in the New Zealand moth genus *Izatha* (Lepidoptera: Xyloryctidae). *Systematic Entomology* 41: 309-322.

Berthier P, Excoffier L, Ruedi M. 2006. Recurrent replacement of mtDNA and cryptic hybridization between two sibling bat species *Myotis myotis* and *Myotis blythii*. *Proceedings of the Royal Society B: Biological Sciences* 273: 3101-3109.

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution* 22: 148-155.

Boulard M. 1988. Les *Lyristes* d'Asie Mineur (Homoptera Cicadidae). I. Sur deux formes éthospécifiques syntopiques et description de deux espèces nouvelles. *L'Entomologiste* 44(3): 153-167.

Brower AVZ. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences USA* 91: 6491-6495.

Bundesamt für Umwelt. 2011. *Liste der national prioritären Arten. Arten mit nationaler Priorität für die Erhaltung und Förderung, Stand 2010*. Bern: Umwelt-Vollzug Nr. 1103, 132 pp.

Butlin RK, Galindo J, Grahame JW. 2008. Sympatric, parapatric or allopatric: the most important way to classify speciation? *Philosophical Transactions of the Royal Society B: Biological Sciences* 363: 2997-3007.

Carstens BC, Pelletier TA, Reid NM, Satler JD. 2013. How to fail at species delimitation. *Molecular Ecology* 22: 4369-4383.

Chan KMA, Levin SA. 2005. Leaky prezygotic isolation and porous genomes: rapid introgression of maternally inherited DNA. *Evolution* 59: 720-729.

Cordillot F, Klaus. 2011. *Gefährdete Arten in der Schweiz – Synthese Rote Listen, Stand 2010*. Bern: Bundesamt für Umwelt. Umwelt-Zustand Nr. 1120, 111 pp.

De Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* 56(6): 879-886.

Delarze R. 1987. L'origine des pelouses steppiques valaisannes à la lumière de leurs liens de parenté avec les régions limitrophes. *Bulletin de la Murithienne* 105: 41-70.

Drosopolos S, Eliopoulos E, Tsakalou P. 2006. Is migration responsible for the peculiar geographical distribution and speciation based on acoustic divergence of two cicadas in the Aegean archipelago? In: Drosopoulos S, Claridge M, eds. *Insect sounds and communication: Physiology, behaviour, ecology and evolution.* Boca Raton: CRC Press Taylor & Francis Group, 219-226.

Dugdale JS, Fleming CA. 1978. New Zealand cicadas of the genus *Maoricicada* (Homoptera: Tibicinidae). *New Zealand Journal of Zoology* 5(2): 295-340.

Fivaz FP, Gonseth Y. 2014. Using species distribution models for IUCN Red Lists of threatened species. *Journal of Insect Conservation* 18(3): 427-436.

Gogala M, Trilar T. 2004. Bioacoustic investigations and taxonomic considerations on the *Cicadetta montana* species complex (Homoptera: Cicadoidea: Tibicinidae). *Anais da Academia Brasileira de Ciências* 76(2): 316-324.

Gogala M, Trilar T, Drosopoulos S. 2011. Two new species and a new genus of Cicadettini (Hemiptera, Cicadidae) from the Greek island of Evia. *Deutsche Entomologische Zeitschrift* 58(1): 105-117.

Grant PR, Grant BR. 1995. The founding of a new population of Darwin's Finches. *Evolution* 49: 229-240.

Hertach T. 2007. Three species instead of only one: Distribution and ecology of the *Cicadetta montana* species complex (Hemiptera: Cicadoidea) in Switzerland. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 80: 37-61.

Hertach T. 2011. Spectacular song pattern from the Sicilian Mountains: The new cicada species *Cicadetta anapaistica* sp. n. (Hemiptera: Cicadidae). *Zootaxa* 2771: 25-40.

Hertach T. 2015. *Singzikaden (Cicadidae) in der Schweiz – Ergänzende Stichprobe als Basis einer Roten Liste.* Hedingen, unpublished, 16 pp.

Hertach T, Nagel P. 2013. Cicadas in Switzerland: a scientific overview of the historic and current knowledge of a popular taxon (Hemiptera: Cicadidae). *Revue suisse de Zoologie* 120(2): 229-269.

Hertach T, Trilar T, Wade EJ, Simon C, Nagel P. 2015. Songs, genetics, and morphology: revealing the taxonomic units in the European *Cicadetta cerdaniensis* cicada group, with a description of new taxa (Hemiptera: Cicadidae). *Zoological Journal of the Linnean Society* 173(2): 320-351.

Hewitt GM. 1999. Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* 68: 87-112.

Hey J, Pinho C. 2012. Population genetics and objectivity in species diagnosis. *Evolution* 66: 1413-1429.

