AMPHIBIAN DIVERSITY, DISTRIBUTION AND CONSERVATION IN THE ETHIOPIAN HIGHLANDS

MORPHOLOGICAL, MOLECULAR AND BIOGEOGRAPHIC INVESTIGATION ON *LEPTOPELIS* AND *PTYCHADENA* (ANURA)

Inauguraldissertation

zur
Erlangung der Würde eines Doktors der Philosophie
vorgelegt der
Philosophisch-Naturwissenschaftlichen Facultät
der Universität Basel

von

Abebe Ameha Mengistu

Basel 2012

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Genehmigt von der Philosophisch-Naturwissenschaftlichen Facultät

auf Antrag von

Prof. Dr. Peter Nagel, Dr. Simon Loader, Dr. David Gower und Prof. Dr. Walter Salzburger

Basel, den 18 October 2011

Prof. Dr. M. Spiess

Dekan

To Dejen Amaha, Selamawit Fetene and Sofonyas Abebe.

With love and respect!

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The current phylogeographic, taxonomic and conservation assessment of Ethiopian amphibians required inputs of funding, collaboration of relevant institutions and team work of experts and local people.

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PREFACE

Since the first description of amphibians from Ethiopia in the first half of the 19th Century, there were occasional reports on species until the turn of the 20th Century and further until the 1940s. These studies were based on collections made by researchers and expatriate travelers making short visits at some places. Field collection was not reported for about thirty years until the 1970s, and description of many new species was made in the years then after (Largen, 2001). After the three-week Harenna Forest Expedition in 1986, there was another hiatus on field work for about thirty years. With a thorough ten years analysis of available specimens, literature, and field itineraries, a comprehensive monograph of Ethiopian amphibians was published by Malcolm J. Largen in 2001.

Following Largen's report on Ethiopian amphibians and John C. Poynton's discussion on the biogeography of the Ethiopian Highlands, David G. Gower and Simon P. Loader of the NHM (London) made a short visit to Ethiopia in 2006. This pilot survey helped to plan future research projects. Simultaneously, Breda Zimkus (Harvard University) surveyed the Bale Mountains and Central Shoa highlands, collecting over 350 amphibians and tissue samples. In 2007, Loader (now staff of UB) organized an institutional collaboration for research and capacity building between the CNS (AAU) and the NLU (UB). In July 2008, a joint field work was conducted by Simon Loader, Abebe Ameha Mengistu (ZNHM, AAU), Samy Saber and Fikirte Gebresenbet (Environmental Sciences Program, AAU), and Roman Kassahun (EWCA). Many amphibian specimens and tissues were collected from Gughe Mountains, Awash National Park and Bale Mountains. A National Geographic funded field survey led by Simon Loader and including Abebe A. Mengistu (now PhD candidate at the UB), David Gower (NHM, London), Rafael de Sá (Richmond University) and Michele Menegon (NHM, Trento) was conducted in the Kaffa forests and Bale Mountains for one month in July 2009. More amphibian specimens and tissue samples were collected during this survey. Preliminary analysis of the new specimens and tissues collected so far for the current PhD research program, and visits to the NHM (London) and MCZ (Harvard) indicated further needs to sample from some type localities. In July-August 2010, Abebe A. Mengistu conducted a two-month field survey in Gojam (with Mohamed Adem Gizaw), Gamo Gofa (with Xenia Freilich, Queens College, New York), Arsi (with Abebe Getahun, AAU), and West Shoa (with Alemayehu Negasa, Ambo University) and Alemu Asefa; and also studied specimens at the ZNHM (AAU). About 175 new specimens and tissues were collected from these areas (mainly from type localities of some species of *Leptopelis* and *Ptychadena*).

Given the bad reputation and negative attitude that many local people had towards amphibians, it is not surprising that no single Ethiopian herpetologist followed the footsteps of Malcolm Largen during his eleven years (1966-1977) stay in the Department of Biology, Addis Ababa University. Now over three decades after he left Ethiopia, his wish stated in the introduction part of the 'Catalogue of the Amphibians of Ethiopia' seems to have come true:

"…If the information distilled into the present monograph provides some benefit and encouragement to the first Ethiopian herpetologist at the beginning of his or her career, the effort will have been amply rewarded…" (M.J. Largen, 2001).

It is almost two centuries since the beginning of amphibian taxonomy in Ethiopia; but unfortunately, until now, the rich amphibian diversity of the country has not been properly examined. Several internal and external political, socio-economic and cultural factors have hindered local capacity and foreign access for research. Most of the earlier Ethiopian amphibian collections were not deposited at the natural history museum in Addis Ababa University, but in museums in Europe and North America. M.J. Largen underlines on this issue stating, "*While valuable information in old literature and important specimens are deposited very far in other countries, it will be difficult for local researchers to show rapid progress*". Unlike most of the pre-1986 Ethiopian amphibian collections (including all holotypes) housed in Europe and North America, the recent (2006-2010) collections are stored at the ZNHM (AAU). Previous practices have made the existing wildlife protection and research rules stricter, but smooth and well-organized (FDRE Proclamation No. 541/2007). Collaboration of local and foreign scientists and institutions will be required to make better progress in research. Collaborative capacity building of the natural history museum in AAU and upgrading its organization to a national level is also necessary.

Some young Ethiopian researchers are becoming interested in studying Ethiopian amphibians at the School of Graduate Studies in AAU. As there are relatively many Ethiopian specialists in other related fields such as fisheries and aquatic sciences, mammalogy, ornithology, and entomology, we expect that some graduate students will be interested in studying amphibian biology in the future. With the current sharp increase in the number of college students joining newly established universities and programs, interest and research on the vacant field of Ethiopian amphibians will improve. This inevitably requires establishment of departments (or sections) of herpetology in different universities, foundation of a national professional association, and media of professional networking such as journals and internet web pages.

The organization of this thesis is structured in three parts. The first part (Chapter 1) gives general introductory notes (literature review) on past and ongoing research on Ethiopian amphibians, associated gaps and problems, future directions for research, and an overview of the current research project on Ethiopian *Leptopelis* and *Ptychadena*. The second part has two chapters dealing with the phylogeography of Ethiopian *Leptopelis* (Chapter 2) and *Ptychadena* (Chapter 3), discussing molecular phylogeny, morphological assessment and biogeographic patterns. The third part (Chapter 4) focuses on conservation of endemic highland amphibians, discussing biogeographic categories and prioritizing species for conservation. A summary of the whole thesis is given at the end with a translation in Amharic (Ethiopian official language).

A lot of effort has been put to achieve the results reported in the current study, with more than ten researchers involved in the field and laboratory work, six years of engagement in field and laboratory work, and financial support provided from over five sources. The current work appears to be the first phylogeographic assessment of Ethiopian amphibians and the broadest taxonomic assessment of these fauna since the publication of the 'Catalogue' by Malcolm Largen in 2001. Two relatively widespread genera (*Leptopelis* and *Ptychadena*) comprising over 25% of the country's species of amphibians were assessed. The techniques applied for taxonomic assessment included modern phylogenetic and GIS techniques during field collection and in the laboratory. Reference collections of specimens in different natural history museums were used for classical morphological comparisons with new specimens. Data analysis was conducted using efficient software designed for molecular and biogeographic assessment. All in all, substantial commitment has been made by the authors, friends, families, collaborating institutions and funding agencies to achieve the current results.

I am optimistic that training of herpetologists in Ethiopia is feasible given my own experience. My exposure to practical herpetological studies started very recently (2006), while I was working at the ZNHM (AAU). Previous field and laboratory experiences in marine biology, fish biology, fisheries and aquatic sciences, and biodiversity assessment during the past twenty years helped as a strong foundation for the current work. It would therefore not be difficult for other zoologists to conduct research on Ethiopian amphibians after having basic herpetological and methodological training. Furthermore, although research is relatively limited in Ethiopia so far, Largen's monograph of Amphibians of Ethiopia provides an excellent resource for gaining a good basic understanding of Ethiopian species. Several issues can be addressed, among many other topics, as amphibian-related research problems: taxonomy, evolutionary history, biogeography, ecology, behavior (breeding, feeding, etc.), physiology, development, population dynamics, medicine, pest management (biological control), environmental indicators, and conservation.

Abebe Ameha Mengistu

October 2011, Basel

ACRONYMS

Institutions

AAU	Addis Ababa University (Ethiopia)
AMNH	American Museum of Natural History (New York)
ANP	Awash National Park (Ethiopia)
BMNP	Bale Mountains National Park (Ethiopia)
BSE	The Biological Society of Ethiopia (Addis Ababa)
CITES	Convention on International Trade in Endangered Species
CNS	College of Natural Sciences (Addis Ababa University)
EPA	Environmental Protection Authority (Addis Ababa)
EWCA	Ethiopian Wildlife Conservation Authority (Addis Ababa)
EWNHS	Ethiopian Wildlife and Natural History Society (Addis Ababa)
EWNRA	Ethio-Wetlands and Natural Resources Association (Addis Ababa)
FAG	Freiwilligen Akademischen Gesellschaft (Basel) (Voluntary Academic Society)
FDRE	Federal Democratic Republic of Ethiopia
FfE	Forum for Environment (Addis Ababa)
IBC	Institute of Biodiversity Conservation (Addis Ababa)
IUCN	International Union for Conservation of Nature
MCZ	Museum of Comparative Zoology (Harvard University)
MSNG	Museo Civico di Storia Naturale di Genova Giacomo Doria (Genoa)
NGO	Non-governmental organizations
NHM	Natural History Museum (London)
NLU	Institut für Natur-, Landschafts und Umweltwissenschaften (University of Basel) (Institute for Nature-, Landscape- and Environmental Sciences)
NMA	National Meteorology Agency (Addis Ababa)
SNE	Stipendiencommission für Nachwuchskräfte aus Entwicklungsländern (Basel) (Scholarship Commission for Trainees from Developing Countries)
UB	University of Basel (Switzerland)
UTA	University of Texas Arlington (Arlington)
ZMB	Universität Humboldt Zoologisches Museum (Berlin)
ZNHM	Zoological Natural History Museum (Addis Ababa University)

Molecular and Biogeographic

AOO	Area of occupancy
BLAST	Basic Local Alignment Search Tool
EBEOO	Elevation-based extent of occurrence
EOO	Extent of occurrence
DNA	Deoxyribonucleic acid
GPS	Global Positioning System
ML	Maximum Likelihood
MP	Maximum Parsimony
mtDNA	Mitochondrial DNA

Mya	Millions of years ago
NCBI	National Center for Biotechnology Information
PCR	Polymerase chain reaction

Morphometric characters:

DLR	Dorso-lateral ridge
DSkF	Dorsal skin folds
DT4D	Disc of toe-4 diameter
ED	Eye diameter
ENL	Eye-nare length
FmL	Femur length
FtL	Foot length
HW	Head width
IMTL	Inner meta-tarsal tubercle length
INL	Inter-narial length
IOW	Inter-orbital width
OMT	Outer meta-tarsal tubercle
SnM	Snout top marking
Spc	Spicules on skin
SVL	Snout-vent length
T1L	Toe-1 length
T4-webbing	Number of phalanges free of webbing on toe 4
T5-webbing	Number of phalanges free of webbing on toe 5
TbL	Tibia length
TbTM	Tibia top marking
ThTM	Thigh top marking
TyD	Tympanum diameter
TyM	Tympanum marking
VeM	Vertebral marking
VSC	Vocal sac color

PART I:

INTRODUCTION

Current Status of Amphibian Diversity, Distribution and Conservation in Ethiopia: with emphasis on *Leptopelis* and *Ptychadena* (Anura)

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ABSTRACT

Ethiopia has a diverse amphibian fauna occurring in many ecosystems, from savanna to alpine highlands. However, little is known about the taxonomy, evolution, population biology and conservation status of the different species. The Ethiopian Highlands are particularly important habitats as several endemic amphibian genera and species are restricted to these highly fragmented areas. In total for Ethiopia, two of the 15 families, five of the 24 genera, and 26 of the 64 known species are endemic. As is the case for amphibians worldwide, the survival of these species faces threats from habitat degradation, climate change, and a pathogenic fungal disease. Several factors might explain our low level of knowledge on Ethiopian amphibians; this includes culture and belief, education and training, and economic value. Taxonomic and biogeographic re-assessment and field surveys in unexplored areas is needed to understand the natural history and population status of Ethiopian amphibians. This paper tries to summarize existing knowledge on the amphibians of Ethiopia pertaining mainly to their systematics and conservation. We discuss past and ongoing studies, existing gaps of knowledge, future needs in the area, and the application of phylogeography to resolve taxonomic complexities and outline the distribution patterns of some problematic groups such as *Leptopelis* and *Ptychadena*.

Key words: Amphibian diversity; Biodiversity conservation; Ethiopian Highlands; *Leptopelis*; Molecular systematic; Phylogeography; *Ptychadena*.

I) INTRODUCTION: EXISTING KNOWLEDGE

Amphibian biology has been one of the zoological disciplines to which little attention was given in Ethiopia until recently. This is evident from the fact that despite having diverse amphibian fauna in its varied ecosystems, little is known about the taxonomy, evolution, population biology and conservation status of the different species. The relative lack of research on amphibians can be attributed to several factors that are discussed below. Any current effort to review the status of amphibian biology in Ethiopia would to the most part be simply repeating the information discussed in the catalogue compiled by Largen (2001a) or the same information summarized in the book by Largen and Spawls (2010). There are few recent publications that appeared during the past decade with some updates on a few taxonomic groups. Therefore, in this paper, we will try to briefly discuss some of the background information on earlier works, main updates from recent and ongoing research, existing gaps of knowledge, future needs and potential methods, as well as an introduction to the phylogeography and conservation of Ethiopian *Leptopelis* and *Ptychadena*.

A) Amphibian Biology in Ethiopia

The comprehensive review by Largen (2001a) summarizes field collection of amphibians in Ethiopia by earlier European and North American visitors and researchers (including some Ethiopian academicians) from the first half of the nineteenth century until 1986, and ten years of analytical work referencing museum collections. This review provides a background for all the necessary taxonomic information, and is therefore an excellent starting point for future research, including the ongoing studies outlined here. Earlier and recent field collections of amphibians from Ethiopia are kept in natural history museums in Ethiopia and elsewhere, the major ones including the ZNHM (AAU), NHM (London), MCZ (Harvard), AMNH (New York), MSNG (Genoa), ZMB (Berlin), the Field Museum (Chicago), and UTA (Arlington).

The few previous studies conducted on Ethiopian amphibians were mainly focused on their taxonomy, geographic distribution, conservation (e.g., Poynton, 1999; Largen, 2001a), and a few other biological aspects such as feeding behavior and natural history (Drewes and Roth, 1981; Grandison, 1978, 1981; Wake, 1980). Previous taxonomic descriptions were based in the most part on morphology, complemented by basic understanding of the ecology of each species (Largen, 2001a). Larger analyses of mainly molecular data using samples from around the world, mostly of non-Ethiopian taxa) have resulted in various taxonomic changes, including the taxonomic position of some species and genera of Ethiopian amphibians (Frost et al., 2006; Frost, 2010). We have extracted and compiled a summary of the complete list of the currently recognized amphibian species of Ethiopia from the IUCN online database (IUCN, 2010) (Table 1.1); some major nomenclatural changes are applied from Frost et al. (2006) and Wilkinson et al. (2011). In this regard, there are currently 64 amphibian species recorded as occurring in Ethiopia. These belong mostly to the Order Anura, with a single species representing Caecilians (Apoda), and no representative of salamanders (Caudata).

Two of the 15 families, five of the 24 genera, and 26 of the 64 species are endemic to Ethiopia. Seven of the genera (with their respective families) are monotypic (Table 1.2).

B) The Ethiopian Highlands and their Biogeographic Relevance

The Ethiopian Highlands are clusters of highly fractured chains of mountains surrounded by vast areas of lowlands, and are part of the Eastern Afromontane Biodiversity Hotspot in Eastern Africa (Poynton, 1999; Myers et al., 2000; CI and McGinley, 2009), and constitute 50% of the land above 2000 m (Seleshi and Demarée, 1995) and about 80% of the land above 3000 m (Yalden, 1983; WWF, 2010) in Africa. There are several peaks in excess of 4000 m, the highest being Ras Dejen at 4550 m. The Great East African Rift Valley splits these highlands into the northwestern and the southeastern parts, which are further split by several major drainage systems (Fig. 1.1). The highlands are characterized by vast plateaus, deep gorges, steep escarpments, highland lakes, Afroalpine moorlands, Afromontane forests, riverine forests, and highland grasslands. Many of these areas have been sites of ancient civilizations that were accompanied by age-old farming practices, which have resulted in severe erosion of the landscapes (Tadesse, 2001). The fractures in the landscapes have through time created diverse micro- and macro-habitats for a variety of fauna and flora. Extremes such as isolated peaks of high mountains have become homes for many globally unique endemics, such as the Ethiopian Wolf and Mountain Nyala.

The Ethiopian Highlands are climatically important in trapping moist air that mainly comes from the Indian Ocean, and providing precipitation to the country. Average annual rainfall varies between 600 mm per year in Tigray (the north) and more than 2000 mm per year in the southwestern highlands (Krauer, 1988). The rainfall in this area has, however, been subject to historical variability, facing declines during the second-half of the 20th Century (Seleshi and Demarée, 1995; Osman and Sauerborn, 2002).

Demographically, the highlands harbor over 80% of Ethiopia’s human population, engaged in agriculture and urban activities. In general the national population is increasing at a high rate – about ten fold in the past 60 years (CI and McGinley, 2009). Pressure from increased human population usually leads to expansion of settlements to uninhabited natural areas, thereby disturbing wildlife and natural habitats. For instance, a study made in the Belete-Gera Forest in the southwestern forests showed that coffee plantations and encroachment negatively influenced up to 49% of the accessible natural forest (Cheng et al., 1998). Historically, the northern and central parts of the Ethiopian Highlands were subject to ancient civilization and agricultural practices for thousands of years, negatively impacting the vegetation cover and faunal diversity of the area (Reader, 2009); in recent times, despite varying figures, many reports agree on the very high level of deforestation that took place in the highlands in the past few decades (EFAP, 1994; Reusing, 2000).

Table 1.1. List of amphibian species of Ethiopia with their conservation status (extracted and compiled from online records of IUCN, 2010).

Family	**Scientific name**	**Taxonomic authority**	**Common name**	**Endemicity**	**IUCN Red List status**
Indotyphlidae Lescure, Renous & Gasc 1986	*Sylvacaecilia grandisonae*	(Taylor 1970)	Aleku Caecilian	Endemic	Data Deficient
Arthroleptidae Mivart 1869	*Leptopelis bocagii*	(Günther 1865)		Non-endemic	Least Concern
	Leptopelis gramineus	(Boulenger 1898)	Badditu Forest Treefrog	Endemic	Least Concern
	Leptopelis ragazzii	(Boulenger 1896)	Shoa Forest Tree Frog	Endemic	Vulnerable
	Leptopelis susanae	Largen 1977	Susana's Forest Tree Frog	Endemic	Endangered
	Leptopelis vannutellii	(Boulenger 1898)	Dimme Forest Tree Frog	Endemic	Vulnerable
	Leptopelis yaldeni	Largen 1977	Grassland Forest Tree Frog	Endemic	Near Threatened
Brevicipitidae Bonaparte 1850	*Balebreviceps hillmani*	Largen & Drewes 1989	Bale Mountains Tree Frog	Endemic	Endangered
Bufonidae Gray 1825	*Altiphrynoides malcolmi*	(Grandison 1978)	Malcolm's Ethiopian Toad	Endemic	Endangered
	Altiphrynoides osgoodi	(Loveridge 1932)	Osgood's Ethiopian Toad	Endemic	Vulnerable
	Amietophrynus asmarae	(Tandy *et al.* 1982)	Asmara Toad	Non-endemic	Least Concern
	Amietophrynus blanfordii	(Boulenger 1882)	Blanford's Toad	Non-endemic	Least Concern
	Amietophrynus garmani	(Meek 1897)		Non-endemic	Least Concern
	Amietophrynus kerinyagae	(Keith 1968)		Non-endemic	Least Concern
	Amietophrynus langanoensis	(Largen *et al.* 1978)	Lake Langano Toad	Endemic	Data Deficient
	Amietophrynus maculatus	(Hallowell 1855)		Non-endemic	Least Concern
	Amietophrynus regularis	(Reuss 1833)	African Common Toad	Non-endemic	Least Concern
	Amietophrynus steindachneri	(Pfeffer 1893)		Non-endemic	Least Concern
	Amietophrynus xeros	(Tandy *et al.* 1976)	Subdesert Toad	Non-endemic	Least Concern
	Bufo dodsoni	Boulenger 1895		Non-endemic	Least Concern
	Poyntonophrynus lughensis	(Loveridge 1932)		Non-endemic	Least Concern
Dicroglossidae Anderson 1871	*Hoplobatrachus occipitalis*	(Günther 1858)	Crowned Bullfrog	Non-endemic	Least Concern
Hemisotidae Cope 1867	*Hemisus marmoratus*	(Peters 1854)	Marbled Snout-burrower	Non-endemic	Least Concern
	Hemisus microscaphus	Laurent 1972	Ethiopian Snout-burrower	Endemic	Least Concern
Hyperoliidae Laurent 1943	*Afrixalus clarkei*	Largen 1974	Clark's Banana Frog	Endemic	Vulnerable

Family	**Scientific name**	**Taxonomic authority**	**Common name**	**Endemicity**	**IUCN Red List status**
	Afrixalus enseticola	Largen 1974	Ethiopian Banana Frog	Endemic	Vulnerable
	Afrixalus quadrivittatus	(Werner 1907)		Non-endemic	Least Concern
	Hyperolius acuticeps	Ahl 1931		Non-endemic	Least Concern
	Hyperolius balfouri	(Werner 1908)		Non-endemic	Least Concern
	Hyperolius kivuensis	Ahl 1931	Kivu Reed Frog	Non-endemic	Least Concern
	Hyperolius viridiflavus	(Duméril & Bibron 1841)	Common Reed Frog	Non-endemic	Least Concern
	Kassina maculifer	(Ahl 1924)	Spotted Kassina	Non-endemic	Least Concern
	Kassina senegalensis	(Duméril & Bibron 1841)	Senegal Kassina	Non-endemic	Least Concern
	Kassina somalica	Scortecci 1932		Non-endemic	Least Concern
	Paracassina kounhiensis	(Mocquard 1905)	Kouni Valley Striped Frog	Endemic	Least Concern
	Paracassina obscura	(Boulenger 1894)	Ethiopian Striped Frog	Endemic	Least Concern
Microhylidae Günther 1858	*Phrynomantis somalicus*	(Scortecci 1941)	Somali Rubber Frog	Non-endemic	Least Concern
Petropedetidae Noble 1931	*Conraua beccarii*	(Boulenger 1911)		Non-endemic	Least Concern
Phrynobatrachidae Laurent 1941	*Phrynobatrachus bullans*	Crutsinger *et al.* 2004		Non-endemic	Least Concern
	Phrynobatrachus inexpectatus	Largen 2001	Bore River Frog	Endemic	Data Deficient
	Phrynobatrachus minutus	(Boulenger 1895)		Endemic	Least Concern
	Phrynobatrachus natalensis	(Smith 1849)	Natal Dwarf Puddle Frog	Non-endemic	Least Concern
Pipidae Gray 1825	*Xenopus clivii*	Peracca 1898	Peracca's Clawed Frog	Non-endemic	Least Concern
	Xenopus largeni	Tinsley 1995	Largen's Clawed Frog	Endemic	Data Deficient
Ptychadenidae Dubois 1987	*Hildebrandtia macrotympanum*	(Boulenger 1912)		Non-endemic	Least Concern
	Ptychadena anchietae	(Bocage 1868)	Anchieta's Ridged Frog	Non-endemic	Least Concern
	Ptychadena cooperi	(Parker 1930)	Cooper's Grassland Frog	Endemic	Least Concern
	Ptychadena erlangeri	(Ahl 1924)	Erlanger's Grassland Frog	Endemic	Near Threatened
	Ptychadena filwoha	Largen 1997	Filwoha Grassland Frog	Endemic	Data Deficient
	Ptychadena harenna	Largen 1997	Bale Grassland Frog	Endemic	Data Deficient
	Ptychadena mascareniensis	(Duméril & Bibron 1841)	Mascarene Grassland Frog	Non-endemic	Least Concern
	Ptychadena nana	(Perret 1980)	Arussi Grassland Frog	Endemic	Data Deficient
	Ptychadena neumanni	(Ahl 1924)	Neumann's Grassland Frog	Endemic	Least Concern

Family	**Scientific name**	**Taxonomic authority**	**Common name**	**Endemicity**	**IUCN Red List status**
	Ptychadena porosissima	(Steindachner 1867)		Non-endemic	Least Concern
	Ptychadena pumilio	(Boulenger 1920)		Non-endemic	Least Concern
	Ptychadena schillukorum	(Werner 1907)	Sudan Grassland Frog	Non-endemic	Least Concern
	Ptychadena tellinii	(Peracca 1904)	Central Grassland Frog	Non-endemic	Least Concern
	Ptychadena wadei	Largen 2000	Tisisat Grassland Frog	Endemic	Data Deficient
Pyxicephalidae Bonaparte 1850	*Amietia angolensis*	(Bocage 1866)	Angola River Frog	Non-endemic	Least Concern
	Cacosternum boettgeri	(Boulenger 1882)	Boettger’s Dainty Frog	Non-endemic	Least Concern
	Ericabatrachus baleensis	Largen 1991	Bale Mountains Frog	Endemic	Endangered
	Tomopterna cryptotis	(Boulenger 1907)	Common Sand Frog	Non-endemic	Least Concern
Ranidae Rafinesque 1814	*Hylarana galamensis*	(Duméril & Bibron 1841)		Non-endemic	Least Concern
Rhacophoridae Hoffman 1932	*Chiromantis kelleri*	(Boettger 1893)	Keller's Foam-nest Frog	Non-endemic	Least Concern

Hyperolius nasutus Gunther 1865 has been included in the catalogue by Largen (2001a), but has been excluded from the list by Frost (2010) and IUCN (2010). *Phrynobatrachus bullans* Crutsinger *et al.* 2004 was not included in Largen (2001a), but has been recently recorded in Ethiopia by Zimkus (2008), but not yet included in the IUCN records. After Frost (2010), the following changes have been made on the taxonomic placement of some Ethiopian genera: *Bufo* to *Amietophrynus* or to *Poyntonophrynus* (except for *Bufo dodsoni*); *Spinophrynoides* to *Altiphrynoides*; *Euphlyctis* to *Hoplobatrachus*; *Rana* to *Amietia*; and *Afrixalus clarkeorum* to *A. clarkei*. There are eight species listed in Frost (2010) but for which information on their distribution in Ethiopia is not given either by Frost and AMNH or by IUCN. To avoid confusion, we have omitted these species from our revised list until further updates confirm their occurrence in Ethiopia.

Table 1.2. Summary of the number of families, genera and species of Ethiopian amphibians (number of endemics in parentheses).

Order	Family	Number of Genera	Number of Species
Gymnophiona	Indotyphlidae Lescure, Renous & Gasc 1986	1(1)	1(1)
Anura	Arthroleptidae Mivart 1869	1	6(5)
	Brevicipitidae Bonaparte 1850	1(1)	1(1)
	Bufonidae Gray 1825	4(1)	13(3)
	Dicroglossidae Anderson 1871	1	1
	Hemisotidae Cope 1867	1	2(1)
	Hyperoliidae Laurent 1943	4(1)	12(4)
	Microhylidae Günther 1858	1	1
	Petropedetidae Noble 1931	1	1
	Phrynobatrachidae Laurent 1941	1	4(2)
	Pipidae Gray 1825	1	2(1)
	Ptychadenidae Dubois 1987	1	14(7)
	Pyxicephalidae Bonaparte 1850	4(1)	4(1)
	Ranidae Rafinesque 1814	1	1
	Rhacophoridae Hoffman 1932	1	1
Total number:	**15 families (2 endemics)**	**24 (5)**	**64 (26)**

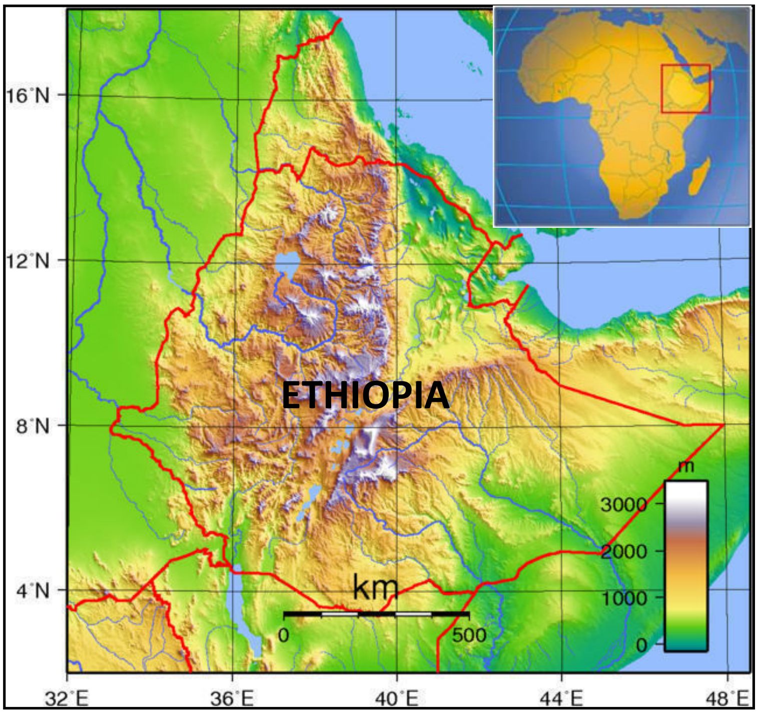

Fig. 1.1. Topographic map of the Ethiopian Highlands showing the Great East African Rift Valley and further fractured mountain ranges. (Modified from source: Sadalmelik, I. (2007); downloaded from http://en.wikipedia.org/wiki/File:Ethiopia_Topography.png).

The highlands of Ethiopia are the main repositories of moist forests and wetlands in Ethiopia, which are known to be home for, among others, a diverse and unique amphibian fauna. In general, mountain ranges in tropical regions are seen to be important because they harbor much diversity at species, lineage and allelic levels (Hewitt, 2004), with the reasons for this being unclear. Because of the nature of their delicate skin that is used for respiration, most amphibians dwell in moist habitats, such as swamps/bogs, streams, lake shores, and moist forests (Pough et al., 2003). Drying or decreased moisture of such habitats brings in fragmentation into micro-habitats and local disappearance of populations of amphibians. At its extreme, this could lead to extinction of species that have narrow geographic distribution and specialized resource requirements (IBC, 2005).

So far, about 32.5% of the roughly 6,000 known amphibian species in the world are reported to be globally threatened (Stuart et al., 2004). Habitat destruction, together with climate change, is accounted as being the main reason for the decline. In addition, chytridiomycosis, a pathogenic disease caused by the fungus *Batrachochytrium dendrobatidis*, is another emerging cause for the global decline of amphibian populations and extinctions (Rödder et al., 2009, Lötters et al., 2010). A recent study on the prevalence of this disease in Kenya has confirmed its widespread occurrence in various habitats – but more pronounced in areas where the temperature is relatively lower (Kielgast et al., 2010). This work concludes that detailed study of the nature and distribution of the fungus is necessary in different parts of Africa, especially in the high altitudes. As inferred from predictive models that combine ecological niches, climate envelope and known occurrences of the fungus, the Ethiopian Highlands are expected to be one of the potentially most suitable sites to harbor *B. dendrobatidis*, implying that amphibians in this area could be highly susceptible for the disease (Rödder et al., 2009; Lötters et al., 2010). A preliminary study on the prevalence of the fungus in amphibians of the Ethiopian Highlands indicated that about one-half of the studied specimens tested positive for the fungus (Gower et al., 2012). The correspondence of high level of diversity in mountain areas and high chytrid presence makes concerns for Ethiopian fauna a high priority.

Mountain ranges remain ideal places for the survival of lineages through climatic changes, and hence for genome divergence (Hewitt, 2004). The Ethiopian Montane has the highest rank of percentage of endemic genera and species of amphibians within biogeographic provinces of the Intertropical Montane region in Africa (Poynton, 1999). It is also understandable that many undiscovered species of amphibians still remain in species-rich tropical countries (Köhler et al., 2005). Therefore, the Ethiopian Highlands require high priority research on amphibian systematics for focused conservation. Timely action is needed when one considers the value of amphibians as indicators of habitat change, and the current scale of human interference in these habitats.

C) The Ethiopian Lowlands

The Ethiopian lowlands are relatively warm regions having altitudes as low as -115 m (below sea level). Contrasting to the uplifted Ethiopian plateau, the underlying lowlands form part of the Horn of Africa Biodiversity Hotspot (CI and McGinley, 2007). The lowlands are contained in one of three broader types of areas: the Rift Valley, border lowlands, or major river gorges. The Rift Valley runs through the Ethiopian Highlands from northeast (wider and deeper part) to southwest (narrower and elevated part). The northeastern part in Ethiopia is a terrestrial dead end blocked by the Red Sea, whereas the southwestern end connects with lowland areas in western and southern Ethiopia, northwestern Kenya and South Sudan. The border lowlands surround Ethiopia in the west, south and east connecting it with Sudan, South Sudan, Kenya, Somalia and Djibouti. Within the major river basins and sub-basins of Ethiopia run several river gorges slicing the mountain ranges and connecting the highlands with the Rift Valley and the border lowlands. For instance, the Abay (Blue Nile) Gorge starts at Tisisat Falls some 30 km south of Lake Tana and runs close to the northwestern border with Sudan, having at some places a depth of about 1.6 km. Omo, Gibe, and Awash are some of the other major rivers that have deep gorges.

The lowlands of Ethiopia are generally characterized by warmer and drier climate that is continuous with other parts of eastern, southern, central and northern Africa. Many of the species of amphibians occurring in these areas therefore appear to have wide and continuous distribution. Although these habitats are suitable for some species of amphibians, they are not renowned as places for high endemicity as seen in the highlands.

II) MAJOR GAPS IN AMPHIBIAN RESEARCH AND CONSERVATION IN ETHIOPIA

Several factors contribute to the low level of understanding and concern on Ethiopian amphibians. These can be broadly summarized as, 1) the bad reputation that many local people had towards amphibians and reptiles (based on our field observation in the past two decades), and 2) so far no known commercial importance of these animals in Ethiopia (e.g., no report of export of amphibians from Ethiopia; Earth Trends, 2003). Accordingly, until recently, little coverage has been given to the study of amphibians by higher education and research institutions and conservation organizations in the country. The following discussion elaborates the abovementioned points based on our observation on the local practices of various communities and education/research institutions.

Culture, belief, and economic value: To our knowledge, amphibians are not used as sources of food for humans in Ethiopia, in particular in the highlands where most of the population lives. Age-old and conserved culture and beliefs as well as availability of easily-accessed alternative sources of protein (such as beef, lamb, fish, cereals and other crops) have made amphibians to be excluded from the dishes. Ornamental uses or aquaculture practices also are not known. There are however some uses of amphibians (e.g., toads) for traditional medicine by local practitioners. Otherwise, it seems there is no clearly reported economic benefit gained from amphibians in Ethiopia. In general, Ethiopian amphibians are the least focused of all other major groups of vertebrates in terms of economic and social contribution.

Education and research: basic, secondary and college: The existing effort of zoologists who specialized on non-amphibian vertebrate groups or other biological fields (mammalogists, ornithologists, ichthyologists, geneticists, museum curators) to train students on amphibian biology is highly appreciated. Although there is basic knowledge on the biology of amphibians in general among students at different levels of education in Ethiopian schools and universities, there is very limited or no understanding on the taxonomy and conservation status of the country's amphibians. The curricula seem to give less coverage to Ethiopian amphibians than the big-sized, more visible and reputed wildlife such as mammals, birds, and fish. Absence of local professional herpetologists specifically skilled as amphibian biologists appears to be a factor for this gap of knowledge. Recently, the Environmental Sciences Program of the School of Graduate Studies of AAU had one of the co-authors of this paper (Samy Saber) employed for a few years as the only herpetologist. The ZNHM of this university has some collections of amphibians that are taken care of by volunteer non-amphibian zoologists and a curator. The ZNHM and vertebrate laboratory of the Department of Biology (AAU) appear to be the only educational and research places where Ethiopian amphibians are given some space and attention; however, courses on amphibian biology (or herpetology) are not given to students as compulsory requirements. Therefore, the current little coverage of amphibian biology in Ethiopia mainly sees the field as small component of trainings given in broader fields such as biology and zoology.

As is the case in the formal education system of Ethiopia mentioned above, professional associations (such as the BSE and the EWNHS) give limited attention to amphibians. If there may be an issue to deal with research or publication on Ethiopian amphibians, professional support would be possible mainly from foreign experts. Existing local and foreign professional associations have not established links between professionals, the general public and several government organizations in the transfer and exchange of ideas and knowledge on amphibians and their relevance for development and conservation in Ethiopia. We do not see any public awareness programs on the values of amphibians through mass media, posters, publications in local journals and other means of communication that can serve as informal means of educating the broader non-scientific community.

Conservation of amphibians and their habitats: The main factors that might prevent effective conservation of threatened amphibians in Ethiopia are lack of knowledge on their biology (Largen, 2001a), and ever-increasing habitat degradation - mainly deforestation (Gole et al., 2002; Seleshi and Demarée, 1995; EFAP, 1994) (Fig. 1.2). The remaining refugia for some of the threatened highland species of amphibians are confined to small areas (some protected) and fragments of highland forests (example, Bale Mountains, Southwest forests) (Largen, 2001a; Weinsheimer et al., 2010). EWCA, as a national overseer of wildlife protection, is playing a direct role in conservation of amphibians through recognizing the value of these fauna, promoting research, and indirectly through protecting areas that are mainly reputed for their mammal and bird species. Some other local organizations such as IBC, EWNRA, EPA, and FfE are providing direct or indirect contribution towards conserving amphibians and their habitats. These organizations try to protect, among others, suitable amphibian habitats such as forests, grasslands, wetlands and aquatic habitats; but this not directly intended to protect the amphibians there. However, the effort of all governmental and non-governmental institutions is constrained by the absence of skilled amphibian biologists in the country and inadequate baseline data. Historical aspects of some of the problems in research and conservation are pointed out in the Preface of this thesis.

Fig. 1.2. Protected swampy grassland and forest amphibian habitat in the Bale Mountains (top), and habitat degradation in the south-west highlands (bottom) in Ethiopia. (Photo credit: Abebe A. Mengistu, 2009 and 2010).

III) ONGOING RESEARCH, FUTURE NEEDS AND METHODS

A) Recent Activities

There are some recently communicated works on Ethiopian amphibians focused on systematics and distribution (Zimkus, 2008; Zimkus and Blackburn, 2008; Zimkus and Schick, 2010; Zimkus et al., 2010; Evans et al., 2011), distribution modeling (Weinsheimer et al., 2010), as well as conservation of threatened endemics (Gower et al., in press) and prevalence of chytridiomycosis (Gower et al., 2012). Graduate students from AAU (Ethiopia) and the UB (Switzerland) are addressing research topics focused on preliminary studies on the genetics, occurrence, distribution and conservation of some species of Ethiopian amphibians (Missale, 2008; Schwaller, 2009; Kassahun, 2009; Tito, 2009).

For a more complete knowledge on Ethiopian amphibians and to recommend more reliable conservation options, we need to understand not only the taxonomic status and distribution of each species, but also their life history, including among others, habitat, behavior (feeding, breeding, etc) and interactions with parasites. This in turn requires a multi-disciplinary approach and concerted effort of all stakeholders. In this line, as a higher learning and research institution, the Section of Biogeography (UB), in collaboration with the College of Natural Sciences (AAU) and EWCA, is conducting biogeographic studies on Ethiopian amphibians. This will help to improve the skills of local scientists, to build institutional capacity, and to recommend prioritized conservation options. One part of this collaborative project deals with the diversity, geographic distribution and conservation of some amphibians in the Ethiopian highlands (see below).

B) Future Needs

Future research, development and protection of amphibians in Ethiopia require a systematic approach where more urgent needs and appropriate methods are identified and prioritized, and research resources utilized efficiently. Some geographic areas and species are less surveyed than others; most research topics other than taxonomic issues have not been well addressed for most species. Even for taxonomic assessment, most of the earlier taxonomic methods relied on morphology only with some reference to ecology (Largen, 2001a). All of the existing professional herpetologists and laboratories working on Ethiopian amphibians are based in Europe and North America; and natural history museums in these two continents hold all of the holotypes of the known species of Ethiopian amphibians. These kinds of institutional and professional capacities need to be established in Ethiopia to enable more feasible research locally (Largen, 2001), and for a better implementation of practical conservation actions.

Upcoming project activities should focus on prioritized research problems that could be based on taxa, geographic areas, or habitats. The methodologies to be used should incorporate a combination of classical and modern techniques. As the basis for all other studies on amphibian biology, we will discuss the suitable taxonomic research needs and modern approaches required to achieve a better understanding of amphibians in this region.

Taxa and Geographic Areas: Taxonomic units with diverse and complex, endemic, threatened, or data deficient populations should take priority over others for taxonomic assessment. Taxonomic groups with diverse species and populations contain a lot of genetic information pertaining to the evolutionary history of the group; endemic, threatened or data deficient taxa also are the only representatives of their kind to preserve their respective evolutionary histories. Most of the 26 known endemic amphibians of Ethiopia are found in the highlands, which are surrounded by vast lowlands linked with other parts of eastern and central Africa. In an effort to re-assess the taxonomy of some of these taxa, there are ongoing studies on various families and genera. For instance, the taxonomic status of species within the Genus *Phrynobatrachus* in Ethiopia has been assessed by Largen (2001b) and is being revised at a continental level for Africa (Zimkus, 2008; Zimkus and Blackburn, 2008; Zimkus et al., 2010; Zimkus and Schick, 2010). These taxonomic assessments were achieved using molecular techniques, in conjunction with traditional morphological approaches. The biogeography of *Phrynobatrachus* was also considered (e.g., Zimkus et al., 2010). Further assessment of other highland groups such as many members of the families Bufonidae (*Altiphrynoides*), Brevicipitidae (*Balebreviceps*), Pyxicephalidae (*Ericabatrachus*), and Hyperolidae (*Kassina*, *Paracassina*, *Afrixalus, Hyperolius*) is underway as part of a broader project for the Eastern Afromontane amphibian biodiversity (SPL, pers. comm.). The mysterious fossorial life of *Sylvacaecilia grandisonae* (Taylor 1970) (Indotyphilidae) and its aquatic-breeding behavior (accounted for having a larval stage), unlike other African caecilians, has attracted attention of researchers (David Gower, pers. comm.) (Fig. 1.3). This Ethiopian endemic has been encountered in the wild in the southwest forests of the country a few decades ago and only in a few occasions recently.

The genera that are diverse and have wide geographic distribution in Ethiopia include *Amietophrynus* (Bufonidae), *Leptopelis* (Arthroleptidae), *Ptychadena* (Ptychadenidae), and members of the family Hyperoliidae. These are groups to which priority can be given based on their diversity and endemicity. Other less-diverse families and genera may need priority because of their endemicity, monotypic representations, and threatened conservation status (e.g., *Ericabatrachus*, *Balebreviceps*; Gower et al., in press).

Geographically, the Ethiopian Highlands are home for most of the endemic and threatened species of amphibians as contrasted with the lowlands that have widespread populations of species shared with other parts of Africa. Deforestation, habitat degradation agriculture and urbanization are increasing and replacing natural forested, grassland and aquatic habitats. The majority of the country’s human population also dwells in the highlands. Within the highlands themselves, some areas have been relatively better surveyed than others, and some highland segments have more suitable amphibian habitats than other fragments.

These scenarios mean that it is necessary and timely to conduct research on amphibian species in the highland parts of Ethiopia.

Fig. 1.3. Holotype of *Sylvacaecilia grandisonae* (Gymnophiona), BM 1969.1589. Photo credit: Abebe A. Mengistu, 2010.

C) Highlighting the Methods

Implementing modern techniques such as molecular systematic of Ethiopian amphibians in local institutions would require establishment of a broader-scope laboratory for animal systematics. Until availability of facilities and funding permits, it is possible to work on taxonomy using traditional morphological and ecological data, as well as through collaboration with other overseas institutions. In this regard, phylogenetic and biogeographic investigation would benefit from data from genetic, bioacoustics, behavioral, morphological, and/or geographic/ecological studies. As a modern tool to understand the evolutionary history, relationship and distribution of organisms, the following section discusses the potential and prospects of phylogeography.

1) Phylogeography as a Tool

Phylogeography is a field that deals with the geographical distribution of genealogical lineages, their spatial relationships and evolutionary history as populations, sub-species and species (Avise, 2000). As a new and rapidly developing field, phylogeography is nowadays serving a great deal in the study of systematic, biogeography and conservation of many organisms. This has been enabled through an easier access to mtDNA sequences of most animal species (Avise, 1998). Because mtDNA has a relatively fast rate of nucleotide divergence, it is well suited to examine events over the last few million years (Hewitt, 2004).

In particular, anuran amphibians have several advantages in phylogeographic studies; that is, they have generally low individual mobility – which consequently results in genetically highly structured populations (Beebee, 1996), are relatively easy to sample, and have a worldwide distribution and substantial species diversity (Zeisset and Beebee, 2008). Evaluation of the recent status of genetic exploration of amphibians indicated that there are reported taxonomic and geographic disparities. In 2006, there were no gene sequences for 86% of species of Gymnophiona and 76% of Anura, and for 76% of African species (Vences and Köhler, 2006). In view of this, intensified sampling in remote tropical regions and specific research for key taxa and highly endangered species were necessary.

For the African amphibian species for which genetic exploration has been conducted, it has been found to be helpful to understand phylogenetic relationships at different taxonomic levels, and to correct previous misplacement of taxa outside of their close relatives (paraphyly). For instance, the molecular phylogeny of hyperoliid tree frogs of Africa has been assessed using the 12S and 16S genes of the mtDNA. Based on this data, the family Hyperoliidae was recognized as being a paraphylectic assemblage having the genus *Leptopelis* as a basal group forming a group outside of hyperoloiids (Richards and Moore, 1996; Vences et al., 2003). Later, this has been further refined and *Leptopelis* was taken out of the hyperoliids and placed under the Family Arthroleptidae. Karyological data also supported that *Leptopelis* does not form a monophyletic group with the other hyperoliids (Odierna et al., 2007).

Among Ethiopian amphibians, gene sequences exist only for very few of the known species. Extensive barcoding of as many species of amphibians as possible would enable to test the traditional taxonomy and to see clearer relationships of the populations and species that potentially have been geographically isolated for millions of years. To achieve this, we can use the 16S gene, which is a highly conserved mitochondrial marker, but having common mutations in some variable regions, and ensuring a sufficient amount of mutations among species. This gene has been recommended as an important additional standard DNA barcoding marker for amphibians and other vertebrates (Avise, 2009; Vences et al., 2005). Generating 16S data of many other African taxa would mean placement of Ethiopian taxa on a broad scale is also possible.

Progress is being made in identifying appropriate molecular markers – the 12S and 16S genes more commonly applied – and production of more reliable and taxon-specific primers. Furthermore, the development of software to analyze molecular (sequence) data and geographic information continues rapidly. The prospect of utilizing molecular systematics is a highly valuable tool to solve problems of understanding and conserving amphibian diversity. An example for the useful contribution of molecular data to better understand amphibian diversity has been presented by Vieites et al. (2009) in revealing the underestimated amphibian diversity of Madagascar, and the phylogeography of the cosmopolitan species *Ptychadena mascareniensis* (Vences et al., 2004).

However, as is the case with the use of morphological characters and ecology to make species distinctions, the use of molecular techniques also has the problem of setting threshold values to delimit species boundaries. Therefore, the assignment of a minimum value of genetic distance to make species distinctions remains a subjective decision. Another weakness seen while using mitochondrial genes as genetic markers is that these genes contain phylogenetic information of only the maternal lineage, masking the complete evolutionary history of a species that comes from both parents. Despite the limitations, mitochondrial genes are currently widely used to understand genetic relationships and supplement taxonomic diagnosis of species. In this regard, the techniques that can be used to conduct phylogeographic assessment of Ethiopian amphibians are highlighted below.

2) Field Sampling and Specimen Handling

To conduct studies on the diversity and distribution of amphibians in Ethiopia in general (not specifically for this thesis), it is preferable to organize field surveys and sampling of whole specimens and tissues during the main rainy season (Amharic: *kiremt*, from June to August) and/or the dry season (Amharic: *bega*, from December to February). Various localities and habitats, including tropical forests, Afroalpine moorland, streams, swamps, highland grasslands, forest edges and inhabited/agricultural lands, are suitable places for amphibians. For molecular studies, cutting external body parts or taking tissue samples from the liver by dissecting the animal, would damage potentially useful diagnostic morphological characters. It is therefore preferable to take tissue samples from muscles of adults or tail/toe clips of metamorphs or tadpoles, and store them at -20°C in 96% ethanol. To preserve whole animals, the specimen is first fixed in 5% formaldehyde solution, rinsed with pure water, and then kept in 70% ethanol solution for further morphological study. If conservation-related survey on chytrid fungus infection (prevalence of chytridiomycosis) is needed, swabs can be taken from the skin of sampled specimens. Basic field sampling and museum preservation techniques of amphibians are presented in Getahun and Mengistu (2006). Advertisement calls and the behavior of amphibians are species-specific and are some of the most important data that help in taxonomic studies. Recording geographic data such as GPS coordinates, elevation, habitat type, and locality name and other details, as well as taking high resolution photographs of specimens and habitats should accompany field sampling of specimens. The date and time of collection, name(s) of collector(s), and tag numbers are also important information to have during field collection. Proper documentation and organization of the raw data in natural history museums and/or printed or electronic databases helps to produce good quality results and makes future access to the data easier.

In addition, museum collections of Ethiopian amphibians can be used to deal with historical materials for morphological comparison and referencing with fresh/new collections. The majority of these collections are housed in the ZNHM-AAU (Addis Ababa), NHM (London), MCZ (Harvard), ZMB (Berlin), and MSNG (Genoa). Most of these museums have kept very old collections and type materials that are important to revise the taxonomy of the complex and less-studied Ethiopian amphibians. These specimens can be accessed through study visits and loans arranged between collaborating institutions and the museums.

3) Molecular Work

Basic preparations for laboratory work should be followed using guidelines such as those outlined in Hillis et al. (1996) and DeSalle et al. (2002). Collection of genetic data in molecular laboratories is conducted first by extracting and purifying total DNA, and then by amplifying the DNA using PCR. DNA sequences of small parts of the mitochondrial DNA (mtDNA) and nuclear genes provide phylogenetic information that can give estimates of evolutionary relationships, genetic distances and divergence times of populations and species. Analysis of molecular data requires several steps and analytical models incorporated in different computer programs. The commonly used software include MEGA (Tamura et al., 2011), PAUP* (Swofford, 2002), and jModeltest (Guindon and Gascuel 2003; Posada, in press). Analysis of biogeographic data (mapping distributions, estimating areas, predicting potential habitats) incorporates the use of software such as ArcGIS (Environmental Systems Research Institute, Inc., Redlands, California) and DIVA-GIS (Hijmans et al., 2005).

The results of analysis of molecular, morphological and geographic/ecological data provide various results. That is, the results could verify/validate the taxonomic status of known taxa, reveal cryptic taxa (morphological conservatism), or conspecifics (morphological plasticity). Accordingly, assessment of geographic distribution data could provide with range retention, extension or shrinkage, thereby changing implications for conservation. As there could be taxonomic discrepancies between molecular and morphological assessments, the general definition for candidate species (Vieites et al., 2009) can be applied to evaluate the taxonomic status of newly revealed groups among populations. In this definition, 1) **Unconfirmed Candidate Species (UCS)** are deep genealogical lineages of unknown status, data deficient for morphology, ecology, and distribution; 2) **Confirmed Candidate Species (CCS)** show genetic divergence combined with a distinct difference in either morphology or in a character that mediates premating isolation; and 3) **Deep Conspecific Lineage (DCL)** are deep genealogies above a threshold value typical for comparisons among closely related species in the group of animals under study. These definitions can be modified and applied for different taxa (e.g., genera) depending on the respective results of genetic, morphological and geographic data. Overall, these approaches serve to make estimates of species diversity or evolutionary significant units.

4) Evaluation of Conservation Status

The outputs of the phylogeographic analyses are important inputs to assess the conservation status of the species under consideration. If a previously single species becomes split into several phylo- or morpho-species, or if a presumed widely distributed species turns out to have very limited geographic range and/or if the habitat of a species deteriorates through time, then these would imply that the conservation status of the species in question may need revision and prioritization. Conversely, species or populations that genetically prove to be conspecifics, or those for which new records indicate extension of previously known geographic ranges, or when habitats remain intact or become protected, then the conservation status of the species can be revised to have lower ranks. These evaluations are based on the standard criteria set for the IUCN Red List of species (IUCN, 2010). In addition to geographic ranges, it would be important to consider, if any, information on the trend and status of populations or habitats, prevalence of amphibian diseases, possible threats and opportunities pertinent in a specific range. Based on these assessments, the researcher can prioritize the studied taxa for further research and more practical conservation planning.

In summary, effective preservation of Ethiopian amphibians and proper utilization by humans as living natural resources can be achieved through more complete understanding of their taxonomy and natural history. Concerted and well-organized research and conservation programs on amphibians in unexplored areas, coupled with training of amateurs, semi- and highly-skilled professionals, could serve as means of building the capacity of local education institutions and wildlife conservation organizations. Proper handling of old museum specimens and curation of newly collected materials will enable future researchers to make more detailed studies, and for students to have suitable reference materials.

IV) PHYLOGEOGRAPHY AND CONSERVATION OF ETHIOPIAN *LEPTOPELIS* AND *PTYCHDENA*

Leptopelis and *Ptychadena* (Fig. 1.4) together comprise 30% of all known species of Ethiopian amphibians. Five of the six *Leptopelis* and five of the thirteen *Ptychadena* species are endemic to the Ethiopian Highlands, mainly to the Arsi-Bale massifs and the southwest highlands (Largen, 1977, 1997, 2001a). Species belonging to these genera have been reported to be some of the most problematic groups for taxonomy due to their complexity and similarities in morphology and geographic distribution (Largen, 1977, 1997, 2001a; Richards and Moore, 1996; Vences et al., 2004).

Within *Leptopelis*, for instance, *Leptopelis ragazzii* appears to have a wide geographic range extending from the central highlands south to the south-west forests and across the Rift Valley to the Arsi-Bale massifs (Fig. 1.5). Some of the distributional range for *L. ragazzii* is also thought to overlap with that of *L. vannutellii* in the south-west forests. Within *Ptychadena*, the “*P. neumannii* and allies” is a good example for the difficulties in identifying its member species. In this complex, Largen (2001a) includes *P. erlangeri*, *P. neumannii* and *P. nana*. In particular the altitudinal range proposed for *P. neumannii* (820-3,800 m) does not agree with the relatively narrower elevation ranges (mostly restricted either to higher or lower altitudes) reported for most of its closest relatives. As is the case for much of the species-rich tropical African amphibian biodiversity (Hewitt, 2004), five species of Ethiopian *Leptopelis* and *Ptychadena* are threatened at different levels, while four others are data deficient (IUCN, 2010; Table 1.1). Therefore, these groups require rigorous examination of additional materials and appropriate revision of their taxonomy for effective conservation plans.

Fig. 1.4. Top-left: *Leptopelis yaldeni* from Debre Markos, type locality (AM-016); top-right: Ventral view of *L. ragazzii*, from Ghedo (BM 1976.971 and BM 1976.974); bottom-left: *Ptychadena wadei* from Andassa, near Tis-Abay area (AM-021); bottom-right: ventral side of *P. cooperi*, near Bore (BM 1975.1817). (Photo credit: Abebe A.Mengistu, 2010).

A) Approach

This thesis deals with the systematics, biogeography and conservation of Ethiopian *Leptopelis* and *Ptychadena*, in particular those dwelling in the highland areas (Appendix 1.1). As indicated above, these highlands are important in terms of amphibian diversity and conservation. Ethiopia's few remaining pristine forests are concentrated in the southern and southwestern parts of the country; hence, our assessment focuses in these areas – namely, the Arsi-Bale Mountains and Southwest Forests in Kaffa and Gemu Gofa areas. The central part of the Ethiopian Highlands (Shoa area) and the northwestern region (Gojam area) are included in the survey. For comparison purpose, some lowland species in parts of the Rift Valley will also be assessed. The Ethiopian Highlands are split apart by the Rift Valley into northwestern and southeastern mountain ranges; this geologic event has been happening for a long period of time (since around 30 Mya, Arndt and Menzies, 2005). Further separation of mountain ranges by deep gorges to form smaller segments such as the Gughe Mountain, Kaffa highlands, and central Shoa plateau could imply the possibility of having vicariant taxa across mountain ranges (as discussed in Cox and Moore, 2005). That is, the patchy mountain ranges might possess their own separate assemblages of species belonging to separate taxa (at least for some species) instead of the wide distribution previously proposed (Largen, 1977, 2001a; Weinsheimer et al., 2010, for *Leptopelis ragazzii;* Largen 1997, 2001a for *Ptychadena neumannii*). A similar (but not same) case has been reported for amphibian diversity in different segments of the Eastern Arc Mountains in Tanzania (Poynton et al., 2007), that are part of the Eastern Afromontane Biodiversity Hotspot, to which the Ethiopian Highlands also belong to. Therefore, assessment of any association between phylogenetic, morphological, and/or geographic groupings of species will enable to outline clear biogeographic patterns at species or population level. Further assessment of the time of diversification of Ethiopian species of *Leptopelis* and *Ptychadena* in association with major geo-climatic events that formed the Ethiopian Highlands will be made to preliminarily demonstrate the evolutionary history of these taxa.

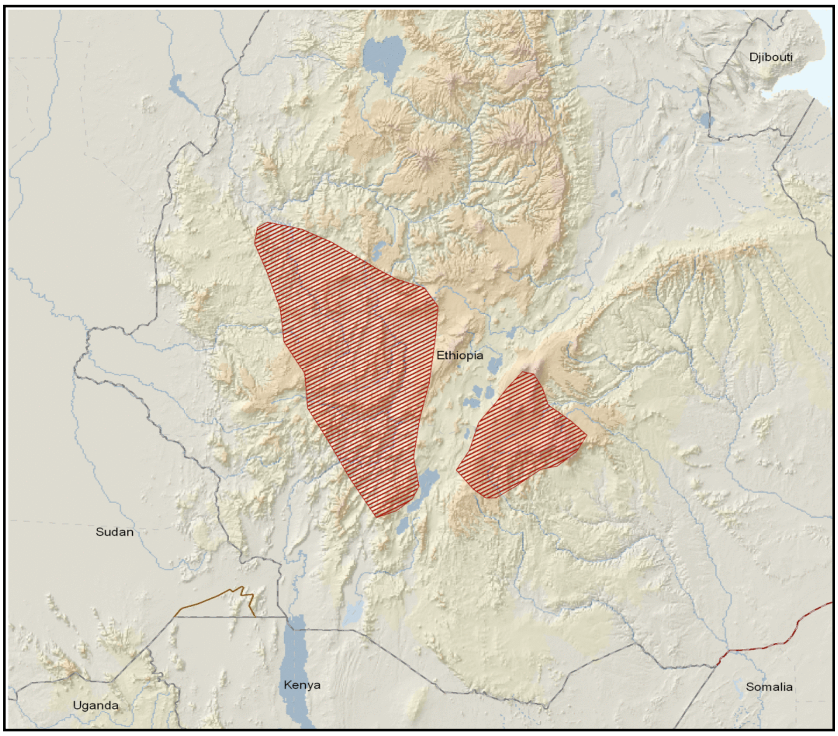

Fig. 1.5. Currently recognized geographic distribution of *Leptopelis ragazzii* in the Ethiopian Highlands across the Rift Valley (source: IUCN, 2010).

B) Research Questions and Hypotheses

The main objectives of this study are, using *Leptopelis* and *Ptychadena* as model taxa, 1) to explore the diversity and phylogeography of some amphibians in the Ethiopian Highlands and parts of the Rift Valley; 2) to preliminarily explain the geo-climatic events that are associated with the evolutionary history of these taxa; and 3) to re-evaluate the conservation status of species and their habitats by associating phylogenetic, biogeographic and ecological information.

To achieve the intended goals of this study pertaining to the systematics, biogeography, evolutionary history and conservation of *Leptopelis* and *Ptychadena* in Ethiopia, we will test the following hypotheses:

1) Current (traditional) taxonomy based on morphological characters and ecology corresponds to phylogeny using mtDNA markers;

2) Single species do not occur on both sides of major lowland barriers (e.g., the Rift Valley and major river gorges);

3) Diversification of extant highland species in Ethiopia started after the formation of the Ethiopian Highlands by uplifting and volcanic activities about 30 Mya; and

In addition, we will prioritize species for conservation by applying a new approach of estimation of geographic ranges of species using altitude-enhanced EBEOO instead of the current simple polygon estimates of EOO used by IUCN.

C) Methodology

To answer the abovementioned research questions, we will explore available information on the diversity and distribution of the largely endemic and relatively speciose Ethiopian *Leptopelis* and *Ptychadena*. New information will be compiled using recent field data and molecular approaches that would enable to produce improved estimates of diversity and distribution. Diversification of species in the Ethiopian Highlands will be assessed using preliminary estimates of evolutionary divergence timescale. We will re-evaluate the conservation status of species and their habitats using elevation-based geographic ranges and associated land cover data.

1) Field Sampling and Handling of Specimens

Voucher specimens and tissues were collected during field sampling programs conducted in the Ethiopian Highlands across the Rift Valley in 2006, 2008, 2009 and 2010. The surveys were made during part of the main rainy season (June to September) to increase chances of collecting more specimens under moist and humid conditions. Searches were made on grass fields, under rocks and bases of shrubs, around swamps, along creeks, forest-edges, and on branches of trees and shrubs. The samples were collected from the Eastern Rift (Arsi, Bale Mountains, and Bore areas), the Western Rift (Gughe Mountain, Soddo, Kaffa, West Shoa and Gojam areas) and for some species of *Ptychadena*, within the Rift Valley (Ziway, Langano, and Arba Minch areas) (Fig. 1.1). Locality information (including elevation in meters above sea level) and GPS coordinates were recorded for each sampled site. Specimens of *L.*

graminues and many specimens of *Ptychadena* were collected by hand at daytime and in the evening, while arboreal species of *Leptopelis* were caught mostly in the evening using headlamps and by triangulating calls of the males. Live animals were temporarily kept in air-filled moist polyethene bags before they were anaesthetized with solution of MS222. Tissue samples were taken (see below) and voucher specimens fixed with 5% formaldehyde for 24 hrs, rinsed with tap water for 24 hrs, and then kept in 70% ethanol indefinitely for further morphological studies. All voucher specimens were stored at the ZNHM of AAU. High resolution (5 to 15 megapixels) color photographs of live or preserved specimens were taken using Canon G10 digital camera.

2) Morphological Data

Morphometric measurements were done for all adult specimens of the recent collections (2008-2010) and for old specimens (pre-1977) kept in different natural history museums: MCZ (Harvard), NHM (London), ZNHM (Addis Ababa), ZMB (Berlin), and MSNG (Genoa). These museums were selected for housing type specimens and almost all of the historical collections of Ethiopian species of amphibians. We were not able to get travel grants to see some specimens at the AMNH (New York), the Field Museum (Chicago) and UTA (Arlington). The quantitative measurements taken (in mm or counts) include, 1) for *Leptopelis*: snout-vent length (SVL), head width (HW), femur length (FmL), tibia length (TbL), foot length (FtL), toe-1 length (T1L), inner meta-tarsal tubercle length (IMTL), disc of toe-4 diameter (DT4D), inter-orbital width (IOW), eye diameter (ED), tympanum diameter (TyD), number of phalanges free of webbing on toe 5 (T5-webbing); and 2) for *Ptychadena*: SVL, HW, FmL, TbL, FtL, T1L, IMTL, ED, TyD, eye-nare length (ENL), inter-narial length (INL), number of phalanges free of webbing on toes 4 (T4) and 5 (T5). Studied non-metric morphological characters for *Ptychadena* include: presence/absence of outer meta-tarsal tubercle (OMT), vocal sac color (VSC), tympanum marking (TyM), snout top marking (SnM), spicules on skin (Spc), vertebral marking (VeM), dorsal skin folds (DSkF), thigh top marking (ThTM), tibia top marking (TbTM), and dorso-lateral ridge (DLR). Body coloration and patterns were noted as non-morphometric morphological characters. All appendicular and non-axial measurements were taken consistently on the right side of the animal. The studied museum specimens included holotypes or lectotypes for all known species of Ethiopian *Leptopelis* and most Ethiopian species of *Ptychadena,* as well as several paratypes or non-type materials.

Absolute measurements (counts or metric) or ratios of sizes were computed for the following physical features: 1) for *Leptopelis*: SVL, HW:SVL, FmL:SVL, TbL:SVL, FtL:SVL, TbL:FmL, IMTL:T1L, IMTL:SVL, DT4D:T1L, and TyD:ED; 2) for *Ptychadena*: SVL, HW:SVL, FmL:SVL, TbL:SVL, FtL:SVL, SVL:HiLi, TbL:FmL, TbL:FtL, T1L:FtL, IMTL:T1L, IMTL:SVL, TyD:ED, T4-webbing and T5-webbing. We did pairwise comparisons of group means using t-tests to see if groupings based on morphological features will be congruent with phylogenetic (mtDNA) and biogeographic (distribution and elevation) groupings of populations. Principal component analyses (multivariate assessments) were done to show correlations of various character states and populations. Principal component analyses and other statistical tests were done in The Unscrambler® X version 10.0.1.

3) Molecular Data and Phylogenetic Assessment

Tissue samples were taken from sub-samples of fresh materials of recently collected (2006-2010) adult (muscle or liver), juvenile (muscle or toe tip), or tadpole (tail) specimens of *Leptopelis* and *Ptychadena* from localities in Bale, Arsi, Gemu Gofa, Kaffa, Soddo, Wenchi, Debre Markos, and the Rift Valley (Appendix 2.1; Appendix 3.2). We attempted to sample all known Ethiopian highland species of *Leptopelis* and *Ptychadena* (*L. gramineus, L. ragazzii, L. susanae, L. vannutellii, L. yaldeni, P. cooperi, P. erlangeri, P. nana, P. neumanni* and *P. wadei*). We also included some non-endemic lowland forms of these genera (*L. bocagii, P. anchietae* and *P. mascareniensis*) for comparison with species from the Ethiopian Highlands and haplotypes elsewhere in Africa. Additional sub-samples of tissues of some *Ptychadena* from Bore (Eastern Rift), ANP (Rift Valley) and Yadot River (southern base of Bale Mountains) were obtained from separate collections of one of the co-authors (B. Zimkus) in 2006. The tissues were kept in 96% ethanol at -20°C for further laboratory analysis.

Most of the molecular work was conducted in the facilities of the molecular laboratory at the Section of Biogeography (Department of Environmental Sciences, UB), while some of the tissue samples of *Ptychadena* from Bale and Bore areas were purified and sequenced in the laboratories of the MCZ (Harvard University) by B. Zimkus. DNA extraction and purification, amplification of the 12S and 16S genes, and purification of PCR products conducted in the UB were done as follows: Total DNA was extracted from 25 mg of tissue by using 20 µl Proteinase-K and 180 µl Buffer ATL, and incubating the mixture at 55°C and 1400 rpm for at least three hours. The digested product has been purified by using QUIAGEN®/DNeasy® Blood and Tissue Kit following the manufacturer's procedure. We checked for successful purification of total DNA by running gel electrophoresis at 91 V for 40 minutes. A sub-sample of 2 µl of template DNA from each sample was used to amplify the 16S and 12S genes of the mitochondrial DNA (mtDNA) through polymerase chain reaction (PCR) by using illustra™ puReTaq Ready-To-Go PCR Beads following the manufacturer's procedure. The primers used were 16S forward (5'-CGC CTG TTT ATC AAA AAC AT-3') and reverse (5'-CCG GTC TGA ACT CAG ATC ACG T-3'), as well as 12SA forward (5'-AAA CTG GGA TTA GAT ACC CCA CTA T-3') and reverse (5'-GAG GGT GAC GGG CGG TGT GT-3') manufactured by Eurofins MWG Operon. For the 16S PCR we conducted 35 cycles of denaturation (94°C, 1 min), annealing (51°C, 1 min), and extension (72°C, 1.5 min). For the 12S PCR we followed the same steps as for 16S, changing only the annealing temperature to 62°C for *Leptopleis* and to 47 °C for *Ptychadena*. PCR products were purified using SIGMA™ GenElute PCR Clean-Up Kit following the manufacturer's procedure. Sequence PCR and sequencing were conducted at Macrogen Inc. (Seoul, South Korea) following the company's procedures.

Each of the sequences was aligned, and gaps and traces were checked and uncertain nucleotides were corrected by eye in CodonCode Aligner version 2.0 (by CodonCode Corp., Dedham, MA, U.S.A.). Assembled sequences were BLAST-searched and checked online (NCBI-BLAST) for closeness with previously studied species of *Leptopelis* or *Ptychadena*.

Additional sequence data of *Leptopelis* that we used from other sources include: S. Loader's unaccessioned sequences of *L. flavomaculatus* (Günther, 1864)*, L. ulugurensis* Barbour and Loveridge, 1928, *L. vermiculatus* (Boulenger, 1909) from their type localities, as well as available GenBank sequences for non-Ethiopian populations of *L. argenteus* (Pfeffer, 1893), *L. bocagii, L. vermiculatus, L. palmatus* (Peters, 1868) and *L. kivuensis* Ahl, 1929. Additional sequence data and alignments of *Ptychadena* that we used from other sources include: 26 sequences (16S) of *P. mascareniensis* (2), *P. filwoha* (2), *P. anchietae* (1), *P. harenna* (4), *P. cooperi* (4), *P. erlangeri* (6) and *P. neumanni* (7) aligned (nexus format) by B. Zimkus; and available GenBank sequences for non-Ethiopian specimens of *P. anchietae*, *P. porosissima*, and *P. mascareniensis.* The outgroups used in this study were GenBank sequences for *Arthroleptis palava* Blackburn, Gvoždík, and Leaché, 2010 and *Astylosternus schioetzi* Amiet, 1978, as well as *Leptopelis kivuensis* Ahl, 1929 in the case of *Ptychadena* (Table 2.2; Table 3.2).

All fasta files (including outgroups) were grouped within their respective genera for further processing. For sequences within each genus, complete alignment was done for all assembled (contig) sequences combined using the default parameters in CLUSTALX 2.0.12 (Thompson et al., 1997). Model selection for phylogenetic analysis in Maximum Likelihood (ML) was conducted using Akaike Information Criterion in Modeltest 3.7 (Posada and Crandall, 1998), jModeltest (Guindon and Gascuel, 2003; Posada, in press) as well as Bayesian Information Criterion in MEGA 5 (Tamura et al., 2011). Heuristic searches were made for optimal tree scores under Maximum Parsimony (MP) as well as ML criteria. To test specific hypotheses we conducted topology tests to compare whether constrained sub-optimal scores of trees are significantly different from the most parsimonious (or optimal) tree solutions. Topology tests were conducted in PAUP* 4 b10 (Swofford, 2002) using MP (Non-parametric test (Templeton, 1983)) as well as ML (KH-test (Kishino and Hasegawa, 1989) with full optimization, two-tailed, 100 replicates settings).

Bootstrap phylogeny (1000 replicates; Felsenstein, 1985) for the most parsimonious trees was conducted by complete deletion of gaps and missing data with Nearest-Neighbor-Interchange heuristic method in MEGA 5 (Tamura et al., 2011).

Evolutionary divergence over sequence pairs between groups was estimated using p-distance in MEGA 5 (Tamura et al., 2011). Pairwise comparison was done using Distance methods to compare the degree of variability between different populations and species. To test specific hypotheses we conducted topology tests to compare whether constrained trees are significantly different from most parsimonious (or the best ML) tree solutions. Topology tests were conducted in PAUP* 4b10 (Swofford, 2002) using Maximum Parsimony (MP) (non-parametric test; Templeton, 1983) as well as ML (KH-test; Kishino and Hasegawa, 1989). Phylogenetic inferences were then made by assessing the resulting relationships within and outside Ethiopian populations of *Leptopelis* and *Ptychadena*.

Preliminary estimates of evolutionary divergence times were made using the MP consensus tree in MEGA 5 (Tamura et al., 2011) with secondary calibrations applied from divergence time estimates for the split of Astylosterninae and Leptopelinae around the K-T boundary about 99.3-43.3 Mya (time corresponding to node 22 in Table 2 and timescale in Fig. 3 in Bossuyt et al., 2006). All aligned nucleotide sequence data generated in this study for Ethiopian *Leptopelis* and *Ptychadena* are deposited in the database of NCBI GenBank (accession numbers: JX464828 to JX465107).

The taxonomic statuses of newly revealed groups among the studied populations were evaluated for Ethiopian *Leptopelis* and *Ptychadena* with modification of the general definition for candidate species as outlined in Vieites et al. (2009); see Section III-C-3 in this chapter for general definitions. In this evaluation, we integrated results of assessments on morphological similarities, phylogenetic relations and biogeographic patterns.

4) Biogeography and Conservation

The context of 'Ethiopian Highlands' in this thesis reflects 'the geo-climatic area in Ethiopia that is part of the Eastern Afromontane Biodiversity Hotspot, excluding the Rift Valley, deep gorges and warmer lowlands in the western, southern and eastern parts of the country, generally exceeding 1500 m.' The lower altitudinal limit of the Eastern Afromontane Biodiversity Hotspot is usually taken as between 1,500 and 2,000 meters, although this is lower away from the equator (CI and McGinley, 2009). As Ethiopia is located slightly north of the equator (roughly between 4°N and 14°N), considering the 1,500 m lower altitudinal limit is reasonable. But, it should be noted that the altitudinal limits of some species of Ethiopian *Leptopelis* and *Ptychadena* goes higher or lower than 1,500 m.

The geographic distribution of the known amphibian species in Ethiopia has been presented in the catalogue by Largen (2001a). However, with upcoming revision of the taxonomy of the different taxa and additional records of species from unexplored localities, it will be important to map the new and revised geographic distribution of the species in consideration. To achieve this, we gathered and organized coordinate and locality data for all of the recognized species. These data arise either from historical records of old museum collections or recent records by various researchers.

GPS coordinate data were sorted following currently revised phylogenetic groupings, and mapped for each population and species. Point maps, along with elevation ranges for each population, were then used to define the geographic ranges of populations. This new approach of estimating elevation-based extent of occurrence of species is aimed to produce better estimates of distribution areas than the crude polygons used in the IUCN criteria. To preliminarily evaluate the status of species and their respective habitats, the coordinate points and/or estimated distribution areas were overlay on country-, elevation- and vegetation cover maps in DIVA-GIS (Hijmans et al., 2005). We examined existing historical and recent climatic and habitat data to note substantial changes happening in the distribution areas of the taxa under consideration. Special emphasis is given to those species that are endemic to the Ethiopian Highlands. More details of the methodology on biogeography and conservation aspects are discussed in Chapter 4. Outcomes of the taxonomic, biogeographic and habitat data, as inputs in the IUCN criteria to categorize status of species, will potentially aid to prioritize species for conservation.

D) Expected Outputs

The main outputs expected from the current study for *Leptopelis* and *Ptychadena* in the Ethiopian Highlands are:

1) An understanding of the level of correspondence of current taxonomy of Ethiopian amphibians with phylogeny, thereby revealing phylogenetic relationships and morphological characterization of species and populations;

2) Definition of distinct biogeographic patterns for the distribution of species and populations within segmented mountain ranges across lowland barriers;

3) Preliminary explanation of the history of events for diversification of populations spread in the Ethiopian Highlands across the Rift Valley and other lowland barriers; and

4) Revised conservation status of species using better estimates of elevation-based geographic ranges and incorporating information on land cover and local climate.

The next chapters of this thesis are organized to address phylogeographic aspects and conservation issues focused on *Leptopelis* and *Ptychadena* in Ethiopia. Chapter 2 deals with morphological, molecular and biogeographic investigation of *Leptopelis*, while Chapter 3 addresses similar issues for *Ptychadena*. The methodology followed and organization of the topics in these two chapters is outlined in a similar fashion. Each and every geographically and genetically distinct population is discussed in detail. Chapter 4 focuses on integrating phylogeographic results with potential distribution, climate and habitat data to make inference on the conservation status of populations and species included in this study. In Chapter 5, a short summary will synthesize the overall results of phylogeographic and conservation assessments.

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REFERENCES

Arndt, N. and Menzies, M.A. (2005). The Ethiopian large igneous province. Accessed online on 31 August 2010. http://www.largeigneousprovinces.org/05jan.html

Avise, J.C. (1998). The history and purview of phylogeography: a personal reflection. *Molecul. Ecol.* 7: 371-379.

Avise, J.C. (2000). Phylogeography: The History and Formation of Species. Harvard University Press. Cambridge. 447 p.

Avise, J.C. (2009). Phylogeography: retrospect and prospect. *J. Biogeogr.* 36: 3-15.

Beebee, T.J.C. (1996). Ecology and Conservation of Amphibians. Chapman and Hall. London. 224 p.

Bossuyt, F., Brown, R.M., Hillis, D.H., Cannatella, D.C. and Milinkovitch, M.C. (2006). Phylogeny and biogeography of a cosmopolitan frog radiation: Late Cretaceous diversification resulted in continent-scale endemism in the family Ranidae. *Systematic Biology* 55 (4): 579-594.

Cheng, S., Hiwatashi, Y., Imai, H., Naito, M. and Numata, T. (1998). Deforestation and degradation of natural resources in Ethiopia: Forest management implications from a case study in the Belete-Gera Forest. *J. For. Res.* 3: 199-204.

CI (Conservation International, Content Partner) and McGinley, M. (Topic Editor) (2007). "Biological diversity in the Horn of Africa." In: Encyclopedia of Earth. Eds. Cutler J. Cleveland (Washington, D.C.: Environmental Information Coalition, National Council for Science and the Environment). [Published in the Encyclopedia of Earth October 26, 2007; Retrieved February 16, 2010].

CI (Conservation International, Content Partner) and McGinley, M. (Topic Editor) (2009). "Biological diversity in the Eastern Afromontane." In: Encyclopedia of Earth (Cleveland, C.J., ed. (Washington, D.C.: Environmental Information Coalition, National Council for Science and the Environment). [First published in the Encyclopedia of Earth October 13, 2008; Last revised June 25, 2009; Retrieved February 16, 2010]. <http://www.eoearth.org/article/Biological_diversity_in_the_Eastern_Afromontane>

Cox, C.B. and Moore, P.D. (2005). Biogeography: An Ecological and Evolutionary Approach. 7th Ed. Blackwell Publishing. 428 p.

DeSalle, R., Giribert, G. and Wheeler, W. (2002). Techniques in Molecular Systematics and Evolution. Birkhäuser. Basel. 450 p.

Drewes, R.C. and Roth, B. (1981). Snail-eating frogs from the Ethiopian highlands: a new anuran specialization. *Zool. J. Linn. Soc*. 72(3): 267-287.

Earth Trends (2003). Biodiversity and Protected Areas: Ethiopia. Earth Trends Country Profiles. Pp. 1-7. Accessed online at http://earthtrends.wri.org/

EFAP (1994). Ethiopian Forestry Action Program. The Challenge for Development. Volume II. Ministry of Natural Resources Development and Environmental Protection, Addis Ababa.

Evans, B., Bliss, S.M., Mendel, S.A. and Tinsley, R.C. (2011). The Rift Valley is a major barrier to dispersal of African clawed frogs (*Xenopus*) in Ethiopia. *Molecular Ecology* 20(20): 4216-4230. DOI: 10.1111/j.1365-294X.2011.05262.x

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.

Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., de Sá, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., L. Blotto, B., Moler, P.E., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M. and Wheeler, W. (2006). The Amphibian Tree of Life. *Bulletin of the American Museum of Natural History* 297: 1-370.

Frost, D.R. (2010). Amphibian Species of the World: an Online Reference. Version 5.3 (12 February, 2009). Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/ American Museum of Natural History, New York, USA.

Getahun, A. and Mengistu, A.A. (Eds.) (2006). Field and Laboratory Techniques for Zoological Museum Collections: Manual on Methods of Collection, Preservation and Management. The Zoological Natural History Museum, Addis Ababa University, Addis Ababa. 91p.

Gole, T.W., Denich, M., Teketay, D. and Vlek, P.L.G. (2002). Human impacts on the *Coffea arabica* genepool in Ethiopia and the need for its in situ conservation. In: Managing Plant Genetic Diversity, pp. 237–247 (Engels, J.M.M., Rao, V.R., Brown, A.H.D., Jackson, M.T., eds.) CABI Publishing, Oxon.

Gower D.J., Aberra R.K., Schwaller S., Largen M.J., Collen B., Spawls S., Menegon M., Zimkus B.M, de Sá R., Mengistu A.A., Gebresenbet F., Moore R.D., Saber S.A. and Loader S.P. (in press). Long-term data for endemic frog genera reveal potential conservation crisis in the Bale Mountains, Ethiopia. *Oryx*.

Gower D.J., Doherty-Bone T.M., Aberra R.K., Mengistu A.A., Schwaller S., Menegon, de Sá R., Saber S.A., Cunningham A.A., and Loader S.P. (2012). High prevalence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) across multiple taxa and localities in the highlands of Ethiopia. *Herpetological Journal* 22: 225-233.

Grandison, A.G.C. (1978). The occurrence of *Nectophrynoides* (Anura Bufonidae) in Ethiopia. A new concept of the genus with a description of a new species. *Monit. Zool. Ital. New. Ser. Suppl*. 11: 119-172.

Grandison, A.G.C. (1981). Morphology and phylogenetic position of the west African *Didynampius sjoestedti* Anderson, 1903 (Anura: Bufonidae). *Monit. Sool. Ital. New. Ser. Suppl*. 15: 187-215.

Guindon, S. and Gascuel, O. (2003). A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* 52: 696-704.

Hewitt, G.M. (2004). The structure of biodiversity – insights from molecular phylogeography. *Front. in Zool.* 1: 4.

Hijmans, R.J., Guarino, L., Jarvis, A., O'Brrien, R., Mathur, P., Bussink, C., Cruz, M., Barrantes, I. and Rojas, E. (2005). DIVA-GIS Version 5.2: Manual.

Hillis, D., Moritz, C. and Mable, B.K. (1996). Molecular Systematics, 2nd Ed. Sinauer Associates, Inc. Sunderland. 655 p.

IBC (2005). Nationl Biodiversity Strategy and Action Plan. Institute of Biodiversity Conservation, Government of the Federal Democratic Republic of Ethiopia. Addis Ababa.

IUCN (2010). IUCN Red List of Threatened Species. Version 2010.4. <www.iucnredlist.org>. Downloaded on 20 February 2010.

Kassahun, R. (2009). An investigation of amphibian diversity and abundance in relation to environmental change in Harenna Forest, Bale Mountains National Park, Ethiopia. Unpublished MSc thesis, School of Graduate Studies, Addis Ababa University. Addis Ababa.

Kielgast, J., Rödder, E., Veith, M. and Lötters, S. (2010). Widespread occurrence of the amphibian chytrid fungus in Kenya. *Anim. Conserv*. 13 Suppl. 1: 1-8.

Kishino, H. and Hasegawa, M. (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *Journal of Molecular Evolution* 29: 170- 179.

Köhler, J., Vietes, D.R., Bonett, R.M., Hita, G.F., Glaw, F., Steinke, D. and Vences, M. (2005). New amphibians and global conservation: a boost in species discoveries in a highly endangered vertebrate group. *BioScience* 55: 693-696.

Krauer, J. (1988). Rainfall, Erosivity and Isoerodent Map of Ethiopia. Soil Conservation Research Project, Research Report 15. University of Bern, Bern. 132 p.

Largen, M.J. (1977). The status of the genus *Leptopelis* (Amphibia, Anura, Hyperoliidae) in Ethiopia, including descriptions of two new species. *Monit. Zool. Ital.,* N.S. Supplemento 9: 85-136.

Largen, M.J. (1997). Two new species of *Ptychadena* Boulenger 1917 (Amphibia, Anura, Ranidae) from Ethiopia, with observations on other members of the genus recorded from this country and a tentative key for their identification. *Trop. Zool.* 10: 223-246.

Largen, M.J. (2001a). Catalogue of amphibians of Ethiopia, including a key for their identification. *Trop. Zool.* 14: 307-402.

Largen, M.J. (2001b). The status of the genus *Phrynobatrachus* Günther 1862 in Ethiopia and Eritrea, including description of a new species (Amphibia, Anura, Ranidae). *Trop. Zool.* 14: 287-306.

Lötters, S., Kielgast, J., Bielby, J., Schmidtlein, S., Bosch, J., Veith, M., Walker, S., Fisher, M.C. and Rödder, D. (2010). The link between rapid enigmatic amphibian decline and the globally emerging chytrid fungus. *EcoHealth*, *International Association for Ecology and Health*. Published online on 12 March 2010.

Mengistu, A.A., Loader, S., Getahun, A., Saber S. and Nagel, P. (2009). Amphibian Diversity and Conservation in the Ethiopian Highlands. Abstract and Poster. Symposium on Biodiversity Hotspots: Evolution and Conservation. March 2009, Luxembourg.

Missale, A.T. (2008). Chromosome study of some Ethiopian amphibian species. Unpublished MSc thesis, School of Graduate Studies, Addis Ababa University. Addis Ababa.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. and Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858. doi:10.1038/35002501.

Nei, M. and Kumar, S. (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Odierna, G., Aprea, G., Andreone, F., Böhme, W. and Vences, M. (2007). Cytosystematics of hyperoliid frogs: Phylogeny of *Heterixalus*, low karyotypic variability in hyperoliines and separate phylogenetic position of *Leptopelis*. *Ital. J. Zool.* 74: 71-81.

Osman, M. and Sauerborn, P. (2002). A Preliminary Assessment of Characteristics and Long-term Variability of Rainfall in Ethiopia - Basis for Sustainable Land Use and Resource Management. Conference on International Agricultural Research for Development. Deutscher Tropentag. Witzenhausen, October 9-11, 2002. Pp. 1-8.

Posada, D. (in press). jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution*.

Posada, D. and Crandall K.A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* 14 (9): 817-818.

Pough, F.H., Andrews, R.M., Cadle, J.E., Crump, M.L., Savitsky, A.H. and Wells, K.D. (2003). Herpetology. 3rd Edition. Benjamin Cummings, San Francisco, CA. 736 p.

Poynton, J.C. (1999). Distribution of amphibians in Sub-Saharan Africa, Madagascar and Seychelles, pp. 483-539. In: Patterns of Distribution of Amphibians: A Global Perspective (Duellman, W.E., ed.). 633 p. The John Hopkins University Press, Baltimore.

Poynton, J.C., Loader, S.P., Sherratt, E. and Clarke, B.T. (2007). Amphibian diversity in East African biodiversity hotspots: altitudinal and latitudinal patterns. *Biodivers. Conserv.* 16:1103–1118.

Reusing, M. (2000). Change detection of natural high forests in Ethiopia using remote sensing and GIS techniques. *International Archives of Photogrammetry and Remote Sensing*. Vol. XXXIII, Part B7: 1253-1258.

Richards, C.M. and Moore, W.S. (1996). A phylogeny for the African treefrog Family Hyperoliidae based on mitochondrial rDNA. *Molec. Phylog. Evol.* 5(3): 522-532.

Rödder, D., Kielgast, J., Bielby, J., Schmidtlein, S., Bosch, J., Garner, T.W.J., Veith, M., Walker, S., Fisher, M.C. and Lötters, S. (2009). Global amphibian extinction risk assessment for the panzootic chytrid fungus. *Diversity* 1: 52-66.

Schwaller, S. (2009). Environmental change in Harenna Forest, Ethiopia. Unpublished MSc thesis, Swiss Tropical and Public Health Institute, University of Basel, Basel.

Seleshi, Y. and Demarée, G.R. (1995). Rainfall variability in the Ethiopian and Eritrean highlands and its links with the southern Oscillation Index. *J. Biogeogr.*, Vol. 22, No. 4/5, Terrestrial Ecosystem Interactions with Global Change, Volume 2 (Jul. - Sep., 1995), pp. 945-952.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodriguez, A.S.L., Fishman, D.L. and Waller, R.W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1783-1786.

Swofford, D. (2002). PAUP*: Phylogenetic Analysis Using Parsimony (*and other Methods). Version 4.0b10. Sinauer Associates, Sunderland, Mass.

Tadesse, G. (2001). Land degradation: A challenge to Ethiopia. *Environ. Manag*. 27(6): 815–824.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011**).** MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* (submitted).

Templeton, A.R. (1983). Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* 37: 221-244.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876-4882.

Tito, W. (2009). Amphibian community in the Abijata-Shalla Lakes and Awash National Park as indicator of the health of the environment. Unpublished MSc thesis, School of Graduate Studies, Addis Ababa University. Addis Ababa.

Vences, M., Kosuch, J., Rödel, M-O, Lötters, S., Channing, A., Glaw, F. and Böhme, W. (2004). Phylogeography of *Ptychadena mascareniensis* suggests transoceanic dispersal in a widespread African Malagasy frog radiation. *J. Biogeogr*. 31: 593-601.

Vences, M. and Köhler, J. (2006). The current status of genetic exploration in amphibians: taxonomic and geographical disparities (pp. 193-195). In: Herpetologia Bonnensis II - Proceedings of the 13th Congress of the Societas Europaea Herpetologica. Vences, M., J. Köhler, T. Ziegler, W. Böhme (Eds.).

Vences, M., Kosuch, J., Glaw, F., Böhme, W. and Veith, M. (2003). Molecular phylogeny of hyperoliid treefrogs: biogeographic origin of Malagasy and Seychellean taxa and re-analysis of familial paraphyly. *J. Zool. Syst. Evol. Res.* 41: 205-215.

Vences, M., Thomas, M., van der Meijden, Chiari, Y. and Vietes, D.R. (2005). Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Front. Zool.* 2: 5.

Vieites, D.R., Wollenberg, K.C., Andreone, F., Köhler, J., Glaw, F. and Vences, M. (2009). Vast underestimation of Madagascar’s biodiversity evidenced by an integrative amphibian diversity. *PNAS*. Pp. 1-6.

Wake, M.H. (1980). The reproductive biology of *Nectophrynoides malcolmi* (Amphibia: Bufonidae), with comments on the evolution of reproductive modes in the genus *Nectophrynoides*. *Copeia* 1980(2): 193-209.

Weinsheimer, F., Mengistu, A.A. and Rödder, D. (2010). Potential distribution of threatened *Leptopelis* spp. (Anura, Arthroleptidae) in Ethiopia derived from climate and land-cover data. *Endang. Species Res*. 9: 117-124.

Wilkinson, M., San Mauro, D., Sherratt, E., Gower, D.J. (2011). A nine-family classification of caecilians (Amphibia: Gymnophiona). *Zootaxa* 2874: 41-64.

WWF (2010). Ethiopian Montane Moorlands. Online accessed on 09 June 2010. http://www.worldwildlife.org/wildworld/profiles/terrestrial/at/at1008_full.html

Yalden, D.W. (1983). The extent of high ground in Ethiopia compared to the rest of Africa. *SINET: Ethiopian Journal of Science*, 6: 35-39.

Zeisset, I. and Beebee, T.J.C. (2008). Amphibian phylogeography: a model for understanding historical aspects of species distributions. *Heredity* 101: 109-119.

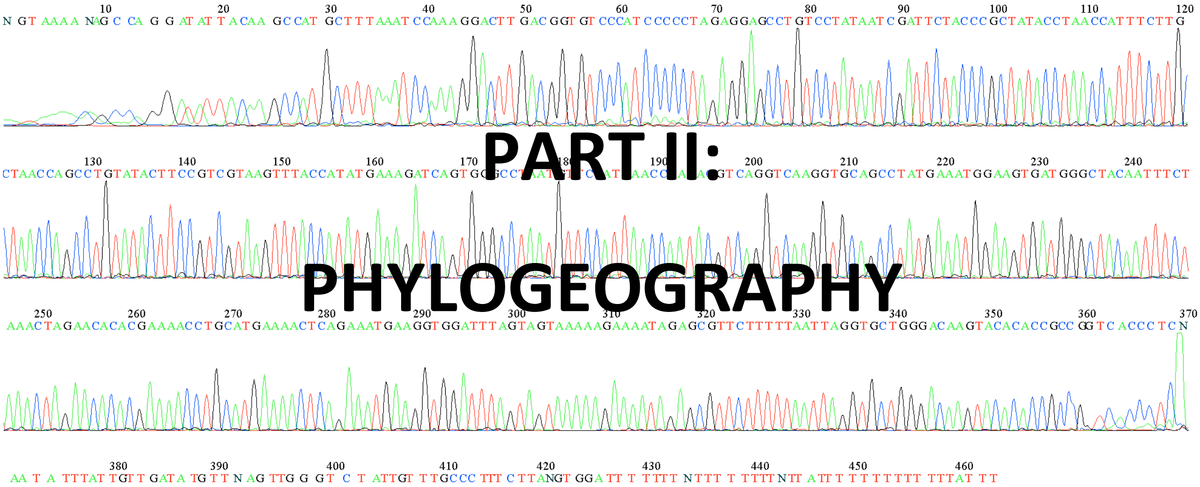
Zimkus, B. (2008). *Phrynobatrachus bullans*: geographic distribution (Tanzania, Kenya, and Ethiopia). *Herpet. Rev.* 39(2): 235.

Zimkus, B. and Blackburn, D.C. (2008). Distinguishing features of the sub-Saharan frog genera *Arthroleptis* and *Phrynobatrachus*: a short guide for field and museum researchers. *Breviora* 513: 1-12.

Zimkus, B. and Schick, S. (2010). Light at the end of the tunnel: insights into the molecular systematics of East African puddle frogs (Anura: Phrynobatrachidae). *System. Biodiv.* 8(1): 39-47.

Zimkus, B., Rodel, M-O. and Hillers, A. (2010). Complex patterns of continental speciation: Molecular phylogenetics and biogeography of sub-Saharan puddle frogs (*Phrynobatrachus*). *Molec. Phylogen. Evol.* 55: 883-900.

PART II: PHYLOGEOGRAPHY



Phylogeography of *Leptopelis* (Arthroleptidae, Amphibia) in the Ethiopian Highlands: Molecular and Morphological Investigation

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ABSTRACT

Six of the 52 known solely sub-Saharan African species of *Leptopelis* are found in Ethiopia, five being endemic to the highlands. These are currently distinguished on the basis of morphological and ecological studies. Using material collected during our field surveys between 2006 and 2010 from several localities on plateaus across the Ethiopian Rift Valley, we conducted a phylogenetic analysis using partial mitochondrial gene sequences of the 12S and 16S genes. Phylogenetic relationships and divergence times were estimated using Maximum Likelihood and Maximum Parsimony methods. Morphological observations were also made on fresh collections, holotypes and non-type museum material. The reconstructed phylogenetic relationships show endemic monophyletic species and populations of *Leptopelis* restricted to the Ethiopian highlands. Cryptic diversity of highland endemics was revealed, highlighting at least two potentially new candidate species. Wide overlaps in several morphological features made identification of specimens of geographically and genetically distinct populations difficult. Relatively small evolutionary distances were found among species, suggesting relatively recent radiation. An estimation of divergence times of species corresponded with timing of major geological events that happened in the area. The ancestor of all highland species of Ethiopian *Leptopelis* split from other African clades approximately 30.3-13.2 Mya, while diversification occurred very late at about 10.4-2.1 Mya. Range extension is revealed for some species in the current study, and there is unfortunately a substantial reduction in others from what has been previously proposed. We observed congruence of phylogenetic groups with clear patterns of geographic distribution, allowing us to potentially identify distinct biogeographic categories separated by barriers.

Key words: Bale Mountains; Biogeography; Central Ethiopian Highlands; Grass (Ridged) Frogs; Kaffa forests; *Leptopelis*; Molecular systematic; Phylogeny.

I) INTRODUCTION

The African tree frogs belonging to the genus *Leptopelis* Günther 1859 have previously been grouped, on morphological basis, under Family Hyperoliidae Laurent 1943. However, *Leptopelis* appeared as a basal group among all genera of hyperolids (Vences et al., 2003; Richards and Moore 1996). Cytosystematic study on hyperolids showed that species of *Leptopelis* have a different karyological structure as compared with species of other hyperolid genera (Odierna et al., 2007), suggesting a divergent relationship. With the advancement of molecular systematics and a thorough revision of amphibian phylogeny, *Leptopelis* is currently grouped under family Arthroleptidae Mivart 1869 (Frost et al., 2006) and not a hyperolid.

There are 52 species of *Leptopelis* distributed solely in sub-Saharan Africa (Amphibiaweb, 2011), six species recognized in Ethiopia (Table 2.1, summarized from Largen, 2001). The taxonomic status of these species has been revised several times in the past 100 years.

Table 2.1. Known species of *Leptopelis* in Ethiopia assessed in the current study, with the respective synonyms, type localities and geographic areas (summarized from Largen, 2001 and IUCN, 2011).

Species	**Synonyms**	**Type locality**	**Distribution and altitude**
Leptopelis bocagii (Günther 1864)	*Cystignathus bocagii* Günther 1864 *Rana ornata* (not Peters 1878) Boulenger 1896b *Leptopelis angolensis* (not Bocage 1893); Ahl 1931 *Leptopelis gramineus* (not Boulenger 1898) Scortecci 1940 *Leptopelis bocagei* Largen 1977 *Leptopelis bocagii* Largen 2001	Duque de Braganca (Angola)	Eastern Africa from Ethiopia to Zimbabwe and northern Namibia. In Ethiopia, in southern and western lowlands. 500-1900 m.
Leptopelis gramineus (Boulenger 1898)	*Megalixalus gramineus* Boulenger 1898 *Pseudocassina ocellata* Ahl 1924 *Pseudocassina rugosa* Ahl 1924 *Leptopelis rugosus* Ahl 1931 *Leptopelis gramineus* Parker 1930; Urban 1967; Largen 1977	Between Badditu and Dime (Ethiopia)	Ethiopian plateau. 1900-3900 m.
Leptopelis ragazzii (Boulenger 1896)	*Hylambates ragazzii* Boulenger 1896a *Leptopelis ragazzii* Largen 1977 *Leptopelis yaldeni* (not Largen 1977) Largen 1977	Shoa Province (Ethiopia)	Ethiopian plateau. 1930-3100 m.
Leptopelis susanae Largen 1977	*Leptopelis susanae* Largen 1977	9 km N of Chencha, Gemu Gofa Province (Ethiopia)	Gughe Mountain in southern Ethiopia. 2600-3000 m
Leptopelis vannutellii (Boulenger 1898)	*Hylambates vannutellii* Boulenger 1898 *Leptopelis vannutellii* Largen 1977	Between Badditu and Dime (Ethiopia)	Southwestern Ethiopia. 1500-2200 m.
Leptopelis yaldeni Largen 1977	*Leptopelis yaldeni* Largen 1977	Debre Markos, Gojam Province (Ethiopia)	Gojam Province in Ethiopia. 2000-2700 m.

With the exception of *L. bocagii*, all are endemic to Ethiopia, in particular to the highlands in Bale, Arsi, Gemu Gofa, Kaffa, Shoa and Gojam areas (Largen, 1977). The known distribution record of *L. bocagii* in Ethiopia is patchy at lower altitudes (Loveridge, 1957). Populations of this species are believed to form several distinct geographic races or even complex species in different parts of Africa (Schiøtz, 1975). The other species of Ethiopian *Leptopelis* mainly inhabit altitudes ranging from 1900 up to 3900 m. *Leptopelis* are referred to as 'leaf-', 'tree-'or 'forest tree-frogs' by different authors (Frost, 2011); however, *L. gramineus* and *L. bocagii*, unlike their arboreal Ethiopian counterparts, naturally lead fossorial or semi-fossorial modes of life (Largen, 1977).

Although there is a better understanding of phylogenetic relations of some species of *Leptopelis* as compared with other African genera of tree frogs (Drewes, 1984; Channing, 1989; Richards and Moore, 1996; Vences et al., 2003), the taxonomic status and evolutionary relationship of the species in Ethiopia remains unexplored. There is little or no information and data on non-taxonomic biological aspects (e.g., behavior, natural history) of these fauna. These research gaps, coupled with the ever-increasing human impact on natural habitats of these amphibians, are thought to threaten the existence of most of these species (Largen, 1977, 2001; Largen and Spawls, 2010; IUCN 2011). An account of the conservation status of these species categorizes *L. susanae* as Endangered, *L. yaldeni* as Near Threatened, *L. ragazzii* and *L. vannutellii* as Vulnerable, and *L. bocagii* and *L. gramineus* as Least Concern (IUCN, 2011).

So far, the most comprehensive assessment of the taxonomy of Ethiopian *Leptopelis* has been made by Malcolm Largen (Largen, 1977, 2001), with the same results reprinted recently (Largen and Spawls, 2010). Previous taxonomy in general has been based on morphological, acoustic and ecological grounds (Günther, 1864; Boulenger, 1896a, 1896b, 1898; Bocage, 1893; Ahl, 1924, 1931; Parker, 1930; Scortecci, 1940; Urban, 1967; Largen, 1977). Earlier findings serve as important background for ongoing and future research on Ethiopian *Leptopelis*; however, presentation of only morphometric data and the corresponding identification key has made field and museum identification of several specimens to be difficult. Wide overlaps are seen in measured quantitative characters as well as qualitative descriptions such as body color and pattern (see Largen, 1977). This problem is emphasized especially in the arboreal forms that occupy varied and patchy habitats.

The geomorphology of the Ethiopian Highlands (Fig. 2.1) is exceptional when compared to the remaining vast physical surface of Africa. It is not only the high density of elevated ground (Yalden, 1983) that makes these highlands impressive, but also the rugged nature of the mountain ranges divided into smaller geographic units. Some observers referred to these landscapes as "the fractured dome" (Kingdon, 1990). In addition to the split into two northwestern and southeastern parts by the Great East African Rift Valley, deep gorges of the Abay (Blue Nile), Awash, Omo, Genale, and other major rivers twist around and slice the hills. Existence of several major river basins, fed by a number of capillary-like tributaries has resulted in a fine network of drainage systems. The valleys or gorges are in most cases wide and are characterized by warmer (and relatively drier) habitats than those found at higher altitudes.

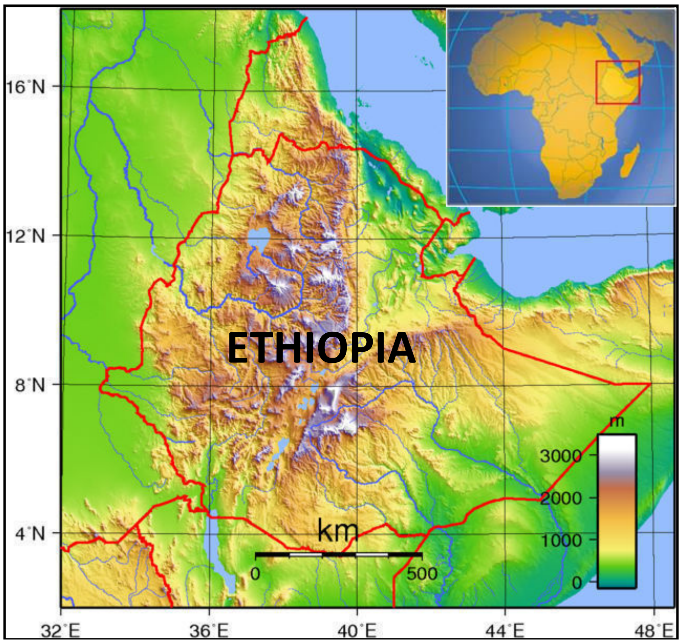

Fig. 2.1. Location map of Ethiopia in the Horn of Africa (inset) and topographic map of Ethiopia showing mountain ranges bisected by the Rift Valley and fragmented by major rivers, and encircled by vast areas of lowlands. (Modified from source: Sadalmelik, I. (2007); downloaded from http://en.wikipedia.org/wiki/File:Ethiopia_Topography.png).

Prediction of the biogeographic distribution of *L. ragazzii* in Ethiopia was made by using a climate envelope model (Weinsheimer et al., 2010). Such prediction models and efforts of conservation prioritization require well-refined input data from taxonomic studies (Hillis et al., 1996). Some species of tree frogs (e.g., *L. ragazzii* and *L. gramineus*) are thought to be distributed across the Ethiopian Rift Valley, a temporally and spatially considerable barrier for genetic exchange to take place between populations of highland-restricted species of amphibians. There are also cases where morphologically similar species are believed to have overlapping ranges (e.g., *L. ragazzii* and *L. susanae* in southwest Ethiopia) (Largen, 1977). Therefore, when one considers the potential role of such barriers as a major factor to facilitate vicariant processes among populations (Cox and Moore, 2005; Poynton, 1999), an understanding of the evolutionary relationship and phylogeographic aspects of Ethiopian *Leptopelis* will become important. Major river gorges could have contributed to further separation of one population or species from the other; for instance, the range of *L. yaldeni* is totally encircled by the Abay Gorge.

Studies that incorporate both molecular and morphological data will provide much better descriptions and interpretations of biological diversity than those that focus on just one approach (Hillis et al., 1996). We have therefore conducted a thorough assessment of the molecular phylogeny and biogeography of *Leptopelis* in Ethiopia for the first time to test the hypotheses that, 1) current (traditional) taxonomy based on morphological characters and ecology corresponds to phylogeny using mtDNA markers; 2) single species do not occur on both sides of major lowland barriers (e.g., the Rift Valley and major river gorges); and 3) diversification of extant highland species in Ethiopia started after the formation of the Ethiopian Highlands by uplifting and volcanic activities about 30 Mya. To test these important research questions, we tried to refine the taxonomic status of *Leptopelis* in Ethiopia based on molecular, morphological and geographic distribution data. The outcomes will help to elucidate diversification patterns, and ultimately this will contribute to prioritizing which threatened endemic species require conservation actions.

II) MATERIALS AND METHODS

A) Field Sampling and Handling of Specimens

Voucher specimens and tissues were collected during field sampling programs conducted in the Ethiopian Highlands across the Rift Valley in 2006, 2008, 2009 and 2010. The samples were collected from the Eastern Rift (Arsi, Bale Mountains, and Bore areas) and the Western Rift (Gughe Mountain, Soddo, Kaffa, West Shoa and Gojam areas) (Fig. 2.1). Details of the methods used in field sampling and handling of specimens are given in Chapter 1, Section IV-C-1.

B) Morphological, Molecular and Biogeographic Data

Morphometric measurements were done for all adult specimens of the recent collections (2006, 2008-2010) and for old specimens (pre-1977) kept in different natural history museums. The studied museum specimens included holotypes or lectotypes for all known species of Ethiopian *Leptopelis,* as well as several paratypes or non-type materials. Principal component analyses and other statistical tests were done in The Unscrambler® X version 10.0.1. See details in Chapter 1, Section IV-C-2 for the list of studied morphological characters and data analysis.

Tissue samples were taken from sub-samples of fresh materials of recently collected (2006-2010) adult (muscle or liver), juvenile (muscle or toe tip), or tadpole (tail) specimens of *Leptopelis* from localities in Bale, Arsi, Gemu Gofa, Kaffa, Soddo, Wenchi, and Debre Markos (Appendix 2.1). We attempted to sample at least all known species of *Leptopelis* in Ethiopia: *L. bocagii, L. gramineus, L. ragazzii, L. susanae, L. vannutellii,* and *L. yaldeni*. Molecular work was conducted using the 12S and 16S mtDNA genes in the facilities of the molecular laboratory at the Section of Biogeography (Department of Environmental Sciences, UB). Details of the methods used for tissue sampling, DNA extraction, PCR amplification and sequencing are presented in Chapter 1, Section IV-C-3.

Locality names and coordinate data (in degrees) were recorded during our field surveys, and additional data were obtained and compiled from historical records of museum specimens reported in the gazzetter in Largen (2001). These data were mapped for each population and species in DIVA-GIS (Hijmans et al., 2005). See details on methodology for biogeographic data collection and analysis in Chapter 1, Section IV-C-4.

C) Molecular Data Analysis

Additional sequence data were included from other sources (Table 2.2), including: SL's unaccessioned sequences of *L. flavomaculatus* (Günther, 1864)*, L. ulugurensis* Barbour and Loveridge, 1928, *L. vermiculatus* (Boulenger, 1909) from their type localities; and available GenBank sequences for the non-Ethiopian *L. argenteus* (Pfeffer, 1893), *L. bocagii, L. vermiculatus, L. palmatus* (Peters, 1868), *L. kivuensis* Ahl, 1929. The outgroups used in this study were GenBank sequences for *Astylosternus schioetzi* Amiet, 1978 and *Arthroleptis palava* Blackburn, Gvoždík, and Leaché, 2010.

Table 2.2. Additional non-Ethiopian species used in the current study for phylogenetic analyses of mitochondrial DNA sequences of the 12S and 16S genes.