Hubbs CL. 1955. Hybridization between fish species in nature. *Systematic Zoology* 4: 1-20.

IUCN. 2012. *IUCN Red List categories and criteria version 3.1.* Second edition. Gland, Switzerland and Cambridge, UK: IUCN. iv + 32pp.

Keller V, Bollmann K. 2004. From Red Lists to species of conservation concern. *Conservation Biology* 18(6): 1636-1644.

Lecocq T, Dellicour S, Michez D, Lhomme P, Vanderplanck M, Valterová I, Rasplus JY, Rasmont P. 2013. Scent of a break-up: phylogeography and reproductive trait divergences in the red-tailed bumblebee (*Bombus lapidarius*). *BMC Evolutionary Biology* 13, 263.

Lee YJ. 2008. Revised synonymic list of Cicadidae (Insecta: Hemiptera) from the Korean Peninsula, with the description of a new species and some taxonomic remarks. *Proceedings of the Biological Society of Washington* 121: 445-467.

Mallet J. 2005. Hybridization as an invasion of the genome. *Trends in Ecology & Evolution* 20(5): 229-237.

Mallet J. 2008. Hybridization, ecological races and the nature of species: empirical evidence for the ease of speciation. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 363: 2971-2986.

Marshall DC, Hill KBR, Cooley JR, Simon C. 2011. Hybridization, mitochondrial DNA phylogeography, and prediction of the early stages of reproductive isolation: Lessons from New Zealand cicadas (Genus *Kikihia*). *Systematic Biology* 60: 482-502.

Melo-Ferreira J, Boursot P, Suchentrunk F, Ferrand N, Alves PC. 2005. Invasion from the cold past: extensive introgression of mountain hare (*Lepus timidus*) mitochondrial DNA into three other hare species in northern Iberia. *Molecular Ecology* 14: 2459-2464.

Moore W, Robertson JA. 2014. Explosive adaptive radiation and extreme phenotypic diversity within ant-nest beetles. *Current biology* 24: 2435-2439.

Nicholls JA, Challis RJ, Mutun S, Stone GN. 2012. Mitochondrial barcodes are diagnostic of shared refugia but not species in hybridizing oak gallwasps. *Molecular Ecology* 21: 4051-4062.

Owen CL, Marshall DC, Hill KBR, Simon C. 2015. The phylogenetic utility of acetyltransferase (ARD1) and glutaminyl tRNA synthetase (QtRNA) for reconstructing Cenozoic relationships as exemplified by the large Australian cicada *Pauropsalta* generic complex. *Molecular Phylogenetics and Evolution* 83: 258-277.

Papadopoulou A, Anastasiou I, Vogler AP. 2010. Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. *Molecular Biology and Evolution* 27: 1659-1672.

Paterson HEH. 1985. The recognition concept of species. In: Vrba ES, ed. *Species and Speciation.* Pretoria: Transvaal Museum, Monograph No. 4, 21-29.

Pfenninger M, Schwenk K. 2007. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology* 7: 121.

Pillet JM. 1993. Les Cigales du Valais. *Bulletin de la Murithienne* 111: 95-113.

Pinto-Juma GA, Quartau JA, Bruford MW. 2009. Mitochondrial DNA variation and the evolutionary history of the Mediterranean species of *Cicada* L. (Hemiptera, Cicadoidea). *Zoological Journal of the Linnean Society* 155: 266-288.

Podnar M, Mayer W, Tvrtković N. 2005. Phylogeography of the Italian wall lizard, *Podarcis sicula*, as revealed by mitochondrial DNA sequences. *Molecular Ecology* 14: 575-588.

Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* 55: 595-609.

Popple LW. 2013. A revision of the *Pauropsalta annulata* Goding & Froggatt species group (Hemiptera: Cicadidae) based on morphology, calling songs and ecology, with investigations into calling song structure, molecular phylogenetic relationships and a case of hybridisation between two subspecies. *Zootaxa* 3730(1): 1-102.

Puissant S. 2006. *Contribution à la connaissance des cigales de France: Géonémie et écologie des populations (Hemiptera, Cicadidae).* Bédeilhac/Aynat: Association pour la Caractérisation et l'Etude des Entomocénoses, 193 pp.

Puissant S, Sueur J. 2010. A hotspot for Mediterranean cicadas (Insecta: Hemiptera: Cicadidae): new genera, species and songs from southern Spain. *Systematics and Biodiversity* 8(4): 555-574.

Quartau JA, Simões PC. 2005. *Cicada cretensis* sp. n. (Hemiptera, Cicadidae) from southern Greece. *Biologia, Bratislava* 60(5): 489-494.

Quartau JA, Simões PC. 2006. Acoustic evolutionary divergence in cicadas: the species of *Cicada* L. in Southern Europe. In: Drosopoulos S, Claridge M, eds. *Insect sounds and communication: Physiology, behaviour, ecology and evolution.* Boca Raton: CRC Press Taylor & Francis Group, 227-237.

Regato P, Salman R. 2008. *Mediterranean Mountains in a Changing World: Guidelines for developing action plans*. Malaga: IUCN Centre for Mediterranean Cooperation, xii + 88 pp.

Reid WV. 1992. How many species will there be? In: Whitmore TC, Sayer JA, eds. *Tropical deforestation and species extinction.* New York: Chapman and Hall, 55-73.

Schlick-Steiner B, Steiner FM, Seifert B, Stauffer C, Christian E, Corzier RH. 2010. Integrative taxonomy: A multisource approach to exploring biodiversity. *Annual Review of Entomology* 55: 421-438.

Seabra SG, Genage A, Quartau JA. 2008. Variation in the acoustic properties of the calling song of *Cicada barbara* and *C. orni* (Hemiptera: Cicadidae) at the individual and population levels. *Zoological Studies* 47(1): 1-10.

Sites JW, Marshall JC. 2004. Operational criteria for delimiting species. *Annual Review of Ecology, Evolution, and Systematics* 35: 199-227.

Solís-Lemus C, Knowles LL, Ané C. 2015. Bayesian species delimitation combining multiple genes and traits in a unified framework. *Evolution* 69: 492-507.

Sueur J. 2006. Insect species and their songs. In: Drosopoulos S, Claridge M, eds. *Insect sounds and communication: Physiology, behaviour, ecology and evolution.* Boca Raton: CRC Press Taylor & Francis Group, 207-217.

Sueur J, Aubin T. 2003. Specifity of cicada calling songs in the genus *Tibicina* (Hemiptera, Cicadidae). *Systematic Entomology* 28: 481-492.

Sueur J, Puissant S, Simões PC, Seabra SG, Boulard M, Quartau JA. 2004. Cicadas from Portugal: revised list of species with eco-ethological data (Hemiptera: Cicadidae). *Insect Systematics and Evolution* 35: 177-187.

Sueur J, Vanderpool D, Simon C, Ouvrard D, Bourgoin T. 2007. Molecular phylogeny of the genus *Tibicina* (Hemiptera, Cicadidae): rapid radiation and acoustic behaviour. *Biological Journal of the Linnean Society* 91: 611-626.

Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* 7: 453-464.

Tobias JA, Seddon N, Spottiswoode CN, Pilgrim JD, Fishpool LDC, Collar NJ. 2010. Quantitative criteria for species delimitation. *Ibis* 152: 724-746.

Trilar T, Hertach T. 2008. Three species of Mountain Cicadas *Cicadetta montana* (sensu lato) found in northern Italy. *Bulletin of Insectology* 61(1): 185-186.

Wade EJ, Hertach T, Gogala M, Trilar T, Simon C. 2015. Molecular species delimitation methods recover most song-delimited cicada species in the European *Cicadetta montana* complex. *Journal of Evolutionary Biology* 28: 2318-2336.

Yeates DK, Seago A, Nelson L, Cameron SL, Joseph L, Trueman JWH. 2011. Integrative taxonomy, or iterative taxonomy? *Systematic Entomology* 36: 209-217.

Summary

Cicadas (Cicadidae) are among the most popular insect groups owing to their calling songs. However, they are scientifically relatively poorly known, even in Europe; and the cicada diversity is underestimated crucially, as it was based only on a morphological approach. The songs often evolve more rapidly than the morphology. They are an important component of specific-mate recognition systems and, therefore, excellent markers of the early stages of reproductive isolation among populations. Recently, acoustic characters have been used extensively to reveal hidden taxonomic diversity.

In this thesis the *Cicadetta montana* species complex served as a model group, where morphological traits are missing or weak but high carrier frequency song patterns (10 kHz to 20 kHz) provide a remarkable, recently discovered and taxonomically relevant richness. The three species *Cicadetta montana* s. str., *Cicadetta brevipennis* Fieber, 1876 (sensu Gogala & Trilar, 2004) and *Cicadetta cerdaniensis* Puissant & Boulard, 2000 were known to occur in the two countries focussed on, namely Italy (records from very few locations) and Switzerland (a fairly good dataset) at the beginning of this thesis. We aimed to elaborate a modern and complete systematics for the Italian and Swiss taxa of the *Cicadetta montana* species complex. The elaboration was based on extensive field work (e.g. 27 weeks in Italy) and the analysis of numerous data sets dealing with acoustics, morphology, genetics (basically *COI* and *COII*) and ecology. With this ‘integrative’ approach we elevated the evidence of species delimitation in a taxonomically challenging group. Later, we evaluated scenarios explaining evolutionary and biogeographical aspects. Furthermore, habitat preferences and the impact on conservation strategies are discussed. Finally, the first national synthesis of the entire Cicadidae (sensu Moulds), beyond the model group, is presented for Switzerland.