Species name	**Locality (Country)**	**GenBank Accession no.**
*Arthroleptis palava**	Cameroon	HM238195.1
*Astylosternus schioetzi**	Cameroon	DQ283349
Leptopelis argenteus	Kenya	DQ283226.1
Leptopelis bocagii	Tanzania	DQ283418.1
Leptopelis kivuensis	Uganda	FJ151060
Leptopelis flavomaculatus	Tanzania	Unaccessioned
Leptopelis palmatus	Sao Tome and Principe	AY603992
Leptopelis ulugurensi	Tanzania	Unaccessioned
Leptopelis vermiculatus	Tanzania	Unaccessioned
Leptopelis vermiculatus	Tanzania	DQ283242.1

* *Arthroleptis palava* and *Astylosternus schioetzi* were used as outgroups, and *Astylosternus schioetzi was used to calibrate divergence time.*

Complete alignment was done for all assembled (contig) sequences combined in CLUSTALX 2.0.12 using the default parameters (Thompson et al., 1997). Model selection for phylogenetic analysis in Maximum Likelihood (ML) was conducted using Akaike Information Criterion in jModeltest (Guindon and Gascuel, 2003; Posada, in press) suggesting the General Time Reversible model based on discrete Gamma Distribution (GTR+G) (Nei and Kumar, 2000) as the best model. Using the Bayesian Information Criterion in MEGA 5 (Tamura et al., 2011), the best method fitting our data was the General Time Reversible model based on discrete Gamma Distribution with Invariant sites (GTR+G+I) (Nei and Kumar, 2000). Topology tests were conducted in PAUP* 4 b10 (Swofford, 2002) using MP (Non-parametric test (Templeton, 1983)) as well as ML (KH-test (Kishino and Hasegawa, 1989) with full optimization, two-tailed, 100 replicates settings). Further details on phylogenetic data analyses and preliminary estimates of evolutionary divergence times are presented in Chapter 1, Section IV-C-3. All aligned nucleotide sequence data generated in this study for Ethiopian *Leptopelis* are deposited in the database of NCBI GenBank (accession numbers: for 12S, JX464968 to JX465036; for 16S, JX464899 to JX464967).

The taxonomic status of newly revealed groups among the studied populations were evaluated for Ethiopian *Leptopelis* based on the general definition for candidate species outlined in Vieites et al. (2009); see Chapter 1, Section III-C-3 in this thesis for definitions.

III) RESULTS

A) Evolutionary relationships and divergence times

A total of 80 mtDNA (12S and 16S) sequences (69 Ethiopian and nine none-Ethiopian *Leptopelis*, and two outgroups) were included in the final analysis. The resulting concatenated sequence alignment for the combined dataset was on average a total of about 959 base pairs long (412 for the 12S, and 583 for the 16S) including gapped sites. The percent nucleotide composition was T(U)=23.7, C=24.0, A=32.9, G=19.4. There were a total of 849 positions included in the final dataset for phylogenetic analysis. Evolutionary distances between species from Ethiopian Highlands and other African species are generally large (5.2 to 9.7%), whereas relatively small distances (1.3 to 3.8%) are seen among the species/populations in the Ethiopian Highlands. Only one putative new Ethiopian species, *Leptopelis* sp. (Soddo) has large divergence values of 6.2-7.4% from other species in the Ethiopian Highlands. Details of the evolutionary distances and variance between groups are given in Table 2.3.

The reconstructed evolutionary relationship for relationships between groups is broadly similar using both ML and MP methods; the MP and ML trees are presented in Fig. 2.2 and Fig. 2.3, respectively. The relationship is consistent with the validity of the five endemic species of Ethiopian *Leptopelis*. This study also reveals putative new taxa that are closely related to some of the known species. All of the studied species of *Leptopelis* from Ethiopia, with the exception of *Leptopelis* sp. (Soddo), form a well-supported monophyletic group (98%). Other species of *Leptopelis* from other parts of Africa, together with *Leptopelis* sp. (Soddo) from Ethiopia, are placed outside the highland cluster. The specimen from Soddo is the only Ethiopian representative that clusters with other species of this genus elsewhere in Africa. The minor inconsistent placement of sample numbers T1099, T1277 and T1280 outside the expected geographic localities could be due to very slight individual variation of the specimens; the genetic difference within each of the groups to which these samples belong is very low (0.001-0.002%).

Table 2.3. Estimates of genetic distance. The analysis involved 80 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 849 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011). A) Between phylogenetic groups. The number of base differences per site averaged over all sequence pairs between groups are shown below the diagonal. Standard error estimate(s) are shown above the diagonal. Analyses were conducted using the Maximum Composite Likelihood model (Tamura et al., 2004). Shaded colors: green = endemic monophyletic species of the Ethiopian Highlands, blue = endemic non-monophyletic with populations of Ethiopian Highlands, yellow = distances lower than 2%. B) Within each phylogenetic group. The numbers of base differences per site from averaging over all sequence pairs within each group are shown. The presence of n/c in the results denotes cases in which it was not possible to estimate evolutionary distances.

	A) Between Groups	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	*L. vannutellii* (Gughe-Kaffa)		0.004	0.006	0.007	0.008	0.007	0.009	0.009	0.016	0.015	0.014	0.016	0.015	0.018	0.017	0.015	0.017	0.033	0.044
2	*L. yaldeni* (Debre Markos)	0.015		0.005	0.007	0.009	0.007	0.008	0.009	0.015	0.015	0.014	0.016	0.016	0.017	0.017	0.017	0.017	0.033	0.045
3	*L.* cf. *ragazzii* (Bale-Bore)	0.021	0.020		0.007	0.008	0.006	0.008	0.008	0.014	0.014	0.014	0.014	0.015	0.017	0.017	0.015	0.018	0.031	0.043
4	*L.* cf. *gramineus* (Shoa)	0.029	0.028	0.024		0.006	0.004	0.005	0.006	0.014	0.012	0.013	0.016	0.015	0.016	0.017	0.015	0.018	0.030	0.043
5	*L.* cf. *gramineus* (Bale)	0.036	0.038	0.029	0.020		0.006	0.006	0.007	0.016	0.013	0.014	0.017	0.016	0.018	0.019	0.017	0.019	0.031	0.044
6	*L. gramineus* (Arsi-Bale-Bore)	0.027	0.030	0.023	0.013	0.020		0.006	0.006	0.015	0.013	0.013	0.016	0.015	0.017	0.018	0.016	0.019	0.032	0.043
7	*L.* sp. (Saja)	0.036	0.035	0.032	0.019	0.023	0.021		0.008	0.016	0.014	0.014	0.018	0.017	0.018	0.019	0.017	0.020	0.032	0.044
8	*L. susanae* (Gughe-Kaffa)	0.035	0.035	0.031	0.021	0.028	0.022	0.031		0.015	0.014	0.014	0.016	0.016	0.016	0.018	0.016	0.020	0.032	0.044
9	*L.* sp. (Soddo)	0.070	0.068	0.063	0.062	0.074	0.067	0.074	0.068		0.007	0.008	0.011	0.011	0.012	0.015	0.017	0.018	0.032	0.043
10	*L. bocagii* (TAN)	0.066	0.064	0.060	0.052	0.058	0.057	0.061	0.059	0.026		0.006	0.011	0.009	0.012	0.013	0.016	0.017	0.030	0.043
11	*L. flavomaculatus* (TAN)	0.061	0.061	0.062	0.057	0.060	0.057	0.064	0.061	0.030	0.022		0.012	0.011	0.014	0.015	0.018	0.017	0.030	0.043
12	*L. vermiculatus* (TAN)	0.070	0.072	0.064	0.072	0.080	0.071	0.081	0.074	0.046	0.049	0.049		0.011	0.014	0.015	0.019	0.019	0.033	0.047
13	*L. palmatus* (SAO)	0.069	0.071	0.070	0.069	0.076	0.068	0.080	0.071	0.049	0.038	0.048	0.049		0.012	0.014	0.017	0.017	0.032	0.042
14	*L. kivuensis* (UGA)	0.080	0.080	0.077	0.074	0.082	0.076	0.086	0.074	0.053	0.054	0.064	0.063	0.050		0.017	0.019	0.018	0.033	0.043
15	*L.* sp. (CAM)	0.080	0.080	0.079	0.081	0.090	0.083	0.091	0.084	0.070	0.062	0.068	0.072	0.066	0.083		0.019	0.020	0.035	0.044
16	*L. argenteus* (KEN)	0.071	0.079	0.073	0.070	0.080	0.075	0.079	0.076	0.084	0.080	0.086	0.092	0.078	0.092	0.097		0.015	0.033	0.041
17	*L. uluguruensis* (TAN)	0.082	0.084	0.084	0.087	0.091	0.090	0.097	0.097	0.088	0.081	0.082	0.090	0.080	0.089	0.103	0.068		0.034	0.044
18	*Astylosternus schioetzi* (CAM)	0.166	0.163	0.157	0.154	0.159	0.160	0.161	0.162	0.160	0.155	0.152	0.169	0.166	0.170	0.178	0.173	0.175		0.041
19	*Arthroleptis palava* (CAM)	0.225	0.229	0.222	0.216	0.220	0.216	0.226	0.223	0.221	0.225	0.220	0.243	0.218	0.224	0.225	0.211	0.225	0.214	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	B) Within Group	0.005	0.002	0.002	0.001	0.001	0.004	n/c	0.005	n/c	n/c	n/c	0.002	n/c	n/c	n/c	n/c	n/c	n/c	n/c


VN34(T1069) - Doshka (Gughe)
VN43(T1073) - Angle (Gughe)
VN38(T1077) - Angle (Gughe)
VN40(T1082) - Angle (Gughe)
VN36(T1070) - Doshka (Gughe)
VN44(T1075) - Angle (Gughe)
VN37(T1072) - Doshka (Gughe)
VN45(T1081) - Angle (Gughe)
VN39(T1078) - Angle (Gughe)
VN42(T1080) - Angle (Gughe)
VN41(T1079) - Angle (Gughe)
VN47(T1074) - Angle (Gughe)
VN46(T1068) - Dorze (Gughe)
VN53(T1067) - Dorze (Gughe)
VN35(T1089) - Mankira (Kaffa)
VN52(T1093) - Koma (Kaffa)
VN51(T1092) - Koma (Kaffa)
VN48(T1090) - Mankira (Kaffa)
VN49(T1096) - Saja (Kaffa)
VN50(T1097) - Saja (Kaffa)
Leptopelis vannutellii
Y57(T1277) - (Debre Markos)
Y56(T1274) - (Debre Markos)
Y54(T1276) - (Debre Markos)
Y55(T1275) - (Debre Markos)
Leptopelis yaldeni
R59(T1099) - Shawe (Bale)
R74(T1011) - Alayu (Bore)
R76(T1197) - (Wendo Genet)
R78(T1196) - (Wendo Genet)
R77(T1010) - Alayu (Bore)
R75(T1012) - Alayu (Bore)
R72(T909) - WWF camp (Bale)
R73(T1212) - Katcha (Bale)
R64(T827) - Katcha (Bale)
R58(T931) - Fute stream (Bale)
R60(T1207) - Rira (Bale)
R70(T1100) - Shawe (Bale)
R71(T917) - WWF camp (Bale)
R66(T921) - Katcha (Bale)
R65(T939) - Rira (Bale)
R61(T938) - Rira (Bale)
R69(T923) - Katcha (Bale)
R62(T941) - Rira (Bale)
R63(T907) - WWF camp (Bale)
R67(T943) - Rira (Bale)
R68(T944) - Rira (Bale)
Leptopelis cf. ragazzii
SS11(T1071) - (Gughe)
SS12(T1199) - (Gughe)
SS13(T1202) - Saja (Kaffa)
Leptopelis susanae
Gsh22(T1281) - Wenchi (Shoa)
Gsh23(1282) - Wenchi (Shoa)
Gsh24(T1280) - Wenchi (Shoa)
Leptopelis gramineus (Shoa)
Gb16(T910) - WWF camp (Bale)
Gb17(T927) - Rira (Bale)
Gb18(T937) - Rira (Bale)
Gb15(T905) - WWF camp (Bale)
Gb20(T1211) - Katcha (Bale)
Gb19(T1210) - Katcha (Bale)
Gb21(T940) - Rira (Bale)
Leptopelis gramineus (Bale)
SJ14(T1098) - Saja (Kaffa)
Leptopelis sp. (Saja)
Gab31(T1279) - Didaa (Arsi)
Gab27(T815) - (-)
Gab30(T831) - Dinshu (Bale)
Gab33(T821) - Rira (Bale)
Gab25(T996) - Gutu (Bore)
Gab26(T998) - Bidika (Bore)
Gab29(T1002) - Koti Balcha (Bore)
Gab28(T1009) - Irba Moda (Bore)
Gab32(T997) - Gutu (Bore)
Leptopelis gramineus (Arsi-Bale-Bore)
Ethiopian Highlands
X3(T947) - Cameroon
Leptopelis sp.
P5(AY603992.1) - Sao Tome and Principe
Leptopelis palmatus
K4(FJ151060.1) - Uganda
Leptopelis kivuensis
VR7(--) - E.Usambara (Tanzania)
VR6(DQ283242.1) - Tanzania
Leptopelis vermiculatus
SD9(T1278) - (Soddo)
Leptopelis sp. (Soddo)
B10(DQ283418.1) - Tanzania
Leptopelis bocagii
F8(--) - E.Usambara (Tanzania)
Leptopelis flavomaculatus
A2(DQ283226.1) - Kenya
Leptopelis argenteus
U1(--) - Uluguru (Tanzania)
Leptopelis uluguruensis
As79(DQ283349.1) - Cameroon
Astylosternus schioetzi
Ar80(HM238195.1) - Cameroon
Arthroleptis palava
95
60
64
59
68
89
99
77
96
94
98
99
99
72
94
74
76
99
79
55
71
60
86
63
61
80
100
81
72
99
52

Fig. 2.2 (previous page). Maximum Parsimony tree: Phylogenetic relationship of Ethiopian species of *Leptopelis* inferred using partial mtDNA of the 12S and 16S genes. The most parsimonious bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). The percentages of replicate trees in which the associated taxa clustered together with more than 50% values in the bootstrap (1000 replicates) are shown next to the branches (Felsenstein, 1985). The MP tree was obtained using the Close-Neighbor-Interchange algorithm (pg. 128 in ref. Nei and Kumar, 2000) with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). The analysis involved 80 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 849 positions in the final analyzed dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011).

Two major clusters are formed by populations of *Leptopelis* from the Ethiopian Highlands. The first cluster consists of the strictly arboreal *L. vannutellii* (Kaffa-Gughe), and the semi-arboreal *L. yaldeni* (Debre Markos) and *L. ragazzii* (Bale-Bore) in a weakly-supported grouping (77%). The second unexpected cluster is formed by the ground-dwelling *L. gramineus,* and the arboreal species *L. susanae* (Gughe and Kaffa) and *Leptopelis* sp. (Saja) in a weakly-supported grouping (74%).

Patterns of arboreality and fossoriality in this radiation of Ethiopian *Leptopelis* tested with suboptimal topologies of a monophyletic arboreal, and fossorial grouping could be rejected as being significantly suboptimal using Templeton Tests (Templeton, 1983) and KH tests (Kishino and Hasegawa, 1989).

One of the major outcomes revealed in the evolutionary relationship of populations of *Leptopelis* in Ethiopia is the split seen in *L. gramineus*. In most cases this split is associated with widely separated geographic areas, namely, Bale (Eastern Rift), Arsi-Bore (Eastern Rift), and Shoa (Western Rift). An exception is that there are two populations of *L. gramineus* (from Arsi-Bale-Bore, and Bale only) sympatrically occurring in Bale. However, the genetic distances estimated among these populations are relatively small (1.3-2.0%).

Evolutionary divergence times estimated for Ethiopian and non-Ethiopian species of *Leptopelis* indicate that continental-level diversification within the sampled *Leptopelis* probably started about 35.3-15.4 Mya (Table 2.4 and Fig. 2.4). Following this, about 30.3-13.2 Mya, species of the Ethiopian Highlands were separated from other African species. The radiation and diversification of the species in the Ethiopian Highlands probably began about 10.4-4.5 Mya and all of the extant species were formed by about 4.8-2.1 Mya. This indicates that the Ethiopian species are a relatively recent radiation, contrasting with the appearance of the first *Leptopelis* around the K-T boundary (about 71.3 Mya).

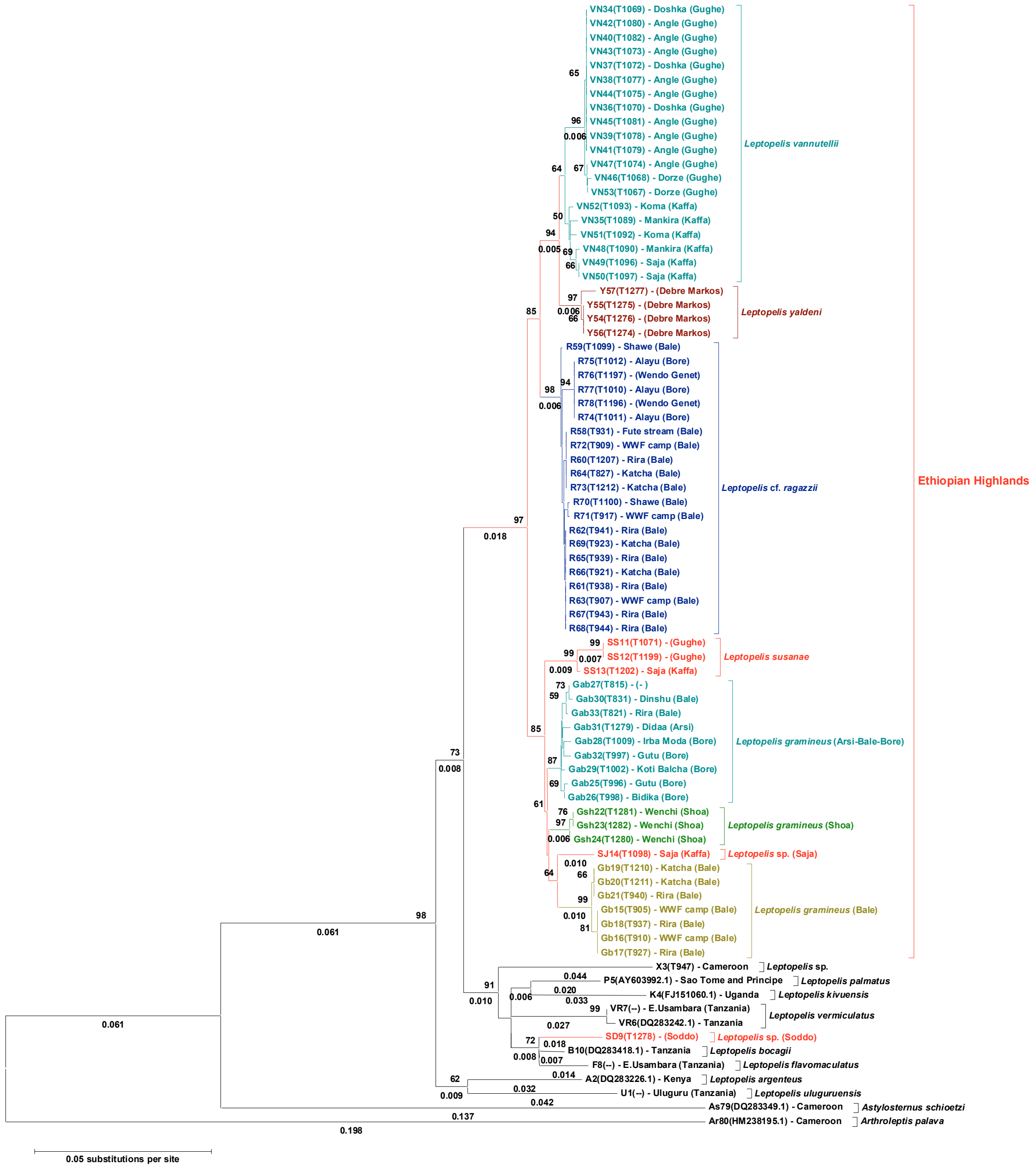

Fig. 2.3. Maximum Likelihood tree: Phylogenetic relationship of Ethiopian species of *Leptopelis* inferred using partial mtDNA of the 12S and 16S genes. The evolutionary history was inferred by using the Maximum Likelihood method based on the Data specific model (Nei and Kumar, 2000). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap (500 replicates) is shown above the branches (Felsenstein, 1985). Branch support values are shown only when greater than 50%. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (below the branches). Branch length values are not shown when lower than 0.005. The analysis involved 80 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 849 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2007).

Table 2.4. Preliminary estimates of divergence time for Ethiopian *Leptopelis,* inferred from secondary calibrations of the interval of time estimated for the split between Astylosterninae and Leptopelinae at 99.3-43.3 Mya (Bossuyt et al., 2006). Colors of rows (events) correspond to timescale zones in Fig. 2.4.

Events of split and diversification	Interval of time (Mya)	Mid-point (Mya)
Split between *L. vannutellii* and *L. yaldeni*	4.8 – 2.1	3.5
Split between *L. ragazzii* and *L. vannutellii - L. yaldeni*	7.4 – 3.2	5.3
Split between major populations of *L. gramineus*	5.7 – 2.5	4.1
Split between *L. susanae* and *L. gramineus*	7.9 – 3.4	5.7
Split between arboreal and fossorial forms of *Leptopelis* in the Ethiopian Highlands	10.4 – 4.5	7.5
Split between *Leptopelis* in the Ethiopian Highlands and other African regions	30.3 – 13.2	21.8
Start of diversification of *Leptopelis* in Africa	35.3 – 15.4	25.4
Split between Astylosterninae and Leptopelinae	99.3 – 43.3	71.3

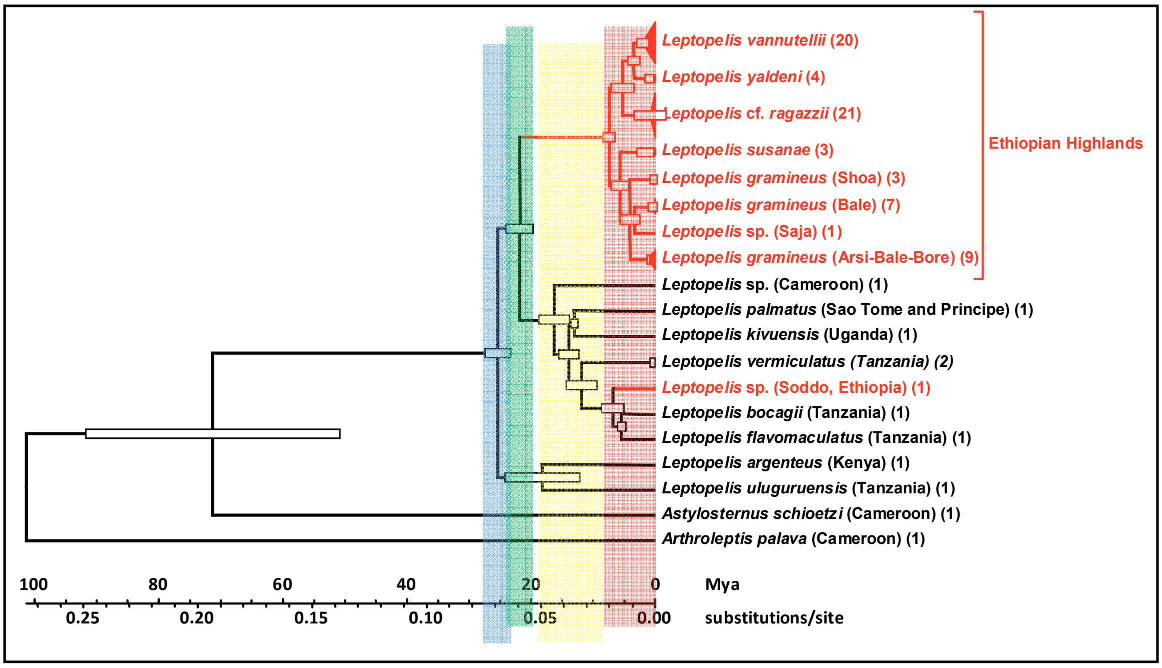

Fig. 2.4. Molecular timescale for evolution of Ethiopian and some other African *Leptopelis,* preliminarily estimated using secondary calibrations of the split between Astylosterinae and Leptopelinae around the K-T boundary (Bossuyt et al., 2006). The evolutionary relationship used for the estimation is reconstructed using MP consensus tree with 1000 bootstrap replicates. Numbers in parentheses indicate number of samples, and Ethiopian taxa are marked in red. Blue, green and red colors of timescale zones correspond to events in Table 2.4; yellow zone shows diversification time of most non-Ethiopian species.

B) Morphology

The majority of comparisons of various morphometric parameters done for different pairs of species did not provide reliable diagnostic characters to distinguish most of the species. Wide overlaps made it difficult to make distinctions among most species (Fig. 2.5). Qualitative characters such as body color, except some patterns, also add to the confusion. The single new specimen of *Leptopelis* from Soddo appears to be a juvenile, and therefore we did not include its morphology in the present analysis.

Principal component analysis of the studied morphometric characters indicates that there is non-ambiguous morphological distinction between two bigger groups, the fossorial populations of *L. gramineus* and the arboreal species of *Leptopelis* as a whole (Fig. 2.5 and Fig. 2.6). As compared with the arboreal species, *L. gramineus* has shorter hind limbs (femur + tibia + foot) (in terms of FmL:SVL, TbL:SVL and FtL:SVL), larger (long, wide and deep) inner metatarsal tubercles (IMTL:SVL and IMTL:T1L), and less extended discs on tips of toes (DT4D:T1L) (Fig. 2.5). Summarized descriptive data and results of all pairwise tests performed for all of the studied morphometric characters are presented in Appendix 2.2.

Among the arboreal species, and their populations, there is overlap of morphometric characters as well as body color and patterns (central positioning of scores in Fig. 2.6). For instance, species occurring in geographically distant areas such as *L. yaldeni* (in Gojam) and *L.* cf. *ragazzii* (in Bale), with over 400 km aerial distance and a series of geographic barriers, did not show consistent and non-overlapping variation in any of the measured characters.

Between populations of *L. ragazzii* across the Rift Valley, the studied specimens appear to show some variation. The eastern population generally has larger TbL:FML ratio and smaller DT4D:T1L than the western. It should be noted that the specimens from the east are fresh materials where as those from the west are old museum collections. Within the fragmented populations of *L. gramineus* across the Rift Valley and segments of mountain ranges, there is hardly any variation in most of the studied morphometric features. Only the population from Wenchi (Shoa) appears to have the shortest hind limbs (smallest TbL:SVL and FtL:SVL), and the biggest inner metatarsal tubercle (largest IMTL:SVL and IMTL:T1L) of all (Fig. 2.6).

Some color patterns, when present, appear to be clearly distinct for a group. *Leptopelis* sp. (Saja) is distinct from others by the bold black and white marbled pattern on the ventrum, dark-grey dorsum with wide posteriorly bifurcating black patterns, and bold black bands on the inter-orbital area and on arms and legs. A brownish dorsal triangular or longitudinal pattern is also characteristic of some individuals of *L. vannutellii* and *L. ragazzii*, followed by three or more posterior transverse stripes. *Leptopelis susane* from Gughe has brick red or green body color with distinct dark lateral blotches and three dorsal longitudinal marks, while a specimen recorded from a new locality is somewhat different, having green body with a wide bold black "M"-shaped mark between the eyes and tip of the snout. Otherwise, the coloration and patterns in the arboreal species are not consistent for all populations and individuals of a species, sometimes present, absent or modified.

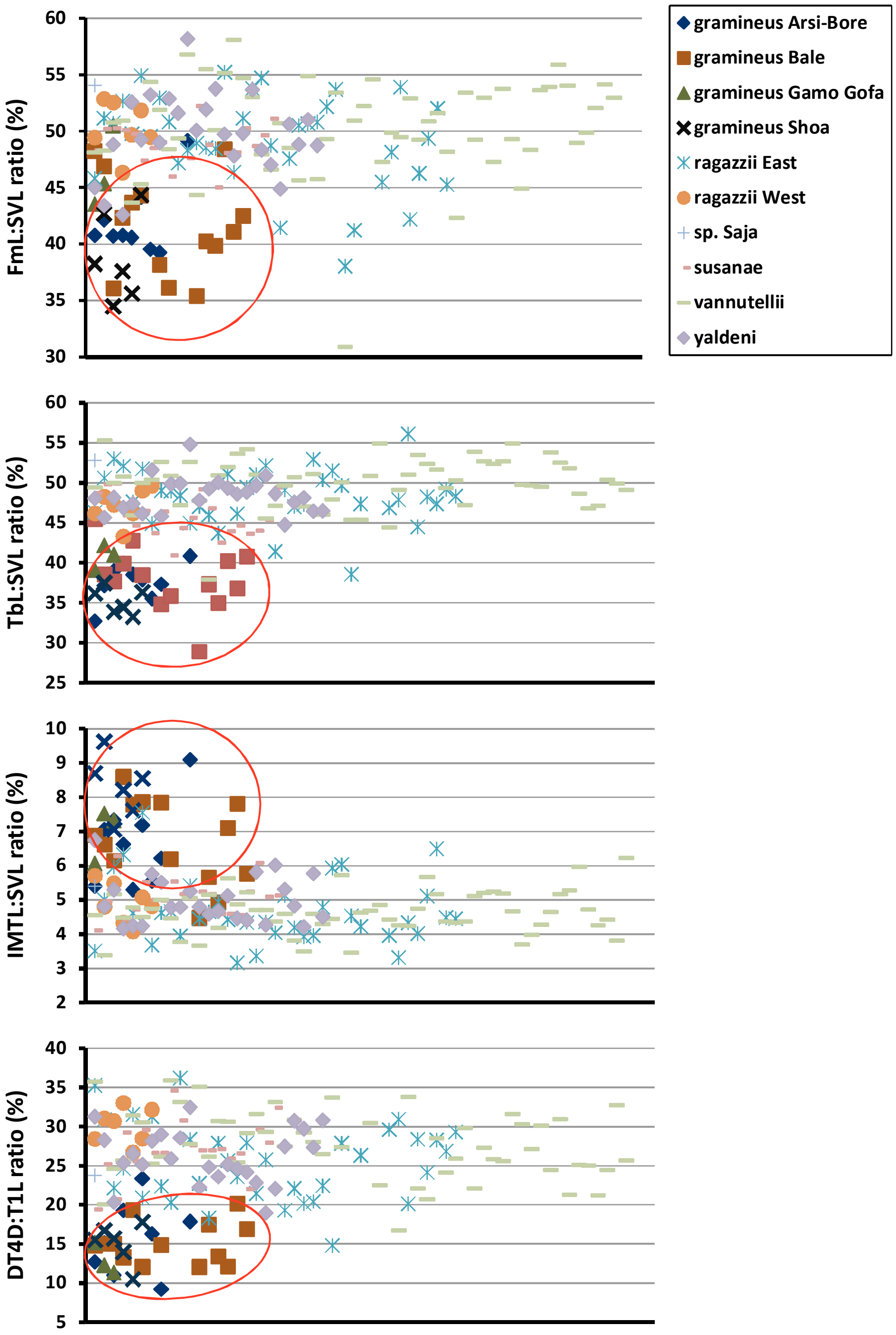

Fig. 2.5. Scatter plots of morphological features that can be used to make distinction between *Leptopelis gramineus* (fossorial) and other (arboreal) species of *Leptopelis* in Ethiopia. Points encircled in red (with some outliers) represent *L. gramineus*, the ones outside being arboreal forms. Features (top to bottom): ratios of FmL:SVL, TbL:SVL, IMTL:SVL, and DT4D:T1L.

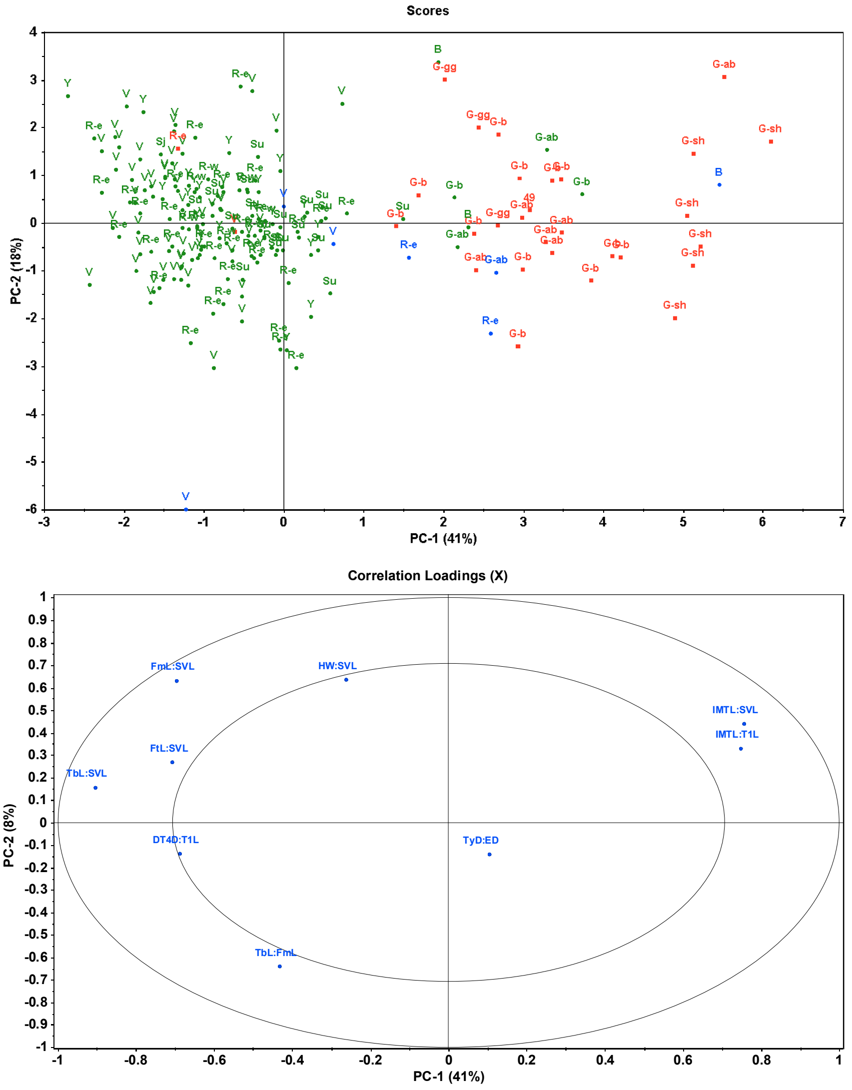

Fig. 2.6. Principal component analysis of some morphometric characters of Ethiopian *Leptopelis*. The scores plot (top) depicts distinct groupings of fossorial *L. gramineus* to the right side (red) and arboreal species to the left (green). Letter abbreviations represent species of *Leptopelis*: B: *bocagii*, G-ab: *gramineus* (Arsi-Bale-Bore), G-b: *gramineus* (Bale), G-gg: *gramineus* (Gamo Gofa), G-sh: *gramineus* (Shoa), R-e: *ragazzii* (Eastern Rift), Su: *susanae*, Sj: sp. (Saja), V: *vannutellii*, Y: *yaldeni*. The loadings plot (bottom) shows the characters associated with the above groupings. The points placed in between the two ellipses represent characters that brought about the principal components for the major groupings.

Two color phases were observed in the three localities where *L. gramineus* was sampled (Fig. 2.7) in the current study. The first one is plain green or with some light brown marks. The second phase is dull light grey to brown background marked with deep dark brown to black patterns, with no trace of green. In both cases, however, we find blue coloration at the posterior side of the thighs. The mtDNAs of these two phases didn't show any difference for specimens sampled from similar or closer localities. The same holds true for the two color phases seen in the other species of *Leptopelis* in Ethiopia.

Fig. 2.7. Color phases of *L.* cf. *gramineus* (Shoa) from Hora village near Lake Wenchi, Shoa (altitude 3100 m). Left: green phase with brown blotches on the sides (ZNHM-A2010-125); right: brown phase with dark blotches on the sides and patterns on dorsum and legs (ZNHM-A2010-126). Note presence of the blue coloration on the posterior of the thighs in both specimens. Photo by: Abebe A. Mengistu 2010.

C) Geographic Distribution

Following new records of localities for some populations and the new groupings that resulted from genetic and morphological data, we can see that valleys and major river systems might have served the main role for the distinction of populations of *Leptopelis* in the Ethiopian Highlands (Table 2.5). In general, we have come across some insights into the geographic distribution and altitudinal ranges of the studied species (Figs. 2.8 – 2.11).

Leptopelis vannutellii is recorded from two mountain ranges separated by the Omo River Valley: forests in Gughe Mountain (Doshka and Angle forests near Dorze), and Kaffa (Mankira, Saja, Koma forests) (Fig. 2.8). The altitudinal range of these populations goes up to about 2500 m, or even higher in Gughe Mountain; Largen (2001) reported the presence of populations of *L. ragazzii* from altitudes above 2200 m in Gughe. There were no records of *L. ragazzii* from Gughe in the current survey; this can be attributed to either a previous misidentification, or incomplete sampling in the current study. One old museum specimen collected from Didesa Valley in Welega has been previously assigned to *L. ragazzii* based on geographic area (Scortecci, 1930; Largen, 2001). This locality is geographically closer to Kaffa area and the recorded altitude of Didesa Valley is 900-1200 m, an elevation range far lower than that known for *L. ragazzii* (see below). Therefore, we put this specimen together with *L. vannutellii*. The type locality of *L. vannutellii* has been documented as ‘between Badditu and Dime’, two localities (ca 05°55’N 37°55’E and 06°18’N 36°16’E, respectively) that are separated by a distance of over 200 km across the Rift Valley. The current result shows that this species solely occupies the southwest highlands and is not recorded from the eastern rift. This could be indicative of the location of the type locality to be in the southwest highlands, but, the exact location remains uncertain.

Table 2.5. Categorization of biogeographic segments* in the Ethiopian highlands identified in the current study based on phylogeny and distribution of species of *Leptopelis* recorded in each segment.

Locality	Category of highland segment	Major Geographic Barrier(s)	Habitat Types	Altitude (m) (Current data only)	Known Records of Species of *Leptopelis*
Arsi-Bale Mountains[2]	South-Central	East African Rift Valley Genale River Gorge Shebele River Gorge Southern lowlands	Montane grassland, forest, Afroalpine moorland, streams, swamps with small shrubs	1680 - 3170	*L. gramineus* (Bale) *L.* cf. *ragazzii* (Eastern Rift)
Bore	Southern	Genale River Gorge East African Rift Valley Southern lowlands	Montane forest, grassland, streams and swamps	2200 - 2700	*L. gramineus* (Bore) *L.* cf. *ragazzii* (Eastern Rift)
Gojam	Northwestern	Abay River Gorge Western lowlands	Montane grassland, with cultivated koba, sensel and undergrowth vegetation	1850 - 2700	*L. bocagii* [1] *L. yaldeni*
Kaffa-Gemu Gofa	Southwestern	East African Rift Valley Western lowlands	Montane forest, streams, swamps, or grassland, cultivated enset vegetation	1750 - 2700	*L.* cf. *gramineus* (Gamo Gofa) [1] *L. susanae* *L. vannutellii* *Leptopelis* sp. (Saja) *Leptopelis* sp. (Soddo)
Shoa	Central	Abay River Gorge East African Rift Valley	Montane grassland, small streams or flooded pools,	2500 - 3100	*L.* cf. *gramineus* (Shoa) *L. ragazzii* (Shoa) [1]

* Parts of the Ethiopian Highlands that have never been or were less sampled before include: the some parts of the Northwestern (Gondar), Northern (Tigray), Northeastern (Wollo and North Shoa), Western (Wellega), and Eastern (Hararge) segments.

[1] Samples not represented in our data, but included here with reference to previous records as presented in Largen (1977).

[2] The affinity of *L. gramineus* from Dida'a Plateau (Arsi massifs) is not clear as genetic data lump it together with the population from Bore; however, geographically it can be grouped with those of the Bale Mountains, the closest area across Shebele River gorge.

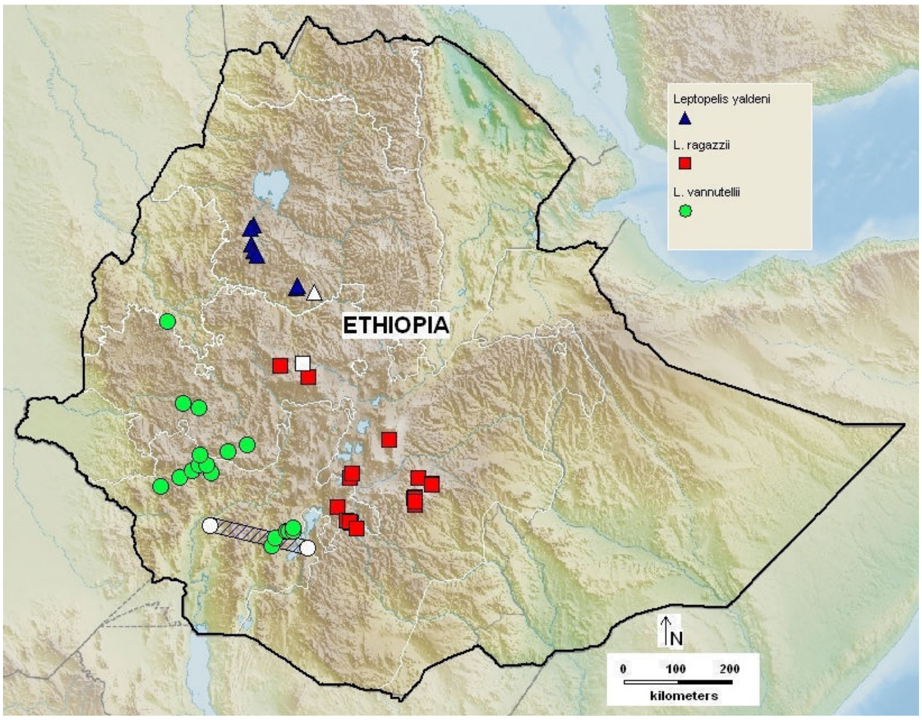

Fig. 2.8. Revised biogeographic distribution of *Leptopelis yaldeni* (triangles), *L. ragazzii* (squares) and *L. vannutellii* (circles) in Ethiopia. Type localities: white triangle = *L. yaldeni*; white square = *L. ragazzii*; white circles and shaded lines = uncertain type locality of *L. vannutellii* between coordinate points of Badditu (right) and Dime (left) areas. White lines indicate regional boundaries, green lines indicate major rivers, and light blue areas indicate lakes.

For ***L. ragazzii***, our sampling was unfortunately limited only to the eastern rift, and did not cover the full range of the species' distribution (Fig. 2.8). We were unable to collect tissue samples from its type locality in the western rift. The type locality has been documented as 'Wourambulchi', but we were not able to find a place with this name following the documented coordinates and elevation in Largen (1977). However, its population in Arsi-Bale-Bore areas (Eastern Rift) is spatially separated from all other arboreal forms of *Leptopelis* without any overlap. Its altitudinal range extends from about 1900 m at Shawe River in Bale (Eastern Rift), to about 3200 m in Arsi, Goba, and Shoa. Old specimens collected from Gughe and Didesa Valley do not appear to belong to this species, and we have put them with *L. vannutellii* (see above).

Leptopelis yaldeni is the northernmost group in the Ethiopian Highlands, occupying the area enclosed by the Abay River Gorge (Fig. 2.8). It has been collected from altitudes ranging between 2000 and 2700 m, and there is no new distribution record for this group. The only other species previously recorded from lower altitudes in this geographic area is the semi-fossorial lowland form *L. bocagii* (see below). Our specimens of *L. yaldeni* were collected from backyard vegetation of koba/enset, *Ensete ventricosum* (Welw.) Cheeseman, and sensel (smiza), *Justicia schimperiana* (Hochst. ex A. Nees) T. Anders, with undergrowth.

Leptopelis gramineus has distinct populations in at least four geographic areas: Shoa, Bale Mountains, Arsi-Bale-Bore and Gamo Gofa (Fig. 2.9). The first population from Shoa is geographically placed in the central part of the western rift. The altitudinal range for this population is between 2000 and 3300 m. The second population from Bale Mountains occupies a wider altitudinal range from about 1950 m at Shawe River to about 3900 m at the Sanetti Plateau. The local climatic conditions and the habitats within this range show abrupt changes on steep sides of the mountain range, from grasslands within montane forests to Afroalpine moorland over the tree line. The third population from Arsi-Bale-Bore area appears as an extension of the population from the Bale Mountains area, occupying an altitude between 1900 and 2900 m around Bore, an area relatively close to the type locality. The fourth region in the southwest (Gughe Mountains, in Gamo Gofa) has been indicated in previous records to cover between 2600 and 3200 m. We did not collect this species from this area in the current study. The overall distribution of *L. gramineus* extends widely on the highlands across the Rift Valley and distinct mountain ranges, and covers a wide altitudinal range with varied climatic conditions (Fig. 2.11). It was difficult to locate the type locality for this species, because it is documented as 'between Badditu and Dime' (see above for *L. vannutellii*). The coordinates of the type locality were therefore not mapped previously. It is not known whether the types of both *L. vannutellii* and *L. gramineus* were collected from exactly the same locality, or not. *Leptopelis gramineus* occupies wide geographic areas on both sides of the Rift Valley. Therefore, unlike that of *L. vannutellii*, it is difficult to suggest the general location of the type locality for *L. gramineus*, and the exact location remains uncertain.

A single specimen representing an undescribed ***Leptopelis* sp. (Saja)** was collected from a broad-leaved montane forest in Saja, about 60 km north of Bonga town (Kaffa area) (Fig. 2.9). It was found on a tree branch near a small permanent river that runs through the forest, and the altitude for this locality is about 1960 m.

A new collection record for ***L. susanae*** extends its previously reported geographic distribution from Gughe Mountain further northwest across the Omo River Valley to Saja forest, Kaffa area (Fig. 2.10). This would give it a fragmented distribution in the southern part of the Western Rift. Our records also extend its altitudinal range lower to about 2270 m at Gughe Mountain, and even lower to about 1950 m at Saja forest.

***Leptopelis* sp. (Soddo)**, a new specimen (clustering close to the non-endemic *L. bocagii* from Tanzania) was collected from an enset tree in a village southwest of Soddo town at an altitude of about 1840 m. This locality is found between the two patches of areas outlined for *L. bocagii* in previous reports. The previously defined altitudinal range for *L. bocagii* therefore remains the same (500-1900 m) (Fig. 2.10).

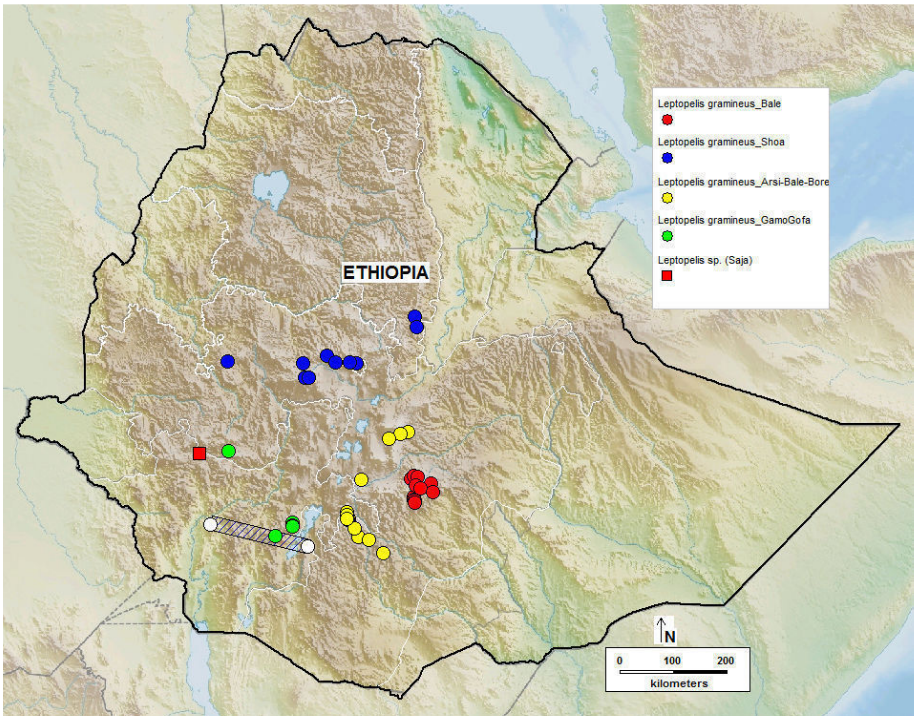

Fig. 2.9. Revised biogeographic distribution of *Leptopelis* sp. (Saja) (square) and *L. gramineus* (circles) in Ethiopia. Different populations of *L. gramineus* are marked with blue, green, yellow and red circles. White circles and shaded lines represent uncertain type locality of *L. gramineus* between coordinate points of Badditu (right) and Dime (left) areas. White lines indicate regional boundaries, green lines indicate major rivers, and light blue areas indicate lakes.

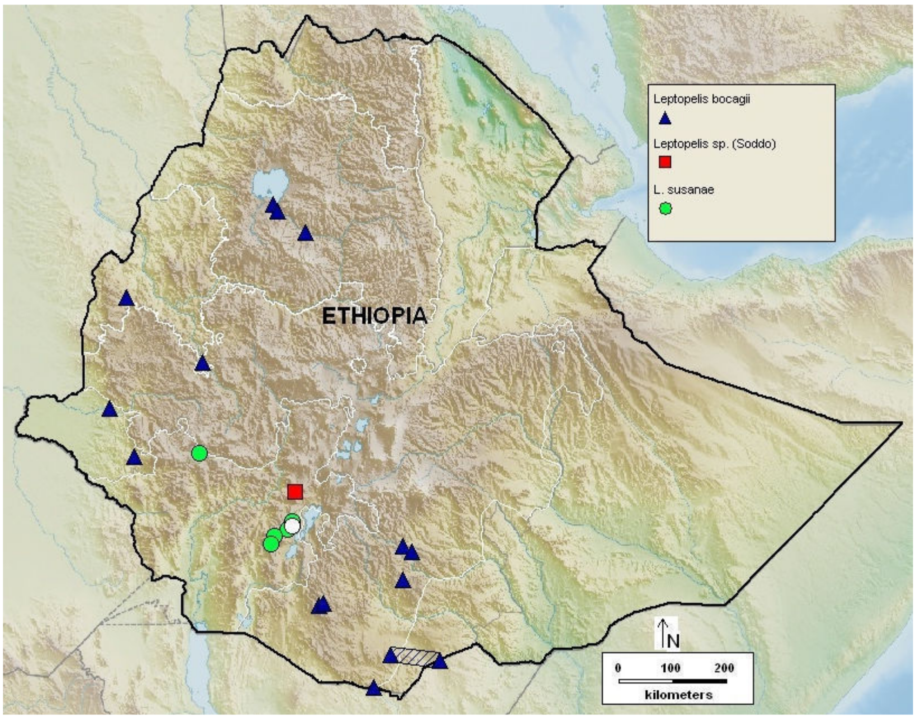

Fig. 2.10. Revised biogeographic distribution of *Leptopelis bocagii* (triangles), *Leptopelis* sp. (Soddo) (square) and *L. susanae* (circles) in Ethiopia. White circle indicate type locality of *L. susanae*; shaded lines indicate uncertain locality between two coordinate points. White lines indicate regional boundaries, green lines indicate major rivers, and light blue indicate areas indicate lakes.

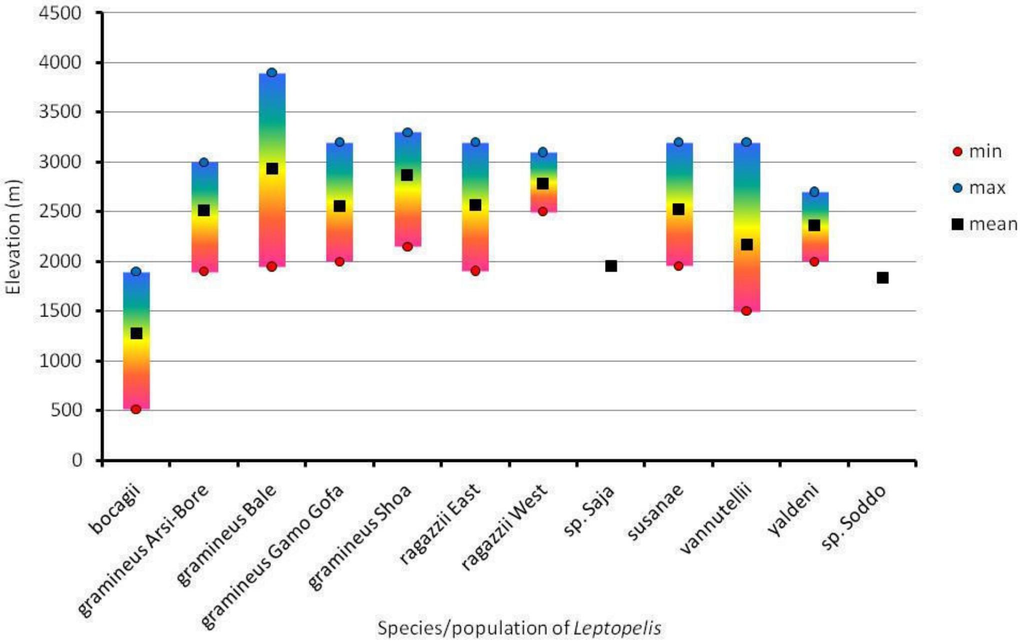

Fig. 2.11. Altitudinal ranges of geographically and genetically disjunct species and populations of *Leptopelis* in Ethiopia. Note that the mean values could be at, above or below the median. Species having only single locality records are shown without elevation ranges. Color gradient: blue-green-yellow-orange-red = cooler high-ground to warmer low-ground.

IV) DISCUSSION

The use of external morphological characters alone for identification of species or populations of *Leptopelis* in Ethiopia appears currently difficult. However, it is possible to apply morphometrics to compare fossorial versus arboreal forms. Within a species, some distinct morphological features, such as brown versus green color phases, could confuse identification of specimens. For instance, the sympatric occurrence of the biphasic coloration within populations of Ethiopian *Leptopelis* has been reported in earlier works (Largen, 1977), and we have observed this feature in fresh specimens as well as a few museum specimens in ZNHM and NHM. The brown color phase of *L. gramineus* has been reported to occur rarely in Bale area (Largen, 1997); in the current survey, however, the brown color phase was recorded in Bale more often. The different color phases have no genetic difference in the currently studied mitochondrial genes.

Most of the morphological characters applied so far might be helpful to distinguish *Leptopelis* from other genera in Arthroleptidae or Hyperoliidae, than for inter-specific diagnosis within Ethiopian *Leptopelis*. Our data strengthen the similar problem that was previously encountered and discussed for *Leptopelis* by Largen (1977). At higher taxonomic levels, when comparing Leptopelinae (*Leptopelis*) with Hyperoliinae (*sensu* Hyperoliidae), Vences et al. (2003) stated that in general the former are known to lack any autapomorphic character. It is likely that this has focused earlier efforts for the diagnosis of species towards supplementary use of coloration and ecological specializations. In the future, given the morphological conservatism in this group, it will be important to look for other possible diagnostic characters including further studies on external morphology, osteology, physiology, bio-acoustics, behavior or life history (in particular larval stages) for more reliable field identification of species of *Leptopelis* in Ethiopia.

In the meantime, it is necessary to suggest a practical means for tentative field identification of specimens in future research programs. That is, using knowledge on existing biogeographic information for known species/populations, newly collected specimens can be temporarily assigned to specific species/population(s). Molecular phylogenetic assessment can then be used in conjunction with available morphological and ecological data to reveal evolutionary relationship and appropriate taxonomic placements (Vieites et al., 2009).

For the purpose of common understanding and relevance to practical conservation plans, we suggest the following criteria for species distinction for Ethiopian *Leptopelis.* These criteria are modified from the general definitions of categories of candidate species proposed for amphibians of Madagascar (Vieites et al., 2009), and specifically based on the current results of genetic, morphological and geographic distribution data that work to distinguish distinct species that were previously diagnosed using morphology. That is, populations of *L. gramineus* have 1.9-3.8% (average ≈ 2.6%) mtDNA gene divergence to all arboreal species. Although there is vague morphological variation within the arboreal forms, their genetic divergence is 1.5-3.6% (average ≈ 2.9%). A genetic variation greater than 2.5% appears appropriate to make species distinctions; but, based on 1) the lowest observed genetic variation among valid species of the studied populations of *Leptopelis* (1.5-2.0% genetic distance seen among some valid arboreal species), and 2) the geographic separation of the studied populations, we propose to adjust the minimum threshold value for phylo-species distinction at 1.5% (Table 2.6). The following discussion is outlined in this context to elaborate the current results.

A) Phylogenetic Grouping

We have identified seven phylogenetic groups (or species), including putative new species, of *Leptopelis* in the Ethiopian Highlands (Fig. 2.12). While there is separate clustering of arboreal and fossorial forms for the majority of the studied populations, two arboreal groups, *Leptopelis* sp. (Saja) and *L. susanae*, exceptionally cluster with the fossorial populations of *L. gramineus*. Unless these relationships change through additional sampling of either individuals or gene fragments, this would suggest the evolution of arboreal and fossorial modes of life in the Ethiopian radiation once, followed by a questionable reversal in *Leptopelis* sp. (Saja). Unlike all other studied populations, there is substantial population-level genetic variation within *L. gramineus* occupying widely fragmented geographic areas. Taxonomic assignment and biogeographic category of each of the studied specimens is given in Appendix 2.1. We summarized the phylogentic structure and the respective geographic distributions of *Leptopelis* in Ethiopia as follows.

1) ***Leptopelis vannutellii*** **(Kaffa-Gamo Gofa)**

These are composed of two major populations that dwell in pristine dense tropical forests in the southwest highlands across the Omo Valley, that is, one from Gughe Mountain and the other from highlands of Kaffa. The two populations clearly show distinct genetic clusters, however their inter-population genetic divergences are probably too low to assign them as distinct species. These two populations were probably separated by the presence of the Omo valley very recently. There are no records of *L. vannutellii* from the eastern side of the Rift Valley. In previous reports that were based on morphology alone, the population from Gughe Mountain was identified as *L. ragazzii* (Largen, 1977). But, there are no records of *L. ragazzii* from this area in the current sampling. The explanation for this could be either incomplete sampling from Gughe in the current survey, or previous misidentification of some specimens of *L. vannutellii* as *L. ragazzii*. Further sampling from Gughe Mountain is needed to confirm the occurrence of *L. ragazzii* in this area; molecular data could help to make distinctions between these closely related and morphologically ambiguous species. Although morphology could not provide good distinction between *L. vannutellii* and other arboreal species, it has at least 1.5% mtDNA gene divergence to all other studied species, and is geographically bound within the southwest highlands of Ethiopia. Therefore, the previously valid taxonomic status of *L. vannutellii* as a species is consistent with the current genetic data.

2) ***Leptopelis yaldeni*** **(Gojam)**

This species forms a distinct group distributed on an isolated highland plateau in Gojam. Specimens collected from different localities within this range show similarities in their morphology, but with varied coloration and patterns. The molecular data used in the current study were collected only from the type locality in Debre Markos town. Whether populations in other nearby localities (Dangila and Enjibara) are conspecific with those from Debre Markos can be clarified by incorporating additional data in the future. But we do not expect significant variation among these populations as their habitats are uniform and continuous without barriers. The sister group of *L. yaldeni* is *L. vannutellii* from across the Abay gorge, but on the same side of the Rift Valley. These two species, along with *L. susanae* from another branch, make up the arboreal forms of *Leptopelis* that are fully restricted to the Western Rift. Morphology could not provide good distinction between *L. yaldeni* and other arboreal species. This species has at least 1.5% mtDNA gene divergence to all other studied species, and is geographically circumscribed by the Abay River gorge encircling Gojam province in the northwest highlands. Therefore, the validity of the previous taxonomic status of *L. yaldeni* as a species is acceptable.

3) ***Leptopelis* cf. *ragazzii* (Eastern Rift)**

When comparing our new samples of *L. ragazzii* with old museum materials, we demonstrated morphological similarities with *L. vannutellii* and *L. susanae.* Therefore, it won't be surprising that earlier reports assumed occurrence of *L. ragazzii* (instead of *L. vannutellii*) in Gughe area. Our specimens of *L. vannutellii* from Gughe actually do not belong to *L. ragazzii*; otherwise we would have found closer phylogenetic relationship between our samples from Gughe (Western Rift) and *L. ragazzii* from the Eastern Rift. The population from Gughe clustered with *L. vannutellii* from Kaffa (Western Rift).

Table 2.6. Definition of criteria for distinction of phylo-species of *Leptopelis* in Ethiopia.

Category	General definition (Vieites et al., 2009)	Definition for Ethiopian *Leptopelis*
Unconfirmed Candidate Species (UCS)	Deep genealogical lineages of unknown status, data deficient for morphology, ecology, and distribution	Uncorrected p-distance in 12S and/or 16S mtDNA gene >1.5% to all other species, coupled with: (i) no data on morphology, and (ii) population(s) isolated in nature from other closely related population(s) by geo-climatic barriers (e.g., deep and warm valleys or gorges, big rivers, or mountain ranges).
Confirmed Candidate Species (CCS)	Show genetic divergence combined with a distinct difference in either morphology or in a character that mediates premating isolation	Uncorrected p-distance in 12S and/or 16S mtDNA gene to all other described species >1.5%. Concordance of this molecular differentiation with: (i) diagnostic difference in at least one morphological character, or (ii) population(s) isolated in nature from other closely related population(s) by geo-climatic barriers.
Deep Conspecific Lineage (DCL)	Deep genealogies above a threshold value typical for comparisons among closely related species in the group of animals under study	Uncorrected p-distance in 12S and/or 16S mtDNA gene >1.5% to all other described species in combination with: (i) no morphological differences, or differences only in characters known to show intraspecific variability: e.g., size, relative hindlimb length, body color and pattern, and/or (ii) population(s) co-occurring with other genetically divergent sister group population(s)

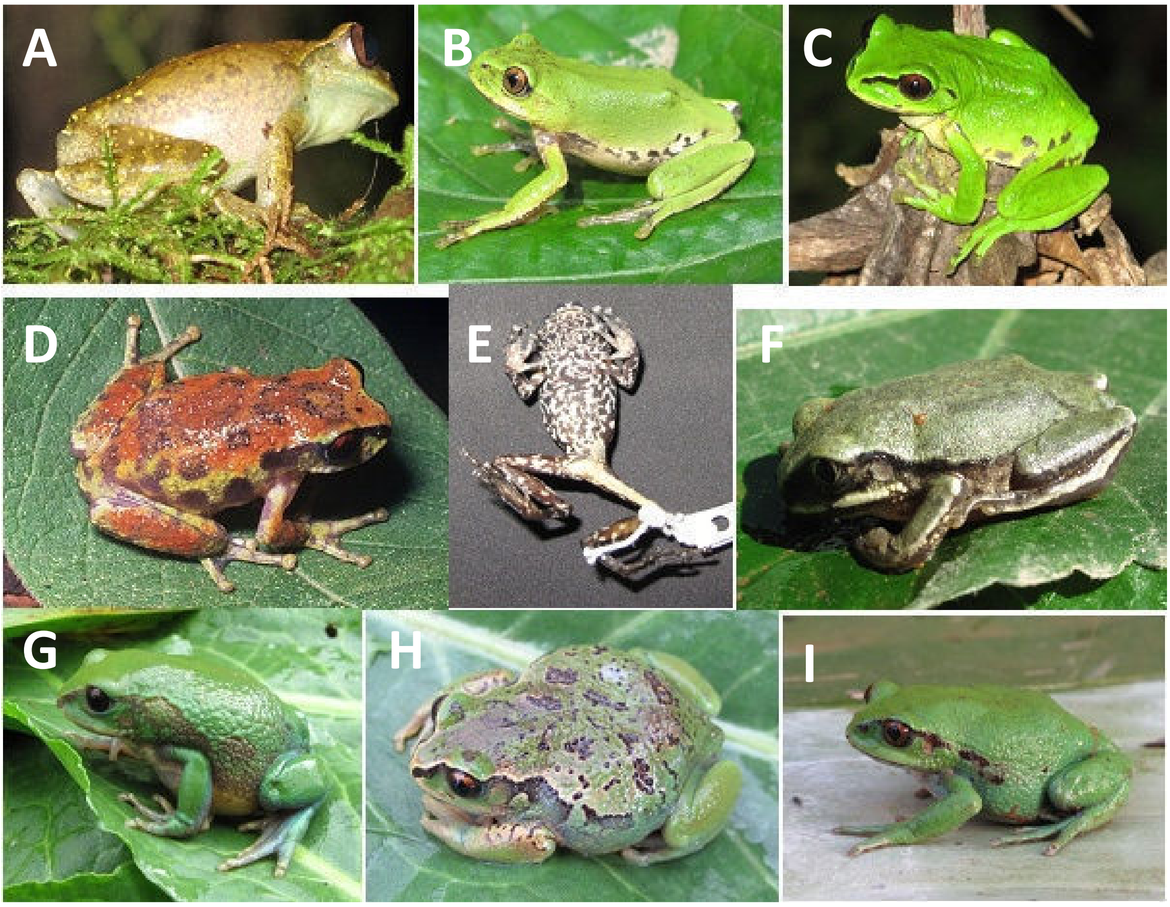

Fig. 2.12. Endemic Ethiopian *Leptopelis*, including putative new species identified in the current study: A) *L. vannutellii* (Koma, AK?); B) *L. yaldeni* (Debre Markos, AM-021); C) *L. ragazzii* (Rira, AK?); D) *L. susanae* (Chencha); E) *Leptopelis* sp. (Saja), ventral view (Saja, AK-1850); F) *Leptopelis* sp. (Soddo) (Soddo, AM-050); G) *L.* cf. *gramineus* (Shoa, AM-125); H) *L.* cf. *gramineus* (Arsi, AM-102); I) *L.* cf. *gramineus* (Bore, AK?). All pictures except (E) taken in life. Photos by: Abebe A. Mengistu 2009-10 (A-C, E-I); Malcolm J. Largen 2008 (D).

The two populations of *L. ragazzii* that reside in Bale and Bore across the Genale gorge show no significant genetic divergence in their 12S and 16S genes; a similar case is observed in one of the populations of *L. gramineus* occupying the same areas (see below). This can indicate that the upper part of Genale River (higher altitude without deep gorge) probably allows continued gene flow between some high altitude populations occurring across the gorge.

Further sampling and molecular studies on populations of *L. ragazzii* from the Western Rift, in particular from its type locality, will reveal whether the population in the Eastern Rift is evolutionarily distant and distinct as a separate entity. The type locality has been documented with disagreeing locality name, coordinates and elevation records, and has to be re-surveyed. Although there is vague morphological distinction between *L. ragazzii* and the other arboreal species of Ethiopian *Leptopelis*, the studied populations in Bale and Bore together have at least 2.0% mtDNA gene divergence to all other studied species, and are geographically restricted to the highlands east of the Rift Valley in Ethiopia. As there is no genetic data from the type locality to support the conspecificity of populations of the eastern rift with *L. ragazzii*, we intend to keep the previous taxonomic status of this species, and provisionally assign the population in the eastern rift as *Leptopelis* cf. *ragazzii* (Eastern Rift).

4) *Leptopelis susanae* (Gughe-Kaffa)

This species has been reported to occur in a tiny and increasingly diminishing forest in the Gughe Mountain area. One specimen collected from Saja forest in Kaffa further west across the Omo valley has genetically clustered with *L. susanae* from Gughe. It might be difficult to conclude with this single record that the range of *L. susanae* extends outside of Gughe Mountain. However, there are instances where range extensions have been reported from records of single specimens, as for *L. karissimbensis* Ahl 1929 in Rwanda (Hölting et al., 2009). In our case, it is possible that unexplored populations of *L. susanae* could exist elsewhere in the southwest highlands at altitudes lower than Gughe. As is the case for *L. vannutellii*, there is the possibility of a recent separation of populations of *L. susanae* across the Omo valley. The dark-brown to brick coloration of this species is its major morphological difference from the other arboreal species of *Leptopelis* in Ethiopia. Additional specimens are needed from the locality in Kaffa to present details of morphological comparison with the population in Gughe. The mtDNA gene divergence of this species to all other studied species is at least 2.1%, and is geographically restricted in the southwest highlands. Therefore, the validity of the previous taxonomic status of *L. susanae* as a species is consistent with the current phylogenetic results.

5) *Leptopelis* sp. (Saja) appears distinct from other related species by its unusual body color pattern. It has a deep dark coloration dorsally, with very deep and clear marbled black and white spotted pattern ventrally. Its feet and toes also appear relatively shorter than those of all other arboreal species. The extended discs on the toes and fingers, its slimmer trunk and legs, as well as the forest area where it was collected are shared attributes with the other arboreal forms. However, the reconstructed phylogenetic relationship (both in ML and MP trees) placed this specimen embedded within populations of the fossorial *L. gramineus*. Since this phylogenetic group is represented by a single specimen, reliable morphological comparison with other species would require collection of additional specimens. But diagnosis of the existing specimen based on morphology is possible. The mtDNA gene divergence of this group to all other studied species is greater than 1.9%, and is geographically restricted to Saja forest (Kaffa) in the southwest highlands. We therefore assigned this material as *Leptopelis* sp. (Saja), a Confirmed Candidate Species awaiting description.

6) *Leptopelis* cf. *gramineus* (Shoa, Bale, and Arsi-Bale-Bore)

One of the morphologically most easily recognized species of tree frogs in Ethiopia is *L. gramineus*. It is usually more robust in body depth and width than the other highland species. Lack of or reduced discs on tips of toes, large inner metatarsal tubercle, and fossorial life, are the most important features to identify this species. Body color has been reported to be bright green with or without some brown patterns, as well as blue coloration on posterior thigh. There is a second color phase that has been reported before (Largen, 1977), with light grey to brown background with broad darker patterns on dorsum, and usually smaller body size. There are also green individuals with similar body size as the brownish ones. Genetically, there is no distinction between different color phases sampled from the same area.

Leptopelis gramineus (Shoa) (western rift) shows some morphological variation from the other two populations studied in the eastern rift. It has the shortest tibia and foot, and the longest inner meta-tarsal tubercle of all. The ranges of these attributes overlap to some extent with those of the other two populations, and could just be population-level variations. Otherwise no significant morphological variation is observed in the studied characters for all populations. Wider morphological assessment of all known populations and those from unexplored areas is needed to strengthen these distinctions.

The three populations of *L. gramineus* sampled from Shoa, Bale, and Arsi-Bale-Bore areas show divergence in the sampled partial mitochondrial genes; but their genetic distances do not appear large enough to assign them as distinct species. Relationships are poorly resolved because of only limited differentiation and corresponding weak support for relationships between the three populations. There are two arboreal species, *L. susanae* and especially the putative new taxon *Leptopelis* sp. (Saja), that make elucidating the evolutionary relationships of populations of *L. gramineus* a complex group to understand. Poor resolution of evolutionary relationships obscures a clear understanding of the relationships. Historical aspects of the distribution of these populations across the Rift Valley requires thorough investigation that takes into account the relevance of the formation of physical and climatic barriers before and after the last glacial maximum. But, when considering the age and extent of the geographic and/or climatic barriers between them (the Rift Valley and Genale River gorge), it at least indicates that these areas have allowed formation of distinct phylogeographic groups. There is no molecular data for the population from the type locality and its reported range in the southwest highlands. As for *L. vannutellii,* the documented type locality for *L. gramineus* (between Badditu and Dime) still remains ambiguous and difficult to specifically locate.

The taxonomic placement of populations of *L. gramineus* is difficult due to, 1) the conserved morphology of the studied populations of *L. gramineus* from Shoa, Bale and Arsi-Bale-Bore areas, 2) sympatric occurrence of the genetically varied (2.0%) Bale and Arsi-Bale-Bore populations as well as geographic separation of the Shoa population, 3) uncertain phylogenetic embedment of the arboreal *Leptopelis* sp. (Saja) within *L. gramineus*, and 4) incomplete sampling of known populations from the southwest highlands. If additional genetic data take out *Leptopelis* sp. (Saja) out of *L. gramineus*, and if morphological, bioacoustic, behavior and/or natural history evidences separate these populations, then we can elevate each of them to the rank of species. For now, based on the considerable mtDNA gene divergence (1.3-2.0%) among most of the studied populations, and the aforementioned reasons, it would be appropriate to assign the populations of *L. gramineus* as Deep Conspecific Lineages.

7) *Leptopelis* sp. (Soddo)

This new specimen was collected in the southwest highlands close to the localities of *L. vannutellii, L. susanae* and *L. gramineus*. It has bright green color with dark marks on the lateral sides, and appears a juvenile that has completely metamorphosed. It was collected from an enset tree in a human-inhabited area. Based on a quick field observation on its morphology (including presence of moderately enlarged discs on the tip of toes and fingers), there is no special character observed to put it outside of *L. ragazzii*, *L. vannutellii*, or even the juvenile green phase of *L. bocagii* (Largen, 1977), that can possibly occur in the same area. There is no previous record of *L. bocagii* from Soddo area to compare our material with. Molecular data, however, grouped this specimen separately outside of any of the highland endemics of Ethiopia. It appears relatively close to *L. bocagii* (from Tanzania), but its evolutionary distance keeps it separate from these non-Ethiopian taxa. There are two options to decide the taxonomic status of this specimen. The *first* option is that, as it is not yet clear whether the Ethiopian populations of *L. bocagii* are conspecific with southern African forms, our material could belong to a distinct Ethiopian population. The *second* option is that it might not fall into any other known species, making it a new species. This specimen does not seem to belong to the *L. bocagii* complex widespread in eastern and southwestern Africa. Referring to the greater than 2.6% mtDNA gene divergence (>4.0% in most cases), and data deficiency in morphology or other aspects, we tentatively assign *Leptopelis* sp. (Soddo) as an Unconfirmed Candidate Species; additional data is required to ascertain its status.

8) *Leptopelis bocagii*

Despite some morphological and habitat similarities between *L. bocagii* and *L. gramineus*, such as robust body size, (partial or complete) fossorial life, lack of or highly reduced discs on fingers and toes, these two groups appear distant in their evolutionary relationships. As has been indicated in earlier works (Largen, 1977, 2001; Largen and Spawls 2010), *L. bocagii* has a patchy distribution at lower altitudes in western and southern Ethiopia, with a more continuous pattern from eastern to southwest Africa. Whether the populations in different African regions (including those in Ethiopia) are conspecifics or form distinct species has not been surveyed genetically. Our study mainly focused on the endemic highland species of *Leptopelis* in Ethiopia, and we have not done morphological observation on *L. bocagii* specimens collected elsewhere. However, adults are sufficiently distinct morphologically on the basis of their body color (cream, golden-brown or red-brown above and associated dark patterns) and sometimes relatively big discs on toe tips (Largen, 1977). Ecologically, unlike *L. gramineus* which is a strictly fossorial high-altitude (1900-3900 m) inhabitant, *L. bocagii* leads a semi-arboreal life at lowland (500-1900 m) savannas and on enset trees (Largen, 1977). The taxonomic status and evolutionary history of Ethiopian populations of *L. bocagii* can be verified upon DNA sampling and morphological comparison with its haplotypes farther south in Africa. Currently, the information at hand is not sufficient to question the presence of *L. bocagii* in Ethiopia.

On Advertisement Calls

In the current study we have encountered in the field individual males of some species of *Leptopelis* making advertisement calls. Several males of the strictly arboreal *L. vannutellii* from Koma Forest were seen calling from high up to 3 m on stems and branches of trees early evening (from about 7:00 pm onwards) in June during the main rainy season in a relatively drier year (2009). A short repeated clack sound is sometimes followed by a completely different repeated squeaking rhythm not observed in any other Ethiopian *Leptopelis*. This rhythm has also been observed earlier and reported in Largen (1977). With detailed acoustics studies, this could be a reliable character to distinguish *L. vannutellii* from other arboreal species, in particular from its closely-related sister group *L. yaldeni*.

The males of *L. yaldeni* were seen calling on the ground covered with thick undergrowth in backyard cultivated vegetation at daytime during the main rainy season in July 2010. A similar feature has been observed for *L. ragazzii* in Bale at night time in June 2009. Some males were seen hiding on the ground under small shrubs, while others were calling on open areas on the ground at the same area close to a slow-moving small river.

A specimen of *L. gramineus* was seen making short and less frequent clack calls from under wet grass close to a small ditch in Dida’a (Arsi-Robe) town early evening (at about 6 pm) in August 2010. Further systematic and detailed acoustics study is needed to look for any possible distinctions between calls of the different species to supplement molecular, morphological and ecological data.

B) Biogeography

The context of ‘Ethiopian Highlands’ in this paper is defined in Chapter 1, Section IV-C-4. In general, our findings indicate that the Ethiopian Rift Valley and other major river gorges seem to have played an important role for influencing diversification patterns in Ethiopian *Leptopelis*. The ‘island’ nature of the segments of mountain ranges partitioned by valleys and major rivers probably has led to the formation of palaeoendemics, a case where populations evolve in their original localities after formation of a geographic or climatic barrier between them (Cox and Moore, 2005). The Ethiopian Rift Valley is believed to have initiated through volcanic and uplifting activities about 30 Mya

(Hofmann et al., 1997; Arndt and Menzies, 2005), developed about 11 Mya (Wolfenden et al., 2004), and extension started about 6-5 Mya (Bonini et al., 2005). Major drainage basins of Ethiopia were established between 30 and 23 Mya (Pik et al., 2003). The massive uplifting and extension of the Ethiopian plateau immediately after about 6 Mya likely resulted in aridification by inhibiting a zonal circulation of moist air and replacement of closed forest woodlands by open grasslands (Sepulchre et al., 2006; Gani et al., 2007).

Corresponding the above geologic events with our preliminary estimates of genetic divergence times for species of Ethiopian *Leptopelis* (Table 2.4), 1) the split between ancestral species of the Ethiopian Highlands and other African regions (30.3-13.2 Mya) probably followed the sequence of geographic isolation events during uplifting, volcanic processes, and formation of major drainage basins; and 2) a further major split between ancestral arboreal (*L. vannutellii*-*L. yaldeni*-*L. ragazzii*) and ancestral fossorial (*L. gramineus*-*Leptopelis* sp. (Saja)-*L. susanae*) clades in the Ethiopian Highlands (10.4-4.5 Mya) probably occurred during the later aridification event that resulted in transition from wooded forests to open grasslands (Sepulchre et al., 2006; Gani et al., 2007). The very recent diversification of populations of highland Ethiopian *Leptopelis* therefore could probably have resulted from reshaped geo-climatic conditions and habitat changes in the past 8 million years, a situation that has probably directed adaptation and evolution of several other organisms, including East African hominins (Gani et al., 2007). After the last split between the *Leptopelis* clade of the Ethiopian Highlands and the non-Ethiopian clades (including *Leptopelis* sp. (Soddo) of Ethiopia), several non-Ethiopian species were formed relatively early (yellow zone in Fig. 2.4). During this period, there was no diversification within the Ethiopian Highlands clade. The appearance of new geo-climatic conditions in this time was followed by formation of new vegetation covers and habitats across mountain segments that were separated by newly formed major river gorges. Later on (10.4 Mya to Recent, red zone in Fig. 2.4), all of the species of *Leptopelis* in the Ethiopian Highlands were formed. Further investigation is needed to fully understand the processes that governed the evolutionary history of species of amphibians in the Ethiopian Highlands in the past 30 million years.

More recently fluctuating historical climatic changes have shaped environmental conditions in tropical Africa, emphasized by cooler and drier conditions during the last glacial maximum (LGM: ca. 23-18 ka) (Street-Perrot et al., 1997; Jolly and Haxeltine, 1997), followed by post-glacial warming and wetting events at the onset of the Holocene around 15-11 ka (Gasse, 2000). There were short-term dry events in Ethiopia around 8.3-8 ka, 7-6.5 ka, and 4.2-4 ka (Said, 1993; Gasse and Van Campo, 1994). In addition, Ethiopia is portrayed as an outlier of the cold habitats that dominated Eurasia during the LGM (Kingdon, 1990). Pollen sequence data from Bale Mountains indicated that repeated alterations between glaciation, Afroalpine vegetation, and extended woody vegetation cover during the Quaternary must have contributed to the high endemic biodiversity of the Ethiopian highlands (Umer et al., 2007). This could supplement the possibility of a very recent diversification of endemic *Leptopelis* in Ethiopia recovered in this study. The current results indicate that the relatively warm and moist habitats in the southwestern segment house the highest diversity of *Leptopelis*. This region could have therefore been a possible historical biogeographic link used for dispersal of populations of *Leptopelis* between western and eastern parts of Africa; in addition, the southwest forests could be potential refugia for amphibians of the Ethiopian Highlands (discussed below).

Accurate estimation of molecular divergences is required in this study, using more suitable gene partitions (e.g. nuclear genes) and multiple calibration points. Once such data are available more robust conclusions regarding correspondence of geographic events and species divergences can be made. Until such data are collected our date estimates remain very preliminary. Regardless of concerns regarding the specific timing of diversification events, the current findings enabled an identification of some major biogeographic areas from the phylogenetic relationships and distribution patterns of *Leptopelis* in Ethiopia (Table 2.5; Fig. 2.13). Such categories can be adjusted with additional information to designate 'amphibian conservation units' (ACUs) and protect species that need conservation priority (discussed in Chapter 4 in this thesis).

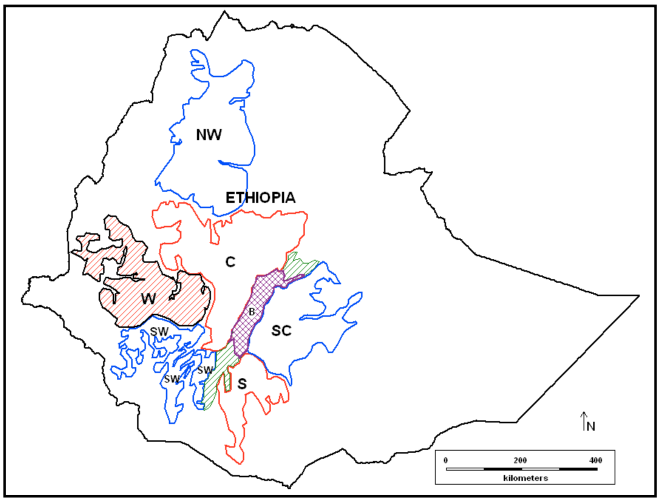

Fig. 2.13. Biogeographic segmentation of the Ethiopian Highlands delimited by the Rift Valley and major river gorges and valleys; figure shows only the areas from which specimens of *Leptopelis* and *Ptychadena* were recorded. NW = Northwestern, C = Central, W = Western (no genetic data from this region), SW = Southwestern, S = Southern, SC = South-central, B = relatively elevated parts of the Rift Valley.

1) Southwestern segment: This part of the Ethiopian Highlands harbors the highest diversity of species of *Leptopelis* in Ethiopia; five of the eight species identified in this study were recorded in this region. It is characterized by evergreen tropical forests, (montane, bamboo, or riverine), montane grasslands, wetlands (rivers, streams, or swamps), and warm humid climate. The natural habitats are highly fragmented by human settlement and 'development' activities rather than by geographic or climatic barriers. As the northern parts of the Ethiopian Highlands have been subject to long-term human impacts, e.g. ancient civilization and intense habitat degradation in the past few thousands of years (Butzer, 1981). The southwest forests could therefore have served as a potential refugia for biodiversity. Historically, this region is assumed to have had Guinio-Congolian faunal and floral link with the central, western and eastern African region during the warming after the last glacial maximum (Gole et al., 2008). It is also important to note that the only Ethiopian specimen of *Leptopelis* sp. (Soddo), that showed closer evolutionary relationship with Eastern African materials, was collected from the southwest highlands. Therefore, after the formation of the Ethiopian Highlands and new habitats, the southwestern region could have served as a transfer area for dispersal of ancestral populations of *Leptopelis* from western and southern Africa to central, northern and eastern segments of the Ethiopian Highlands.

Referring to previous reports (Largen, 1977, 2001; Largen and Spawls, 2010), although *L. bocagii* is regarded as a lowland species, it has been recorded from altitudes reaching up to 1900 m. The only other species reported to get lower down to 1500 m in SW Ethiopia is *L. vannutellii* (Fig. 2.11). Range extensions are revealed in this region for two species, with a new *L. susanae* record from Saja, and a population of *L. vannutellii* from Gughe Mountain. The known distribution of *L. bocagii* in Ethiopia is patchy, forming two lowland blocks, western and southern. If the specimen that we collected from Soddo (southwest) during this study actually belongs to the *L. bocagii* in Ethiopia, then the new record can be indicative of the continuous distribution of this species along the Ethiopian lowlands curving from the west through the southwest to the south. Further sampling from all localities would possibly solve this gap of information.

2) South-Central segment: This part, unlike the southwestern segment, is a more or less unfragmented habitat with a continuum along its axis. Both the montane grasslands in the vast plateaus and pristine forests on steep slopes at higher altitudes provide suitable habitats for fossorial and arboreal species, respectively. Climatically, it is cooler than the southwest highlands. The wide altitudinal range within this area (from 1700 to over 4000 m) and its contoured vegetation belts (from Afroalpine to Montane Forest and to lower grasslands) didn't add much to the diversity of its populations of *Leptopelis*. Only two of the know species inhabit this area, *L.* cf. *gramineus* Bale and *L.* cf. *ragazzii* (Bale-Bore).

3) Southern segment: This relatively small area is located close to and south of the Bale Mountains, but lies lower, and is warmer and drier. To the west of this segment, we find the relatively elevated and narrow part of the Rift Valley (around Lakes Abaya, Chamo and Hawassa). Historically, this area could have served as a possible link for dispersal of post-glacial southwestern populations of *Leptopelis* into Bore and further to Arsi-Bale areas.

4) Central segment: While this area is a continuation of the southwestern segment along the western edge of the Rift Valley, it is highly depleted of its original vegetation due to early human activities and recent establishment of administrative and economic centers in the past few centuries. The populations of *Leptopelis* in this area are therefore recorded mostly from altered and a few natural habitats. For instance, *L. gramineus* has been recorded from the capital Addis Ababa and other nearby towns where amphibian habitats are subject to physical destruction and pollution.

5) Northwestern segment: This is the northernmost area for records of *Leptopelis* in Ethiopia. Most of this area is encircled by Lake Tana and the Abay River and its wide gorge, while the remaining western side is closed by warmer lowlands spanning further west to the Sudan. The geo-morphology of this segment appears relatively uniform throughout, forming a less fractured highland plateau as compared with the northern or southwestern highlands in the country. This area, like the Central segment, has been subject to agricultural and other human activities for thousands of years. This phenomenon is magnified further north. Although the Northwestern segment is geographically fairly distant from the Southern Central segment, we see close relationship of two disjunct species, *L. yaldeni* versus *L.* cf. *ragazzii* (Eastern Rift).

Unexplored segments: In the northern part of Ethiopia, we find an extension of the Central and Northwestern segments, along the western edge of the Rift Valley. There are no records of *Leptopelis* from these areas to date. If there are any populations of *Leptopelis* in these extremes, the major geographic barriers for their distinction would be Lake Tana, and gorges and valleys of the rivers Abay, Beshilo and Tekeze. We will leave the discussion on these two segments until further survey provides data.

C) Implications of the Current Findings

In summary of what has been discussed above on the phylogenetic relationship and biogeography of *Leptopelis* in Ethiopia, it appears that members of this genus in the highlands form a monophyletic group (bootstrap = 99%). They are geographically restricted to the highlands across the Rift Valley. It is evident from the studied mitochondrial genes that at least seven highland species of Ethiopian *Leptopelis* had a common ancestor that diversified into populations and species adapted to geo-climatic conditions in the multi-segmented Ethiopian Highlands. At present these highlands are surrounded by vast and warmer lowlands, and hence geographically completely isolated from other highlands in Africa. The inclusion of more non-Ethiopian species of *Leptopelis* would ensure a more robust test of this endemic Ethiopian phylogenetic pattern. When compared with other species of *Leptopelis* in Africa, the Ethiopian species could be a recent radiation. As to our knowledge, the current findings would be the first such phylogeographic report of one of the widespread genera, (*Leptopelis*) in Ethiopia. The current findings therefore strengthen the value of the Ethiopian Highlands as repositories of endemic diversity of anuran species and as natural laboratories to understand old and recent evolutionary processes. When molecular data on other species of *Leptopelis* in

Africa become more abundant in the future, the current results can be useful as inputs to understand the complete picture of evolutionary history of the genus, as well as to prioritize species for conservation in Ethiopia.

V) CONCLUSION

The Ethiopian Highlands are very important in terms of not only high endemicity of amphibians, but also as home to a recent radiation of species of *Leptopelis*. These species probably diversified from an ancestral population that had its roots in southwestern Ethiopia and dispersed to the central highlands.

Geo-climatic barriers such as relatively warm and wide valleys (e.g., the Ethiopian Rift Valley, Abay Gorge, Omo Valley) associated with relatively warm and dry local climate appear more important than altitudinal variations within the same mountain range for diversification of species and populations of Ethiopian *Leptopelis*.

Our findings for *Leptopelis* demonstrate the complexity of existing taxonomic information for Ethiopian amphibians. To resolve the evolutionary relationships of species and populations within this genus requires further exploration of areas where sampling is needed. Incorporating acoustics studies with molecular and morphometric data will potentially provide clearer ideas on taxonomy of problematic groups.

The current phylogenetic and biogeoographic data, 1) enabled to tentatively suggest a threshold value of 1.5% genetic divergence in the 12S and/or 16S genes to guide species distinctions within Ethiopian *Leptopelis*; 2) will help to refine the biogeography and phylogeny of *Leptopelis* in Africa, emphasizing on the less-assessed Ethiopian region; and 3) could be used for an initiation and designation of geography-based ‘Amphibian Conservation Units’ to protect known amphibian populations that are facing threat from habitat alteration in Ethiopia.

Although the abovementioned findings provide and serve as an important contribution to understanding the taxonomic status and evolutionary relationship of *Leptopelis* in Ethiopia, some important genetic data are still required. These include DNA sequences from type localities of *L. ragazzii* (Shoa), *L. gramineus* (Gamo Gofa) and *L. vannutellii* (Gamo Gofa), as well as from non-type localities of *L. bocagii* (northwest highlands, western and southern lowlands), *L. gramineus* (southwest highlands). As discussed above, more field sampling is also needed for the newly recorded candidate species from Saja and Soddo areas, and for the new locality of *L. susanae* in Saja (Kaffa).

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REFERENCES

Ahl, E. (1924). Über eine Froschsammlung aus Nordost-Afrika und Arabien. *Mitteilungen aus dem Zoologischen Museum in Berlin* 11 (1): 1-12.

Ahl, E. (1931). Amphibia, Anura III, Polypedatidae. *Das Tierreich* 55: I-XVI + 1-477.

Amphibiaweb (2011). Information on Amphibian Biology and Conservation. [web application]. Berkeley, California: Available: http://amphibiaweb.org/. (Accessed: Mar 17, 2011).

Arndt, N. and Menzies, M.A. (2005). The Ethiopian Large Igneous Province. Accessed online on 31 August 2010. http://www.largeigneousprovinces.org/05jan.html

Bonini, M., Corti, G., Innocenti, F. and Manetti, P. (2005). Evolution of the Main Ethiopian Rift in the frame of Afar and Kenya rifts propagation: *Tectonics*, v. 24, doi: 10.1029/2004TC001680.

Bossuyt, F., Brown, R.M., Hillis, D.H., Cannatella, D.C. and Milinkovitch, M.C. (2006). Phylogeny and biogeography of a cosmopolitan frog radiation: Late Cretaceous diversification resulted in continent-scale endemism in the family Ranidae. *Systematic Biology* 55 (4): 579-594.

Boulenger, G.A. (1896a). A list of the reptiles and batrachians collected by Dr. Ragazzi in Shoa and Eritrea. *Annali del Museo Civico di Storia Naturale Giacomo Doria* 36: 545-554.

Boulenger, G.A. (1896b). Second report on the reptiles and batrachians collected by Dr. A. Donaldson Smith during his expedition to Lake Rudolf. *Proceedings of the Zoological Society of London*: 212-217.

Boulenger, G.A. (1898). Concluding report on the late Capt. Bottego's collection of reptiles and batrachians from Somaliland and British East Africa. *Annali del Museo Civico di Storia Naturale Giacomo Doria* 38: 715-723.

Bremer, K. (1988). The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42:795-803.

Butzer, K.W. (1981). Rise and fall of Axum, Ethiopia: a geo-archaeological interpretation. *Am. Antiq.* Vol. 46 (3), pp. 471-495.

Channing, A. (1989). A re-evaluation of the phylogeny of Old World treefrogs. *South African Journal of Zoology* 24: 116–131.

Cox, C.B. and Moore, P.D. (2005). Biogeography: An Ecological and Evolutionary Approach. 7th Ed. Blackwell Publishing. 428 p.

Drewes, R.C. (1984). A phylogenetic analysis of the Hyperoliidae (Anura): treefrogs of Africa, Madagascar, and the Seychelles Islands. *Occ. Pap. Calif. Acad. Sci*. 139: 1–70.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.

Frost, D.R. (2011). Amphibian Species of the World: an Online Reference. Version 5.3 (12 February, 2011). Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/ American Museum of Natural History, New York, USA.

Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., de Sá, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., L. Blotto, B., Moler, P.E., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M. and Wheeler, W. (2006). The Amphibian Tree of Life. *Bulletin of the American Museum of Natural History* 297: 1-370.

Gani, N.D.S., Gani, M.R. and Abdelsalam, M.G. (2007). Blue Nile incision on the Ethiopian Plateau: Pulsed plateau growth, Pliocene uplift, and hominin evolution. *GSA Today*: v. 17, no. 9, doi: 10.1130/GSAT01709A.1.

Gasse, F. (2000). Hydrological changes in the African tropics since the Last Glacial Maximum. *Quaternary Science Reviews* 19: 189-211.

Gasse, F. and Van Campo, E. (1994). Abrupt post-glacial climate events in West Asia and North Africa monsoon domains. *Earth and Planetary Science Letters* 126: 435-456.

Gole, T.W., Borsch, T., Denich, M. and Teketay, D. (2008). Floristic composition and environmental factors characterizing coffee forests in southwest Ethiopia. *Forest Ecology and Management* 255: 2138-2150.

Guindon, S. and Gascuel, O. (2003). A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* 52: 696-704.

Hillis, D.M., Moritz, C., and Mable, B.K. (1996). Molecular Systematics, Second Edition. Sinauer Associates, Inc. Sunderland. 655 p.

Hofmann, C., Courtillot, V., Feraud, G., Rochette, P., Yirgu, G., Ketefo, E. and Pik, R. (1997). Timing of the Ethiopian flood basalt event and implications of Plume birth and global change: *Nature* 389: 838–841, doi: 10.1038/39853.

Hölting, M., Böhme, W. and Schmitz, A. (2009). Range extension for the tree frog *Leptopelis karissimbensis* Ahl 1929 in Rwanda (Anura: Arthroleptidae). *Herpetology Notes* 2: 455-47.

IUCN (2011). IUCN Red List of Threatened Species. Version 2010.4. <www.iucnredlist.org>. Downloaded on 18 March 2011.

Jolly, D. and Haxeltine, A. (1997). Effect of low glacial atmospheric CO2 on tropical African montane vegetation. *Science* 276: 786-788.

Kingdon, J. (1990). Island Africa; the Evolution of Africa's Rare Animals and Plants. Collins, London.

Kishino, H. and Hasegawa, M. (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *Journal of Molecular Evolution* 29:170- 179.

Largen, M.J. (1977). The status of the genus *Leptopelis* (Amphibia, Anura, Hyperoliidae) in Ethiopia, including descriptions of two new species. *Monit. Zool. Ital.,* N.S. vx1 *Supplemento* 9: 85-136.

Largen, M.J. (2001). Catalogue of amphibians of Ethiopia, including a key for their identification. *Tropic. Zool.* 14: 307-402.

Largen, M. and Spawls, S. (2010). The Amphibians and Reptiles of Ethiopia and Eritrea. Edition Chimaira, Frankfurt am Main. 692 p.

Loveridge, A. (1957). Check list of the reptiles and amphibians of East Africa (Uganda; Kenya; Tanganyika; Zanzibar). *Bulletin of the Museum of Comparative Zoology, Harvard* 117 (2): 153- 362 + I-XXXVI.

Nei, M. and Kumar, S. (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Odierna, G., Aprea, G., Andreone, F., Böhme, W. and Vences, M. (2007). Cytosystematics of hyperoliid frogs: Phylogeny of *Heterixalus*, low karyotypic variability in hyperoliines and separate phylogenetic position of *Leptopelis*. *Ital. J. Zool.* 74: 71-81.

Parker, H.W. (1930). Report on the Amphibia collected by Mr. J. Omer-Cooper in Ethiopia. *Proceedings of the Zoological Society of London*: 1-6.

Pik, R., Marty, B., Carignan, J. and Lave, J. (2003). Stability of Upper Nile drainage network (Ethiopia) deduces from (U–Th)/He thermochronometry: Implication of uplift and erosion of the Afar plume dome. *Earth and Planetary Science Letters* 215: 73–88, doi: 10.1016/S0012-821X(03)00457-6.

Posada, D. (in press). jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution*.

Poynton, J.C. (1999). Distribution of amphibians in sub- Saharan Africa, Madagascar, and Seychelles. In: Duellman, W.D. (Ed.), Patterns of Distribution of Amphibians, a Global Perspective. John Hopkins University Press, Baltimore, pp. 483-539.

Richards, C.M. and Moore, W.S. (1996). A Phylogeny for the African treefrog Family Hyperoliidae based on mitochondrial rDNA. *Molec. Phylog. Evol.* 5(3): 522-532.

Said, R. (1993). The River Nile. Pergamon, Oxford, 320p.

Schiøtz A. (1975). The treefrogs of Eastern Africa. *Copenhagen: Steenstrupia*, 232 pp.

Scortecci, G. (1930). Contributo alla conoscenza dei rettili e degli anfibi della Somalia, dell’Eritrea e dell’Abissinia. *Bollettino dei Musei di Zoologia e di Anatomia Comparata della R. Università di Torino* (3) 41 (10): 1-26.

Scortecci, G. (1940). Amphibia, pp. 151-160. In: Missione Biologica nel paese dei Borana. Raccolte zoologiche, Vol. 2, parte I. *Roma: Reale Accademia d’Italia, Centro Studi per l’Africa Orientale Italiana*, 791 p.

Sepulchre, P., Ramstein, G., Fluteau, F., Schuster, M., Tierchelin, J. and Brunet, M. (2006). Tectonic uplift and eastern African aridification. *Science* 313: 1419–1423, doi: 10.1126/science.1129158.

Sorenson, M.D. and Franzosa, E.A. (2007). TreeRot, version 3. Boston University, Boston, MA.

Swofford, D. (2002). PAUP*: Phylogenetic Analysis Using Parsimony (*and other Methods). Version 4.0b10. Sinauer Associates, Sunderland, Mass.

Street-Perrott, F.A., Huang, Y., Perrott, R.A., Eglinton, G., Barker, P., Ben Khelifa, L., Harkness, D.A. and Olago, D.D. (1997). Impact of lower atmospheric CO2 on tropical mountain ecosystems. *Science* 278: 1422-1426.

Tamura, K., Nei, M., and Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*) 101:11030-11035.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* (submitted).

Templeton, A.R. (1983). Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* 37: 221-244.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876-4882.

Umer, M., Lamb, H.F., Bonnefille, R., Lézine, A.-M., Tiercelin, J.-J., Gibert, E., Cazet, J.-P. and Watrin, J. (2007). Late Pleistocene and Holocene vegetation history of the Bale Mountains, Ethiopia. *Quat. Sci. Rev.* 26: 2229-2246.

Urban, E.K. (1967). Notes on a collection of amphibians from Ethiopia. *Journal of the East Africa Natural History Society and National Museum, Nairobi* 26 (2) (114): 85-86.

Vences, M., Kosuch, J., Glaw, F., Böhme, W. and Veith, M. (2003). Molecular phylogeny of hyperoliid treefrogs: biogeographic origin of Malagasy and Seychellean taxa and re-analysis of familial paraphyly. *J. Zool. Syst. Evol. Res.* 41: 205-215.

Vieites, D.R., Wollenberg, K.C., Andreone, F., Köhler, J., Glaw, F. and Vences, M. (2009). Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proc. Natl. Acad. Sci. U.S.A*. 106: 8267-8272.

Weinsheimer, F., Mengistu, A.A. and Rödder, D. (2010). Potential distribution of threatened *Leptopelis* spp. (Anura, Arthroleptidae) in Ethiopia derived from climate and land-cover data. *Endang. Species Res*. 9: 117-124.

Wolfenden, E., Yirgu, G., Ebinger, C., Deino, A. and Ayalew, D. (2004). Evolution of the northern Main Ethiopian rift: Birth of a triple junction. *Earth and Planetary Science Letters* 224: 213–228, doi: 10.1016/j.epsl.2004.04.022.

Yalden, D.W. (1983). The extent of high ground in Ethiopia compared to the rest of Africa. *SINET: Ethiopian Journal of Science* 6: 35-39.

Phylogeography of *Ptychadena* (Ptychadenidae, Amphibia) in the Ethiopian Highlands and Rift Valley: Molecular and Morphological Investigation

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ABSTRACT

The genus *Ptychadena* is widespread in Africa, Madagascar, Seychelles Islands, and the Mascarene Islands with 53 species mainly distributed in sub-Saharan Africa. Thirteen species are known from Ethiopia, with seven endemics (five occupying the Ethiopian Highlands) and six non-endemics widely distributed in the mid-altitudes and the lowlands. Many of the highland species are difficult to distinguish in the field and in museums. We conducted field surveys in the Ethiopian Highlands and the Rift Valley to assess phylogenetic relationships and distribution patterns of all five highland and four lowland species. Evolutionary relationships were reconstructed using mtDNA sequences of the 12S and 16S genes; phylogenetic assessment and estimates of divergence times were conducted using Maximum Parsimony and Maximum Likelihood methods. Biogeographic distribution patterns were analysed using both new and historic locality records. Morphological assessment was made on new specimens, holotypes and non-type museum collections. The reconstructed phylogenetic relationship resulted in groupings that confirmed the validity of known species, revealed cryptic taxa within complex groups and identified clades that question the taxonomic status of some species. Preliminary evolutionary timescale estimates indicated that endemic monophyletic highland species group began diversification recently (21.6-9.4 Mya), corresponding with major geologic events that altered the climate and habitats in the studied area. Distributional ranges that were previously documented in IUCN database were reduced for many species due to the currently proposed revised taxonomic status of species. Gorges and valleys were found to be the major barriers to delimit species boundaries in the highlands, while lowland species show continuous distribution within and outside the Ethiopian Rift Valley.

Key words: Bale Mountains; Biogeography; Central Ethiopian Highlands; Grass (Ridged) Frogs; Kaffa forests; Molecular systematic; Phylogeny; *Ptychadena.*

I) INTRODUCTION

The genus *Ptychadena* Boulenger 1917 (Anura), comprising amphibians commonly called Grassland Frogs or Ridged Frogs, has 53 species distributed in Africa, mainly in the sub-Saharan region (Amphibiaweb, 2011; Frost, 2011). A recent revision of the taxonomic status of amphibians has removed this genus from the family Ranidae Rafinesque 1814 and placed it under Ptychadenidae Dubois 1987 (Frost et al., 2006) along with *Hildebrandtia* Nieden 1907 and *Lanzarana* Clarke 1982 (Frost, 2011). Assigning individual specimens collected in the field or preserved specimens held in museum collections, to species in the genus *Ptychadena* can be difficult. Morphological variation among species of *Ptychadena* is poorly understood for many species, and there are few clear diagnostic characters for determining species (Perret, 1980, 1994; Poynton and Broadley, 1985; Largen, 1997; Bwong et al., 2009). Furthermore, the wide geographic distribution of many species adds to the complexity of this diverse group.

In Ethiopia, thirteen species of *Ptychadena* are currently recognized based on morphology and ecological grounds (Largen, 2000, 2001; Largen and Spawls, 2010). Eight of these are either lowland forms or occupy mid-altitudes: *P. anchietae* (Bocage 1867), *P. filwoha* Largen 1997, *P. harenna* Largen 1997, *P. mascareniensis* (Duméril & Bibron 1841), *P. porosissima* (Steindachner 1867), *P. pumilio* (Boulenger 1920), *P. schillukorum* (Werner 1907), and *P. tellinii* (Peracca 1904). Five are highland forms including *P. cooperi* (Parker 1930), *P. erlangeri* (Ahl 1924), *P. nana* Perret 1980, *P. neumanni* (Ahl 1924), and *P. wadei* Largen 2000. All of the highland forms and two lowland species (*P. filwoha* and *P. harenna*) are endemic to Ethiopia. The remaining lowland species are confined to the Ethiopian Rift Valley and lowlands that border the country, and are thought to be distributed in other sub-Saharan countries. *Ptychadena mascareniensis* is the only species ranging further north into Egypt along the Nile River. The distribution and type localities of the focal species in the current study are summarized from Largen (2001) and presented in Table 3.1. The status of populations of all species is not properly surveyed. The conservation status of one species, *P. erlangeri* is documented as Near Threatened (NT), while *P. filwoha, P. harenna, P. nana* and *P. wadei* are Data Deficient (DD). The status of the remaining species of Ethiopian *Ptychadena* is Least Concern (LC) (IUCN, 2011).

Some *Ptychadena* species have distinct morphological features and precise type localities enabling easy identification. *Ptychadena wadei* can be recognized by having an outer metatarsal tubercle and five pairs of essentially complete skin folds, and is located south of Lake Tana near the Abay (Blue Nile) River. Extensive webbing of the toes, a pale triangle on the snout and dark markings on the dorsum are characteristic of *P. anchietae*. The features used to distinguish the other Ethiopian species are problematic, and their geographic distribution ranges are overlapping and extensive. Morphologically complex populations of *P. neumanni* are thought to be widely distributed across the Rift Valley at altitudes ranging between 820 and 3800 m. All lowland species of Ethiopian *Ptychadena* are in general believed to have continuous distribution with other populations elsewhere in central, western or eastern and southern Africa. All previous taxonomy of this genus in Ethiopia has been solely based on morphological and ecological observations.

Table 3.1. Species of *Ptychadena* in Ethiopia assessed in the current study, with the respective synonyms, type localities and geographic areas (summarized from Largen, 2001 and IUCN, 2011).

Species of *Ptychadena*	**Other names (synonyms) used earlier**	**Type locality**	**Distribution and altitude**
Ptychadena cooperi (Parker 1930)	*Rana (Ptychadena) cooperi* Parker 1930 *Rana cooperi* Loveridge 1936 *Ptychadena cooperi* Largen 1997	Wouramboulchi, Shoa (Ethiopia)	Ethiopian plateau. 2500-3100 m.
Ptychadena erlangeri (Ahl 1924)	*Rana erlangeri* Ahl 1924 *Rana mascareniensis* (not Duméril & Bibron 1841); Boulenger 1896b; Loveridge 1936 *Ptychadena erlangeri* Perret 1980; Largen 1997	East side of Lake Abaya (Ethiopia)	Ethiopian plateau. 1500-2500 m.
Ptychadena nana Perret 1980	*Ptychadena nana* Perret 1980 *Rana nemanni* Ahl 1924; Largen 1997	Didda plateau, Arsi (Ethiopia)	Arsi Mts, Ethiopia. 2000-3000 m.
Ptychadena neumanni (Ahl 1924)	*Rana nemanni* Ahl 1924 *Rana mascareniensis* (not Duméril & Bibron 1841); Boulenger 1896a; Boulenger 1898; Ahl 1924; Parker 1930; Scortecci 1930; Loveridge 1936 *Rana cooperi* (not Parker 1930); Urban 1967 *Ptychadena neumanni* Perret 1980; Largen 1997 *Ptychadena largeni* Perret 1994	Gadat, Gofa (Ethiopia)	Ethiopian plateau, and western lowlands 820-3800 m.
Ptychadena wadei Largen 2000	*Ptychadena wadei* Largen 2000	Tisisat, Gojam (Ethiopia)	South of Lake Tana, Ethiopia. 1800-1850 m.
Ptychadena filwoha Largen 1997	*Ptychadena filwoha* Largen 1997 *Ptychadena taenioscelis* (not Laurent 1954); Lanza 1972 *Rana angolensis* (not Bocage 1866); Lanza 1972	Filwoha, Awash National Park (Ethiopia)	Northern Rift Valley, Ethiopia. 800-1000 m.
Ptychadena harenna Largen 1997	*P. harenna* Largen 1997	W. bank of Yadot R., close to its confluence with Shisha River, Bale Mountains National Park (Ethiopia)	Southern slopes of Bale Mts., Ethiopia. 1550 m.
Ptychadena anchietae (Bocage 1867)	*Rana anchietae* Bocage 1867 *Rana abyssinica* Peters 1881 *Rana mascareniensis* (not Duméril & Bibron 1841); Boulenger 1895a; Boulenger 1895b; Boulenger 1896b; Boulenger 1896c; Ahl 1924 *Rana gondokorensis* Werner 1907 *Rana aberae* Ahl 1923 *Rana mascareniensis schillukorum* (not Werner 1907); Ahl 1924 *Rana oxyrhinchus* (not Smith 1849); Parker 1930; Loveridge 1936; Roux 1936 *Rana (Ptychadena) oxyrhinchus* (not Smith 1849); Scortecci 1943 *Rana oxyrhinchus oxyrhinchus* (not Smith 1849); Battersby 1954 *Ptychadena anchietae* Lanza 1972; Largen 1997	Benguella (Angola)	Eritrea, Ethiopia, Somalia, South Africa, Congo Kinshasa, Angola, Congo Brazzaville. Savanna areas in Ethiopia. Up to 1800 m.
Ptychadena mascareniensis (Duméril & Bibron 1841)	*Rana mascareniensis* Duméril & Bibron 1841; Roux 1936; Urban 1967 *Rana mascareniensis mascareniensis* Loveridge 1936 *Rana oxyrhinchus* (not Smith 1849); Loveridge 1936 Ptychadena mascareniensis Largen 1997	Mascarene Islands and Seychelles Islands	Sierra Leone to Ethiopia, along the Nile to Egypt, South Africa, Madagascar, Seychelles, Mascarene Islands. Rift Valley and other lowlands in Ethiopia. 0- >2000 m

Thorough assessment and relatively frequent revision of the taxonomic status of species of *Ptychadena* in Ethiopia has been made by Perret (1980, 1994) and Largen (1997, 2000, 2001). These studies relied on morphological observations on relatively old museum collections dating back to the 1970s or earlier. The outcomes of these works are the basis for ongoing and future research on Ethiopian *Ptychadena*. However, there are important aspects that need further consideration pertaining to several taxonomic ambiguities.