As a result of these studies six species as well as two subspecies for Italy and four species for Switzerland, instead of three, are reported newly within the *Cicadetta montana* species complex. Two species (*Cicadetta sibillae* Hertach & Trilar, 2015, *Cicadetta anapaistica* Hertach, 2011) and two subspecies (*C. anapaistica lucana* Hertach, 2015 and *C. brevipennis hippolaidica* ssp. n.*) are new to science. The findings of this thesis have an impact on the nomenclature, diversity and conservation of cicadas in many (other) European countries. Ten autochthonous cicada species are present in Switzerland; this is demonstrated with detailed distribution maps.

*The new subspecies name is not yet available at the submission date of the present thesis.

Songs are inherited and nuclear-encoded; they can serve as predictors of the most parsimonious evolution and as indicators of recent hybridisation. We profited from the remarkable complexity of these songs and introduced relative power differences as a character to the taxonomy of cicadas. The importance of perch temperature measurements for temporal characters, when delimiting species, was also demonstrated.

However, the song patterns did not suffice to explain the diversity within the *C. montana* species complex in Switzerland and Italy. This group is intricate and has an unusual evolution, especially in the – herewith identified – song groups *brevipennis* and *cerdaniensis.* Hybridisation among taxa of these two song groups is obviously present to different degrees. We found at least three intra-group hybridisation zones between taxa, inferred from intermediate song patterns: Twice populations mixed to a degree that obliterated the differences over vast regions (subspecies described) and once the intermediate patterns affected only a few peripheral populations (species described). Gene flow is obviously also present between the *cerdaniensis* and the *brevipennis* groups themselves, but at a low level.

We were able to understand the *cerdaniensis* and the *brevipennis* song groups only step-by-step. These taxa are characterised by two clearly different basic song patterns and three well supported, but interdigitated mtDNA clades. The acoustic song patterns are similar in some taxa with relevantly different haplotypes, but clearly different in some taxa with closely related or identical haplotypes. The closest relative of each of the four *cerdaniensis* group species is a *brevipennis* group taxon. A colouration character supports the molecular differences and opposes the acoustic groups. Only by making use of an integrative approach and searching for “separately evolving metapopulation lineages” (De Queiroz’ ‘Unified species concept’), were we able to find surprising and interesting results: Mitochondrial molecular clades are most probably diagnostic for glacial refugia in the Pleistocene (central and southern Apenninian, Iberian, Balkan) and as a result of inter-group introgression not for species. Where *brevipennis* and *cerdaniensis* song groups met postglacially they exhibit an unusual and puzzling distribution: Inter-group taxa pairs for which we predict common glacial refugia and origins are nowadays not distributed sympatrically but parapatrically or allopatrically. Parapatric inter-group distribution areas end sharply within a few kilometres in the Apennine Mountains. When populations of the two groups occur in sympatry (three regions known), or even in syntopy, they stem from different mtDNA geographical clades.

A particularly good example of song evolution is the *cerdaniensis* song group. The patterns within this group show stepwise increases in complexity from *C. cantilatrix* to *C. anapaistica anapaistica.* None of the elements of this song evolution seem to have been lost until now. High complexity allows higher intraspecific variability to be identified still as conspecific. *Cicadetta anapaistica* songs are variable among males in a way that many of them within a population can be recognised supposedly individually.

The intraspecific variability of morphology is high and overlaps among species. While the calling songs are clearly different, a supposed threshold of minimally two million years of reproductive isolation in *Cicadetta montana* s. str. (which is not part of the *cerdaniensis* and *brevipennis* groups) was not sufficient to evolve one morphological or morphometric character valid for the correct separation of all specimens from other species within the complex. Many younger species within the complex are even more difficult to determine morphologically. Sexual selection is based on the acoustics; this favours morphological stasis.

Cicadas in Italy and Switzerland, as well as in many other European countries, occur predominantly in ecotone and woodland habitats. Closely related *Cicadetta montana* complex species prefer different vertical niches in the vegetation structure (treetops, bushes, herb layer); some are slightly more mesophilic than others.

Eight of the ten Swiss cicada species are part of the Red List, which will soon be issued for the first time. Only *Cicada orni* and *Cicadetta montana* s. str. are recognised as “not endangered” species. In Italy, grassland species (owing to agriculture) and mountainous species (owing to changing species’ interactions induced by global warming) have the highest risk to become extinct. Nature protection as well as land use management programmes should focus here especially on the endemic mountainous cicada species (e.g. *Cicadetta anapaistica*) in the future.

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