First, as indicated in Largen (1997, 2001), there is difficulty in using most of the morphological features used to diagnose some superficially similar species or populations of Ethiopian *Ptychadena*. Morphometric (measured or counted physical) characters as well as ecological and altitudinal preferences of some species show large overlap or wide range in some groups. For instance, *P. erlangeri*, *P. neumanni* and *P. nana* form a complex group mainly diagnosed from each other by their snout-vent length as bigger, smaller and smallest, respectively. This would be difficult for identification of juveniles of a ‘larger’ species that might be confused with adults of a ‘smaller’ species. A summarized list of the currently used diagnostic characters for Ethiopian *Ptychadena* is given in Appendix 3.1.

Second, some holotypes are represented by very old specimens that have deteriorated over time and made it difficult to do reliable morphological comparisons with fresh collections. When the holotypes of two closely related and morphologically less distinct species (complex groups) have different sexes, comparison of new specimens with the holotypes will be difficult. One example would be the female holotype of *P. erlangeri* for which sex-specific diagnostic characters such as body size, gular slits, or vocal sacs can’t be compared with its close relative *P. neumanni,* represented by a male holotype.

Third, there are no detailed molecular data to date to support previous taxonomic groupings of Ethiopian *Ptychadena*. The importance of additional taxonomic criteria (acoustic, chromosomal, molecular data) has been suggested to resolve evolutionary relationships of Ethiopian amphibians (Largen, 2001), and the status of problematic groups such as ‘*P. neumanni* complex’ in particular (Largen, 1997). Preliminary chromosomal study on *P. cooperi, P. nemanni* and *P. anchietae* in Ethiopia indicated that all of these species have similar karyological composition of 2n=24, including 22 metacentrics and 2 submetacentrics (Missale, 2008). Currently, the only existing GenBank data for Ethiopian *Ptychadena* (DQ283067.1, DQ283066.1, DQ283792.1 and DQ283475.1) are those of two specimens (A158394 and A158395) collected from the Bale Mountains and kept at the American Museum of Natural History (AMNH) (Frost et al., 2006). These materials have been identified as ‘*P. cooperi*’ based on morphological features and the geographic area of the species’ distribution. However, it would be premature to refer to these sequences as *P. cooperi* before comparing them with other sequences from the type locality, which is located further north on a highland plateau some 200 km across the Rift Valley.

The endemism pattern of the amphibians of Ethiopia has been linked to the geomorphology of the Ethiopian Highlands (Poynton, 1999; Largen, 2001; Largen and Spawls, 2010). As depicted in Fig. 3.1, the East African Rift Valley splits these highlands into northwestern and southeastern blocks, with further segmentation seen by major river gorges and valleys (Kingdon, 1990). It has been proposed that the diversification and endemicity of amphibian populations correspond to these narrower segments of mountain geographic barriers, as well as the past climatic history of the area (Chapter 2, this thesis). Largen (1997) suggested that the diversification process of highland populations of *Ptychadena* was 'comparatively recent' in the Ethiopian Highlands given their gross morphological similarity.

Fig. 3.1. Location map of Ethiopia in the Horn of Africa (inset) and topographic map of Ethiopia showing mountain ranges bisected by the Rift Valley and fragmented by major rivers, and encircled by vast areas of lowlands. (Modified from source: Sadalmelik, I. (2007); downloaded from http://en.wikipedia.org/wiki/File:Ethiopia_Topography.png).

Constructing reliable morphology-based phylogenetic relationships in ranoid amphibians is difficult because of high levels of phenotypic homoplasy (van der Meijden et al., 2005). Bwong et al. (2009) proposed a list of 35 morphological characters used to diagnose species of *Ptychadena* in Kakamega Forest (Kenya). In addition, recent advancements in molecular and GIS techniques have revealed complex and cryptic taxonomic elements for various amphibian taxa in areas with high diversity of amphibian species or populations (Vences et al., 2005; Vieites et al., 2009; Loader et al., 2010). A good example is the phylogenetic structure and biogeographic aspect of the cosmopolitan *P. mascareniensis* which revealed how morphologically similar populations of a species could be cryptically varied (Vences et al., 2004).

The current study aims to elucidate the evolutionary relationship and biogeography of *Ptychadena* in Ethiopia. We used molecular, morphological and geographic distribution data to reveal the evolutionary history of these fauna for the first time. We tested the following hypotheses based on our current understanding of Ethiopia's biogeography: 1) current (traditional) taxonomy based on morphological characters and ecology corresponds to phylogeny using mtDNA markers; 2) single species do not occur on both sides of major lowland barriers (e.g., the Rift Valley and major river gorges); and 3) diversification of extant highland species in Ethiopia started after the formation of the Ethiopian Highlands by uplifting and volcanic activities about 30 Mya. The expected results will show future directions for research, contribute to defining geographic units for amphibian conservation and prioritize threatened endemic species that need immediate conservation actions.

II) MATERIALS AND METHODS

A) Field Sampling and Handling of Specimens

Voucher specimens and tissues were collected during field work conducted in the Ethiopian Highlands and the Rift Valley in 2006, 2008, 2009 and 2010. The samples were collected from the Eastern Rift (Arsi, Bale Mountains, and Bore areas), the Western Rift (Gughe Mountain, Soddo, Kaffa, West Shoa and Gojam areas), and within the Rift Valley (Ziway, Langano, and Arba Minch areas). Details of the methods used in field sampling and handling of specimens are given in Chapter 1, Section IV-C-1.

B) Morphological, Molecular and Biogeographic Data

Morphometric measurements were taken for all adult specimens of the recent collections (2006, 2008-2010) and for historic specimens (pre-1977) kept in various natural history museums: The studied museum specimens included holotypes for all known highland species of Ethiopian *Ptychadena,* as well as several paratypes or non-type materials. Principal component analyses and other statistical tests were done in The Unscrambler® X version 10.0.1. See details in Chapter 1, Section IV-C-2 for the list of studied morphological characters and data analysis.

Tissue samples were taken from sub-samples of recently collected (2006-2010) adult (muscle or liver), juvenile (muscle or toe tip), or tadpole (tail) specimens of *Ptychadena* from several localities in Bale, Arsi, Bore, Gamo Gofa, Kaffa, Wolayita Soddo, the Rift Valley, Shoa, and Gojam (Appendix 3.2). We attempted to sample all highland populations of *Ptychadena*, including: *P. neumanni, P. erlangeri, P. nana, P. wadei,* and *P. cooperi.* We also included tissues of two lowland forms (*P. anchietae* and *P. mascareniensis*) from the Rift Valley (Ziway, Langano and Arba Minch localities) for comparison with species from the Ethiopian Highlands and haplotypes elsewhere in Africa. Additional sub-samples of tissue from Bore (Eastern Rift), Awash National Park (Rift Valley) and Yadot River (southern base of Bale Mountains) were obtained from separate collections of B. Zimkus in 2006. Molecular work was conducted using the 12S and 16S mtDNA genes, mostly in the facilities of the molecular laboratory at the Section of Biogeography (Department of Environmental Sciences, UB); some of the tissue samples from Bale and Bore areas were purified and sequenced in the laboratories of the MCZ, Harvard University. Details of the methods used for tissue sampling, DNA extraction, PCR amplification and sequencing are presented in Chapter 1, Section IV-C-3.

Locality names and coordinate data (in degrees) were recorded during our field surveys, and additional data were obtained and compiled from historical records of museum specimens reported in the gazzetter in Largen (2001), as well as from collections made by B. Zimkus in 2006. These data were mapped for each population and species in DIVA-GIS (Hijmans et al., 2005). See details on methodology for biogeographic data collection and analysis in Chapter 1, Section IV-C-4.

C) Molecular Data Analysis

Additional sequence data and alignments that we used from other sources include: 26 sequences (16S) of *P. mascareniensis* (2), *P. filwoha* (2), *P. anchietae* (1), *P. harenna* (4), *P. cooperi* (4), *P. erlangeri* (6) and *P. neumanni* (7) aligned (nexus format) by B. Zimkus; and available GenBank sequences for non-Ethiopian specimens of *P. anchietae*, *P. porosissima*, and *P. mascareniensis.* The outgroups used in this study were GenBank sequence data for *Arthroleptis palava* Blackburn, Gvoždík, and Leaché, 2010, *Astylosternus schioetzi* Amiet, 1978, and *Leptopelis kivuensis* Ahl, 1929 (Table 3.2).

Table 3.2. Additional non-Ethiopian species used in the current study for phylogenetic analyses of mitochondrial DNA sequences of the 16S genes and combined 12S and 16S genes.

Species	Country	GenBank Accession no.
*Arthroleptis palava**	Cameroon	HM238195.1
*Astylosternus schioetzi**	Cameroon	DQ283349
*Leptopelis kivuensis**	Kenya	DQ283226.1
*Leptopelis vermiculatus**	Tanzania	DQ283242.1
Ptychadena anchietae	Somalia	DQ525921.1
Ptychadena porosissima	Tanzania	DQ525941.1
Ptychadena mascareniensis	Reunion Islands	AY517587.1
Ptychadena mascareniensis	Madagascar	AY517590.1
Ptychadena mascareniensis	Madagascar	DQ283031.1
Ptychadena mascareniensis	Kenya	AY517599.1
Ptychadena mascareniensis	Benin	AY517597.1
Ptychadena mascareniensis	Egypt	AY5175 96.1
Ptychadena newtoni	Sao Tome and Principe	GU457591.1

* *Arthroleptis palava, Astylosternus schioetzi,* and *Leptopelis kivuensis* (or *L. vermiculatus* in the combined dataset) were used as outgroup.

Complete alignment was done for all assembled (contig) sequences combined in CLUSTALX 2.0.12 using the default parameters (Thompson et al., 1997). Model selection for phylogenetic analysis in Maximum Likelihood (ML) was conducted using Akaike Information Criterion in Modeltest 3.7 (Posada and Crandall, 1998) as well as Bayesian Information Criterion in MEGA 5 (Tamura et al., 2011). Both criteria suggested the best model fitting our data to be the General Time Reversible model, based on discrete Gamma Distribution with Invariant sites (GTR+G+I) (Nei and Kumar, 2000). Topology tests were conducted in PAUP* 4 b10 (Swofford, 2002) using MP (Non-parametric test (Templeton, 1983)) as well as ML (KH-test (Kishino and Hasegawa, 1989) with full optimization, two-tailed, 100 replicates settings). Further details on phylogenetic data analyses and preliminary estimates of evolutionary divergence times are presented in Chapter 1, Section IV-C-3. All aligned nucleotide sequence data generated in this study for Ethiopian *Ptychadena* are deposited in the database of NCBI GenBank (accession numbers: for 12S, JX465037 to JX465107; for 16S, JX464828 to JX464898).

The taxonomic status of newly revealed groups among the studied populations were evaluated for Ethiopian *Ptychadena* based on the general definition for candidate species as outlined in Vieites et al., 2009; see Chapter 1, Section III-C-3 in this thesis for general definitions.

III) RESULTS

A) Evolutionary Relationships and Divergence Times

For the 16S mtDNA alone, a total of 113 sequences (102 Ethiopian and eight non-Ethiopian *Ptychadena*, plus three outgroups) were included in the final analysis. The combined dataset of 12S and 16S genes included a total of 77 sequences (71 Ethiopian and three non-Ethiopian *Ptychadena*, and three outgroups). The resulting concatenated sequence alignment of the combined dataset had a total of 995 base pairs (412 12S, and 583 16S). The percent nucleotide composition was T(U)=25.5, C=22.4, A=31.6, G=20.6. Phylogenetic relationships and genetic distances analyzed using combined or separate datasets of the 12S and 16S genes gave similar results. Because there are more 16S data for non-Ethiopian species to compare our samples with, we presented the results for the 16S genes alone. There were a total of 452 positions included (16S genes alone) in the final dataset for phylogenetic analysis.

Using the 16S genes, evolutionary divergence within each of the studied phylogenetic groups was low (< 1%). Between-group distances are generally high between different pairs of the highland populations (> 3%), the maximum being approximately 13% when *P. wadei* is included. The only exception was the relatively low (2%) divergence between *P. nana* and *Ptychadena* sp. Bore2-Wenchi. Species from the Ethiopian highlands have significantly large (> 8%) genetic distance from the lowland species and those of other African regions. Details of evolutionary distances and variance among groups are given in Table 3.3.

The affinity of *P. wadei* appears closest to *P. porosissima* (Tanzania) (about 8%), with relatively high divergence values of up to 14% from other sampled *Ptychadena*. *Ptychadena anchietae* from the Ethiopian Rift Valley is distinctive by having high genetic distance (over 8%) from all other studied species, except from the haplotype from Somalia and *P. harenna* (Ethiopia), which both exhibited less than 0.5% divergence. The Ethiopian population of *P. mascareniensis* has a minimum divergence of 5% from all other studied populations and species, with the exception of the Egyptian specimen and *P. filwoha* from northern part of the Ethiopian Rift Valley (~0.4%). The Ethiopian population of *P. mascareniensis* is substantially distant from haplotypes of the type locality (Reunion Islands) by about 6%. For the combined dataset of the 12S and 16S genes, genetic distances were slightly less, but congruent with the above results for the 16S genes alone.

The overall structure of the reconstructed evolutionary relationship between groups is broadly similar using both ML and MP methods; the MP and ML trees are presented in Fig. 3.2 and Fig. 3.3, respectively. The optimal tree supports the validity of many of the Ethiopian species of *Ptychadena*. However, the analysis also indicates contrasting taxonomic conclusions, potentially revealing the presence of cryptic taxa, and synonyms of currently recognized species. In the MP tree, *P.* cf. *mascareniensis* (Ethiopian Rift Valley), *P. wadei* (Andasa) and *P. anchietae* (Bale and Ethiopian Rift Valley) are the only species that clustered with other non-Ethiopian species.

Eight strictly highland and closely related groups were identified: *P.* cf. *cooperi* (Arsi-Bale), *Ptychadena* sp. (Debre Markos), *P. neumanni* (Gughe-Kaffa), *P. erlangeri* (Bore1-Bale), *Ptychadena* sp. (Bada Buna - Akaki), *Ptychadena* sp. (Goba), *P. nana* (Arsi-Bale), and *Ptychadena* sp. (Bore2-Wenchi). All of these populations form a monophyletic group (bootstrap value: 94%). Topology tests also indicated that sub-optimal trees that combined the highland and lowland Ethiopian populations vary significantly from the optimal tree (KH-test p-value = 0.0000; Non-parametric test p-value < 0.0001). While most geographically distinct clusters of phylo-groups are strongly supported (bootstrap = 90-99%), sister group relationships for most of the highland populations are weakly supported (< 80%). *Ptychadena* cf. *cooperi* (Arsi-Bale) and *Ptychadena* sp. (Debre Markos) cluster together, but the monophyly of these two groups is weakly supported (bootstrap = 61%; KH-test p-value = 0.1400; non-parametric test p-value < 0.8185). *Ptychadena nana*, formerly known only from the type locality in Arsi, clustered with another population from Bale. One of the newly identified taxa, *Ptychadena* sp. (Bore2-Wenchi) is the only highland clade shown to have populations with little genetic differentiation across the Rift Valley.

One highland species did not cluster within the monophyletic group that contained the above eight species. *Ptychadena wadei* clustered close to *P. porosissima* (from Tanzania); the latter is represented in Ethiopia by only two very old museum specimens from Lake Tana area. *Ptychadena porosissima* is reported to co-exist with *P. wadei* at this locality, and the two species have morphological similarities (see section B below).

Table 3.3. Estimates of genetic distance. The analysis involved 113 nucleotide sequences (three outgroups not shown here). All positions containing gaps and missing data were eliminated; a total of 452 positions were present in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011). A) Between phylogenetic groups. The number of base differences per site averaged over all sequence pairs between groups are shown below the diagonal. Standard error estimate(s) are shown above the diagonal. Shaded colors: green = endemic species of Ethiopian Highlands, blue = non-endemic species from Ethiopian lowlands, yellow = distances lower than 3%. B) Within each phylogenetic group. The numbers of base differences per site from averaging over all sequence pairs within each group are shown. The presence of n/c in the results denotes cases in which it was not possible to estimate evolutionary distances.

	A) Between Groups	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	*P. nana* (Arsi-Bale)		0.006	0.008	0.009	0.006	0.008	0.010	0.009	0.013	0.013	0.013	0.014	0.015	0.014	0.014	0.015	0.014	0.015	0.014	0.014	0.014
2	*P.* sp. (Bore2-Wenchi)	0.020		0.009	0.009	0.008	0.008	0.010	0.009	0.013	0.013	0.013	0.014	0.015	0.014	0.015	0.015	0.015	0.015	0.014	0.014	0.014
3	*P.* sp. (Goba)	0.041	0.040		0.009	0.008	0.008	0.010	0.011	0.014	0.014	0.014	0.014	0.015	0.016	0.016	0.016	0.016	0.015	0.015	0.015	0.015
4	*P.* sp. (Bada Buna - Akaki)	0.046	0.048	0.045		0.008	0.009	0.010	0.010	0.014	0.014	0.014	0.014	0.015	0.015	0.015	0.015	0.015	0.015	0.014	0.015	0.015
5	*P. erlangeri* (Bore1-Bale)	0.023	0.031	0.035	0.032		0.007	0.010	0.009	0.013	0.013	0.013	0.014	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.014	0.014
6	*P. neumanni* (Gughe-Kaffa-?Dolo Mena)	0.034	0.035	0.037	0.043	0.032		0.009	0.009	0.013	0.013	0.013	0.013	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
7	*P.* sp. (Debre Markos)	0.054	0.056	0.053	0.052	0.047	0.049		0.009	0.014	0.014	0.014	0.014	0.015	0.016	0.016	0.016	0.016	0.015	0.015	0.016	0.016
8	*P.* cf. *cooperi* (Arsi-Bale)	0.046	0.050	0.061	0.061	0.048	0.046	0.043		0.013	0.013	0.013	0.014	0.015	0.016	0.016	0.016	0.015	0.016	0.015	0.015	0.015
9	*P. anchietae* (Bale - Rift Valley)	0.092	0.088	0.099	0.107	0.093	0.097	0.098	0.093		0.003	0.000	0.013	0.016	0.015	0.015	0.016	0.016	0.016	0.016	0.016	0.016
10	*P. anchietae* (SOM)	0.095	0.091	0.102	0.110	0.095	0.099	0.100	0.095	0.005		0.003	0.014	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016
11	*P. harenna* (Bale)	0.092	0.088	0.100	0.107	0.093	0.097	0.098	0.093	0.000	0.004		0.013	0.016	0.015	0.016	0.016	0.016	0.016	0.016	0.016	0.016
12	*P. porosissima* (TAN)	0.116	0.120	0.117	0.118	0.117	0.106	0.116	0.107	0.101	0.104	0.102		0.012	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
13	*P. wadei* (Andasa)	0.130	0.132	0.134	0.137	0.127	0.132	0.134	0.137	0.140	0.143	0.140	0.079		0.016	0.016	0.016	0.016	0.017	0.016	0.016	0.016
14	*P. mascareniensis* (S. Rift Valley)	0.115	0.118	0.133	0.121	0.117	0.126	0.134	0.133	0.135	0.137	0.135	0.135	0.158		0.003	0.003	0.010	0.010	0.010	0.011	0.011
15	*P. filwoha* (N. Rift Valley)	0.115	0.118	0.133	0.121	0.117	0.126	0.134	0.133	0.135	0.137	0.135	0.133	0.158	0.004		0.003	0.010	0.010	0.010	0.010	0.010
16	*P. mascareniensis* (EGY)	0.119	0.122	0.137	0.125	0.122	0.130	0.138	0.136	0.139	0.142	0.139	0.135	0.160	0.004	0.004		0.010	0.010	0.010	0.010	0.010
17	*P. mascareniensis* (KEN)	0.117	0.124	0.137	0.125	0.122	0.126	0.138	0.138	0.139	0.142	0.139	0.135	0.163	0.051	0.051	0.051		0.008	0.011	0.011	0.011
18	*P. mascareniensis* (BEN)	0.130	0.129	0.133	0.125	0.126	0.130	0.138	0.147	0.139	0.142	0.139	0.142	0.176	0.051	0.055	0.055	0.038		0.011	0.013	0.013
19	*P. mascareniensis* (MAD2)	0.119	0.118	0.124	0.121	0.122	0.122	0.134	0.133	0.141	0.144	0.142	0.126	0.160	0.053	0.049	0.051	0.058	0.064		0.007	0.007
20	*P. mascareniensis* (MAD1)	0.115	0.118	0.128	0.132	0.117	0.126	0.145	0.141	0.143	0.146	0.144	0.128	0.154	0.060	0.055	0.058	0.062	0.082	0.024		0.002
21	*P. mascareniensis* (REU)	0.115	0.115	0.128	0.132	0.117	0.126	0.145	0.141	0.141	0.144	0.142	0.128	0.154	0.062	0.058	0.060	0.062	0.082	0.027	0.002	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	B) Within Group	0.005	0.003	0.000	0.002	0.000	0.007	0.002	0.006	0.001	n/c	0.000	n/c	0.002	0.000	0.000	n/c	n/c	n/c	n/c	n/c	n/c

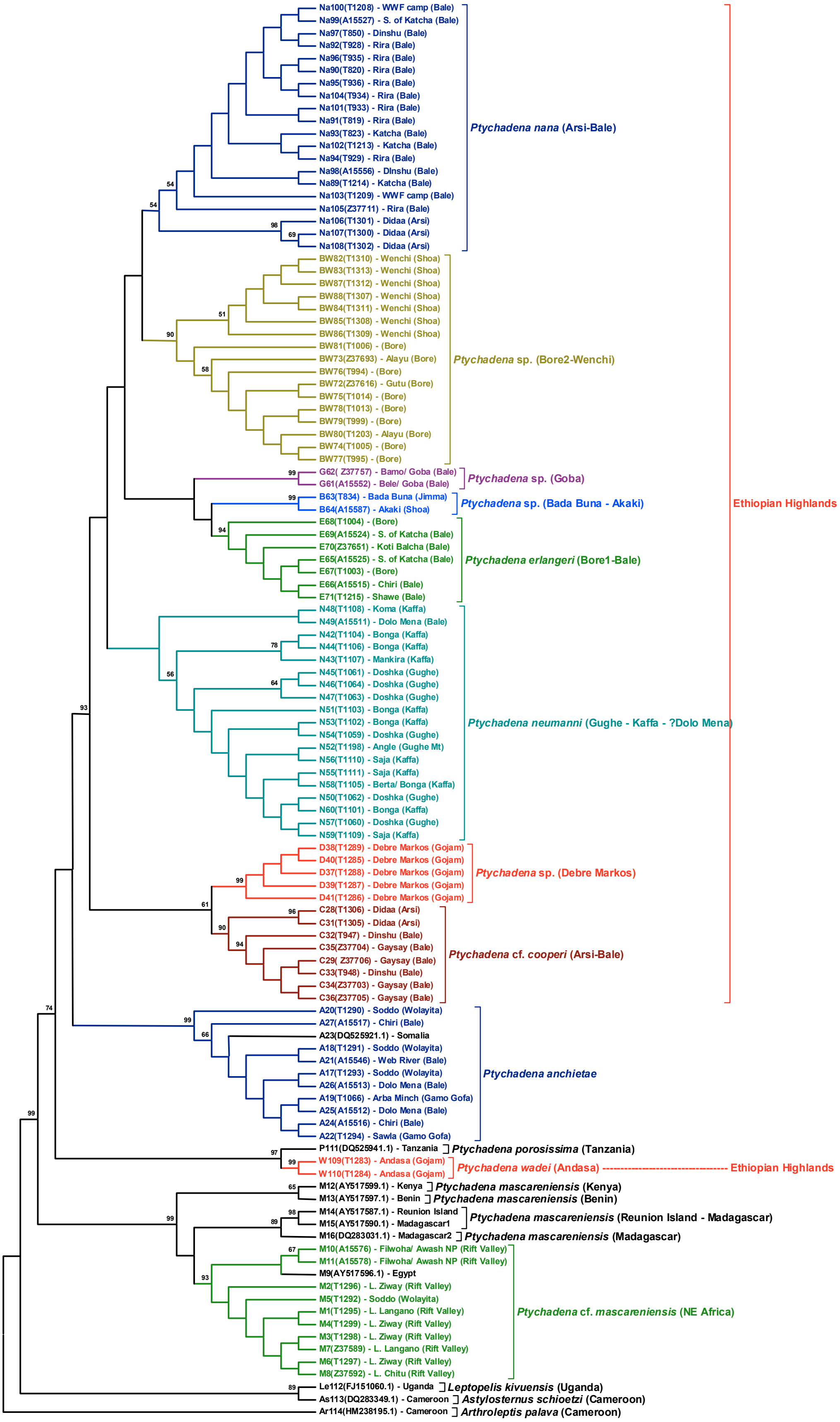

Na100(T1208) - WWF camp (Bale)
Na99(A15527) - S. of Katcha (Bale)
Na97(T850) - Dinshu (Bale)
Na92(T928) - Rira (Bale)
Na96(T935) - Rira (Bale)
Na90(T820) - Rira (Bale)
Na95(T936) - Rira (Bale)
Na104(T934) - Rira (Bale)
Na101(T933) - Rira (Bale)
Na91(T819) - Rira (Bale)
Na93(T823) - Katcha (Bale)
Na102(T1213) - Katcha (Bale)
Na94(T929) - Rira (Bale)
Na98(A15556) - Dinshu (Bale)
Na89(T1214) - Katcha (Bale)
Na103(T1209) - WWF camp (Bale)
Na105(Z37711) - Rira (Bale)
Na106(T1301) - Didaa (Arsi)
Na107(T1300) - Didaa (Arsi)
Na108(T1302) - Didaa (Arsi)
Ptychadena nana (Arsi-Bale)
54
54
98
69
BW82(T1310) - Wenchi (Shoa)
BW83(T1313) - Wenchi (Shoa)
BW87(T1312) - Wenchi (Shoa)
BW88(T1307) - Wenchi (Shoa)
BW84(T1311) - Wenchi (Shoa)
BW85(T1308) - Wenchi (Shoa)
BW86(T1309) - Wenchi (Shoa)
BW81(T1006) - (Bore)
BW73(Z37693) - Alayu (Bore)
BW76(T994) - (Bore)
BW72(Z37616) - Gutu (Bore)
BW75(T1014) - (Bore)
BW78(T1013) - (Bore)
BW79(T999) - (Bore)
BW80(T1203) - Alayu (Bore)
BW74(T1005) - (Bore)
BW77(T995) - (Bore)
Ptychadena sp. (Bore2-Wenchi)
51
90
58
G62(Z37757) - Bamo/ Goba (Bale)
G61(A15552) - Bele/ Goba (Bale)
Ptychadena sp. (Goba)
99
B63(T834) - Bada Buna (Jimma)
B64(A15587) - Akaki (Shoa)
Ptychadena sp. (Bada Buna - Akaki)
99
E68(T1004) - (Bore)
E69(A15524) - S. of Katcha (Bale)
E70(Z37651) - Koti Balcha (Bale)
E65(A15525) - S. of Katcha (Bale)
E67(T1003) - (Bore)
E66(A15515) - Chiri (Bale)
E71(T1215) - Shawe (Bale)
Ptychadena erlangeri (Bore1-Bale)
94
N48(T1108) - Koma (Kaffa)
N49(A15511) - Dolo Mena (Bale)
N42(T1104) - Bonga (Kaffa)
N44(T1106) - Bonga (Kaffa)
N43(T1107) - Mankira (Kaffa)
N45(T1061) - Doshka (Gughe)
N46(T1064) - Doshka (Gughe)
N47(T1063) - Doshka (Gughe)
N51(T1103) - Bonga (Kaffa)
N53(T1102) - Bonga (Kaffa)
N54(T1059) - Doshka (Gughe)
N52(T1198) - Angle (Gughe Mt)
N56(T1110) - Saja (Kaffa)
N55(T1111) - Saja (Kaffa)
N58(T1105) - Berta/ Bonga (Kaffa)
N50(T1062) - Doshka (Gughe)
N60(T1101) - Bonga (Kaffa)
N57(T1060) - Doshka (Gughe)
N59(T1109) - Saja (Kaffa)
Ptychadena neumanni (Gughe - Kaffa - ?Dolo Mena)
78
56
64
93
D38(T1289) - Debre Markos (Gojam)
D40(T1285) - Debre Markos (Gojam)
D37(T1288) - Debre Markos (Gojam)
D39(T1287) - Debre Markos (Gojam)
D41(T1286) - Debre Markos (Gojam)
Ptychadena sp. (Debre Markos)
99
61
C28(T1306) - Didaa (Arsi)
C31(T1305) - Didaa (Arsi)
C32(T947) - Dinshu (Bale)
C35(Z37704) - Gaysay (Bale)
C29(Z37706) - Gaysay (Bale)
C33(T948) - Dinshu (Bale)
C34(Z37703) - Gaysay (Bale)
C36(Z37705) - Gaysay (Bale)
Ptychadena cf. cooperi (Arsi-Bale)
96
90
94
Ethiopian Highlands
A20(T1290) - Soddo (Wolayita)
A27(A15517) - Chiri (Bale)
A23(DQ525921.1) - Somalia
A18(T1291) - Soddo (Wolayita)
A21(A15546) - Web River (Bale)
A17(T1293) - Soddo (Wolayita)
A26(A15513) - Dolo Mena (Bale)
A19(T1066) - Arba Minch (Gamo Gofa)
A25(A15512) - Dolo Mena (Bale)
A24(A15516) - Chiri (Bale)
A22(T1294) - Sawla (Gamo Gofa)
Ptychadena anchietae
99
66
74
P111(DQ525941.1) - Tanzania
Ptychadena porosissima (Tanzania)
97
W109(T1283) - Andasa (Gojam)
W110(T1284) - Andasa (Gojam)
99
Ptychadena wadei (Andasa) ---------------------------------- Ethiopian Highlands
99
M12(AY517599.1) - Kenya
Ptychadena mascareniensis (Kenya)
M13(AY517597.1) - Benin
Ptychadena mascareniensis (Benin)
65
M14(AY517587.1) - Reunion Island
M15(AY517590.1) - Madagascar1
Ptychadena mascareniensis (Reunion Island - Madagascar)
98
89
99
M16(DQ283031.1) - Madagascar2
Ptychadena mascareniensis (Madagascar)
M10(A15576) - Filwoha/ Awash NP (Rift Valley)
M11(A15578) - Filwoha/ Awash NP (Rift Valley)
67
M9(AY517596.1) - Egypt
93
M2(T1296) - L. Ziway (Rift Valley)
M5(T1292) - Soddo (Wolayita)
M1(T1295) - L. Langano (Rift Valley)
M4(T1299) - L. Ziway (Rift Valley)
M3(T1298) - L. Ziway (Rift Valley)
M7(Z37589) - L. Langano (Rift Valley)
M6(T1297) - L. Ziway (Rift Valley)
M8(Z37592) - L. Chitu (Rift Valley)
Ptychadena cf. mascareniensis (NE Africa)
Le112(FJ151060.1) - Uganda
Leptopelis kivuensis (Uganda)
89
As113(DQ283349.1) - Cameroon
Astylosternus schioetzi (Cameroon)
Ar114(HM238195.1) - Cameroon
Arthroleptis palava (Cameroon)

Fig. 3.2 (previous page). Maximum Parsimony tree: Phylogenetic relationship of some Ethiopian species of *Ptychadena* inferred using partial mtDNA of the 16S gene. The most parsimonious bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). The percentage of replicate trees in which the associated taxa clustered together with more than 50% values in the bootstrap (1000 replicates) are shown next to the branches (Felsenstein, 1985). The MP tree was obtained using the Close-Neighbor-Interchange algorithm (pg. 128 in Nei and Kumar, 2000) with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). The analysis involved 113 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 452 positions in the final analyzed dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011).

Topology tests were used to determine whether constrained sub-optimal trees of western or eastern rift populations were significantly different from the optimal trees. The western rift cluster included *P. wadei*, *Ptychadena* sp. (Debre Markos), *Ptychadena* sp. (Bada Buna - Akaki), *Ptychadena* sp. (Wenchi) and *P. neumanni*; the eastern rift cluster included *Ptychadena* sp. (Goba), *P. erlangeri*, *Ptychadena* sp. (Bore2), *P. nana* and *Ptychadena* cf. *cooperi*. Accordingly, the optimal tree was significantly different from the sub-optimal for the western rift cluster (KH-test p-value = 0.000; non-parametric test p-value = 0.003), but inconsistent results for the eastern rift cluster (KH-test p-value = 0.021; non-parametric test p-value = 0.454). Therefore, the hypothesis that a monophyletic western rift grouping is present is rejected, and monophyly of those in the eastern rift can not be ruled out.

Two major groups of lowland species were identified: *P. anchietae* (including *P. harenna*), and *P. mascareniensis* from Ethiopian Rift Valley (including *P. filwoha*). Populations of *P. anchietae* from the Ethiopian Rift Valley show close affinities towards those from Somalia, as evidenced by a very low genetic divergence (<0.5%). In addition, *P. harenna* from a lowland area at the southern base of the Bale Mountains is found to be conspecific with *P. anchietae* (genetic distance <0.5%). The other lowland group from the Rift Valley, *P. mascareniensis*, is genetically very similar to the Egyptian population. Specimens presumed previously to have been *P. filwoha* from the hot spring in ANP (Rift Valley) are found to be conspecific with *P. mascareniensis* from southern Ethiopian Rift Valley. However, the evolutionary divergence between the northeast African *P. mascareniensis* and those from western, eastern and southern Africa is significantly large (>5%). As a whole, between-group divergences are relatively low among the monophyletic highland endemics of Ethiopia as compared with the non-endemic lowland forms or species elsewhere in Africa.

Evolutionary divergence times estimated from secondary calibration made for the studied species of *Ptychadena* indicate that the ancestral highland Ethiopian form probably split from other groups of *Ptychadena* between 46.1 and 20.1 Mya (Table 3.4 and Fig. 3.4). The diversification of the highland endemics probably began between 21.6 and 9.4 Mya. The northeast African *P. mascareniensis* also probably split from other haplotypes between 29.6 and 12.9 Mya. This indicates that the Ethiopian species are relatively young as contrasted with the appearance of other *Ptychadena* elsewhere.

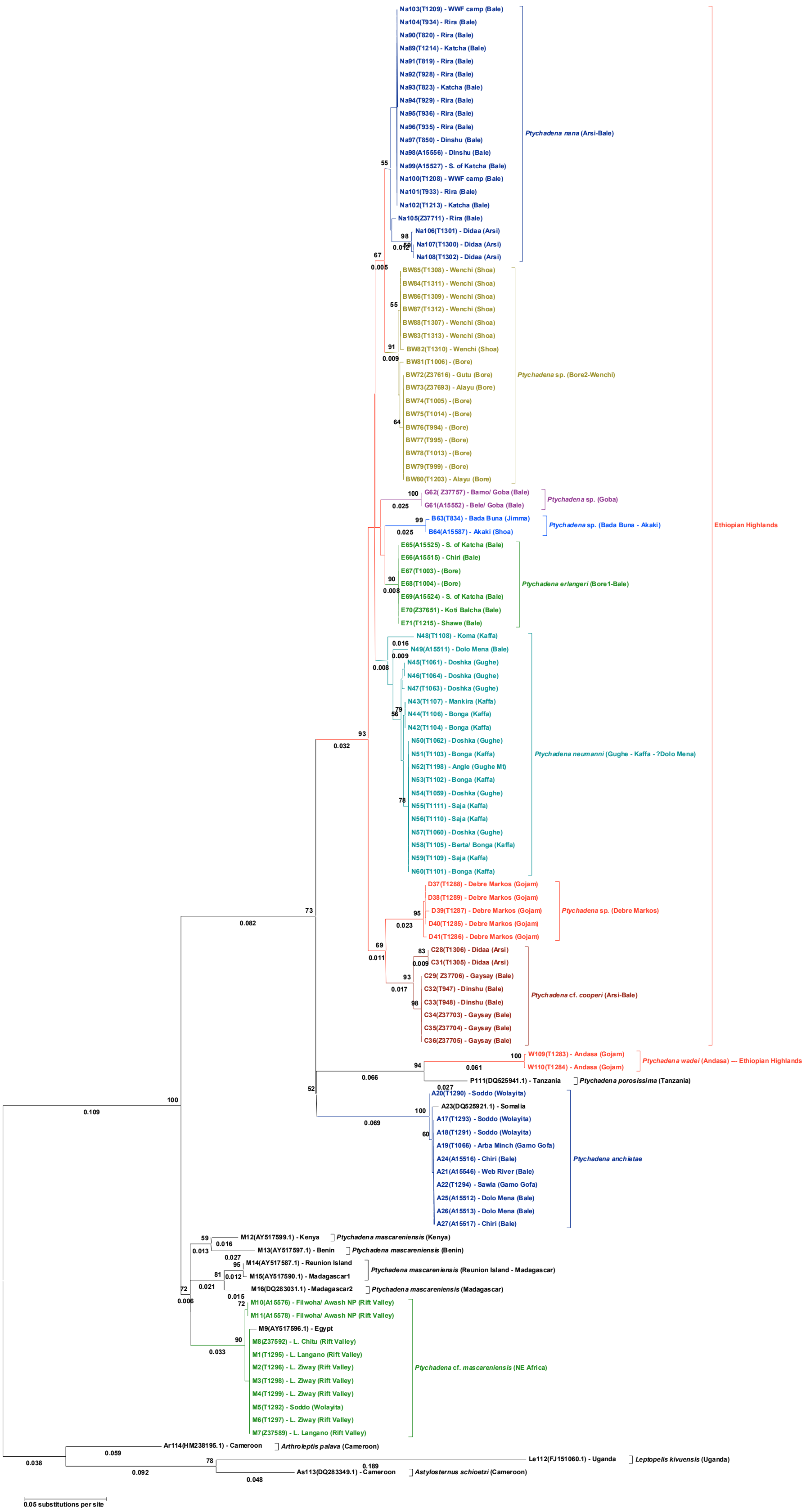

Na103(T1209) - WWF camp (Bale)
Na104(T934) - Rira (Bale)
Na90(T820) - Rira (Bale)
Na89(T1214) - Katcha (Bale)
Na91(T819) - Rira (Bale)
Na92(T928) - Rira (Bale)
Na93(T823) - Katcha (Bale)
Na94(T929) - Rira (Bale)
Na95(T936) - Rira (Bale)
Na96(T935) - Rira (Bale)
Na97(T850) - Dinshu (Bale)
Na98(A15556) - DInshu (Bale)
Na99(A15527) - S. of Katcha (Bale)
Na100(T1208) - WWF camp (Bale)
Na101(T933) - Rira (Bale)
Na102(T1213) - Katcha (Bale)
Na105(Z37711) - Rira (Bale)
Na106(T1301) - Didaa (Arsi)
Na107(T1300) - Didaa (Arsi)
Na108(T1302) - Didaa (Arsi)
55
98
0.012
59
67
0.005
Ptychadena nana (Arsi-Bale)
BW85(T1308) - Wenchi (Shoa)
BW84(T1311) - Wenchi (Shoa)
BW86(T1309) - Wenchi (Shoa)
BW87(T1312) - Wenchi (Shoa)
BW88(T1307) - Wenchi (Shoa)
BW83(T1313) - Wenchi (Shoa)
BW82(T1310) - Wenchi (Shoa)
BW81(T1006) - (Bore)
BW72(Z37616) - Gutu (Bore)
BW73(Z37693) - Alayu (Bore)
BW74(T1005) - (Bore)
BW75(T1014) - (Bore)
BW76(T994) - (Bore)
BW77(T995) - (Bore)
BW78(T1013) - (Bore)
BW79(T999) - (Bore)
BW80(T1203) - Alayu (Bore)
55
91
0.009
64
Ptychadena sp. (Bore2-Wenchi)
G62(Z37757) - Bamo/ Goba (Bale)
G61(A15552) - Bele/ Goba (Bale)
100
0.025
Ptychadena sp. (Goba)
B63(T834) - Bada Buna (Jimma)
B64(A15587) - Akaki (Shoa)
99
0.025
Ptychadena sp. (Bada Buna - Akaki)
Ethiopian Highlands
E65(A15525) - S. of Katcha (Bale)
E66(A15515) - Chiri (Bale)
E67(T1003) - (Bore)
E68(T1004) - (Bore)
E69(A15524) - S. of Katcha (Bale)
E70(Z37651) - Koti Balcha (Bale)
E71(T1215) - Shawe (Bale)
90
0.008
Ptychadena erlangeri (Bore1-Bale)
N48(T1108) - Koma (Kaffa)
N49(A15511) - Dolo Mena (Bale)
N45(T1061) - Doshka (Gughe)
N46(T1064) - Doshka (Gughe)
N47(T1063) - Doshka (Gughe)
N43(T1107) - Mankira (Kaffa)
N44(T1106) - Bonga (Kaffa)
N42(T1104) - Bonga (Kaffa)
N50(T1062) - Doshka (Gughe)
N51(T1103) - Bonga (Kaffa)
N52(T1198) - Angle (Gughe Mt)
N53(T1102) - Bonga (Kaffa)
N54(T1059) - Doshka (Gughe)
N55(T1111) - Saja (Kaffa)
N56(T1110) - Saja (Kaffa)
N57(T1060) - Doshka (Gughe)
N58(T1105) - Berta/ Bonga (Kaffa)
N59(T1109) - Saja (Kaffa)
N60(T1101) - Bonga (Kaffa)
0.016
0.009
0.008
79
56
78
Ptychadena neumanni (Gughe - Kaffa - ?Dolo Mena)
93
0.032
D37(T1288) - Debre Markos (Gojam)
D38(T1289) - Debre Markos (Gojam)
D39(T1287) - Debre Markos (Gojam)
D40(T1285) - Debre Markos (Gojam)
D41(T1286) - Debre Markos (Gojam)
95
0.023
Ptychadena sp. (Debre Markos)
69
0.011
C28(T1306) - Didaa (Arsi)
C31(T1305) - Didaa (Arsi)
C29(Z37706) - Gaysay (Bale)
C32(T947) - Dinshu (Bale)
C33(T948) - Dinshu (Bale)
C34(Z37703) - Gaysay (Bale)
C35(Z37704) - Gaysay (Bale)
C36(Z37705) - Gaysay (Bale)
83
0.009
93
0.017
98
Ptychadena cf. *cooperi* (Arsi-Bale)
73
0.082
W109(T1283) - Andasa (Gojam)
W110(T1284) - Andasa (Gojam)
100
0.061
94
Ptychadena wadei (Andasa) --- Ethiopian Highlands
P111(DQ525941.1) - Tanzania
Ptychadena porosissima (Tanzania)
0.066
0.027
52
A20(T1290) - Soddo (Wolayita)
A23(DQ525921.1) - Somalia
A17(T1293) - Soddo (Wolayita)
A18(T1291) - Soddo (Wolayita)
A19(T1066) - Arba Minch (Gamo Gofa)
A24(A15516) - Chiri (Bale)
A21(A15546) - Web River (Bale)
A22(T1294) - Sawla (Gamo Gofa)
A25(A15512) - Dolo Mena (Bale)
A26(A15513) - Dolo Mena (Bale)
A27(A15517) - Chiri (Bale)
100
0.069
60
Ptychadena anchietae
100
0.109
M12(AY517599.1) - Kenya
Ptychadena mascareniensis (Kenya)
59
0.016
0.013
M13(AY517597.1) - Benin
Ptychadena mascareniensis (Benin)
0.027
95
M14(AY517587.1) - Reunion Island
M15(AY517590.1) - Madagascar1
Ptychadena mascareniensis (Reunion Island - Madagascar)
81
0.012
0.021
M16(DQ283031.1) - Madagascar2
Ptychadena mascareniensis (Madagascar)
72
0.006
0.015
72
M10(A15576) - Filwoha/ Awash NP (Rift Valley)
M11(A15578) - Filwoha/ Awash NP (Rift Valley)
M9(AY517596.1) - Egypt
M8(Z37592) - L. Chitu (Rift Valley)
M1(T1295) - L. Langano (Rift Valley)
M2(T1296) - L. Ziway (Rift Valley)
M3(T1298) - L. Ziway (Rift Valley)
M4(T1299) - L. Ziway (Rift Valley)
M5(T1292) - Soddo (Wolayita)
M6(T1297) - L. Ziway (Rift Valley)
M7(Z37589) - L. Langano (Rift Valley)
90
0.033
Ptychadena cf. *mascareniensis* (NE Africa)
Ar114(HM238195.1) - Cameroon
Arthroleptis palava (Cameroon)
0.059
0.038
Le112(FJ151060.1) - Uganda
Leptopelis kivuensis (Uganda)
78
0.189
0.092
As113(DQ283349.1) - Cameroon
Astylosternus schioetzi (Cameroon)
0.048
0.05 substitutions per site

Fig. 3.3 (previous page). Maximum Likelihood tree: Phylogenetic relationship of Ethiopian species of *Ptychadena* inferred using partial mtDNA of the 16S genes. The evolutionary history was inferred by using the Maximum Likelihood method based on the Data specific model (Nei and Kumar, 2000). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap (500 replicates) is shown above the branches (Felsenstein, 1985). Branch support values are shown only when greater than 50%. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (below the branches). Branch length values are not shown when lower than 0.005. The analysis involved 113 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 452 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2007).

Table 3.4. Preliminary estimates of divergence time for Ethiopian *Ptychadena,* inferred from secondary calibrations of the interval of time estimated for the split between Astylosterninae and Leptopelinae at 99.3-43.3 Mya (Bossuyt et al., 2006).

Events of split and diversification	**Interval of time (Mya)**	**Mid-point (Mya)**
Start of diversification of *Ptychadena* in the Ethiopian Highlands	21.6 - 9.4	15.5
Split between *Ptychadena* of Ethiopian Highlands and lowland areas	46.1 - 20.1	33.1
Split between *P. wadei* and *P. porosissima*	23.3 - 10.2	16.8
Start of diversification of *P. mascareniensis* haplotypes	29.6 - 12.9	21.3
Start of diversification of *Ptychadena* in Africa	85.9 - 37.5	61.7
Split between Astylosterninae and Leptopelinae	99.3 - 43.3	71.3

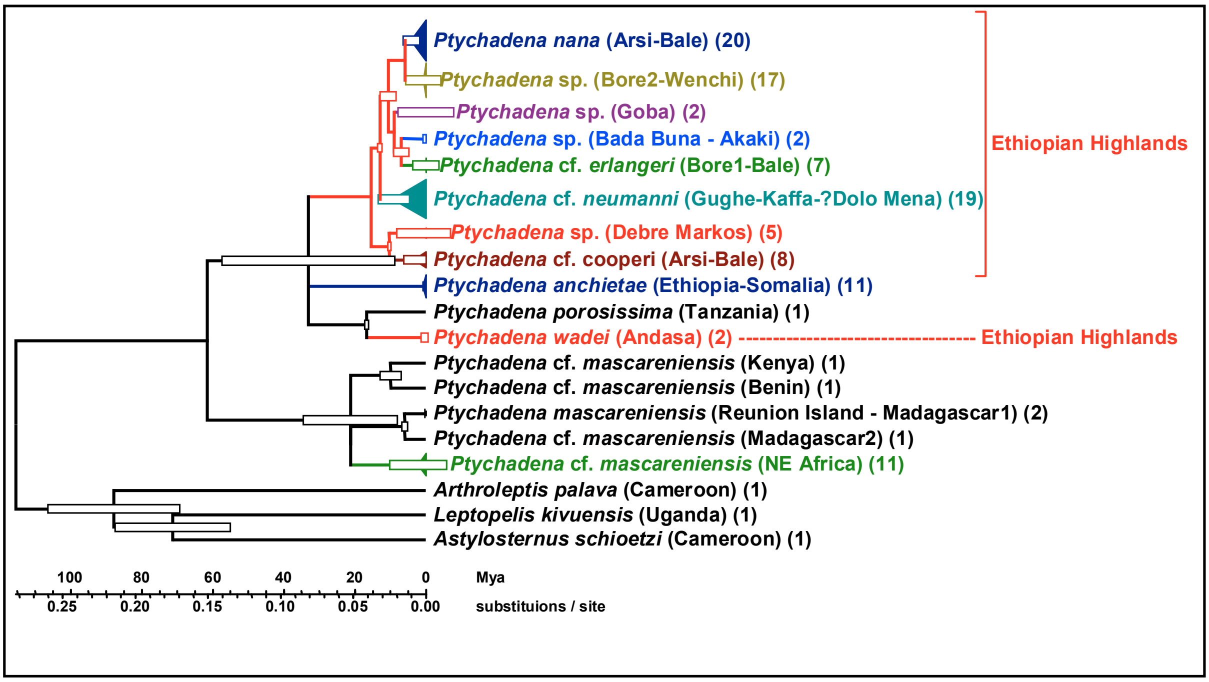


Fig. 3.4. Molecular timescale for evolution of Ethiopian *Ptychadena,* preliminarily estimated using secondary calibrations of the split between Astylosterinae and Leptopelinae around the K-T boundary (Bossuyt et al., 2006). The evolutionary relationship used for the estimation is reconstructed using MP consensus tree with 1000 bootstrap replicates. Numbers in parentheses indicate number of samples.

B) Morphology

Some of the newly studied morphological characters were to some extent informative in distinguishing between species. These include, morphometric (FmL:SVL, TbL:SVL, SVL:HiLi and IMTL:T1L) and non-morphometric (Spc, OMT, SnM, VSC and TyM) morphological characters. The morphometric characters show a wide overlap among species (Fig. 3.5), and are difficult to use as diagnostic features for all studied species. Summarized data for the non-morphometric morphological characters are presented in Appendix 3.3.

Principal component analysis (Figs. 3.6) and pairwise t-test comparisons of means of morphometric data (Appendix 3.4) between different species of *Ptychadena* in Ethiopia indicate that most of the points aggregate close to the center. Proportionally very long hind limbs (SVL:HiLi; or FmL, TbL and FtL against SVL) are characteristics of *P. erlangeri, P. neumanni* and *P. anchietae*, contrasting with very short ones of *Ptychadena* sp. (Debre Markos), *P. wadei* and Ethiopian *P. mascareniensis* (Fig. 3.7, top). Ethiopian *P. mascareniensis* has the smallest IMTL:T1L ratio of all, followed by *P. cooperi*; the largest value is for *Ptychadena* sp. (Bore-Wenchi) (Fig. 3.7, bottom). Relatively broad head width (HW:SVL) is seen in *P. cooperi* and *P. nana*, whereas the narrowest head widths are those of *P. erlangeri*. Summarized descriptive data and results of all pairwise tests performed for all of the studied morphometric characters are presented in Appendix 3.4.

Principal component analysis of some non-morphometric characters resulted in relatively good groupings (Fig. 3.8). Males of *P. neumanni* (including the male holotype) and the single newly collected male specimen of *Ptychadena* sp. (Debre Markos) were found to have their dorsal side covered with spicules (Spc = moderate to extensive), whereas those of *P. erlangeri, P. anchietae* and *P. mascareniensis* were brief or totally absent (holotype of *P. erlangeri* is a female without Spc) (see colored grouping in Fig. 3.8, bottom). Vocal sac color (VSC) of males of *P. erlangeri*, *P. neumanni*, *P. wadei* and the Arsi population of *P. nana* is dark (black or grey), while that of *Ptychadena* sp. (Debre Markos), *P. cooperi*, *Ptychadena* sp. (Bore-Wenchi), and the Bale population of *P. nana* are light (yellow, greenish-yellow) in color. There is no white tympanic marking (TyM) in *P. nana* or *Ptychadena* sp. (Bore-Wenchi), whereas this trait was variable (as absent, dull or white) in all other species and populations.

Some unique morphological features were consistent with earlier reports: presence of an outer meta-tarsal tubercle (OMT) in *P. wadei,* as well as presence of pale triangle on the snout and extensive foot webbing in *P. anchietae* (Fig. 3.8). Measurements and attributes of the remaining features, including SVL alone, ENL, INL, VeM, DSkF, ThTM, TbTM, DLR, and body color, were widely overlapping among species, or variable within a species or population, and are poorly applicable for diagnosis within the studied Ethiopian *Ptychadena*. The male vocal sac apparatus (or its positioning) may not be a good character to distinguish individuals that are not fully matured. Inger (1956) reported that the vocal sac in *P. porosissima* starts to develop (or invaginates) after reaching SVL of 29 mm, and it becomes clearly visible later during full maturity of adults, mostly after reaching SVL 35 mm; vocal sacs were absent for individuals as small as 22 cm and even as big as 40 cm.

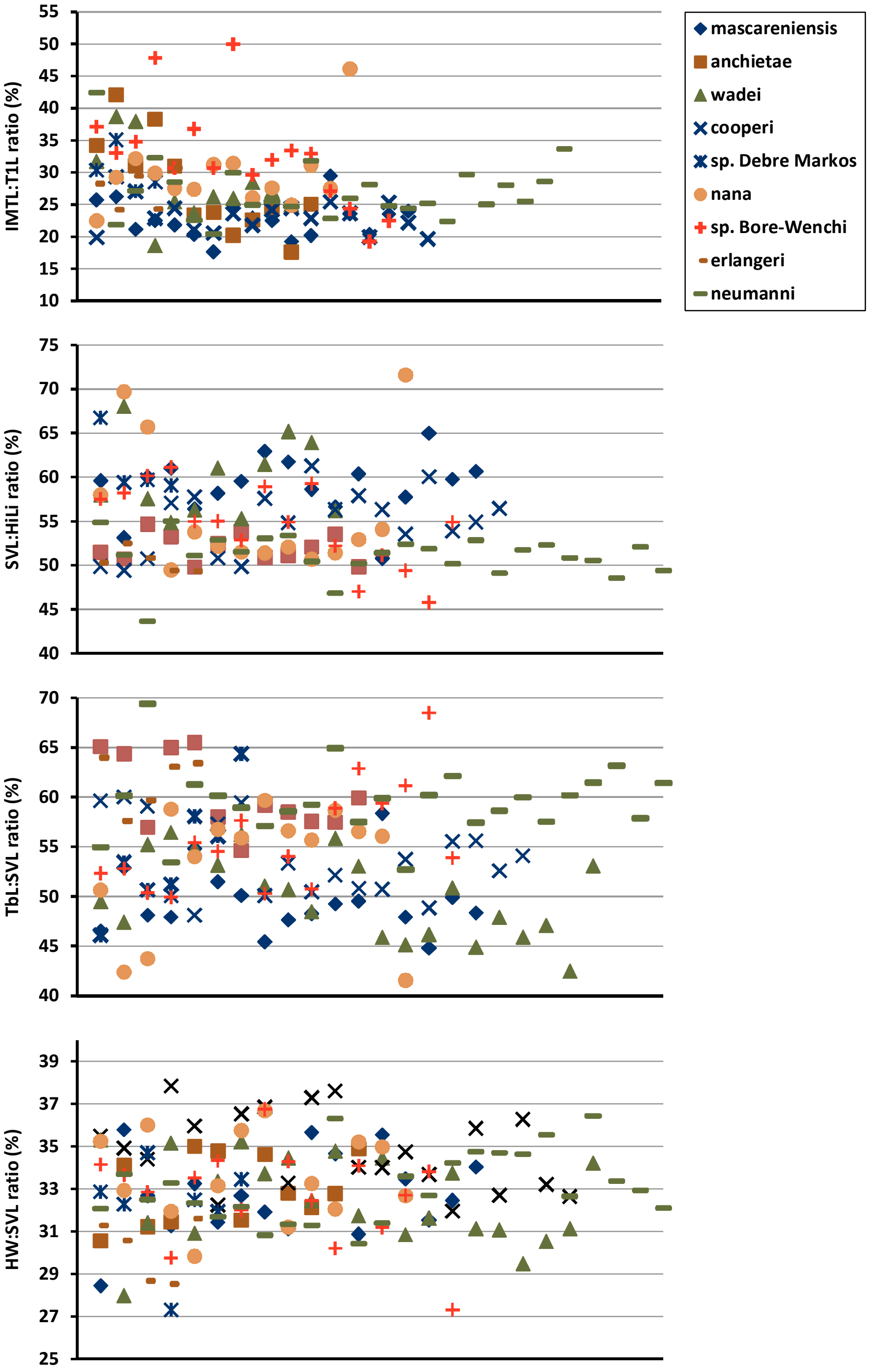


Fig. 3.5. Scatter plots of some morphological features that can be used to make distinction between some species of *Ptychadena* in Ethiopia. Note the wide overlap in all features for most of the species. Features (top to bottom): ratios of IMTL:T1L, SVL:HiLi, TbL:SVL, and HW:SVL.

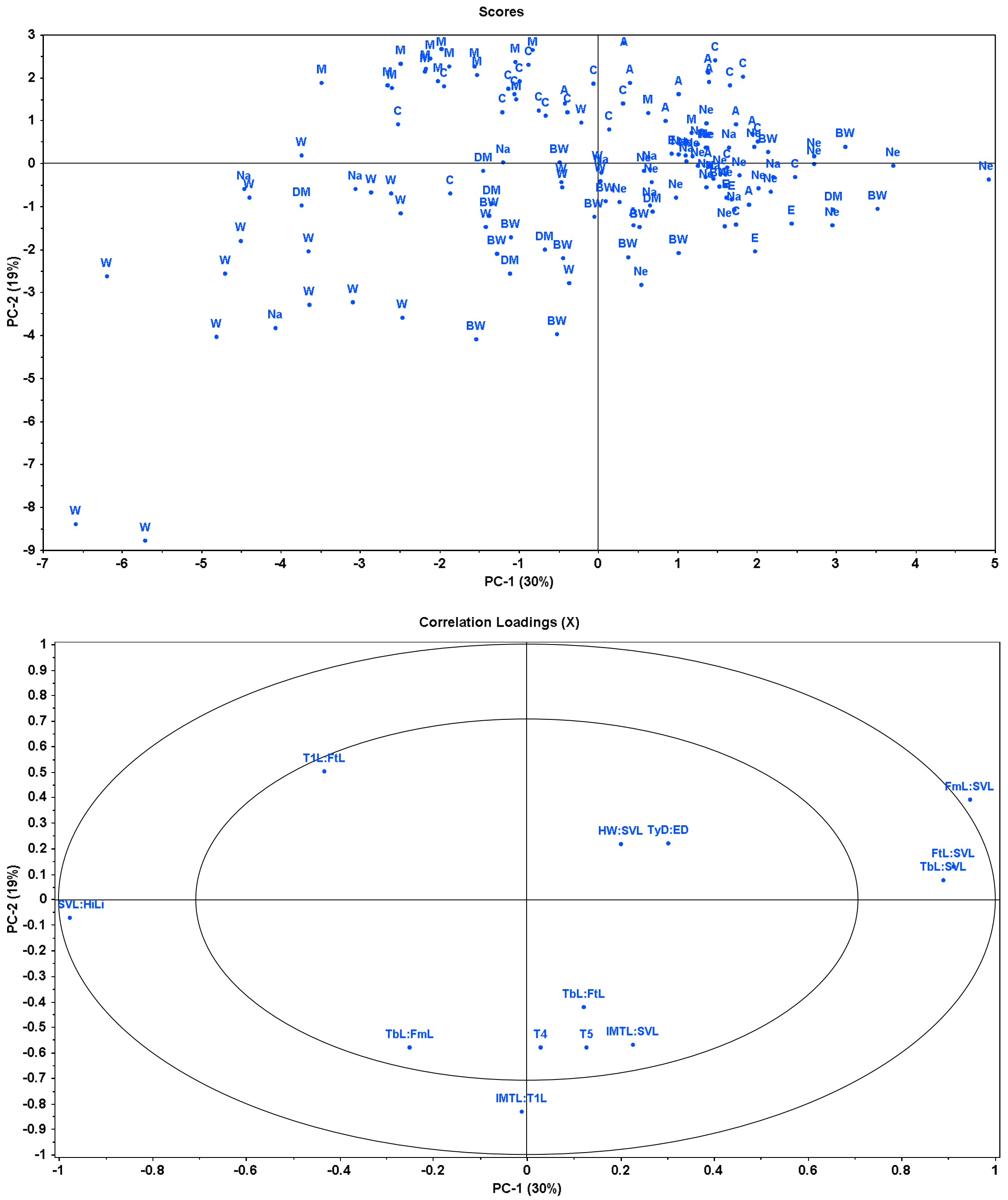
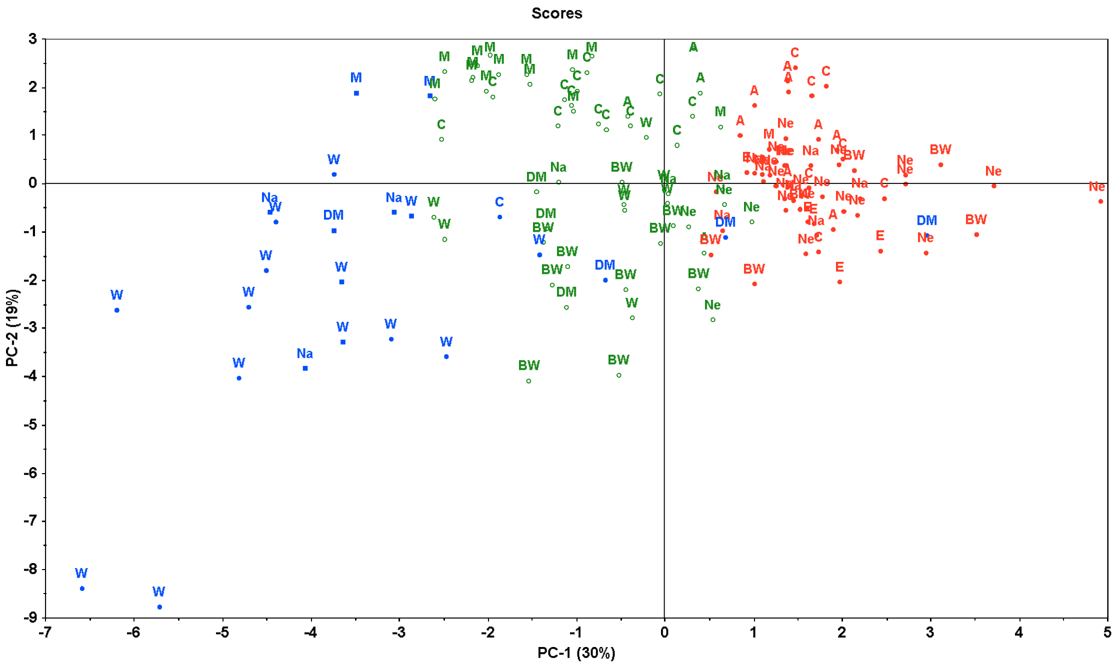


Fig. 3.6. Principal component analysis of some morphometric characters of Ethiopian *Ptychadena*. The scores plot (top) depicts weakly resolved groupings of species and populations. Most of the points are placed close to the center, while few others cluster farther. Letter abbreviations represent species of *Ptychadena*: A: *anchietae*, BW: sp. (Bore-Wenchi), C: *cooperi*, DM: sp. (Debre Markos), E: *erlangeri*, M: *mascareniensis*, Na: *nana*, Ne: *neumanni*, W: *wadei*. The loadings plot (bottom) shows the characters (see Acronyms) associated with the above groupings. The points placed in between the two ellipses represent characters that brought about the principal components for the major groupings; the characters close to the center are less informative to form groupings.



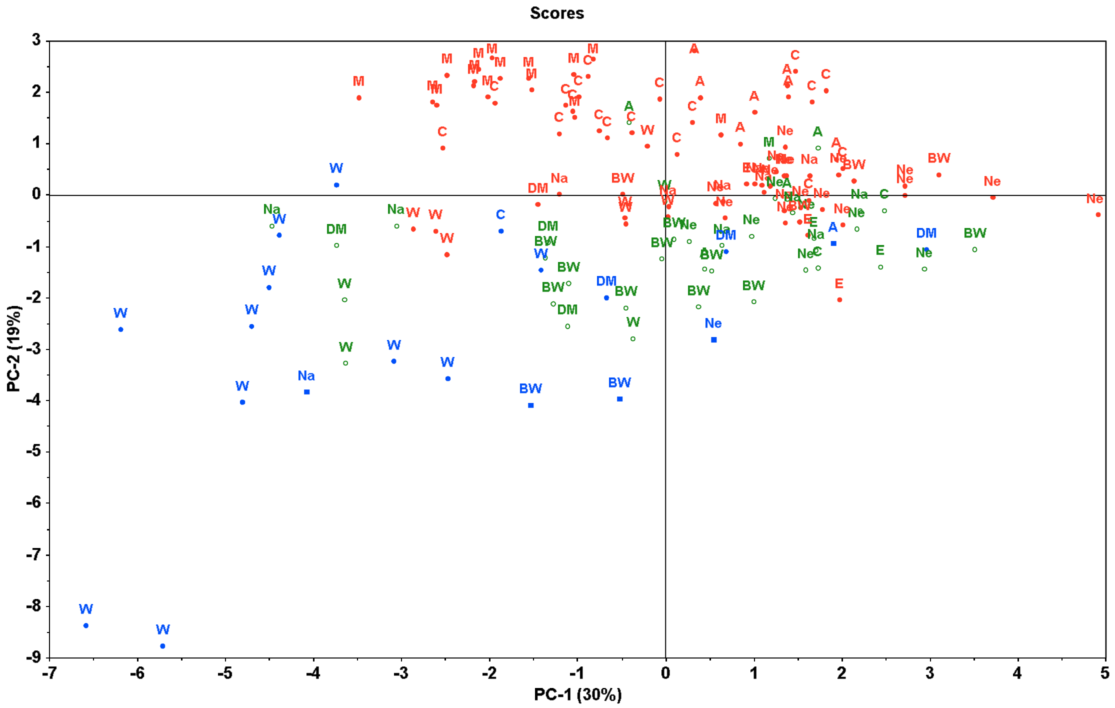


Fig. 3.7. Color separation of major groupings of Ethiopian *Ptychadena* using principal component analysis of two morphometric characters. Letter abbreviations represent species of *Ptychadena*: A: *anchietae*, BW: sp. (Bore-Wenchi), C: *cooperi*, DM: sp. (Debre-Markos), E: *erlangeri*, M: *mascareniensis*, Na: *nana*, Ne: *neumanni*, W: *wadei*. Top plot: ratios of hind limb length (HiLi:SVL), with relatively high (red, to the right), medium (green), and low (blue, to the left) values. Bottom plot: ratios of inner metatarsal tubercle length (IMTL:T1L), with relatively high (blue, to the bottom), medium (green), and red (low, to the top) values. The scores in both plots show weakly resolved groupings of species and populations.

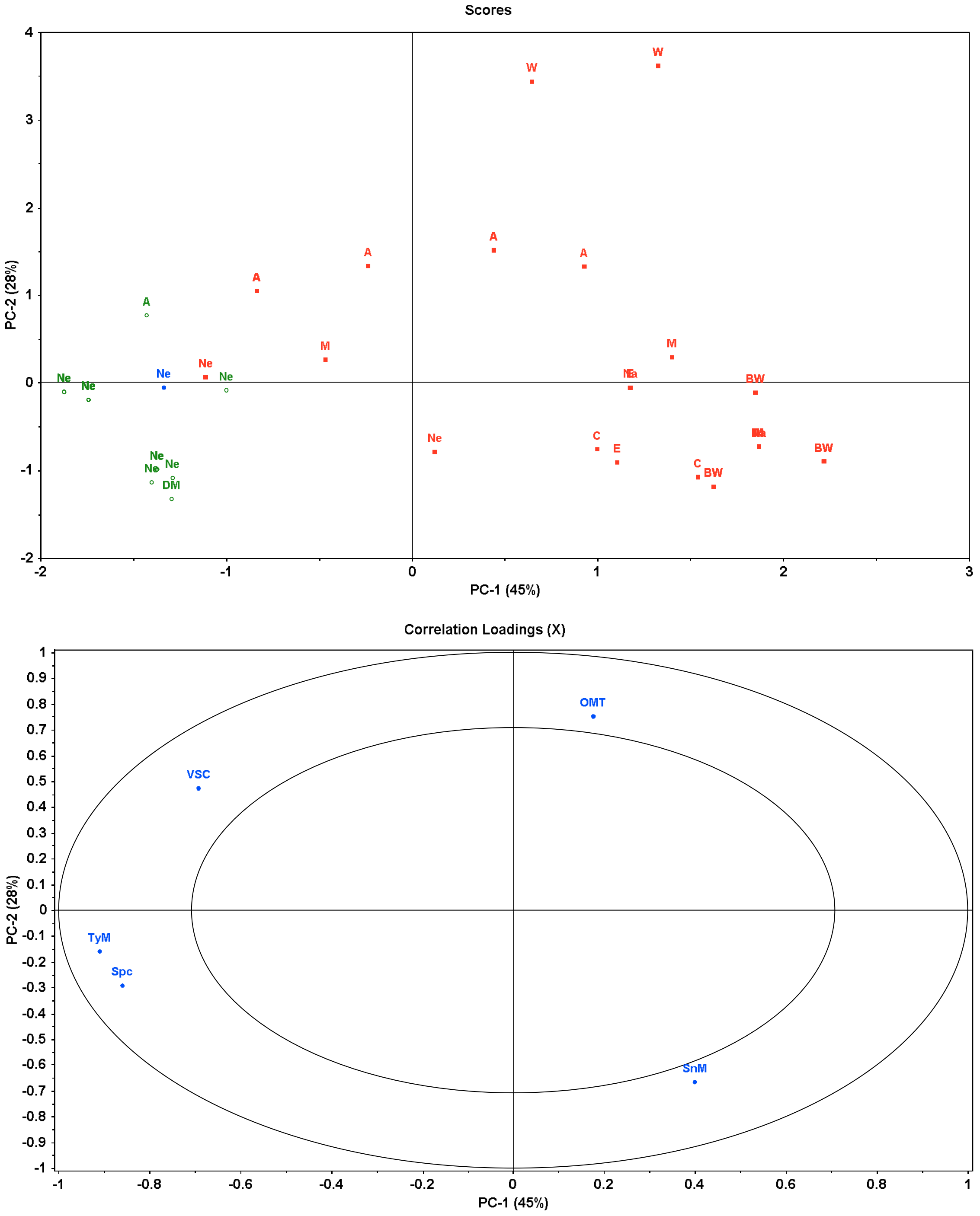


Fig. 3.8. Principal component analysis of five non-morphometric characters of males of Ethiopian *Ptychadena*. The scores plot (top) depicts major groupings that are positioned relatively farther from the center. Letter abbreviations represent species of *Ptychadena*: A: *anchietae*, BW: sp. (Bore-Wenchi), C: *cooperi*, DM: sp. (Debre-Markos), E: *erlangeri*, M: *mascareniensis*, Na: *nana*, Ne: *neumanni*, W: *wadei*. The role of spicules (Spc) to diagnose *P. neumanni* is shown in the color separation, green (to the left) indicating presence, and red (to the right) indicating absence. The loadings plot (bottom) shows the characters that are strongly associated with the above groupings.

C) Geographic Distribution

In general, valleys and gorges of major rivers, associated with warmer climates and habitats different from elevated areas, are the major sources of vicariance for populations of *Ptychadena* in the Ethiopian Highlands (Table 3.5). The geographic distribution and altitudinal ranges of the studied populations of *Ptychadena* in Ethiopia were compiled for each of the identified taxa that resulted from genetic and morphological data (Figs. 3.9 – 3.14). These distribution patterns appear to correspond with the physical characteristics and climatic conditions of the respective localities.

***Ptychadena* cf. *cooperi* (Arsi-Bale):** The type locality of *P. cooperi* (Wouramboulchi, in Shoa, central part of the western rift; Fig. 3.9) could not be located during our survey. The current and historic records from the eastern rift comprise two distinct populations occupying montane grassland habitats, separated by the Shebele River gorge. One group occupies open cattle grazing fields near rivers in Arsi-Robe (Dida'a) plateau at 2410-2450 m, while the other inhabits pristine or partially-modified natural grassland habitats in the Bale Mountains (Dinshu, Rira, and Harenna Forest) at 2920-3170 m. Both geographic areas are part of the central and southern parts of the Eastern Rift. Historic records also include an additional population of *P.* cf. *cooperi* from Bore area in the eastern rift.

***Ptychadena* sp. (Debre Markos):** This isolated population inhabits montane grassland in very old settlement or urbanized areas with grazing fields, agriculture or small-scale industry. The town of Debre Markos is located at 2400 m in an area encircled by the Abay River, in the Northwestern part of the Western Rift (Fig. 3.9). Previous reports most probably identified this population as *P. erlangeri* because of the large size of the adults.

***Ptychadena wadei* (Andasa):** Recorded from grazing fields in human-inhabited areas, this distinct group is known from the type locality at the edges of the Abay River south of Lake Tana near the Tisissat Falls (Fig. 3.9). Our samples were collected near Andassa village located at 1720 m in the Northwestern part of the Western Rift. This species was the only Ethiopian highland species that clustered with species from other parts of Africa.

***Ptychadena* cf. *erlangeri* (Bore1-Bale):** These are specimens recorded in the current study from two localities in the eastern rift (Fig. 3.10). The two populations are relatively close to one another, residing across the Genale River gorge in grasslands near rivers. One population is from lower altitudes in Bale (in pristine montane forest near Shawe River at about 1900 m and Chiri area at about 1500 m); the other population comes from around Bore and Kibre-Mengist towns (altitude: 1670-2680 m). Bore area is located at the southern end of the eastern rift and is geographically very close (about 50 km) to the type locality of *P. erlangeri*.

Table 3.5. Association of phylogenetic groupings of *Ptychadena* in Ethiopia (as used in the current study) with biogeographic categories* of highland segments and the Rift Valley (as discussed in Chapter 2).

Locality (broad region)	**Category of geographic segment**	**Major Geographic Barrier(s)**	**Habitat Types**	**Altitude (m) (Current data only)**	**Known Records of Species of *Ptychadena***
Arsi-Bale Mountains	Southern Central	East African Rift Valley Genale River Gorge Southern lowlands	Montane grassland, forest edge, Afroalpine moorland, streams, swamps with small shrubs	1500 - 3170	*Ptychadena erlangeri* *Ptychadena* cf. *cooperi* *Ptychadena nana* *Ptychadena* sp. (Goba) *Ptychadena neumanni* (Dolo Mena)**
Ethiopian Rift Valley	Rift Valley	Eastern and western rifts	Warmer habitats around permanent lakes or rivers, flood pools, swamps, permanent hotsprings	200 - 1840 (?2500)	*Ptychadena anchietae* [2] *Ptychadena* cf. *mascareniensis* (NE Africa) [3]
Gojam	Northwestern	Abay River Gorge Western lowlands	Montane grassland, with shrubs, swampy fields, flood pools	1720 - 2400	*Ptychadena wadei* *Ptychadena* sp. (Debre Markos) *Ptychadena porosissima* [1]
Kaffa - Jimma - Gamo Gofa	Southwestern	East African Rift Valley Western lowlands	Montane forest, streams, swamps, or grassland, backyard vegetation	1237 - 2520	*Ptychadena neumanni* *Ptychadena* sp. (Bada Buna - Akaki)
Shoa	Central	Abay River Gorge East African Rift Valley	Montane grassland, small streams, flooded pools	2038 - 3080	*Ptychadena cooperi* [1] *Ptychadena* sp. (Bore2-Wenchi) *Ptychadena* sp. (Bada Buna - Akaki)
Sidamo/Bore	Southern	Genale River Gorge East African Rift Valley Southern lowlands	Montane grassland, streams and swamps	1670 - 2680	*Ptychadena cooperi* [1] *Ptychadena erlangeri* *Ptychadena* sp. (Bore2-Wenchi)

* Parts of the Ethiopian Highlands that have never been or less sampled before include the Northern (Gondar, Tigray), Northeastern (Wollo, North Shoa), and Eastern (Hararge) areas.

** We are unsure whether a single DNA sequence (A15511) that clustered with *P. neumanni* is a laboratory mistake or if it actually came from Bale area (eastern rift).

[1] Genetic data not represented in our samples, but included here with reference to old museum specimens as presented in Largen (1977).

[2] *Ptychadena anchietae* has a wide distribution in the southern and western lowlands of Ethiopia (Largen 1977); the population at the southern foothills of the Bale Mountains occupies areas with lower altitudes, previously thought to have been the type locality for *P. harenna*.

[3] The range of *P.* cf. *mascareniensis* (NE Africa) in Ethiopia now extends further north to Awash National Park in the Rift Valley, formerly assumed to have been the type locality of *P. filwoha*.

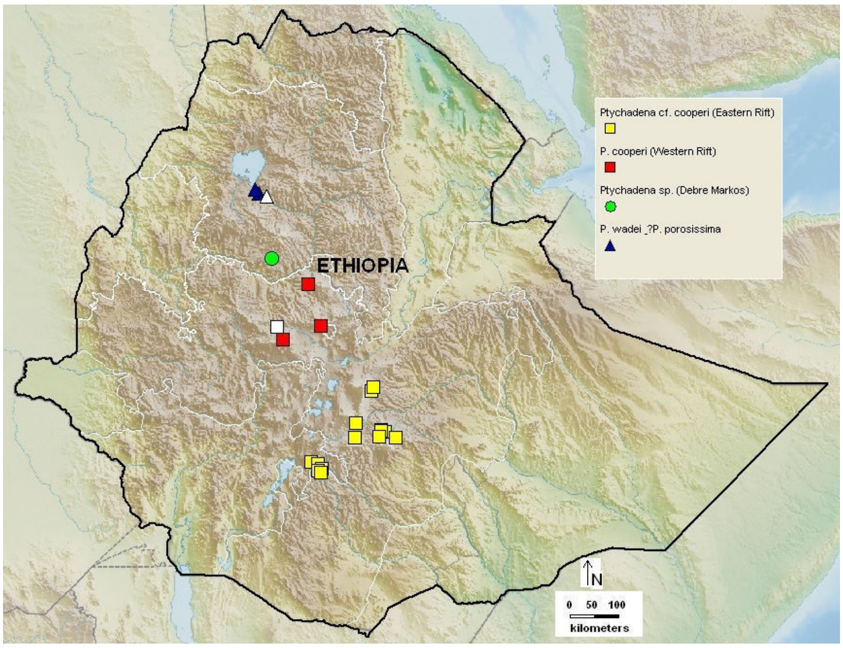


Fig. 3.9. Revised biogeographic distribution of *Ptychadena* cf. *cooperi* (Eastern Rift) (yellow squares), *P. cooperi* (Western Rift) (red squares), *Ptychadena* sp. (Debre Markos) (green circle), *P.wadei* (including *P. porosissima*; blue triangle) in Ethiopia. Type localities: white triangle = *P.wadei*; white square = *P. cooperi*. White lines indicate regional boundaries, blue lines indicate major rivers; light blue areas indicate lakes.

***Ptychadena* cf. *neumanni* (Gughe - Kaffa - ?Dolo Mena):** This species was previously assumed to have had a widespread occurrence in a wide altitudinal range. In the current assessment, its geographic and altitudinal range is reduced substantially. It is now restricted to the southwest highlands in the western rift, with the exception of a single specimen (probably a sample mix up between laboratories) recorded from the eastern rift near Dolo Mena (Fig. 3.10). The recorded altitudinal range for this group generally reaches between 1237 and 2520 m. The population in the western rift is formed of sub-populations occupying adjacent but highly fragmented montane grassland and forest areas with varied habitats such as grasslands or swamps embedded in pristine forests, near rivers, as well as flood pools in towns and villages. These sub-populations are located across the Omo River valley in patchy forest hills in Kaffa (Mankira, Saja, Koma forests, in and around Bonga town) at 1620-2030 m, and Gughe Mountain (Angle and Doshka Forests) at 2270-2520 m, all belonging to the Southern part of the Western Rift. The reported type locality of this species (Gadat, Gemu Gofa, at 2000 m) is between the Kaffa Highlands and Gughe Mountains, but it was not possible for us to find a specific locality and altitude (2000 m) matching the old record of coordinates. The population in the eastern rift dwells in grasslands close to Yadot River (near Dolo Mena) at an altitude of 1237 m at the base of the southern side of the Bale Mountains.

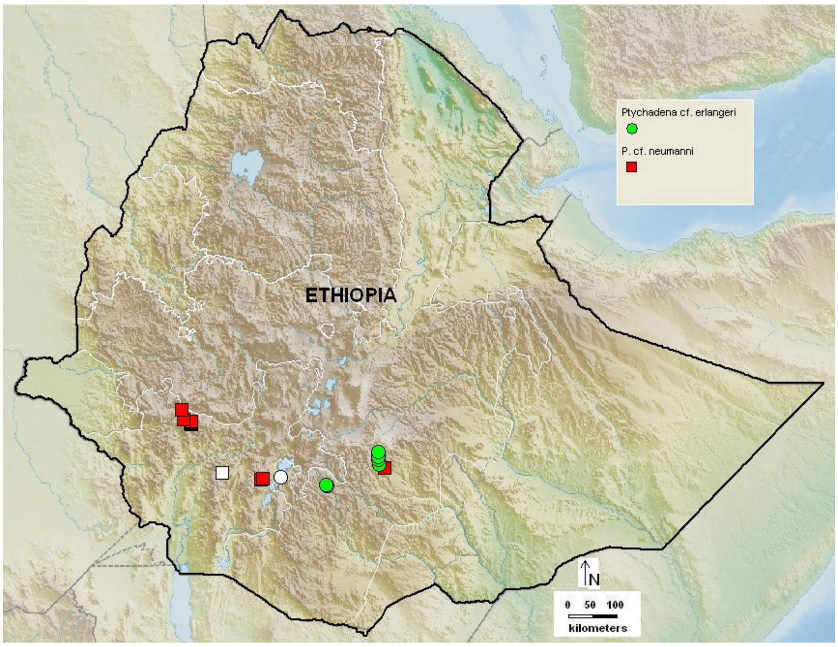


Fig. 3.10. Revised biogeographic distribution of *Ptychadena* cf. *erlangeri* (circles) and *P.* cf. *neumanni* (squares). Type localities: white circle = *P. erlangeri*; white square = *P. neumanni*. White lines indicate regional boundaries; blue lines indicate major rivers; light blue areas indicate lakes.

Ptychadena nana **(Arsi-Bale):** This species was previously recorded only from the type locality in Arsi. In the current study specimens collected from Bale genetically appear close to this species, and both populations occupy areas in the plateaus in the eastern rift (Fig. 3.11). Some of these populations were previously considered to belong to *P. neumanni* that was assumed to have wider altitudinal range. The two major phylogenetic sub-groups of this species come from: **(i) Arsi:** These were caught at the type locality in open, cattle-grazing grasslands near creeks, or along roadside ditches in town, in the highland plateau in Arsi in the central part of the Eastern Rift. Specific localities are in and around Arsi-Robe (Dida'a) town, with altitude records of 2410-2450 m. **(ii) Bale:** These dwell in grasslands with nearby creeks or swamps, embedded within pristine dense tropical forests or partly degraded habitats, in the southern face of the Bale Mountains, Southern part of the Eastern Rift. Specific localities include Rira, Katcha and Harenna Forest, with altitudes ranging from 1900 to 2910 m.

Ptychadena **sp. (Bore2-Wenchi):** This group is recorded from some distantly separated areas across the Rift Valley (Fig. 3.11). These belong to two distantly located sub-populations within human settlement areas. One of them comes from near Bore town in the Southern part of the Eastern Rift (elevation: 2620-2680 m), while the other is from Hora village (elevation: 3080 m) near Lake Wenchi (west Shoa) in the Central part of the Western Rift.

Ptychadena **sp. (Bada Buna - Akaki):** A few specimens were recorded in 2006 from two localities in the western rift (Fig. 3.12). Their habitats are grasslands in the vicinity of Bada Buna

Forest at an altitude of about 1800 m (in the Southern part of the Western Rift), and another wetland area near Akaki (East Shoa) at 2038 m in the Central part of the western highlands.

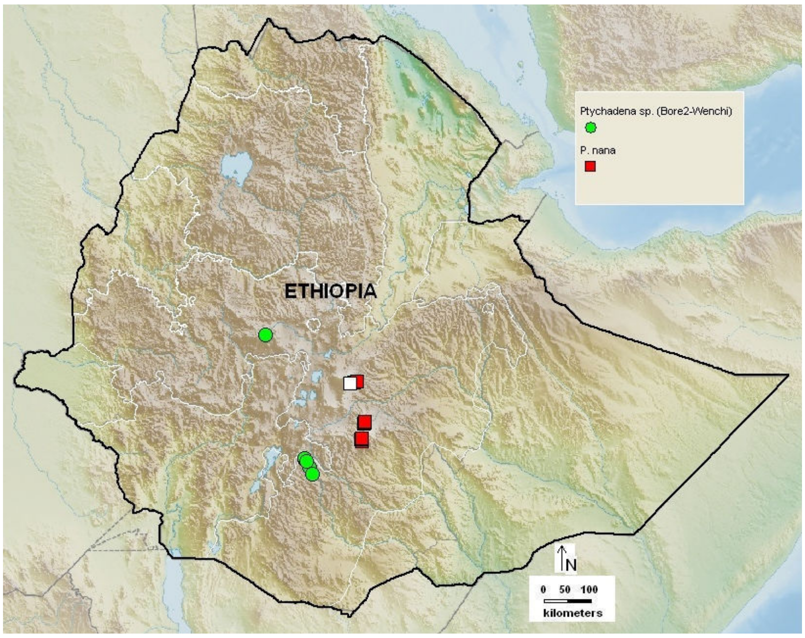


Fig. 3.11. Revised biogeographic distribution of *Ptychadena* sp. (Bore2-Wenchi) (circles) and *P. nana* (squares). Type locality: white square = *P. nana*. White lines indicate regional boundaries; blue lines indicate major rivers; light blue areas indicate lakes.

***Ptychadena* sp. (Goba):** These two specimens were recorded from a swamp and near a river in the highland plateau near Goba town in the Bale Mountains, eastern rift, at altitudes of 2112 and 2711 m (Fig. 3.12). This population could have been mistakenly considered as part of the presumed widespread populations of *P. neumanni*.

***Ptychadena anchietae* (Ethiopian Rift Valley – Dolo Mena):** A distinct group inhabiting lake-shore grasslands as well as flood pools near permanent low altitude waters (elevation: 1260-1800 m) (Fig. 3.13). The localities of the studied materials include vicinities of Soddo and Sawla towns in Gemu Gofa area at the edge of the Rift Valley, shore of Lake Abaya within the Rift Valley, and riverside habitats at Web Valley and the southern foothills of the Bale Mountains. The population from southern Bale area has been previously assumed to belong to *P. harenna*. Earlier Ethiopian records of *P. anchietae* include wide lowland areas (200 - ?2500 m) encircling the highlands and penetrating into several gorges bound by elevated landscapes.

***Ptychadena* cf. *mascareniensis* (Ethiopian Rift Valley – SW Lowlands):** A lowland population from the Rift Valley area, inhabiting similar habitats as that of *P. anchietae,* occurring in shoreline vegetation as well as flood pools near permanent low altitude waters near Soddo town and on shores of Lakes Ziway and Langano at altitudes of 1600-1840 m, and further down to about 800 m at the hot springs in Awash National Park in the northeastern part of the Ethiopian Rift Valley (Fig. 3.13); the later population was previously considered as a distinct species belonging to *P. filwoha*. This reveals horizontal (surface) and vertical (altitudinal) range extension for *P.* cf. *mascareniensis* in Ethiopia, wider than thought before.

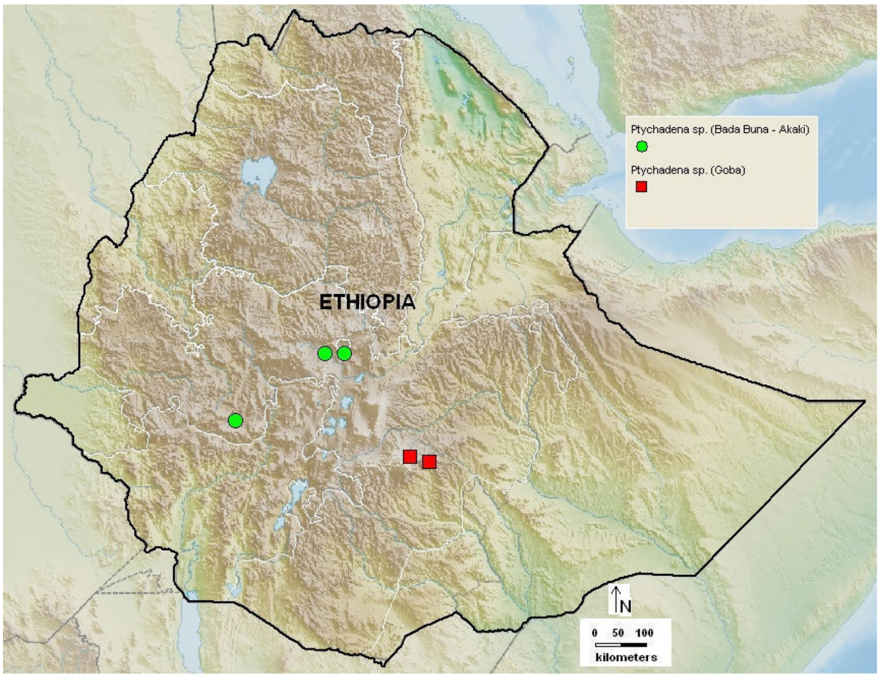


Fig. 3.12. Revised biogeographic distribution of *Ptychadena* sp. (Bada Buna - Akaki) (circles), and *Ptychadena* sp. (Goba) (squares). White lines indicate regional boundaries; green lines indicate major rivers; light blue areas indicate lakes.

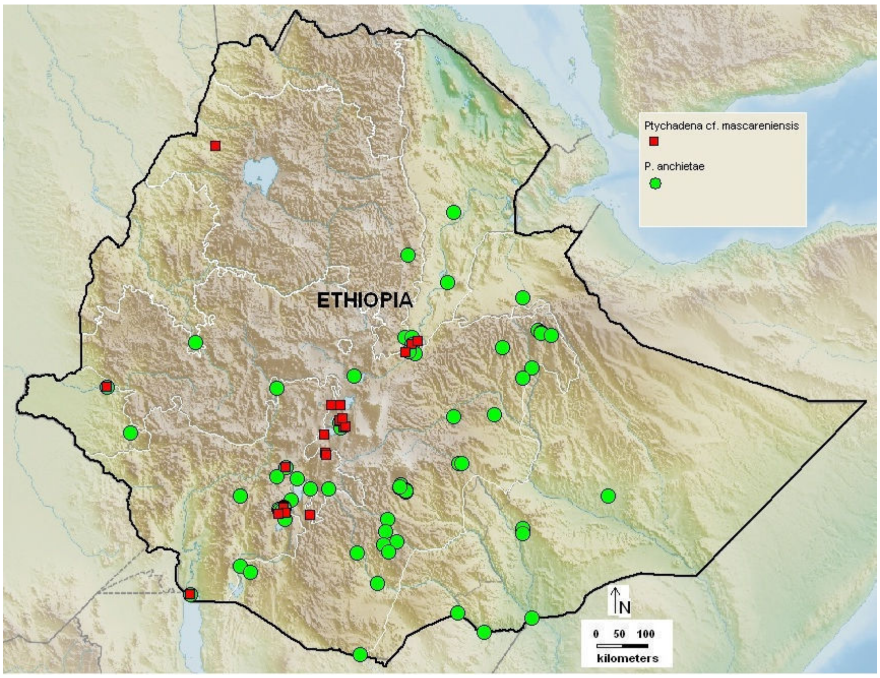


Fig. 3.13. Revised biogeographic distributions of *Ptychadena* cf. *mascareniensis* (squares) and *P. anchietae* (circles). White lines indicate regional boundaries; blue lines indicate major rivers; light blue areas indicate lakes.

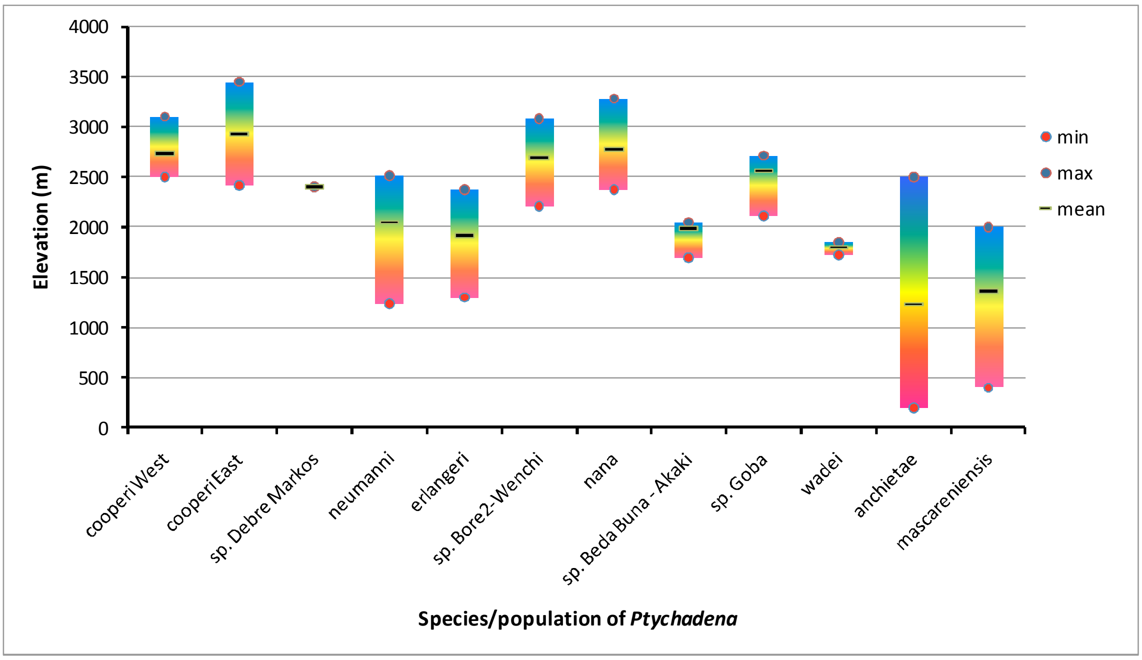


Fig. 3.14. Altitudinal ranges of geographically and genetically disjunct species and populations of *Ptychadena* in Ethiopia. Note that the mean values could be at, above or below the median. Species having only single locality records are shown without elevation ranges. Color gradient: blue-green-yellow-orange-red = cooler high-ground to warmer low-ground.

IV) DISCUSSION

The current assessment of phylogenetic relationships and biogeographic patterns of *Ptychadena* in Ethiopia indicates that there was substantial confusion in assigning specimens to nominal taxa based on morphology. The complexity of the taxonomy of the highland populations of *Ptychadena* was previously discussed in Largen (1997, 2001). A similar problem has also been discussed for *Leptopelis* (Largen, 1977, 2001; Chapter 2 in this thesis). The use of mtDNA data has been proven helpful to reveal cryptic taxa that were previously considered a single species based on morphological studies. A study of populations of the widespread African species *P. mascareniensis* has uncovered that there are at least five distinct species merged as one (Vences et al., 2004). This study reflects how the diversity of species in this genus has been underestimated.

Our investigation on the morphology of this genus is broadly congruent with previous studies on Ethiopian species of *Ptychadena*. Following the identification key in Largen (2001), we encountered several confusing sub-groups within the '*P. neumanni* complex' (*P. erlangeri, P. nana, P. neumanni*). Thus, the use of external morphological characters alone for identification of most highland species or populations of *Ptychadena* in Ethiopia (Fig. 3.15) is problematic. We have identified a few new diagnostic characters to differentiate at least some of the most problematic highland taxa (e.g., presence of spicules in males is shown to differentiate *P. neumanni* and *P. erlangeri)*. We have also demonstrated some of the morphological variation that might allow the subsequent recognition of putative new species (see below). It is relatively simple to use morphology to compare lowland versus highland forms based on webbing of feet. All in all, the current findings highlight what the patterns of distribution and evolutionary relationships of the known populations look like, and show the direction for future research.

Fig. 3.15. Images in life (except D, E and H) of Ethiopian species of *Ptychadena* assessed in the current study; *Ptychadena* sp. (Bada Buna - Akaki) is not included: A) *P. cooperi* (holotype: BM-1927.7.5.15/1947.2.6, Wouramboulchi, Shoa); B) *P.* cf. *cooperi* (AM-112, Dida'a town); C) *Ptychadena* sp. (Debre Markos) (AM-025, Debre Markos); D) *P. erlangeri* (holotype: ZMB-26887, East side of Lake Abaya); E) *P. neumanni* (holotype: ZMB-26879, Gadat, Gamo Gofa); F) *P. nana* (AM-104, Dida'a town); G) *Ptychadena* sp. (Bore2-Wenchi) (AM-122, Hora Village, Lake Wenchi); H) *Ptychadena* sp. (Goba) (Z-37757, Bamo River, Goba, Bale); I) *Ptychadena wadei* (AM-016, Andasa); J) *P. anchietae* (AM-031, Soddo); K) *P.* cf. *mascareniensis* (AM-059, Ziway). Photo credit: Abebe A. Mengistu (A-G, I-K), and Breda Zimkus (H).

The current findings enabled to confirm the validity of some of the previously known species of Ethiopian *Ptychadena* based on morphology and molecular data. While some species appear to be valid, others are conspecific with other widespread species. These taxonomic findings have implications for the distribution of species across Ethiopia. The geographic (distribution and altitudinal) ranges of some species were extended, while those of others were reduced. The role of the Rift Valley and other natural barriers would be interesting to understand the biogeography of Ethiopian populations of *Ptychadena* and their evolutionary history in relation to other species elsewhere in Africa. Relating the geographic isolation of the studied taxa/populations with their evolutionary divergence times, it is reasonable to suggest a 2% divergence in the 16S mtDNA for distinction of species of *Ptychadena* in Ethiopia (see below). Below we document the phylogenetic groupings of *Ptychadena* recovered in our analyses. Although the sampling is not comprehensive, and will require future work, we suggest the following taxonomic implications and associated biogeographic divisions for each population.

A) Phylogenetic Grouping

The major division in the phylogenetic structure of Ethiopian *Ptychadena* is that seen between a diverse monophyletic highland cluster and the mostly non-endemic mid-altitude to lowland groupings. The highland endemics are formed by *P.* cf. *cooperi*, *Ptychadena* sp. (Debre Markos) *P. erlangeri, Ptychadena* sp. (Goba), *Ptychadena* sp. (Akaki – Beda Buna), *P. nana, Ptychadena* sp. (Bore1-Wenchi), and *P. neumanni*. Morphological distinction among the species in this cluster is quite complex, probably indicating, as emphasized in Largen (1997), their very recent diversification. The other cluster comprises of one endemic (*P. wadei*) and two strictly lowland species (*P. anchietae* and *P.* cf. *mascareniensis*). Morphological identification of species in this division is relatively simple. A list of the genetically assessed specimens, their taxonomic assignment and biogeographic category is given in Appendix 3.2.

As indicated in Vieites et al. (2009) and applied after modification for Ethiopian *Leptopelis* in Chapter 2 (this thesis), it would be necessary to have a common understanding of a functional grouping of the studied Ethiopian populations of *Ptychadena*. This grouping would be important to treat disjunct populations as entities for future research and meaningful conservation practices (Agapow, 2005). Unlike the case of *Leptopelis* in the Ethiopian highlands, some morphologically distinct species/populations of *Ptychadena* in this area show relatively large mtDNA gene variation (>3%). Morphologically valid species such as *P. nana* and *P. neumanni* have genetic divergence above 3%, while the lowest genetic distance between valid species such as *P. nana* nd *P. erlangeri* goes as low as 2%. As the populations of *Ptychadena* in the Ethiopian highlands are a recent radiation, a threshold value of 2% appears sufficient to guide species distinctions in this group. Following the same procedure followed to evaluate taxonomic status of populations and species of Ethiopian *Leptopelis*, we suggest to use morphological and geographic distribution evidences as supplementary criteria for taxonomic evaluation of natural groupings of *Ptychadena* in Ethiopia.

1) ***Ptychadena*** **cf.** ***cooperi*** **(Eastern Rift)**

The type locality for *P. cooperi* is Wouramboulchi, west of Addis Ababa in Shoa province, at 2750 m on the western rift. However, the coordinate given in the gazetter for localities in Largen (2001) did not correspond to this locality (Table 3.1). Lake Wenchi (at 2800 m, south of Ambo town in west Shoa) is a crater lake located in the vicinity of the coordinates for the type locality of *P. cooperi*. Largen identified a few specimens collected from Lake Wenchi (but altitude recorded as 3100 m) as *P. cooperi*. The specimens that we collected from Hora Village (at 3080 m, at the top edge of this crater) did not match the morphological description of *P. cooperi* and belonged to a separate phylogenetic grouping (*Ptychadena* sp. (Bore2-Wenchi)). The only specimens we sampled that matched the morphological description of *P. cooperi* were those collected from Arsi and Bale Mountains. There were no earlier records of this species from Arsi, and our samples from this area genetically form a sub-group (population) distinct from those in Bale. Earlier mtDNA sequences (retrieved from GenBank database) of specimens of *P. cooperi* (DQ283066 and DQ283067) from the Bale area also cluster with this group, confirming that our identification of the new specimens is consistent with the description of *P. cooperi*.

Individuals of *P.* cf. *cooperi* usually have distinct dark markings on the ventrum (Largen, 1997), and form one of the two highland taxa that are sister groups to the '*P. neumanni* complex'. Although *P.* cf. *cooperi* genetically clusters with a new cryptic taxon revealed from Debre Markos across the Rift Valley, and even across the Abay Gorge, their monophyly is not well supported. *Ptychadena* cf. *cooperi* can be diagnosed by a combination of its relatively very large HW:SVL and very short IMT:T1L ratios.

As we were not able to sample from the type locality in the western rift, we cannot test the monophyly and divergence of *P. cooperi* populations across the Rift Valley. The mtDNA gene variation of *P.* cf. *cooperi* to other populations is large enough (> 4%), its populations are confined to high altitudes, and is morphologically somewhat distinct. We will therefore refer to the population from the eastern rift as *P.* cf. *cooperi* until additional data can confirm the status of this population.

2) ***Ptychadena*** **sp. (Debre Markos)**

Previously specimens of *Ptychdena* from Debre Markos and its vicinities (Dangila and Injibara) were identified as *P. erlangeri* for having 'large' body size. This can be seen in the distribution map of *P. erlangeri* in Largen (1997, 2001), Largen and Spawls (2010) and IUCN (2011). The population from this area forms a group geographically completely isolated from *P.* cf. *cooperi* or the other highland taxa. It is found in Gojam province that is circumscribed by Lake Tana and the Abay River gorge. Therefore, it is possible that the range of this species could extend within this part of the highland plateaus. Basic morphological data are available for this group, including characters used previously by taxonomists, as well as some newly identified characters from this study. *Ptychadena* sp. (Debre Markos) has the largest SVL:HiLi ratio, that is proportionally the shortest hind limb of all. The only male specimen we collected also exhibits extensive spicules, an attribute shared with *P. neumanni* only. Based on mtDNA data the species is > 4% different from all other populations. Description of the new species awaits detailed analysis of these data, and hence we propose this population as a Confirmed Candidate Species.

3) *Ptychadena* cf. *neumanni* (Southwest highlands and 'Dolo Mena')

The type locality for *P. neumanni* is recorded as Gadat in Gemu Gofa province in the southwest highlands at about 2000 m. It was difficult to locate a place with this name corresponding to documented records of approximate coordinates and elevation (gazzetter in Largen, 2001). We have, however, assessed specimens from areas close to the type locality as well as distant areas in the western and eastern rifts previously thought to have been its ranges.

Ptychadena neumanni has been distinguished from *P. erlangeri* by its small size (but not smaller than *P. nana*; Largen, 1997, 2001). However, there is overlap in body size between these two species, and size could not therefore be a diagnostic character. From our morphological analysis we have found some additional morphological characters that could help to identify *P. neumanni* from its closest allies. Largen (1997) was doubtful on the possibility of subdividing the *P. neumanni* complex group based on morphology, suggesting any such effort would be 'subjective and arbitrary'. We disagree with this comment, and present here some useful diagnostic morphological characters that could enable distinguishing *P. neumanni* from conspecific species. Males of *P. neumanni* exhibit (moderate to extensive) sand-like, white-tipped, fine cone-shaped structures on the dorsal side of their body (sometimes including the top side of legs) and strictly on the lateral side of the body (Fig. 3.16). We termed these grain-like structures as "spicules," a feature that is clearly seen in the type specimen and escaped notice by earlier taxonomists. If one can't see the spicules with the naked eye, alternative use of a hand lens, a dissecting microscope, or a high resolution photograph would make it visible. This possibly sex-specific feature may have reproductive benefits to this species, and therefore physiological and behavioral study is needed to understand its function. In addition, *P. neumanni* can further be distinguished from *P. erlangeri* by having proportionally broad head width (large HW:SVL).

Instead of earlier assumptions of a widespread distribution and altitudinal range (820-3800 m) for this species, we found that it is almost entirely limited to the southwest highlands in the western rift at altitudes of 1620-2520 m. One specimen from Dolo Mena area at the foothills of the Bale Mountains (1237 m) in the eastern rift genetically clustered with *P. neumanni*. If this population (which is genetically 1.7% divergent from *P. neumanni* from SW) is considered taxonomically conspecific with *P. neumanni* then this would lead us to assume an extended lower altitude limit and wider distribution for *P. neumanni*. Otherwise all studied specimens were geographically restricted to the southwest highlands in Kaffa and Gamo Gofa. An earlier record of a *P. erlangeri* specimen from this region (MHNG 1449.71, from Cialla or Challa, west of Jimma in Kaffa) would possibly belong to the current grouping of *P. neumanni*. Broader survey is required to understand the complete distribution of *P. neumanni*.

Fig. 3.16. Extensive presence of conical spicules on dorsal and lateral body parts of a male *P. neumanni* (AK-1803) (top), versus absence in a male *P. erlangeri* (AK-1956) (bottom). Photo credit: Abebe A. Mengistu, 2010.

The type locality for *P. neumanni* is located within the general area where we sampled members of this cluster. Morphological features seen in these materials resemble best to the holotype and description of *P. neumanni*, and mtDNA gene variation (>3%) indicated the distinction of this group from all other taxa. Therefore, we confirm the validity of previous taxonomic designation of this species. The previously assumed widely distributed populations of this species outside of the southwest highlands are currently found to be conspecific with other species discussed in this section.

4) ***Ptychadena* cf. *erlangeri* (Bore1 – Bale)**

The type locality for this species is the eastern shore of Lake Abaya at about 1300 m, a locality at the western foothills of the southernmost segment of the Arsi-Bale massifs. The earlier distribution of this species has been thought to be patchy and distantly spaced (Largen, 1997, 2001; IUCN, 2011). One of the presumed populations thought to exist around Debre Markos across the Abay gorge has been found to be a genetically distinct cryptic species. Members of *P. erlangeri* that are included in the current phylogenetic assessment reside in close proximity, restricted only to the eastern rift. One of the populations in this group is distributed in places (Bore area) and altitudes (1670-2680 m) very close to the type locality of *P. erlangeri*, whereas the other poplation from Bale comes from lower altitudes (1500-1900), avoiding overlap with the Bore population of *Ptychadena* sp. (Bore2-Wenchi) that seems to prefer altitudes over 2600 m.

Morphological features reflected in members of the group identified as *P.* cf. *erlangeri* are consistent with the features used to describe *P. erlangeri*. The species has been earlier distinguished from its allies in the '*P. neumanni* complex' by its 'larger' (but practically widely overlapping with *P. neumanni*) body size. Our phylogenetic, morphological and biogeographic results demonstrate that the actual complex group of Ethiopian *Ptychadena* is formed by '*P. erlangeri* and allies,' rather than the genetically, morphologically and geographically distinct *P. neumanni*. Additional features that can be used to distinguish *P. erlangeri* are very small HW:SVL, and absence (or very brief presence) of spicules.

The sister groups of *P. erlangeri* appear to be two new taxa, one from Goba (eastern Bale) and another from distantly located areas on the western rift (Akaki and Bada Buna; see below). The *P. erlangeri* cluster is embedded within the branch forming the '*P. neumanni* and allies,' forming a sister group to *P. nana* and another putative new species (from Bore2-Wenchi). Therefore, based on morphological similarity of molecular-sampled specimens with the holotype, geographic proximity (distribution in Bore area and lower altitudinal range) to the type locality of *P. erlangeri*, and mtDNA gene variation generally >3%, we confirm the validity of previous taxonomic assignment of this species with major modification on its geographic range.

5) ***Ptychadena* sp. (Bada Buna - Akaki)**

One of the cryptic taxa in the complex highland group, *Ptychadena* sp. (Bada Buna - Akaki) is comprised of two disjunct populations in the central part of the western rift. The possibility of finding other populations in between the two localities cannot be ruled out. The altitudinal range (1800-2038 m) and habitat types (montane grassland, swamps) are overlapping features with other taxa in the highland complex group. Populations from these areas could have possibly been included into either *P. erlangeri* or *P. neumanni* in previous reports. The current genetic data included only two specimens, one from each locality. The mtDNA gene divergence of this group to all other highland groups is large (3-6 %), indicating that it can form a distinct species. The overall physical appearance of the specimens from these areas looks like that of *P. erlangeri*, however there is no morphological data for these specimens. It would be necessary to conduct at least morphological study and collect additional DNA samples from the two localities (and the region in between) to suggest a workable diagnosis for this group. Therefore, we intend to assign this cryptic taxon as an Unconfirmed Candidate Species.

6) *Ptychadena* sp. (Goba)

The molecular data from two specimens, collected in the vicinity of Goba town (Bale Mountains) at altitudes of 2112-2711 m in montane grassland areas in the eastern rift, was found to be one of the cryptic taxa revealed from the highland complex. This new group phylogenetically clusters with *P. erlangeri*, and was earlier thought to be morphologically similar with *P. erlangeri.* Detailed morphological study is needed to diagnose this group. Referring to the significant mtDNA gene variation (>3.5%) between this group and all other studied *Ptychadena*, and its sympatric occurrence in the vicinity of other distinct species, we tentatively assign this group as Unconfirmed Candidate Species.

7) *Ptychadena nana* (Arsi - Bale)

This species is distributed in the montane grasslands of the plateaus of Arsi and steep southern face of the Bale Mountains in the eastern rift. The altitudinal range of this group is less variable in the plateaus of Arsi 2410-2450 m as contrasted with a wider range of 1680-2910 m in Bale. *Ptychadena nana* has previously been known only from its type locality in Dida'a (Arsi-Robe) Plateau; in the current assessment a new cryptic population is revealed from Bale. This new population was previously identified as *P. neumanni*.

Ptychadena nana has been identified based on having the smallest body size and shortest limbs in the '*P. neumanni* complex'. The current additional morphological data supplement that *P. nana* can further be distinguished from *P. neumanni* based on absence of spicules and absence of the white marking on the tympanum (TyM). The most difficult morphological similarity that *P. nana* has is with a cryptic taxon, *Ptychadena* sp. (Bore2-Wenchi), which also appears genetically the closest to this group.

Specimens of *P. nana* collected from the type locality (Arsi) have genetically clustered together, and appear distinct as a population from those in Bale. The genetic distance of this species from *Ptychadena* sp. (Bore2-Wenchi) or *P. erlangeri* (Bore1-Bale) is relatively low (2.0 and 2.3%, respectively), with higher values when compared to other species (>4%). However, if consistent morphological features can be used to distinguish between these populations these might yet represent distinct species. Based on mtDNA gene variation, morphological comparison with the holotype and geographic data, we agree with the previous taxonomic assignment of *P. nana* as a distinct species.

8) *Ptychadena* sp. (Bore2 - Wenchi)

The distribution of this new group looks patchy, occupying montane grasslands in Bore/Sidamo area (eastern rift) to Lake Wenchi area (western rift) across the Rift Valley. The population in the east is recorded from altitudes of 2620-2680 m, as contrasted with a far higher 3080 m in the west. This might be indicative of having other closely related populations distributed in similar habitats at other localities in the central highlands in Shoa. Sampling across these regions would be necessary to clarify this suggestion. The altitudinal range of this phylo-group is almost separate (with marginal overlap) from *P.* cf. *erlangeri* (Bore1-Bale) that has been recorded from lower elevations (1300-2680 m).

The morphology of the new group, although quite distinct from *P. neumanni* by the absence of spicules in the males, is difficult to separate from *P. nana* or *P. erlangeri* and the two newly revealed taxa from Beda Buna - Akaki and Goba. The only diagnostic character we recognized in *Ptychadena* sp. (Bore2-Wenchi) is the proportionally larger inner metatarsal tubercle (large IMTL:T1L). The generally large mtDNA gene variation of this new group (> 3%) from most other studied groups and potentially diagnostic morphological features convinced us to assign this taxon as a Confirmed Candidate Species awaiting description with additional data.

9) *Ptychadena wadei* (Andassa)

This species genetically appears to be most closesly related to *P. porosissima* from Tanzania when compared to all other studied taxa. Given the large genetic divergence (>15%) from all other studied groups in the Ethiopian highlands, there appears to be no doubt that *P. wadei* is a fully distinct species. Its known range is confined to the type locality, a montane grassland south of Lake Tana along the upstream reaches of the Abay River close to Tisisat Falls. It is possible that the distributional and altitudinal range of this species could be wider than what is currently known, given that there are habitats at similar altitudes in the region. As contrasted with the other studied species of Ethiopian *Ptychadena, P. wadei* is unique by having an outer metatarsal tubercle (Largen, 2000, 2001). We have identified an additional feature to characterize this species, a proportionally very short hind limb (very large SVL:HiLi), paralleled only by *Ptychadena* sp. (Debre Markos) and *P.* cf. *mascareniensis*.

Two museum specimens of *P. porosissima* collected about 100 years ago from this locality form the northernmost known distribution range for this mainly eastern and southwest African species (Largen, 1997). Taking into account, 1) the old age (and lower quality) of the Ethiopian specimens to rely on morphological comparison, 2) the extremely wide geographic isolation of the Ethiopian localities from the southern African region, 3) similarity in diagnostic morphological characters such as presence of OMT (checked by SL and DG, 2011), or as reported in Bwong et al. (2009) for *P. porosissima* from southern Kenya), and 4) the genetic affinity of *P. wadei* to Tanzanian *P. porosissima* in our results, it is appropriate to suspect that the Ethiopian specimens of *P. porosissima* could belong to *P. wadei*. The taxonomy and historical link to the origin of these species can be better understood after a wider survey in the surrounding areas. For now, the distinct phylogenetic grouping of *P. wadei* in the current results strengthens the taxonomic status, morphological distinctness and distribution areas known before. The validity of the presence of *P. porosissima* in Ethiopia however is questioned and will require future investigation.

10) *Ptychadena anchietae* (Rift Valley and other lowlands)

This is the most easily recognized species of all Ethiopian *Ptychadena,* which exhibits extensive webbing of the toes and a pale triangle on top of the snout. Localized morphological variations could exist between the widespread populations of *P. anchietae*. However, the genetic divergence among populations of the Ethiopian Rift Valley, Somalia, and *P. harenna* (Ethiopia) was very low. *Ptychadena anchietae* has closer genetic affinity to the endemic highland cluster than has *P. wadei*. This is indicative of a probable separate evolution of different species of *Ptychadena* in Ethiopia. The altitudinal range (<2000 m) and warm habitats preferred by *P. anchietae* in Ethiopia are found within the Rift Valley, in border lowlands, and at the mid-altitudes (1500-2000 m). As we did not assess the morphology or genetic relationship of the Ethiopian *P. anchietae* with its haplotypes in southern Africa, we prefer to keep the known taxonomic assignment of this species in Ethiopia.

11) *Ptychadena* cf. *mascareniensis* (Rift Valley)

The studied populations of *P.* cf. *mascareniensis* in Ethiopia*,* restricted to the Rift Valley and western lowlands, are genetically closest to the haplotypes (or proposed species) from Tanzania, Kenya and Egypt (haplotype A in Vences et al., 2004). Another Kenyan group from Kakamega Forest, haplotype D in Vences et al. (2004), relates with those from western and central Africa (Vences et al., 2004; Bwong et al., 2009). Our molecular data provide supplementary evidence for the cryptic diversity found under the ‘umbrella species’ *P. mascareniensis*, showing a significant mtDNA gene variation (>6%) between the northeast African (including Ethiopian) and the type locality (Reunion Island).

In Ethiopia, the more northern and more lowland-confined (about 800 m) *P. filwoha* is genetically confirmed to be conspecific with *P.* cf. *mascareniensis.* Although ‘*P. filwoha’* lives in a very warm habitat around hotsprings, we found no significant morphological or mtDNA variation to *P.* cf. *mascareniensis*. This would therefore extend the range of *P.* cf. *mascareniensis* further north in the Ethiopian Rift Valley.

The taxonomic placements of the five proposed species within *P.* cf. *mascareniensis* (Vences et al., 2004) require morphological assessment of specimens from various parts of Africa. We have demonstrated that the Ethiopian population belongs to a widely distributed northeastern Africa cluster. Broader understanding of interrelationships between the Ethiopian and other African groups requires rigorous assessment of continental level representative samples. We provisionally assign the Ethiopian group within a Northeastern Africa haplotype A of *P. mascareniensis* (Vences et al., 2004) and suggest the synonym of *P. filwoha* Largen 1997.

B) Biogeography

Suitable habitats for endemic highland species and populations of *Ptychadena* in Ethiopia are found from foothills of mountains (lower than 1800 m such as Dolo Mena area in Bale) to moderate (around 1800 m such as Kaffa and Bahir Dar areas) and high altitudes over 2000 m (e.g., Bale, Gughe, and Wenchi). As indicated in Chapter 2, we prefer to use the context of 'Ethiopian Highlands' as the geographic area included within the Eastern Afromontane Biodiversity Hotspot in this country. All other relatively warm areas such as the Rift Valley, border areas and river gorges comprise the 'lowlands.'

Based on assessment of available elevation data, the current results demonstrate that there are roughly three categories of populations of *Ptychadena*: strictly highland (over 2000 m), mid-to-high altitude (1500-2500 m) and low-to-mid altitude (<2000 m) (Fig. 3.14). The strictly highland species are *P. cooperi* (western rift), *P.* cf. *cooperi* (eastern rift), *Ptychadena* sp. (Bore2-Wenchi), and *P. nana*. The mid-to-high altitude groups are *P. neumanni*, *P. erlangeri*, *Ptychadena* sp. (Bada Buna - Akaki), and *P. wadei*. The low-to-mid altitude category comprises of *P. anchietae* and *P. mascareniensis*. This is indicative of the spectrum of elevation preference by species instead of clear-cut altitudinal demarcations in this recent radiation of amphibians.

The various segments of highlands are separated by warmer gorges and valleys and harbor disjunct populations that are currently potentially reproductively isolated in nature from each other. Most of the genetically assessed species of *Ptychadena* in the Ethiopian Highlands tend to occupy one or more segments of mountain ranges on either the eastern or western side of the Rift Valley. There is only one instance, that of *Ptychadena* sp. (Bore2-Wenchi), where a species has two closely-related populations that are located far apart across the Rift Valley. A similarly potentially patchy group is *P. cooperi*, for which no DNA data are available from its type locality in the western rift. A few other species appear highly localized in narrow geographic areas, although only limited localities were covered in the current field sampling. All in all, the Ethiopian Highlands are home to a complex endemic cluster of *Ptychadena* species. In most cases, the populations of *Ptychadena* in these highlands do not show, to our knowledge, established specialization and geographic differentiation. This could possibly be explained by a combination of the very recent split and radiation of the ancestral populations. Whether other factors (such as a more or less uniform nature of the grassland habitats provided in the montane areas, and high agility of members of the genus enabling potential hybridization as contrasted with other taxa such as species of the tree frog *Leptopelis*) contributed to this low differentiation requires further research.

A biogeographic categorization of the Ethiopian Highlands has been proposed pertaining to the distribution of endemic *Leptopelis* (Chapter 2). This categorization follows a similar pattern in the case of endemic *Ptychadena* in this area (Fig. 3.17). We have therefore revised the geographic distribution of the studied Ethiopian *Ptychadena* using mainly phylogenetic groupings, and some morphological distinctions (Table 3.5, Figs. 3.9 - 3.13).

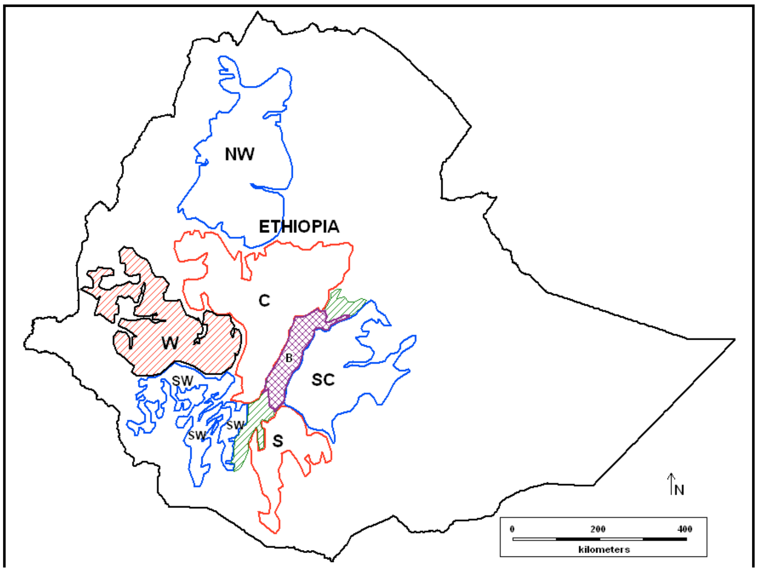


Fig. 3.17. Biogeographic segmentation of the Ethiopian Highlands delimited by the Rift Valley and major river gorges and valleys; figure shows only the areas from which specimens of *Leptopelis* and *Ptychadena* were recorded. NW=Northwestern, C=Central, W=Western (no genetic data from this region), SW=Southwestern, S=Southern, SC=South-central, B=relatively elevated areas in the Rift Valley.

The Ethiopian Rift Valley is believed to have initiated through volcanic and uplifting activities about 30 mya (Hofmann et al., 1997; Arndt and Menzies, 2005), developed about 11 mya (Wolfenden et al., 2004) with extension starting about 6-5 mya (Bonini et al., 2005). Major drainage basins of Ethiopia were established between 30 and 23 mya (Pik et al., 2003). The massive uplifting and extension of the Ethiopian plateau immediately after about 6 mya likely resulted in aridification by inhibiting a zonal circulation of moist air and replacement of closed forest woodlands by open grasslands (Sepulchre et al., 2006; Gani et al., 2007).

Given that *Ptychadena* in the Ethiopian Highlands are a monophyletic group, diversification from a common ancestor probably followed the geologic events that formed the uplifted landscapes and partitioned them by several gorges and valleys. Among the highland taxa, however, we see poorly resolved relationships with low branch support values. Molecular data from additional genes is needed to be collected to attempt to robustly resolve these groupings. The lowland species (*P. anchietae* and *P. mascareniensis*) do not have diverse populational structures, living in a broadly homogenous continuum of geo-climatic conditions.

Further breakdown within species of *Ptychadena* into populations probably occurred very recently. This could be associated with fluctuating historical climatic changes that have shaped environmental conditions in tropical Africa, emphasized by cooler and drier conditions during the last glacial maximum (LGM: ca. 23-18 ka; Street-Perrot et al., 1997; Jolly and Haxeltine, 1997), followed by post-glacial warming and wetting events at the onset of the Holocene around 15-11 ka (Gasse, 2000). There were short-term dry events in Ethiopia around 8.3-8 ka, 7-6.5 ka, and 4.2-4 ka (Said, 1993; Gasse and Van Campo, 1994). In addition, Ethiopia is portrayed as an outlier of the cold habitats that dominated Eurasia during the LGM (Kingdon, 1990). Pollen sequence data from Bale Mountains indicated that repeated alterations between glaciation, Afroalpine vegetation, and extended woody vegetation cover during the Quaternary must have contributed to the high endemic biodiversity of the Ethiopian highlands (Umer et al., 2007). Higher altitudes exceeding 2500 m (e.g., Arsi-Bale massifs and central highlands) tend to have higher diversity of *Ptychadena* as compared to mid-altitudes. Both high endemicity and high diversity exist in the higher habitats than the lower ones. This is contrary to the case for *Leptopelis*, which has its highest diversity in the mid-altitudes (southwest highlands) and not in Arsi-Bale area. This is perhaps associated to different ecological habitats preferred and inhabited by these two genera. Overall, vicariant events might have acted as the prime mechanism for the diversification and endemicity currently seen in the highlands; sympatric speciation could possibly explain co-existence of some taxa (e.g., *Ptychadena* sp. Bore-Wenchi, *Ptychadena* sp. Goba, *P. nana* and *P. erlangeri*).

C) Taxonomic Comments

Using the studied 12S and 16S mtDNA sequences of most Ethiopian and some other African species of *Ptychadena*, we have found that there are monophyletic highland endemics geographically restricted to the Ethiopian part of the Eastern Afromontane Biodiversity Hotspot. This region is surrounded by vast lowlands and the Rift Valley that are relatively warm as compared to the highlands. Our findings highlight the possibility of existence of other species of *Ptychadena* in the Ethiopian Highlands, requiring more extensive survey and genetic studies. To better understand the Ethiopian taxa we require more complete data for related taxa distributed in other regions of Africa. The history and adaptations for diversification of these little known amphibians could be revealed by incorporating additional genetic and morphological data with acoustics and natural history studies.

The '*Ptychadena neumanni* complex,' assumed by Largen (1997) to have contained only three described species, actually appears more complicated than thought before. That is, this complex of three species is paraphyletic and does not form a natural grouping, with very weak branch support (<50%). The sister group of this complex is formed by a branch containing *P.* cf. *cooperi* (Arsi-Bale) and a morphologically presumed *P. erlangeri* population (currently *Ptychadena* sp. Debre Markos; Fig. 3.2). In light of this and the close genetic relationship they have (bootstrap = 94%), the currently identified monophyletic highland species (eight taxa) appear to form a 'species group', that is the simplest form of a species flock (Stölting, 2004; Fouquet et al., 2007). Therefore, referring to the name '*P. neumanni* complex/allies' would create confusion and seems inappropriate. As *P.* cf. *cooperi* and *Ptychadena* sp. (Debre Markos) tend to form the basal group of the highland complex in the reconstructed phylogeny, we intend to assign species of the Ethiopian Highlands (except *P. wadei*) as "*Ptychadena cooperi* species group". The existence of another highland species group of *Leptopelis* in Ethiopia has been discussed in Chapter 2. Treating the Ethiopian highland *Ptychadena* as an entity (of closely related monophyletic species) rather than separate taxonomic units would aid to facilitate an all-inclusive research and conservation strategy in this area. This approach of harmonizing species diversity and distribution with conservation has been reviewed and advocated by Agapow (2005).

The studied lowland species of *Ptychadena* in Ethiopia show a continuous geographic distribution that extends into Somalia, Kenya, Sudan and other lower regions in Eastern and Northeastern Africa. The habitats in these areas appear geo-climatically more continuous than are the cooler aggregation of Ethiopian Highlands. Previous findings for the cryptic diversity of *P. mascareniensis* in Africa (Vences et al., 2004) have helped to evaluate the status of the Ethiopian population of this species. Our recent collections and genetic data could help broader morphological and molecular assessment of these groups at a continental scale.

V) CONCLUSION

Species of *Ptychadena* in the Ethiopian Highlands form a monophyletic highland species group. Preliminary assignment of Ethiopian populations of *Ptychadena* as distinct species is possible using the 16S and/or 12S mtDNA genes. Based on the lowest observed genetic variation among valid species of the studied populations of *Ptychadena*, tentatively we suggest a threshold value of 2% genetic divergence in the 16S genes to guide species distinctions. However, simultaneous morphological, bioacoustic, life history and ecological data, as well as population-level genetic studies will be helpful for valid species description and to further refine the taxonomy of members of this genus in Ethiopia.

Morphological identification of species of *Ptychadena* in the field and at museums will be possible as we continue to make thorough observations. Most of the morphological characters suggested for use (e.g., Largen, 1997; Bwong et al., 2009) appear more helpful for description of species and to distinguish distantly related species. Young species groups such as the *Ptychadena cooperi* species group in the Ethiopian Highlands have very close relationships and can be diagnosed using only a few, probably unique, physical characters.

The biogeographic conclusions drawn from this study are, 1) for most species of *Ptychadena* in the Ethiopian Highlands, the Rift Valley and other major gorges serve as barriers to delimit natural interactions among taxa; 2) the geographic distribution of species of *Ptychadena* in

Ethiopia follows distinctly segmented biogeographic patterns rather than random occurrence; and 3) vicariant forces probably contributed to diversification of ancestral *Ptychadena* originally widespread in the highlands, followed by subsequent fragmentation of populations due to very recent climatic changes associated with the last glacial maximum.

The current findings are a glimpse of the much larger amphibian taxonomy work that has to be done in this biodiversity hotspot in the future. Molecular assessment of populations and taxa through the use of mitochondrial and additional nuclear genetic markers would aid to better understand the evolutionary relationship and history of the complex highland groups and other species in Africa. Although the aforementioned findings provide and serve as a basic clue to the evolutionary relationship of *Ptychadena* in Ethiopia, additional genetic data are required to get a complete picture. The required data in this regard will be DNA sequences from type localities of *P. cooperi* (Wouramboulchi, Shoa), *P. neumanni* (Gadat, Gamo Gofa) and *P. erlangeri* (east of Lake Abaya). As discussed above, more field sampling is also needed in unsurveyed areas. The newly revealed candidate species from Bada Buna - Akaki, and Goba areas require further assessment using both morphological and genetic data.

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REFERENCES

Agapow, P.-M. (2005). Species: demarcation and diversity. In: Purvis, A., Gittleman, J.L. & Brooks, T. (eds) Phylogeny and Conservation: 57–75. Cambridge: Cambridge University Press.

Ahl, E. (1924). Über eine Froschsammlung aus Nordost-Afrika und Arabien. *Mitteilungen aus dem Zoologischen Museum in Berlin* 11 (1): 1-12.

Amphibiaweb (2011). Information on Amphibian Biology and Conservation. [web application]. Berkeley, California: Available: http://amphibiaweb.org/. (Accessed: Mar 17, 2011).

Arndt, N. and Menzies, M.A. (2005). The Ethiopian Large Igneous Province. Accessed online on 31 August 2010. http://www.largeigneousprovinces.org/05jan.html

Battersby, J.C. (1954). Reptiles and amphibians collected in the Northern Frontier division of Kenya in 1951. *Annals and Magazine of Natural History* (12) 7: 241-248.

Bonini, M., Corti, G., Innocenti, F., and Manetti, P. (2005). Evolution of the Main Ethiopian Rift in the frame of Afar and Kenya rifts propagation: *Tectonics*, v. 24, doi: 10.1029/2004TC001680.

Bossuyt, F., Brown, R.M., Hillis, D.H., Cannatella, D.C. and Milinkovitch, M.C. (2006). Phylogeny and biogeography of a cosmopolitan frog radiation: Late Cretaceous diversification resulted in continent-scale endemism in the family Ranidae. *Systematic Biology* 55 (4): 579-594.

Boulenger, G.A. (1895a). An account of the reptiles and batrachians collected by Dr. A. Donaldson Smith in Western Somali-land and the Galla Country. *Proceedings of the Zoological Society of London*: 530-540.

Boulenger, G.A. (1895b). Esplorazione del Giuba e dei suoi affluenti compiuta dal Cap. V. Bottego durante gli anni 1892-93 sotto gli auspici della Società Geografica Italiana. Risultati zoologici. II. Rettili e Batraci. *Annali del Museo Civico di Storia Naturale Giacomo Doria* 35: 9-18.

Boulenger, G.A. (1896a). A list of the reptiles and batrachians collected by Dr. Ragazzi in Shoa and Eritrea. *Annali del Museo Civico di Storia Naturale Giacomo Doria* 36: 545-554.

Boulenger, G.A. (1896b). Second report on the reptiles and batrachians collected by Dr. A. Donaldson Smith during his expedition to Lake Rudolf. Proceedings of the Zoological Society of London: 212-217.

Boulenger, G.A. (1896c). A list of the reptiles and batrachians collected by the late Prince Eugenio Ruspoli in Somaliland and Gallaland in 1893. *Annali del Museo Civico di Storia Naturale Giacomo Doria* 37: 5-14.

Boulenger, G.A. (1898). Concluding report on the late Capt. Bottego's collection of reptiles and batrachians from Somaliland and British East Africa. *Annali del Museo Civico di Storia Naturale Giacomo Doria* 38: 715-723.

Bremer, K. (1988). The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42:795-803.

Bwong, B.A., Chira, R., Schick, S., Veith, M. and Lötters, S. (2009). Diversity of Ridged Frogs (Ptychadenidae: *Ptychadena*) in the easternmost remnant of the Guineo-Congolian rain forest: an analyisis using morphology, bioacoustics and molecular genetics. *Salamandra* 45(3): 129-146.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.

Fouquet, A., Vences, M., Salducci, M.-D., Meyer, A., Marty, C., Blanc, M. and Gilles, A. (2007). Revealing cryptic diversity using molecular phylogenetics and phylogeography in frogs of the Scinax ruber and Rhinella margaritifera species groups. *Molecular Phylogenetics and Evolution* 43: 567-582.

Frost, D. R. (2011). Amphibian Species of the World: an Online Reference. Version 5.3 (12 February, 2011). Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/ American Museum of Natural History, New York, USA.

Frost, D. R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., de Sá, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., L. Blotto, B., Moler, P.E., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M. and Wheeler, W. (2006). The amphibian tree of life*. Bulletin of the American Museum of Natural History* 297: 1-370.

Gani, N.D.S., Gani, M.R. and Abdelsalam, M.G. (2007). Blue Nile incision on the Ethiopian Plateau: Pulsed plateau growth, Pliocene uplift, and hominin evolution. *GSA Today*: v. 17, no. 9, doi: 10.1130/GSAT01709A.1.

Gasse, F. (2000). Hydrological changes in the African tropics since the Last Glacial Maximum. *Quaternary Science Reviews* 19: 189-211.

Gasse, F. and Van Campo, E. (1994). Abrupt post-glacial climate events in West Asia and North Africa monsoon domains. *Earth and Planetary Science Letters* 126: 435-456.

Hofmann, C., Courtillot, V., Feraud, G., Rochette, P., Yirgu, G., Ketefo, E., and Pik, R. (1997). Timing of the Ethiopian flood basalt event and implications of Plume birth and global change. *Nature* 389: 838–841, doi: 10.1038/39853.

Inger, R.F. (1956). Morphology and development of the vocal sac apparatus in the. African frog *Rana (Ptychadena) porossima* Steindachner. *J. Morph.* 99: 57-72.

IUCN (2011). IUCN Red List of Threatened Species. Version 2010.4. <www.iucnredlist.org>. Downloaded on 18 March 2011.

Jolly, D. and Haxeltine, A. (1997). Effect of low glacial atmospheric CO2 on tropical African montane vegetation. Science 276: 786-788.

Kingdon, J. (1990). Island Africa; the Evolution of Africa's Rare Animals and Plants. Collins, London.

Kishino, H. and Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *Journal of Molecular Evolution* 29:170- 179.

Lanza, B. (1972). Il Parco Nazionale dell'Auasc (Scioa, Etiopia). *L'Universo, Firenze* 52 (5): 1035-1070.

Largen, M. and Spawls, S. (2010). The Amphibians and Reptiles of Ethiopia and Eritrea. Edition Chimaira, Frankfurt am Main. 692 p.

Largen, M.J. (1997). Two new species of *Ptychadena* Boulenger 1917 (Amphibia, Anura, Ranidae) from Ethiopia, with observations on other members of the genus recorded from this country and a tentative key for their identification. *Trop. Zool.* 10: 223-246.

Largen, M.J. (2000). Another new species of *Ptychadena* Boulenger 1917 from Ethiopia (Amphibia Anura Ranidae). *Trop. Zool.* 13: 171-178.

Largen, M.J. (2001). Catalogue of amphibians of Ethiopia, including a key for their identification. *Tropic. Zool.* 14: 307-402.

Loader, S.P., Gower, D.J., Muller, H. and Menegon, M. (2010). Two new species of Callulina (Amphibia: Anura: Brevicipitidae) from the Nguru Mountains, Tanzania. *Zootaxa* 2694: 26-42.

Loveridge, A. (1936). African reptiles and amphibians in Field Museum of Natural History*. Field Museum of Natural History Publications (Zoological Series), Chicago* 22 (1): 1-111.

Missale, A.T. (2008). Chromosome study of some Ethiopian amphibian species. Unpublished MSc thesis, School of Graduate Studies, Addis Ababa University. Addis Ababa.

Nei, M. and Kumar, S. (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Parker, H.W. (1930). Report on the Amphibia collected by Mr. J. Omer-Cooper in Ethiopia. *Proceedings of the Zoological Society of London*: 1-6.

Perret, J.-L. (1980). Sur quelques *Ptychadena* (Amphibia Ranidae) d'Éthiopie. *Monitore Zoologico Italiano (Nuova Serie) Supplemento* 13: 151-168.

Perret, J.-L. (1994). Description de *Ptychadena largeni* n. sp. (Anura, Ranidae) d'Èthiopie. *Muséum d'Histoire Naturelle de Genève* 117: 67-77.

Pik, R., Marty, B., Carignan, J. and Lave, J. (2003). Stability of Upper Nile drainage network (Ethiopia) deduces from (U–Th)/He thermochronometry: Implication of uplift and erosion of the Afar plume dome. *Earth and Planetary Science Letters* 215: 73–88, doi: 10.1016/S0012-821X(03)00457-6.

Posada, D. and Crandall K.A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* 14 (9): 817-818.

Poynton, J.C. (1999). Distribution of amphibians in sub- Saharan Africa, Madagascar, and Seychelles. In: Duellman, W.D. (Ed.), Patterns of Distribution of Amphibians, a Global Perspective. John Hopkins University Press, Baltimore, pp. 483-539.

Poynton, J.C. and Broadley, D.G. (1985). Amphibia Zambesiaca, 2. Ranidae. *Annals of the Natal Museum* 27(1): 115-181.

Roux, J. (1936). Mission scientifique de l'Omo. III. Zoologie. Reptilia et Amphibia. *Mémoires du Muséum National d'Histoire Naturelle, Paris* (2) 4: 157-190.

Said, R. (1993). The River Nile. Pergamon, Oxford, pp. 320.

Scortecci, G. (1930). Contributo alla conoscenza dei rettili e degli anfibi della Somalia, dell'Eritrea e dell'Abissinia. *Bollettino dei Musei di Zoologia e di Anatomia Comparata della R. Università di Torino* (3) 41 (10): 1-26.

Scortecci, G. (1943). Amphibia, pp. 307-334. In: Missione Biologica Sagan-Omo. Zoologia 1, Vol. 7. *Roma: Reale Accademia d'Italia, Centro Studi per l'Africa Orientale Italiana*, 373 p.

Sepulchre, P., Ramstein, G., Fluteau, F., Schuster, M., Tierchelin, J. and Brunet, M. (2006). Tectonic uplift and eastern African aridification. *Science* 313: 1419–1423, doi: 10.1126/science.1129158.

Sorenson, M.D. and Franzosa, E.A. (2007). TreeRot, version 3. Boston University, Boston, MA.

Stölting, K.N. (2004). The Midas cichlid species flock: Incipient sympatric speciation? Unpublished Diplomarbeit, Fachbereich Biologie der Universität Konstanz, Konstanz.

Swofford, D. (2002). PAUP*: Phylogenetic Analysis Using Parsimony (*and other Methods). Version 4.0b10. Sinauer Associates, Sunderland, Mass.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution (submitted).

Tamura, K., Nei, M. and Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*) 101:11030-11035.

Templeton, A.R. (1983). Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* 37: 221-244.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876-4882.

Umer*, M.,* Lamb, H.F., Bonnefille, R., Lézine, A.-M., Tiercelin, J.-J., Gibert, E., Cazet, J.-P. and Watrin, J. (2007). Late Pleistocene and Holocene vegetation history of the Bale Mountains, Ethiopia. *Quat. Sci. Rev.* 26: 2229-2246.

Urban, E.K. (1967). Notes on a collection of amphibians from Ethiopia. *Journal of the East Africa Natural History Society and National Museum, Nairobi* 26 (2) (114): 85-86.

van der Meijden, A., Vences, M., Hoegg, S. and Meyer, A. (2005). A previously unrecognized radiation of ranid frogs in Southern Africa revealed by nuclear and mitochondrial DNA sequences. *Mol. Phyl. Evol.* 37: 674-685.

Vences, M., Kosuch, J., Rödel, M-O, Lötters, S., Channing, A., Glaw, F. and Böhme, W. (2004). Phylogeography of *Ptychadena mascareniensis* suggests transoceanic dispersal in a widespread African Malagasy frog radiation. *J. Biogeogr.* 31: 593-601.

Vences, M., Thomas, M., Bonett, R.M. and Vieites, R. (2005). Deciphering amphibian diversity through DNA barcoding: chances and challenges. *Phil. Trans. R. Soc. B.* 360: 1859-1868.

Vieites, D.R., Wollenberg, K.C., Andreone, F., Köhler, J., Glaw, F. and Vences, M. (2009). Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proc. Natl. Acad. Sci. U.S.A.* 106: 8267-8272.

Wolfenden, E., Yirgu, G., Ebinger, C., Deino, A. and Ayalew, D. (2004). Evolution of the northern Main Ethiopian rift: Birth of a triple junction. *Earth and Planetary Science Letters* 224: 213–228, doi: 10.1016/j.epsl.2004.04.022.

PART III:

CONSERVATION

Amphibian Conservation in the Ethiopian Highlands: The Case of Endemic Tree Frogs (*Leptopelis*, Arthroleptidae) and Ridged Frogs (*Ptychadena*, Ptychadenidae)

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ABSTRACT

Until now, forty percent of Ethiopia's 64 species of amphibians are known to be endemics, and nine species (14%) are categorized as threatened in the IUCN Red List. Of the threatened ones, three are *Leptopelis* and none from *Ptychadena*. However, there are no known direct conservation measures exercised to preserve specifically amphibians. New phylogenetic and biogeographic results have enabled to refine earlier taxonomic assignments and distribution of species and populations of *Leptopelis* and *Ptychadena* in the Ethiopian Highlands. Based on these grounds, we mapped new elevation-based estimates of geographic ranges and made preliminary assessment of status of habitats with reference to anthropogenic impacts and spatial maps of vegetation cover. The elevation-based extent of occurrence is proposed to be more meaningful (than the simple polygon in the IUCN criteria) for practical conservation of Ethiopian amphibians. Historical and current rainfall and temperature patterns are assessed to show the potential impact of climate change on amphibian habitats. The conservation status of newly defined phylo-groups (species) was evaluated using the new geographic ranges and information on habitats. Nine (56%) of the sixteen studied known and putative species (three *Leptopelis* and six *Ptychadena*) are proposed to be assigned as threatened at different levels, exceeding the proportion of threatened amphibians in Ethiopia (14%) or the world (32.5%). The current results reveal that taxonomic flaws that were based only on classical morphological methods masked threatened species that need priority for conservation. The extent of problems that shadow conservation of endemic and threatened amphibians in Ethiopia demonstrates the potential biodiversity crisis that can happen in this part of the Eastern Afromontane Biodiversity Hotspot.

Key words: Amphibian conservation; Biodiversity; Climate change; Ethiopian Highlands; Forests; Habitat degradation; Ridged (Grass) Frogs; Tree Frogs; Wetlands.

I) INTRODUCTION

Amphibian diversity in Ethiopia has been underestimated due to lack of research and surveys (Largen and Spawls, 2010; Chapter 3 in this thesis). However, endemicity of amphibians in Ethiopia is high, with 40% of the known species restricted to the region (Largen, 2001; Chapter 1 in this thesis). No other vertebrate group has such a high endemicity in Ethiopia (IBC, 2005). This figure is especially significantly high as contrasted with the endemicity proportion of other vertebrates in Ethiopia. There is also considerable endemicity seen at higher taxonomic levels, with about 28% of the genera and about 13% of the families found solely in Ethiopia (Largen, 2001; Chapter 1 in this thesis). In the northeastern and Horn region of Africa, Ethiopia is the most favorable place for amphibian diversity and endemicity, an area associated with cooler and moist habitats in the highlands, as compared with generally warm and dry surrounding areas (Sudan, northern Kenya, Somalia and Djibouti). Madagascar is the most reputed of all other African regions for its impressive endemic amphibian diversity of about 300 species, with almost 100% endemics (Andreone et al., 2008).

According to Largen (2001), *Leptopelis* and *Ptychadena* comprise 30% of all Ethiopian amphibians, with five of the six *Leptopelis* and five of the thirteen species of *Ptychadena* being highland endemics. Revised phylogeographic and morphological findings (Chapters 2 and 3) revealed some cryptic species (at least two *Leptopelis* and four *Ptychadena*), indicating that the diversity of these two genera has been underestimated.

In the phylogenetic groupings established using mtDNA sequences and discussed in the previous chapters, it has been shown that all known species of *Leptopelis* from the Ethiopian Highlands are valid species. Highly fragmented but wide distributions were observed in populations of *L. gramineus* across the Rift Valley. In contrast, the occurrence of *L. ragazzii* across the rift valley was shown to be incorrect. Largen (2001) noted its occurrence in both the East and in the West (Gughe Mountains). However, we demonstrated that the latter was probably misidentified and might represent populations of *L. vannutellii,* previously restricted to the southwest forests. The range of *L. susanae* potentially has extended to a patchy location further west, and the northwest-restricted range of *L. yaldeni* has been reconfirmed.

The highland species of *Ptychadena* were found to be more diverse than known before, with four putative new species from geographically restricted areas, namely Debre Markos, Bore-Wenchi, Bada Buna – Akaki, and Goba. All the known highland species were genetically reconfirmed as valid species. Based on these phylogenetic findings, the geographic distribution of these species requires reassessment. *Ptychadena erlangeri* has a distribution restricted to the South-central segment (Bore area and southern foothills of Bale Mountains), and two genetically distinct populations (potentially new species) from Goba and Bada Buna – Akaki are close relatives. on the other with a population of a new undescribed species in Bore. The previously presumed widespread occurrence of *P. neumanni* is reduced based on genetic and morphological evidence. The range of *P.*

neumanni is restricted to the southwest highlands (the DNA of one specimen from the foothills of Bale genetically belonging to *P. neumanni* has been reported as an exception, with the suspicion that it could be a result of sample mix up between laboratories). Other populations formerly conceived under this name are likely to represent new species, further sampling is necessary for such an assessment. The species *P. nana,* previously only confirmed as occurring in Arsi, is confirmed to occur in Bale, and a divergent putative new species is identified (Bore 2-Wenchi).

The conservation status of species of *Leptopelis* and *Ptychadena* in the Ethiopian Highlands, as reviewed in the IUCN Red List website (IUCN, 2011) is shown in Table 4.1. There is very limited or no information on their geographic ranges, that is, extent of occurrence (EOO) or area of occupancy (AOO), as well as population status. Some of the species such as *Ptychadena neumanni* had taxonomic complexities, making it difficult to realize existing distribution maps as reliable indicators for status of populations. The highly fractured nature of the Ethiopian Highlands, coupled with human-mediated habitat fragmentation, also makes it more difficult to make good estimates of distribution areas for species with scattered populations. On the other hand, the potential distribution of a species could be overestimated unless the role of geo-climatic barriers is considered with this. In this regard, the potential distribution area of *L. ragazzii* has been predicted using a climate envelope model that was trained using suitable habitats from known occurrence records (Weisenheimer et al., 2010). However, the actual occurrence of this species appears different when assessed using phylogenetic and biogeographic data (Chapter 2). That is, following revised phylogenetic grouping of members of the genus *Leptopelis* (Chapter 2) and *Ptychadena* (Chapter 3), the ranges of some species appeared extended while reduced for others.

The major types of highland habitats associated with *Leptopelis* and *Ptychadena* in Ethiopia include montane grassland, forest edges, Afroalpine moorland, montane forest, dense tropical deciduous forest, long grasses and herbaceous vegetation in forest clearings, flood pools and ditches, inundated fields and swamps, streams and lake shores (Largen, 2001).

Human population in Ethiopia is highly concentrated in the highlands, with large densities seen in the central, southwestern, southern, northwestern, northeastern and eastern parts of the highlands (CIESIN, 2000). These parts of the highlands are subject to vital but intense human activities such as new settlements, urbanization, infrastructure, industries, modern agriculture, cattle breeding, mining and deforestation. A large proportion of the country’s economy relies on agriculture (USAID, 2008). In addition, a recent preliminary study indicates that many of the amphibian species in the Ethiopian highlands, including *Leptopelis* and *Ptychadena*, have (or are potentially prone to) high prevalence of chytrid fungus *Batrachochytrium dendrobatidis* (Gower et al., 2012).

The need for conservation of amphibians rises from their diverse roles in the environment, highest level of threat of extinction among all vertebrates (Regan et al., 2001; Young et al., 2001; Stuart et al., 2004), less attention paid to them (Gascon et al., 2007) and values to humans including medicinal, food sources (Lannoo et al., 1994), indicators of environmental change (Lips, 1998), and pet trade (Behra and Raxworthy, 1991). These values of amphibians are not well recognized by most people in Ethiopia, and little or no attention is given to development and research in this direction (Chapter 1). In addition, amphibians are special in their modes of life, adapted to the aquatic-terrestrial boundary. This has made their life largely dependent on availability of moist and wet habitats, be it on the ground or on trees. Any activity that affects wetlands, grasslands and forested habitats, therefore, could potentially have some negative impact on populations of amphibians in the area.

Table 4.1. The current IUCN Red List status of species of *Leptopelis* and *Ptychadena* in the Ethiopian Highlands (summarized from IUCN, 2011).

Species	**Geographic range (Criterion B): Extent of Occurrence (B1), or Area of Occupancy (B2)**	**Population status (Criteria A, C, D)**	**Quantitative analysis (Criterion E)**	**Red List status**
Leptopelis gramineus	Wide distribution	Large population Unlikely to decline	N/A	Least Concern
Leptopelis ragazzii	AOO < 2,000 km^2	Severely fragmented	N/A	Vulnerable
Leptopelis susanae	EOO < 5,000 km^2 AOO < 500 km^2	In fewer than five locations	N/A	Endangered
Leptopelis vannutellii	AOO < 2,000 km^2	Severely fragmented	N/A	Vulnerable
Leptopelis yaldeni	EOO < 20,000 km^2	Common, stable Restricted range	N/A	Near Threatened (close to Vulnerable)
Ptychadena cooperi	EOO ≈ 20,000 km^2	Large population Common	N/A	Least Concern
Ptychadena erlangeri	EOO ≈ 20,000 km^2	Small populations Widely fragmented	N/A	Near Threatened (close to Vulnerable)
Ptychadena nana	Known from type locality	Unknown	N/A	Data Deficient
Ptychadena neumanni	Wide distribution	Large population Unlikely to decline	N/A	Least Concern
Ptychadena wadei	Known from type locality	Unknown	N/A	Data Deficient

The aim of the current study is to make preliminary assessment of conservation needs for endemic species of *Leptopelis* and *Ptychadena* in the Ethiopian highlands. To estimate the geographic ranges of species, we apply the altitude-enhanced EBEOO instead of the simple polygon estimates of EOO currently used by IUCN. Specific tasks under this will focus on, 1) assessment of geographic ranges using EBEOO and spatial land cover data, 2) observing and comparing historical and current trends in climatic conditions on the highlands, 3) evaluating species for conservation, and 4) creating awareness to researchers, conservationists and administrators about risks facing threatened endemic amphibians.

II) METHODS

A) Data Collection and Analysis

Our own recent field collections and old museum specimens (pre-1977) were used in the geographic analysis. The recent specimens and records were collected during field work conducted in the Ethiopian Highlands (Fig. 4.1) in 2006, 2008-2010. Seven major geographic areas were covered in the field survey: three on the eastern rift (Arsi, Bale, Bore/Sidamo areas) and four on the western rift (Gojam, Shoa, Kaffa, Gemu Gofa areas). A few additional recent records from the eastern rift (in Arsi, Bale and Bore) by Breda Zimkus in 2006 were compiled from the catalogue of the Zoological Natural History Museum (ZNHM), Addis Ababa University. The studied old specimens were collected from the above regions as well as two additional places (Welega and Illubabor); these have been extracted from Largen (2001) and compiled to fit with the current data. For each species (including undescribed new taxa), we recorded and compiled locality name (and description), GPS coordinates (latitude and longitude in degrees), and elevation (meters).

The taxonomy of the species in this study follows the revised phylogenetic grouping of *Leptopelis* and *Ptychadena* discussed in Chapters 2 and 3, respectively. The list of studied taxa includes *L. gramineus, L. ragazzii, L. susanae, L. vannutellii, L. yaldeni, L.* sp. (Saja), *L.* sp. (Soddo), *P. cooperi, P. erlangeri, P. nana, P. neumannii, P.* sp. (Bada Buna – Akaki), *P.* sp. (Bore-Wenchi), *P.* sp. (Debre Markos), *P.* sp. (Goba), and *P. wadei*.

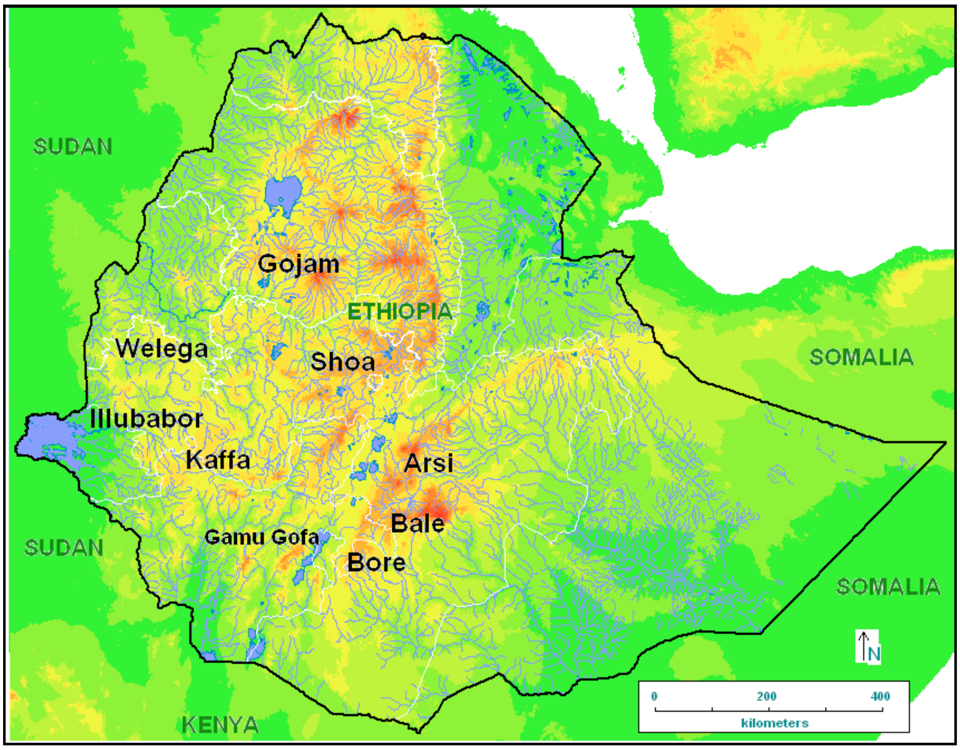

Fig. 4.1. Map of the study area in the Ethiopian Highlands, with names of generalized localities (see details in Appendix 4.1). Red and orange indicate higher altitudes; yellow indicates mid-altitudes; green indicates lower altitudes; blue indicates water areas and lines; white indicates regional administrative boundaries; black indicates international boundary. (Map modified from spatial data in DIVA-GIS).

Country-level spatial data were downloaded for Ethiopia from the website of DIVA-GIS (http://www.diva-gis.org/Data), of which we used vector (area) of the administrative areas (boundary) (Global Administrative Areas, GADM version 1.0), 30-seconds resolution grids of elevation (CGIAR SRTM), and 30 seconds resolution grids of land cover (GLC2000). Topographic maps were geo-referenced online in Map Warper beta (http://warper.geothings.net/) and used in conjunction with spatial data to prepare area grids for each population and species. The area grids were manually traced encompassing coordinate points of adjacent localities within a mountain range following the elevation range of each population. We took caution not to extend the area of a population to similar elevation ranges beyond major gorges/valleys, as the deep and warm stretches of valleys have been reported to be the major barriers between adjacent populations (Chapters 2 and 3). Then, the area of the presumed range of each population was computed (in square kilometers), and summed up to get estimates for each species (hereafter referred to as elevation-based extent of occurrence, EBEOO, to avoid mix up with the IUCN definition of EOO that simply joins shortcuts of recorded peripheral coordinate points. The EOO and EBEOO are defined as follows:

IUCN definition of EOO: the area contained within the shortest continuous imaginary boundary which can be drawn to encompass all the known, inferred or projected sites of present occurrence of a species, excluding cases of vagrancy (IUCN, 2001).

Definition of EBEOO in this research: the sum of areas contained within geographically delimited spans of a taxon where one or more closed boundaries can be drawn to encompass the area in between the known lower and higher elevation limits irrespective of changes in natural habitats, and excluding cases of vagrancy.

The EBEOO can be a more practical means to estimate the geographic range of a species in that it tries to take into account the area within a suitable elevation range rather than an 'imaginary' boundary drawn around known places of occurrence. For this reason, the EBEOO becomes a closer estimate to what climate envelope prediction models do for distribution of species (e.g., Weinsheimer et al., 2010). The EOO appears conservative in that it could exclude suitable areas from which no specimens have been recorded, and conversely could encompass elevation based geographic barriers that may not be suitable for a species. Therefore, for practical amphibian conservation purposes in geographic areas such as the Ethiopian Highlands, EBEOO can be useful in conjunction with other criteria. There are cases where elevation range has been used to draw distribution areas of species (e.g., Randrianantoandro and Hobinjatovo, 2011).

An overlay of the traced area of a species and the land cover within its range was used to show a broader picture of the status of habitats. The highland habitats in our studied areas (with colors as used on the land cover grid map) are characterized by: tree (dark-green), mosaic of tree/other natural vegetation (yellow), shrub (brown), herbaceous (light-green), sparse herbaceous or sparse shrub (light brown), cultivated and managed area (pink), mosaic of cropland/tree/shrub/grass/other natural vegetation (light pink), water bodies (blue), artificial surfaces and associated areas (red). The geographic analyses were conducted in DIVA-GIS version 7.3.0.1 (http://www.diva-gis.org).

The Ethiopian Highlands are cooler as compared with the surrounding lowlands and the Rift Valley. The amount of rainfall on the highlands is crucial for the moist habitats required by *Leptopelis* and *Ptychadena*. Therefore, precipitation and temperature data would be two of the most important climatic inputs to consider in conservation studies. Historical trends and current status of climatic conditions could give clue to the long-term stability of amphibian populations in an area. In this regard, we tried to show any changes in the past climatic history of the Ethiopian highlands (as reported by other workers), and present the most recent rainfall and temperature data (current study). In the current study, monthly data on precipitation and temperature of 16 highland stations was obtained from the National Meteorological Services Agency (NMSE) of Ethiopia for the period 1997-2006. The stations are, **Western rift (11)**: Addis Ababa, Bahir Dar, Bonga, Debre Markos, Dorze, Gondar, Guder, Mekane Selam, Sawla, Wolayita Soddo, Wushwush; **Eastern rift (5)**: Asela, Dollo Mena, Dinshu, Rira, Bale-Robe. The data obtained were monthly precipitation (mm) summed from daily records, mean maximum and mean minimum monthly temperatures (^{o}C) averaged from daily maximum and minimum records, respectively. The data were categorized into two for the eastern and western blocks of the highlands, to see if these blocks have variations in their characteristics in rainfall and temperature.

Mean annual precipitation, mean annual maximum and minimum temperatures per station were calculated by averaging 12 months data for each year, and plotted to show the most recent annual trends over ten years period. Mean monthly precipitation, mean monthly maximum and minimum temperatures per station were calculated by averaging ten years data for each month, and plotted to show seasonal patterns over the year. It should be noted that we did not use total precipitation received on the highlands, as this requires complete record of data from all stations on the highlands; in addition, mean monthly or annual precipitation would characterize habitat preference of amphibian species better than does total precipitation received.

B) Interpretation of Results

The estimated ranges in the form of EOO were evaluated in comparison with previous ranges documented in the IUCN database (IUCN, 2011). EOO (criterion B1) is used as one of the major criteria to evaluate the Red List status of species. As our study did not incorporate studies on the other criteria (A, C, D, E) dealing with abundance, trends and status of populations, we only tried to compare earlier ranges with current results that followed phylogenetic revision of the studied taxa. The grading of the EOO criterion as used to define the categories is (IUCN, 2001):

- Extinct (EX): when there is no reasonable doubt that the last individual has died
- Extinct in the Wild (EW): when a taxon is known only to survive in cultivation

- **Critically Endangered (CR)**: EOO < 100 km^2, severely fragmented or known to exist at only a single location
- **Endangered (EN)**: EOO < 5000 km^2, severely fragmented or known to exist at no more than five locations
- **Vulnerable (VU)**: EOO < 20,000 km^2, severely fragmented or known to exist at no more than ten locations
- Near Threatened (NT): when a taxon is close to qualifying for or is likely to qualify for a threatened category in the near future
- Least Concern (LC):does not qualify for Critically Endangered, Endangered, Vulnerable or Near Threatened
- Data Deficient (DD): when there is inadequate information to make a direct, or indirect, assessment of its risk of extinction based on its distribution and/or population status
- Not Evaluated (NE): when the taxon has not yet been evaluated against the criteria

To show a generalized picture of the status of habitats within the altitudinal range of a species, we will discuss the composition of the geographic range of a species in association with land cover data. Habitat-related socio-economic practices encountered and discussed with the local people during our field work between 2006 and 2010 will be briefly summarized to supplement our data.

III) RESULTS

The number of locality records (GPS coordinates, altitude, and locality name) included in the current analyses was 99 for *Leptopelis* and 69 for *Ptychadena* (Appendix 4.1). Most of the localities are on the western rift that has a relatively large area and fractured landscape.

Some clear patterns are observed in the monthly and annual rainfall and temperature conditions (Table 4.2 and Fig. 4.2). Annual rainfall fluctuates on both rifts, with the east receiving smaller rainfall than the west. In the west, main rains are peak in July-August, with a smaller peak in April; in the east a moderate peak is seen in April-May, with less pronounced difference from another peak in July-October. Steady increase is seen in the average annual temperature (both max and min) on both rifts. The west is generally warmer (both monthly and annual) than the east.

The geographic ranges of some previously known species were estimated to be wider than thought before (range extension), where as those of others appeared far narrower than earlier presumptions (range reduction). These are a result of the taxonomic revision that split some of the taxa (revealed higher diversity), and realization of the fact that human-inhabited areas (changing natural habitats) still serve as home to many of these endemic species. Details of estimates of EBEOO are given for each species and population in Table 4.3.

For all studied species, the vegetation cover characterizing the majority of the geographic ranges of most species is cultivated or managed areas (Figs. 4.3 – 4.6). *Leptopelis gramineus* is a fossorial species occurring in montane grassland habitats with permanent streams or flood pools, or at forest edges. These habitats are subject to cultivation and other ‘development’ activities. Trees or shrubs, which are required by arboreal species of *Leptopelis*, are in most cases scarcely scattered within the managed areas. The only remnant natural tree or shrub covers are located in Kaffa (southwest highlands) and at the southern face of the Bale Mountains (Figs. 4.3 and 4.4).

Table 4.2. Comparison of means of monthly (A) and annual (B) precipitation, maximum- and minimum-temperatures between the eastern and western rifts of the Ethiopian Highlands. Bold figures indicate significant difference between means (two-tailed t-test, unequal variance, p-values < 0.05).

A) Monthly

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Precipitation	0.8875	0.5960	0.5266	0.1145	0.3632	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.3527	0.5472	0.7323
Max. temp.	**0.0001**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0008**	**0.0352**	0.1803	**0.0018**	**0.0000**	**0.0000**	**0.0000**
Min. temp.	**0.0001**	**0.0000**	**0.0000**	**0.0001**	**0.0001**	**0.0002**	**0.0002**	**0.0006**	**0.0002**	**0.0020**	**0.0024**	**0.0004**

B) Annual

	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Precipitation	**0.0130**	0.1262	**0.0096**	**0.0114**	0.1605	**0.0011**	**0.0334**	**0.0111**	**0.0062**	0.0767
Max. temp.	0.2905	0.3017	0.3775	0.3391	0.4989	0.2132	0.5939	0.2355	0.2502	0.2358
Min. temp.	0.4064	0.3348	0.2473	0.2682	0.1588	0.0798	0.3906	0.3854	0.3031	0.3357

The characteristic land cover within the geographic ranges of most species of *Ptychadena* is similar, comprised mainly of cultivated or managed areas as well as mosaic of cropland/tree/shrub/grass/other natural vegetation (Figs. 4.5 and 4.6). The exceptions to this appear *P. neumanni* (in the southwestern highlands) and *P. erlangeri* (in the southern and south-central highlands), occupying grass fields and swamps within or at edges of fragmented forests. In general, we can see on the maps that the actual suitable habitats occupied by the studied species are far smaller than the estimated EBEOO that reflects only altitudinal suitability.

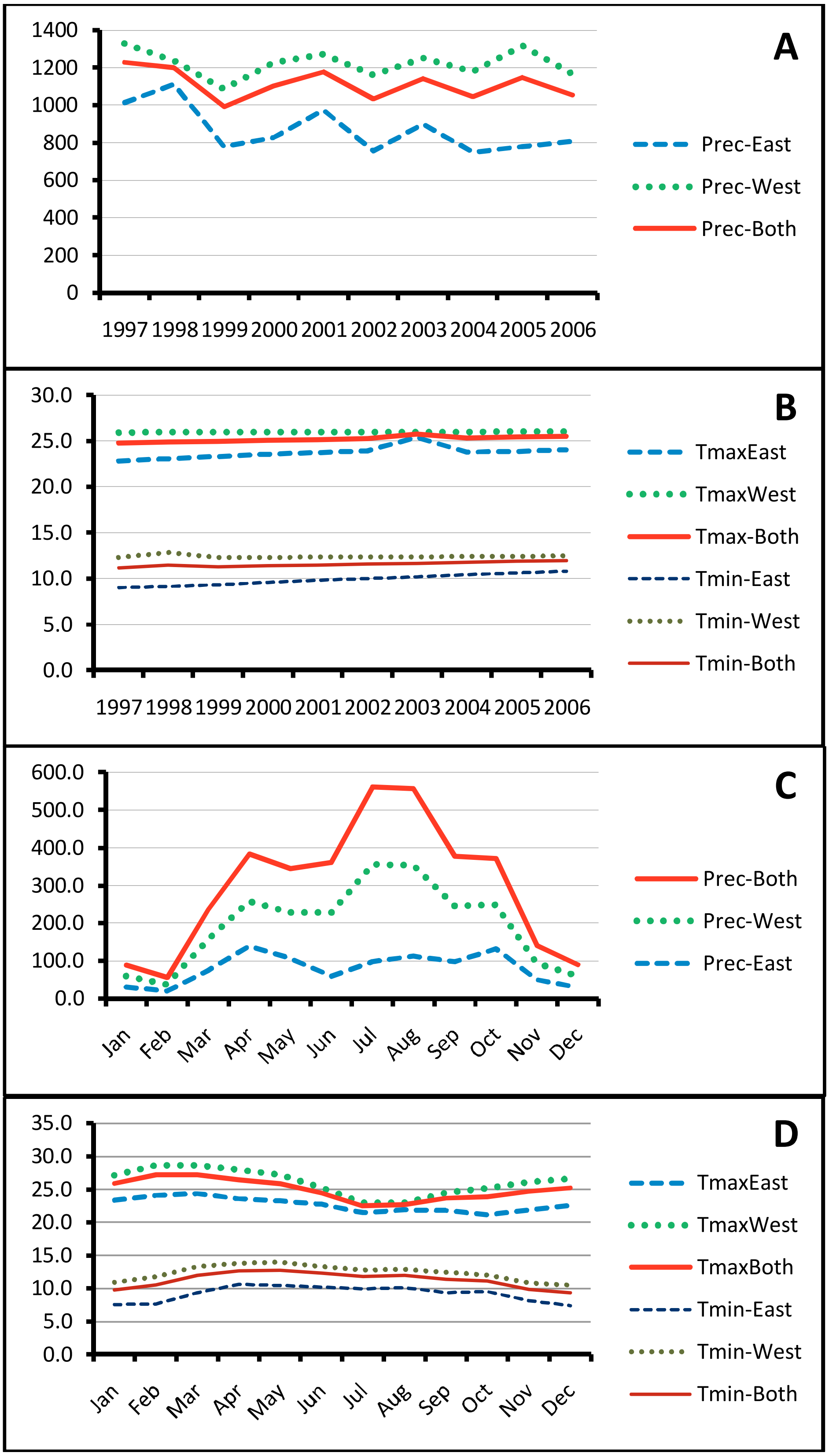

Fig. 4.2. Patterns of annual and monthly rainfall (16 localities) and temperature (12 localities) in the eastern and western rifts in Ethiopia (1997-2006): A) Mean annual precipitation per site over ten years period; B) Mean maximum and mean minimum annual temperature per site over ten years period; C) mean monthly precipitation per site (averaged for ten years); and D) mean maximum and mean minimum monthly temperature per site ((averaged for ten years) (Source for raw data: National Meteorological Services Agency of Ethiopia, 2010).

Table 4.3. Estimated geographic areas in terms of Elevation Based Extent of Occurrence (EBEOO) for the distribution of populations and species of *Leptopelis* and *Ptychadena* in the Ethiopian Highlands. Estimation procedure is discussed in Methods section.

Species	Locality of population	EBEOO for Population (km^2)	EBEOO for Species (km^2)
Leptopelis gramineus	Arsi-Bale-Bore	50,169	128,599
	Gughe	3,058	
	Shoa	56,282	
	Southwest (Kaffa-Illubabor)	17,319	
	West (Welega)	1,771	
Leptopelis ragazzii	Arsi-Bale-Bore	47,280	103,562
	Shoa	56,282	
Leptopelis sp. (Saja)	Saja (Kaffa)	46	46
Leptopelis sp. (Soddo)	Soddo (Wolayita)	3,455	3,455
Leptopelis susanae	Gughe	3,058	3,104
	Kaffa	46	
Leptopelis vannutellii	Southwest	134,453	134,453
Leptopelis yaldeni	Gojam	31,811	31,811
Ptychadena cooperi	Arsi-Bale-Bore	18,771	34,820
	Shoa	16,049	
Ptychadena sp. (Debre Markos)	Gojam	12,275	12,275
Ptychadena erlangeri	Bale	5,165	15,144
	Bore	9,979	
Ptychadena nana	Arsi	10,814	18,413
	Bale	7,599	
Ptychadena neumannii	Dime	1,815	13,218
	Gughe	1,791	
	Kaffa	9,612	
Ptychadena sp. (Bada Buna - Akaki)	Akaki	12,903	26,909
	Bada Buna	14,006	
Ptychadena sp. (Bore2-Wenchi)	Bore	3,155	11,964
	Wenchi (Shoa)	8,809	
Ptychadena sp. (Goba)	Goba	4,602	4,602
Ptychadena wadei	Gojam-Gondar	6,078	6,078

IV) DISCUSSION

A) Geographic Ranges and Climate

The actual area of suitable habitats occupied (AOO) by each of the studied species of *Leptopelis* and *Ptychadena* should be smaller than the estimates of EBEOO. The geographic areas estimated in this study are just indicative of the altitudinal ranges potentially suitable for the species in question, provided that the required vegetation and wet area will be in place. Any conservation plan for these species should primarily focus on the actual (net) area of suitable habitats currently occupied by each species. The EBEOO will be particularly useful in two ways, 1) it would make field searches more focused, saving research resources (time, energy, and money) to collect specimens of patchy populations of a particular species; and 2) any future plans to restore habitats or populations of a threatened species can be targeted within the EBEOO areas defined here.

It should be noted with caution that habitats that are cultivated or used for grazing domestic animals do not necessarily mean that these places are not suitable for some species of amphibians. For instance, *L. yaldeni* is known from human-inhabited areas along the main road from Dejen through Debre Markos to Bahir Dar. The type locality (Debre Markos town) has been a local administrative capital since the 1850s, and through longer period it has been subject to human impact by agriculture and urbanization. *Ptychadena nana* occupies vast plains in the Arsi and Bale Mountains, where huge farms of crops operate and grazing fields are used by large number of domestic animals.

The distribution areas predicted for *L. ragazzii* and *L. vannutellii* using climate envelope models (Weinsheimer et al., 2010) are somehow consistent with the current estimates; but in the former, taxonomic discrepancies about a presumed population of *L. ragazzii* in the Gughe Mountains were not understood and addressed. Now, it appears that this taxonomically misplaced population belongs to *L. vannutellii* (Chapter 2), a strictly arboreal species (Largen, 1977, 2001) occupying fragmented forests in the southwest highlands. *Leptopelis ragazzii* occurs within highly fragmented shrub/trees vegetation remnants in the central (though not recorded since 1975, Largen (2001)), southern and south-central highlands that have rapidly changing habitats.

The reported reductions in the geographic range of some of the species are a result of taxonomic restructuring (Chapters 2 and 3) rather than temporal trends in the populations of the species. It is unfortunately not possible to compare the changes in geographic areas, because of the use of different techniques in previous (Appendix 4.2 and 4.3) and current estimates, as well as absence of absolute figures for earlier estimates (Table 4.1). Historical records of status of populations (when available) need be assessed with new surveys; quantitative studies on abundance and (on-the-ground) status of habitats of species would help to fill these gaps.

There are clear patterns of monthly and annual rainfall and temperature on the highlands across the Rift Valley (Fig. 4.2 and Table 4.2). The western rift generally appears warmer and wetter than the eastern. Significant difference (p-values ≈ 0.0000) in seasonal rainfall of the two rifts is seen during June-September where the western highlands receive a characteristic peak precipitation. Unlike the rainfall patterns, average monthly temperature is significantly different on the two rifts throughout the year. Different trends in seasonal intensity of rainfall in the east and west could mean that different seasons of the year could be suitable for reproduction of amphibians across the Rift Valley. We might also note these seasonal differences to improve timing of field surveys and encounter populations of amphibians for effective sampling.

Trends in annual precipitation consistently fluctuated every year, but did not show substantial decrease. In the western rift, a site on average receives an annual rainfall of around 1200 mm, whereas this goes lower than 1000 mm in the eastern rift; and these differences are significant for most of the years (p-values < 0.05). Mean annual temperatures on the two rifts are not statistically different (p-values > 0.05). However, a general increase in the local temperature of the Ethiopian Highlands by about 0.8^{o}C in just ten years period might be biologically significant, probably influencing the reproductive behavior and habitats of amphibians. On the eastern rift, the increase in temperature is more pronounced, showing an increase of about 1.2-1.8^{o}C over a decade. The local people in Rira village (in the Bale Mountains) explain that recent human settlement, cultivation and animal husbandry in the ericaceous belt (up to 3500 m) is in part a result of warming climate at higher altitudes. It has been reported that one of the endemic species of the Bale Mountains, *Ericabathrachus baleensis*, might have probably been extirpated at its type locality, and other species show declines in their population (Gower et al, in press). A study on temperature sensitivity of amphibians in plantations suggests that diversity of these animals can be affected by climate change (Wanger et al., 2009).

Rainfall pattern in the Ethiopian Highlands shows historical fluctuations. The central highlands were characterized mainly by positive rainfall deviations in the first-, and negative deviations in the second-half of the 20th Century (Seleshi and Demarée, 1995; Osman and Sauerborn, 2002). It was only since the early 1990s that the Ethiopian Highlands experienced successive years with rainfall higher than the long-term average (Osman and Sauerborn, 2002). During the winter in the Northern hemisphere, the western Highlands of Ethiopia receive hot and dry winds from the Sahara, while east winds from the Indian Ocean bring spring rains in the southern part of the country. From end of June onwards, the southeast monsoons bypass the highlands by the south and reach them from the west, initiating the main rainy season that lasts until end of the summer (Goebel and Odenyo, 1984). Two decades ago, mean annual precipitation from 241 sites was found to be 938 mm per year (Yeshanew and Apparo, 1989); our current estimates for the period 1997-2006 are slightly higher than previous decades. Research in the northern Ethiopian Highlands indicates that topographical aspects such as steep overall slope gradients and valley aspect control the spatial distribution of annual rain depth (Nyseen et al., 2005).

The surface area of the western highlands is also far greater than the eastern rift. Thirteen of the sixteen studied endemic species (including putative) occur in the western rift, with nine of these solely restricted to this side of the highlands. Seven species (three solely restricted) occur in the eastern rift. That is, only four species occur on both rifts. Warmer and wetter climate and habitats coupled with larger surface area providing highly fractured suitable habitats could probably have contributed to the relatively high diversity of these endemics in the west.

B) Status of Species and Habitats

Based on the revised taxonomic status of endemic species and populations of *Leptopelis* and *Ptychadena* in the Ethiopian Highlands, estimated geographic ranges (Table 4.3) and status of habitats (criterion B1: EOO in terms of EBEOO), as well as the respective characteristic vegetation of each area (Figs. 4.3 – 4.6), we made preliminary evaluation and indications on the conservation status of species of *Leptopelis* and *Ptychadena* in the Ethiopian Highlands. We contributed a large part of this information to the IUCN Red List re-assessment of African Amphibians organized in Trento, Italy on 01-04 June 2012, an indication of the importance and timeliness of the current results.

***Leptopelis gramineus* (Fig. 4.3A)**: widespread (EBEOO > 129,000 km^2) across highlands on both sides of the Rift Valley; populations residing in different mountain ranges are disjunct, and highly patchy geographically and genetically (Chapter 2). Field searches have to be made in the northern highlands of Ethiopia to check if this species has an extended range, and if it has managed to survive under more severely degraded habitats than the southern part of the country. Potential threats could be agricultural chemicals (mainly pesticides), insecticidal pollutants such as DDT used to control mosquitoes, conversion of grasslands and associated wetlands into crop fields or infrastructure areas. There seems no immediate threat on this species, but further investigation is required to check for possible threats. If the taxonomic status of the disjunct populations of *L. gramineus* proves their distinction as species, the EBEOO and conservation status of each of these populations might be affected. At the current taxonomic status of these populations, the existing **Least Concern** status looks appropriate.

***Leptopelis ragazzii* (Fig. 4.3B)**: widespread (EBEOO > 103,000 km^2) across highlands on both sides of the Rift Valley; populations residing in different mountain ranges are geographically disjunct, but this is not supported by genetic data (Chapter 2). Ranges of *L. ragazzii* overlap widely with that of *L. gramineus*, but the former is found at streams lined with small shrubs; however, it has not been confirmed to occur in the western and southwestern highlands. Field searches have to be made in the central and northern highlands to check its taxonomic status, if it has extended range, and if it has managed to survive under more severely degraded habitats than the southern part of the country. Potential threats could be cutting shrubs for fencing and fuel wood, conversion of shrub covers and associated streams into crop fields or infrastructure areas, herbicides, agricultural chemicals (mainly pesticides), insecticidal pollutants such as DDT used to control mosquitoes. Increased human settlement and habitat loss are major threats; investigation is required to check for other possible threats. Although we did not estimate AOO for this species, the threats on its habitat make the existing **Vulnerable** status appropriate.

***Leptopelis* sp. (Saja) (Fig. 4.3C)**: Known only from a single locality, Saja Forest (EBEOO ≈ 50 km^2) in the southwest highlands; fragmented populations could possibly exist in adjacent forests within these highlands. There are no data on the population status of this undescribed species, but there are expanded deforestation, human settlement and cultivation activities that could threaten this putative new species. Until additional data are obtained, we suggest placing this putative new species as **Data Deficient**. However, if this undescribed species does not occur in wide geographic areas, based on its current EBEOO, single known locality and fragmented habitat, it can be assigned as Critically Endangered.

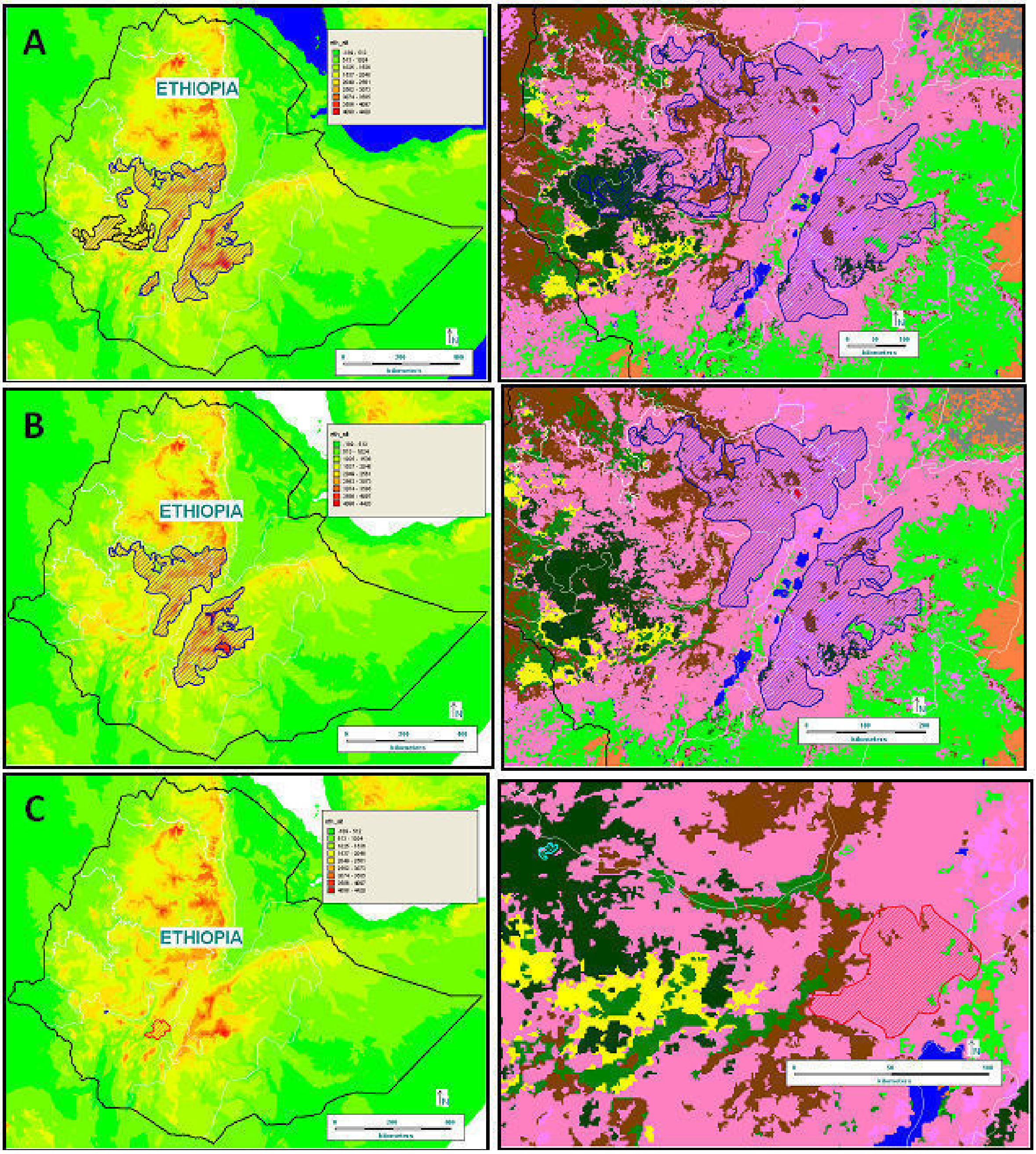

Fig. 4.3. Maps of altitude-based geographic ranges (left) and characteristic land cover (magnified on the right) for species of *Leptopelis* in the Ethiopian Highlands. Diagonal or cross fills = geographic ranges of species: A) *L. gramineus* (blue stripes); B) *L. ragazzii* (blue stripes); C) *Leptopelis* sp. (Saja) (small, blue or cyan stripes) and *Leptopelis* sp. (Soddo) (large, red stripes). Black line = international boundary; white line = regional administrative boundary. Solid color fills in land cover maps: dark-green = tree, yellow = mosaic of tree/other natural vegetation, brown = shrub, light-green = herbaceous, light-brown = sparse herbaceous or sparse shrub, pink = cultivated and managed area, light pink = mosaic of cropland/tree/shrub/grass/other natural vegetation, blue = water bodies, red = artificial surfaces and associated areas.

***Leptopelis* sp. (Soddo) (Fig. 4.3C)**: Known only from a single locality, in backyard enset vegetation west of Soddo town (EBEOO ≈ 3,400 km^2) in the southwest highlands; there is good possibility to find fragmented populations in human-inhabited places within its EBEOO or elsewhere in the southwest highlands. There are no data on the population status of this undescribed species, but the area sees dense human settlement, deforestation, and cultivation activities that could threaten this putative new species. Until additional data are collected, we suggest placing this undescribed species as **Data Deficient**. However, if this taxon does not occur in wide geographic areas, based on its EBEOO, single known locality, and severely fragmented habitat, it can be assigned as Endangered.

***Leptopelis susanae* (Fig. 4.4A)**: Known from two distant localities, with forested habitats in Gughe Mountain and Saja Forest (Kaffa) (EBEOO ≈ 3,000 km^2) in the southwest highlands. The new record from Saja Forest (Chapter 2) is a good indication of a possibly extended geographic range for this species; there may be other fragmented populations in forests characteristic of these highlands. The populations in both major localities are severely fragmented, with threats of deforestation, settlement and cultivation. Considering the estimated EBEOO and fewer than five known localities in severely fragmented habitats, it would be appropriate to keep the existing **Endangered** status.

***Leptopelis vannutellii* (Fig. 4.4B)**: Known from several fragmented localities (EBEOO ≈ 134,000 km^2) in pristine forests in the southwest highlands; the previously known range has been extended to the Gughe Mountain (after phylogeographic studies and taxonomic revision, Chapter 2). There could still be fragmented populations in forests not surveyed within the described EBEOO. However, only a small portion at the western side of the EBEOO is a natural forest; the larger portion of the EBEOO is characterized by cultivated or managed areas, indicating that some populations can thrive in backyard enset trees. Potential threats could be deforestation, settlement and cultivation. Based on the strictly arboreal life of this species, and the possible threats on these areas, as well as new records that could increase its previously known AOO, we suggest revision of its previous Vulnerable status, and assign it as **Near Threatened**.

***Leptopelis yaldeni* (Fig. 4.4C)**: Contrary to IUCN reports of a smaller EOO (Table 1), the geographic range of this species could be larger (EBEOO ≈ 31,000 km^2) but limited to the Gojam area in the northwest highlands. This area is circumscribed by the Abay Gorge where the land cover is characterized mainly by cultivated or managed areas, with patchy shrub cover concentrated at the western edges of the EBEOO. Backyard vegetation with koba (false banana) trees and undergrowth has been noted as an important habitat for this species in this largely human-occupied plateau. Modern agricultural activities, urbanization, cutting of shrubs for fuel wood, could potentially threat this species in the near future. Although the EBEOO appears fairly large, the potential threats could urge a revision of its previous Near Threatened status, which we suggest to be **Vulnerable**.

***Ptychadena cooperi* (Fig. 4.5A)**: Known from several localities in the central, south-central and southern segments (EBEOO ≈ 34,000 km^2). The land cover is mainly characterized partly by cultivated or managed areas, and partly by mosaic of cropland/trees/shrubs/grassland and other natural vegetation; some shrub cover is also included, but forest cover (e.g., in the southern face of Bale Mountains) is excluded. Populations of the eastern and western rifts are isolated in nature by the Rift Valley, and it has not been genetically tested whether these two haplotypes belong to a single or two distinct species. High density of human population practicing cultivation, cattle breeding, expanding urbanization and associated pollution of aquatic habitats are possible threats witnessed in all of its geographic ranges. As the taxonomic status of the two disjunct populations is not known (implying possibly decreased EOO), and as habitat degradation and fragmentation are persistent, we suggest a precautionary assignment of an upgraded **Vulnerable** status.

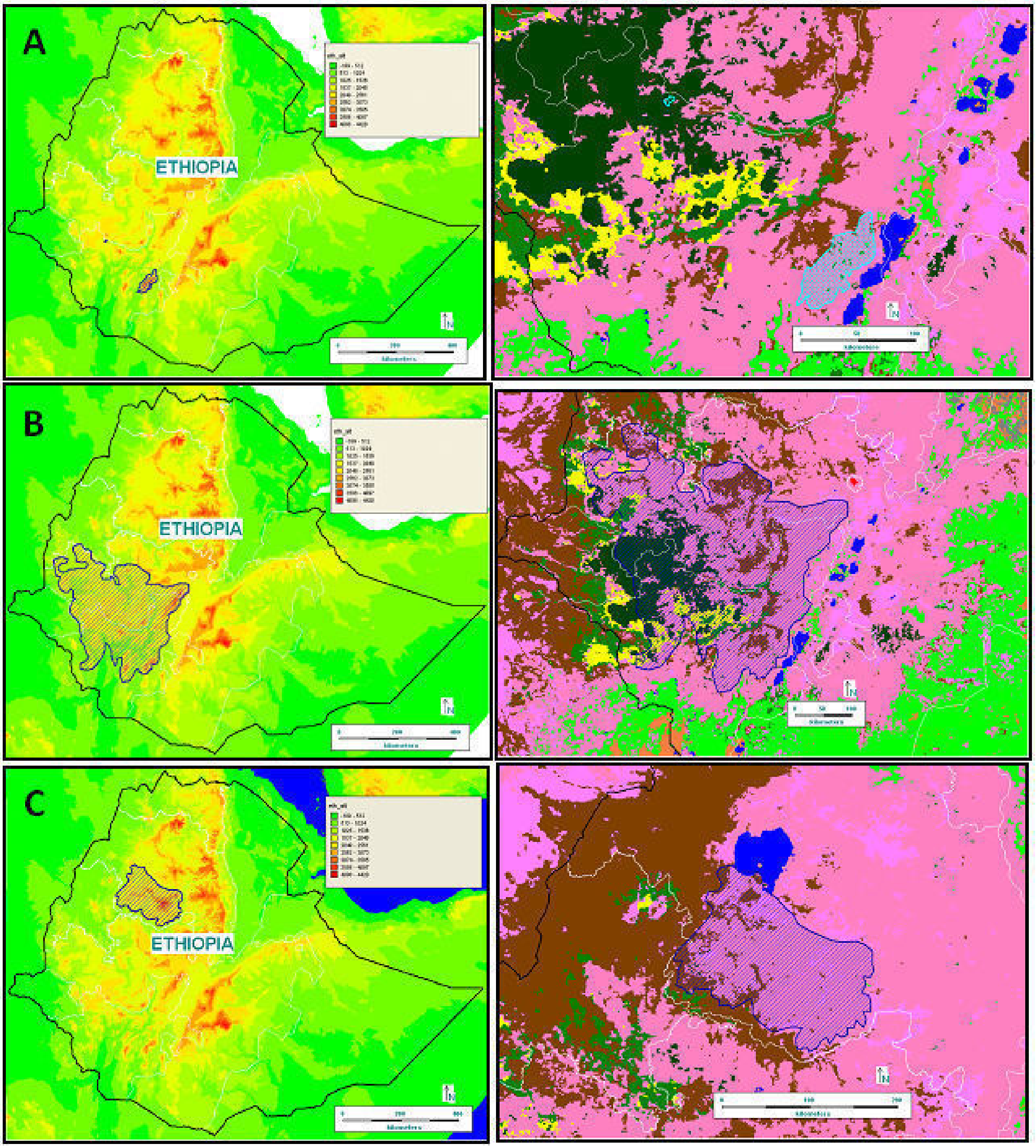

Fig. 4.4. Same as in Fig. 4.3, but for: A) *L. susanae* (blue or cyan stripes); B) *L. vannutellii* (blue stripes); C) *L. yaldeni* (blue stripes).

***Ptychadena* sp. (Bada Buna – Akaki) (Fig. 4.5B)**: Recorded from two localities (Bada Buna and Akaki areas) (EBEOO ≈ 26,000 km^2) in the central highlands; the two localities are close to each other, and tend to form a more or less continuous range. This taxon is phylogenetically distinct, a split from the complex '*P. neumanni* and allies' group, and represented by a few specimens. The range of this putative new species adjoining the southwest highlands is partly covered with cultivated or managed areas, and partly by shrub cover; the other side in the central highlands is almost entirely cultivated or managed area. The common threats characterizing most Ethiopian montane grassland habitats prevail here. The taxonomy, ecology and population status of this undescribed species requires field assessment, and we suggest keeping it for now as **Data Deficient**.

***Ptychadena* sp. (Goba) (Fig. 4.5B)**: Recorded from two proximal localities east of Goba town (EBEOO ≈ 4,000 km^2) in the south-central highlands; this taxon is another phylogenetic split from the complex '*P. neumanni* and allies' group, and is represented by a few specimens. The range of this undescribed species is mostly covered with cultivated or managed areas, and scattered shrub or herbaceous cover. The common threats characterizing most Ethiopian montane grassland habitats also prevail here. The taxonomy, ecology and population status of this taxon requires field assessment, and we suggest keeping it for now as **Data Deficient**.

***Ptychadena* sp. (Debre Markos) (Fig. 4.5C)**: Known from a single locality in Debre Markos town (EBEOO ≈ 12,000 km^2) in the northwest highlands. Its range is circumscribed by a mid-altitude plateau south of Lake Tana, a range of higher mountains in the east, and the Abay Gorge. This putative new species has earlier been thought to be one of the patchy populations of *P. erlangeri* (Largen, 1997, 2001); but phylogeographic studies proved different and suggested to put it as a distinct species (Chapter 3). The land cover is characterized mainly by cultivated or managed areas. Modern agricultural activities, urbanization, and pollution of aquatic habitats could potentially threaten this undescribed species in the near future. The population status of this taxon is not known; based on its EBEOO, the single locality from which it is known, and the possible threats, we suggest to place it as **Vulnerable**.

***Ptychadena wadei* (Fig. 4.5C)**: Known from a few localities along the Abay River between Lake Tana and the Tisissat Falls, and we potentially expect it to occur across the mid-altitude plains forming the Lake Tana Sub-basin in Gojam and Gondar provinces (EBEOO ≈ 6,000 km^2) in the northwestern highlands. The Andasa plains at the type locality are geographically, climatically, and ecologically similar with the Fogera and Dembia plains further north. Most of the range of *P. wadei* is cultivated or managed area, with a few patchy shrub cover. There is large density of human population in these areas, and agricultural development, urbanization, chemical pollutants are potential threats to be considered in the future. However, naturally the Lake Tana Sub-basin is known for being over-flooded for about half of the year during and after the main rainy season. This could ensure availability of reproductive and feeding grounds as an opportunity for sustained population levels of *P. wadei* in these areas. Although there are no data on the population status of this species, based on its EBEOO and under the above ecological considerations, we suggest a **Vulnerable** status to this species.

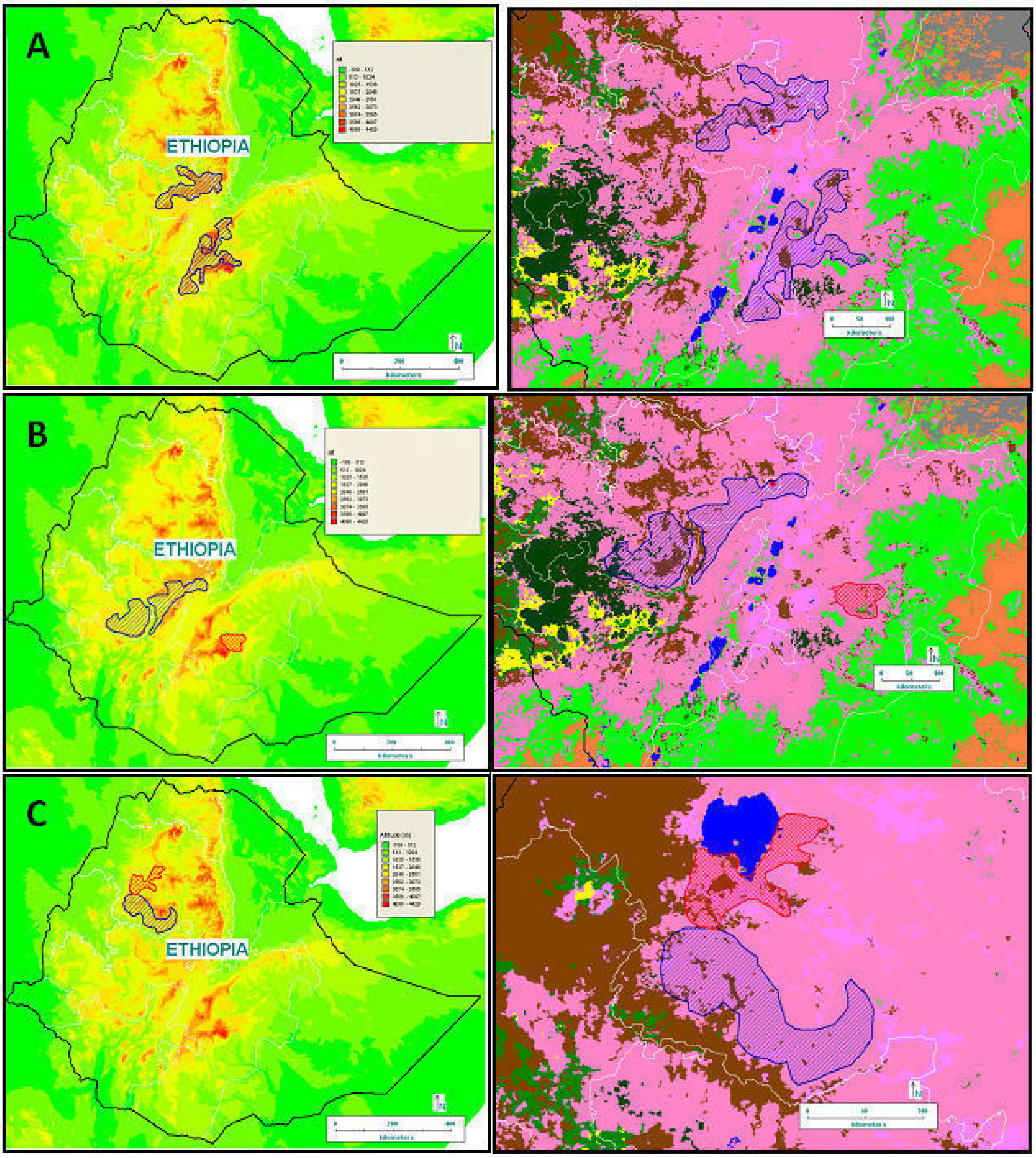

Fig. 4.5. Maps of altitude-based geographic ranges (left) and characteristic land cover (magnified on the right) for species of *Ptychadena* in the Ethiopian Highlands. Diagonal and cross fills = geographic ranges of species: A) *P. cooperi* (blue stripes); B) *Ptychadena* sp. (Bada Buna – Akaki) (blue stripes), and *Ptychadena* sp. (Goba) (red stripes); C) *Ptychadena* sp. (Debre Markos) (blue stripes), and *P. wadei* (red stripes). Black line = international boundary; white line = regional administrative boundary. Solid color fills in land cover maps: dark-green = tree, yellow = mosaic of tree/other natural vegetation, brown = shrub, light-green = herbaceous, light-brown = sparse herbaceous or sparse shrub, pink = cultivated and managed area, light pink = mosaic of cropland/tree/shrub/grass/other natural vegetation, blue = water bodies, red = artificial surfaces and associated areas.

Ptychadena erlangeri **(Fig. 4.6A)**: Contrary to the presumed fragmented and widely fragmented distribution across the Rift Valley (Largen 1997, 2001), *P. erlangeri* is restricted to and known from several localities in Bore/Sidamo and the southern face of the Bale Mountains (EBEOO ≈ 15,000 km^2) in the southern and south-central highlands. In Bale, its habitat is mainly characterized by montane grasslands within and at the edge of forests, while in Bore/Sidamo area, a cultivated or managed areas dominate the forested habitats. The geographic range of *P. erlangeri* appears to have no overlap with either *P. neumaanni* (southwest highlands) or *P. nana* (plateaus of Arsi and the northern face of Bale Mountains), a notable new piece of biogeographic evidence that can help to resolve the complex taxonomy of these three species. The habitat in Bale is mainly a forest cover, and is part of the Bale Mountains National Park and appears relatively less prone to threats on habitats as compared with other species of *Ptychadena* in the highlands. In Bore/Sidamo area, a little part of the range is encompassed within the Nech Sar National Park east of Lakes Abaya and Chamo; otherwise cultivated or managed areas dominate this part of the range. There is a possibility for the EBEOO to extend further south into the Yabelo Wildlife Sanctuary. Although this species occupies habitats associated with forests in protected areas, deforestation, human settlement, and urbanization are viewed as ongoing and potential threats in the future. Based on the estimated EBEOO and sensitivity of the habitat of this species for further fragmentation and degradation, it appears necessary to revise its status, and we suggest an upgrade to **Vulnerable** status.

***P. neumannii* (Fig. 4.6A)**: Previously assumed to have been widespread in the highlands across the Rift Valley, and even at very low altitudes in the southwest (Largen, 1997, 2001); but phylogeographic studies (Chapter 3) revealed that it is restricted mainly to severely fragmented areas in Kaffa and Gemu Gofa (EBEOO ≈ 13,000 km^2) in the southwest highlands. A specimen and tissue sample reported to have been collected from the foothills of Bale Mountains and genetically clustered with *P. neumanni* needs verification through additional field work. The current range of *P. neumanni* is characterized by grasslands occurring at wetland areas or as pockets in moist mid-altitude forests; however, the vast portion of its range, including the area around the type locality, is cultivated or managed area. There is a high density of human population in the area, and deforestation for investment and cultivation, human resettlement, and urbanization are the major threats to these habitats. Giving special attention to the severely fragmented habitats in its range, its highly reduced EOO (due to taxonomic and biogeographic revisions), and the rapidly deteriorating environmental conditions, we suggest to revise the previous Least Concern status and upgrade it to **Vulnerable**.

***Ptychadena* sp. (Bore-Wenchi) (Fig. 4.6B)**: Known from two distantly isolated geographic ranges (Bore/Sidamo and Wenchi) across the Rift Valley (EBEOO ≈ 11,000 km^2) in the southern and central highlands. This undescribed species is one of the four new taxa that resulted from phylogeographic assessment of a previously complex group of species, '*P. neumanni* and allies'. The range in Bore/Sidamo area overlaps with that of *P. cooperi* and *P. erlangeri*, while the population in Wenchi overlaps with the range of the population of *P. cooperi* in Shoa. The habitats in both ranges of this new taxon are composed highly fragmented cultivated or managed areas. Threats imposed on these areas include urbanization, agriculture, and chemical pollutants. The fragmented geographic ranges, the relatively small EBEOO, and the threats on the habitats of this taxon are indicative of a potential threat to the isolated populations, and hence we suggest putting it in a precautionary status as **Endangered (**until additional data are obtained).

***Ptychadena nana* (Fig. 4.6B)**: Known from plateaus of Arsi Mountains, and an extended range further south to the plateaus on the northern side of the Bale Mountains (EBEOO ≈ 18,000 km^2) in the south-central highlands. After phylogeographic studies (Chapter 3), the previously known range at the type locality in Arsi (Dida'a plateau) (Largen, 1997, 2001) is currently expanded to a close by plateau area in the Bale Mountains. Its range is covered mainly with cultivated or managed areas, and a mosaic of cropland-trees-shrubs-grassland-other natural vegetation. Modern agriculture, urbanization, and pollution of aquatic habitats could be possible threats. During field surveys, it appears that individuals of this species are commonly available in populations at different localities. Although additional data is required to understand the population status of this species, realizing the extended range that appears far larger than anticipated before, and considering the potential threats that can affect the populations, we suggest revising the status of this species to **Near Threatened** (close to Vulnerable).

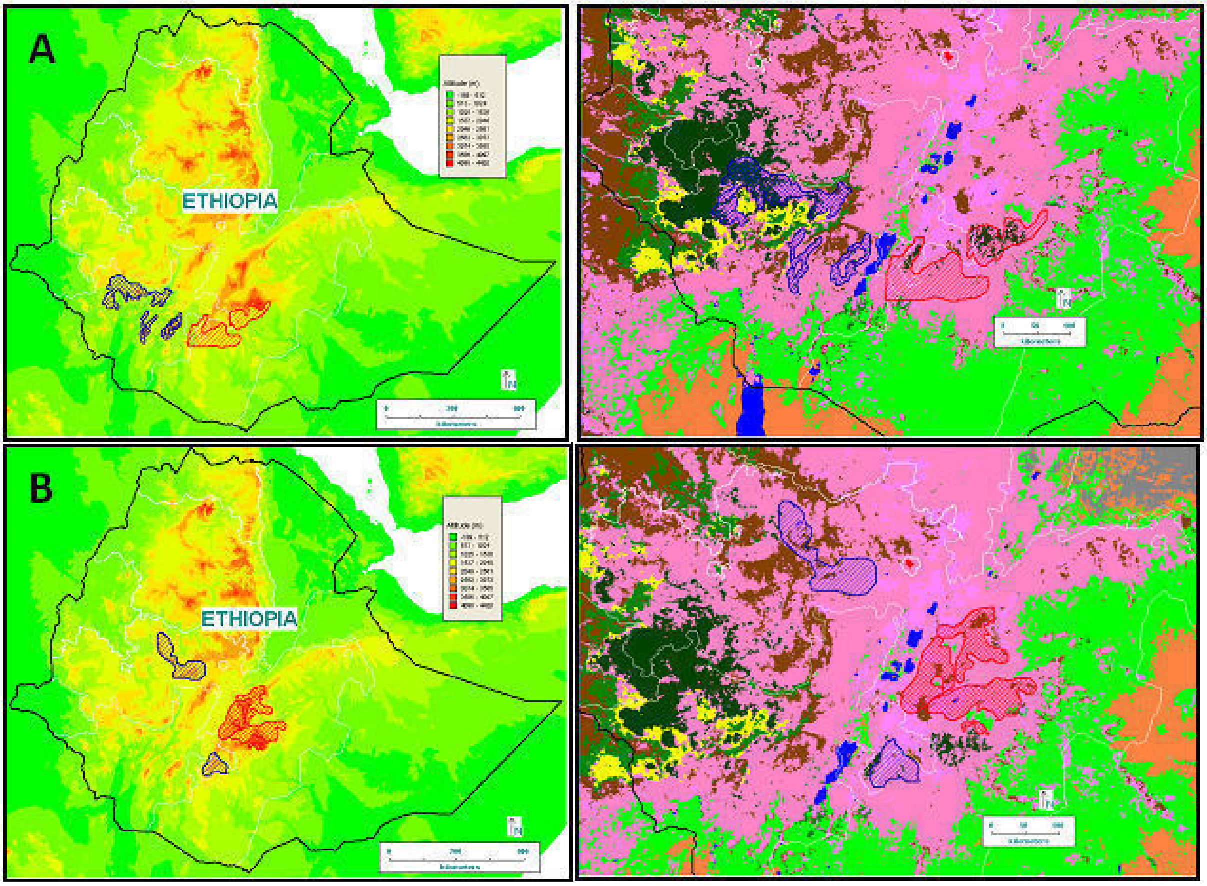

Fig. 4.6. Same as in Fig. 4.5, but for: A) *P. neumanni* (blue stripes), and *P. erlangeri* (red stripes); B) *Ptychadena* sp. (Bore-Wenchi) (blue stripes), and *Ptychadena nana* (red stripes).

C) General Considerations for Conservation

Out of the 16 known and putative new species (representing over 25% of all known Ethiopian amphibians) assessed in the current work, nine fall into the threatened categories, with other two being Near Threatened; four are Data Deficient, and only one is in the Least Concern category. When these values are compared with the proportion of threatened amphibians in the country (14%, IUCN, 2011) or in the world (32.5%, Stuart et al., 2004), our data indicate that 56% of the studied highland endemic species appear threatened. Previously, none of the Ethiopian *Ptychadena* was assigned as threatened. In the current results three (of seven) *Leptopelis* and six (of nine) *Ptychadena* are threatened. That is, local or taxonomic levels of threats could be more meaningful than global indicators to protect threatened diverse endemics. Evaluating local faunas are fundamental to detect long-term changes in vertebrate communities in the tropics (Santos-Barrera, 2008). Clearly the results suggest that there should be greater concern for the long term survival of the known and putative endemic species. The reported high level of diversity and endemicity (Chapters 2 and 3) in this area is confronted by potentially damaging habitat changes. Unless the existing amphibian diversity can be effectively saved and sustained, it might not be too long before we see drastic declines in populations of these amphibians. It has already been noted that declines are evident in amphibian species of Bale Mountains (Gower et al., in press). To underline this idea, we have outlined the existing threats for highland species of Ethiopian amphibians, and the opportunities that can potentially aid to facilitate effective conservation.

1) Threats

Of the six leading hypotheses thought to underline amphibian declines (Collins and Storfer, 2003), alien species and over-exploitation do not currently appear serious problems in the case of Ethiopian amphibians. There is so far no report of a negative effect of either alien species on amphibian species, or practices of harvesting amphibians for commercial purposes in the country. Two species (*Altiphrynoides malcolmi* and *Spinophrynoides osgoodi*) are listed in Appendix 1 of CITES listings. Further assessment of commercial threats on other Ethiopian species is needed to protect threatened species. The other four causes of amphibian declines, namely, land use (or land cover) change, global change, increased use of pesticides and other toxic chemicals, and emerging infectious diseases appear substantially functional in impacting Ethiopian amphibian populations directly or indirectly.

Change in land use is a major factor linked with amphibian declines (Brooks et al., 2002; Collins and Storfer, 2003; Stuart et al, 2004). Habitat fragmentation can affect amphibians by reducing post-metamorphic survival and dispersal success; fragmentation of habitats therefore affects both efficient and less vagile dispersers (Cushman, 2006). Amphibians generally have lower rates of movement per generation than invertebrates, mammals or reptiles (Bowne and Bowers, 2004), and population connectivity is mainly possible through juvenile dispersal (Madison, 1997; Rothermel, 2004). In Ethiopia, extensive habitat degradation is manifested in rural and urban areas. The majority of the over 85 million people of the country live on the highlands, with areas of highest densities more or less fitting with the geographic ranges of the studied endemic amphibians. Consequently, suitable habitats for amphibians are being fragmented, potentially hindering connectivity and interaction among neighboring populations of amphibians.

Deforestation can be aimed for timber production, fuel wood, cultivation and ranch areas, construction sites, human settlement, roads and other infrastructure, irrigation schemes drying rivers and other wetlands. Most Ethiopian species of *Leptopelis* and a few species of *Ptychadena* appear to be dependent on availability of tree or shrub cover; deforestation can therefore directly affect arboreal species of *Leptopelis*. Species of *Ptychadena* dwelling within or around forests (e.g., *P. neumanni* and *P. erlangeri*) need wet grassland habitats for feeding and reproduction. These species might not seem to be exposed to direct effects of deforestation; however, removal of trees and shrubs could indirectly affect nearby grassland habitats by drying streams and pools, changing local patterns of temperature and rainfall, altering diversity and abundance of invertebrate fauna (major prey items for amphibians), and/or facilitating intrusion of invasive species (competing for space or as predators on the frogs and their tadpoles or eggs).

Natural grasslands can be degraded for purposes aimed at agriculture, grazing area (for domestic animals), human settlement, and large scale industrial infrastructure. These and several other factors coupled with changes in local climatic conditions (rainfall, temperature and wind action) could cause soil erosion, changes in natural vegetation, invasion by exotic species, diseases, and ultimately change in the population levels of species and the biodiversity of an area. Many people consider frogs as nuisances in the environment, and most of the time ignore or avoid them. We doubt how many people, mainly in rural areas, might have the knowledge that frogs serve as biological controls for agricultural pest and disease-transmitting insects (e.g., Wanger et al., 2009). The Afro-tropical wetland system of Ethiopia comprises lakes, swamps and flood-plains in the highlands that are the major sources of water for lower areas. Wetland habitats are threatened by conversion of wetlands for intensive irrigation agriculture, the expansion of human settlement, industrial pollution, pesticides and fertilizers and water diversion for drainage and the construction of dams (Abebe and Geheb, 2003).

It has been complex and difficult to associate changes in global temperature and moisture with amphibian declines (Carey and Alexander 2003), and there are no such known correlations reported in Ethiopia. However, indirect effects have been addressed through studies conducted on changed runoff of major drainage systems such as the Blue Nile (Elshamy et al 2008) and Awash (Hailemariam, 1999) that originate in the highlands. Amphibians in the Ethiopian highlands are generally highly dependent on availability of moist habitats with almost constant temperature ranges (roughly 10-25°C, Fig. 4.2), and can thus be sensitive to global warming, as depicted in the current study with roughly 0.8-1.8°C increase over ten years.

The use of pesticides and toxic chemicals in the Ethiopian Highlands has been intensified through agricultural development, rural health extension, urbanization and industrialization programs. Some of the common practices observed in the past decades around aquatic or wetland habitats include, wide use of pesticides for agriculture, spraying DDT for malaria control, washing cars and spilling oil at rivers and lakes, disposal of untreated sewage into aquatic habitats, and the use of toxic powders of seeds of birbira (*Milletia ferruginea* (Hochst.) Baker) for fishing (Mengistu, 2004). These chemical contaminants are washed into streams or wetland habitats and can affect amphibians, their food, and the vegetation. While sprayed chemicals can affect breeding capacity of individuals (eggs, tadpoles or adults) and populations, oil spills and sewage can clog the skin and lungs/gills affecting respiration.

Another emerging cause currently discussed as a potential threat for global declines in populations of amphibians is the infectious disease chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (Rödder et al., 2009; Lötters et al., 2010; Kielgast et al., 2010). Studies conducted using predictive models that combine ecological niches, climate envelope and known occurrences of the fungus, indicated that the Ethiopian Highlands are expected to be one of the potentially most suitable sites to harbor *B. dendrobatidis*, implying that anuran amphibians in this area could be highly susceptible for the disease (Kielgast et al., 2010; Lötters et al., 2010). A recent assessment of Ethiopian amphibians in two geographic areas across the Rift Valley (Bale and Bonga areas) indicated that there is (and potentially could be) a high prevalence of this fungus in many species of frogs, including *Leptopelis* and *Ptychadena* (Gower et al., 2012). There is no reported case of mass mortality of amphibians attributed to chytridiomycosis in Ethiopia. However, chytrid-related declines of amphibian populations at higher elevations elsewhere in the tropics have been reported to be common (Alford et al., 2001; Young et al., 2001).

In general, USAID (2008) underlines on five broad categories linked to threats to Ethiopia's biodiversity, tropical forests, and resource base. These are: limited governmental, institutional, and legal capacity; population growth; land degradation; weak management of protected areas; and deforestation. The most recent large-scale transfer of vast areas of natural land to foreign investors for agricultural business has concerned local communities, researchers, environmentalists, and socio-economic experts (Rahmato, 2011), and such warnings have been addressed earlier (e.g., Abebe and Geheb, 2003). Most of these areas are found in the remaining pristine forest and grassland habitats of wildlife, including amphibians, in the southwest and western parts of the country. Any large-scale infrastructural development geared towards alleviating societal problems should incorporate balanced assessment of environmental impact and socio-economic driven needs. Only from the known amphibian diversity and distribution patterns, we can learn good lesson how ancient civilization and continued human-induced manipulation of natural habitats, coupled with climatic fluctuations, for thousands of years severely degraded faunal biodiversity in the northern and eastern parts of the Ethiopian Highlands.

As a whole, a combination of several factors acting together, rather than a single sole element, is the driving force for habitat degradation (Collins and Storfer, 2003). Prospective solutions and actions to protect and develop threatened biodiversity, therefore, require consideration of multi-directional alternatives with a multi-species (instead of single) approach.

2) Opportunities

Several opportunities exist for prospectively successful conservation of highland endemic amphibians of Ethiopia. The Ethiopian highlands are part of the Eastern Afromontane Biodiversity Hotspot, one of the 34 such places in the world (CI and McGinley 2009). According to the webpage of the Ethiopian Wildlife Conservation Authority (EWCA) (http://www.ewca.gov.et/), accessed on 21-06-2011, there are 52 protected wildlife areas operating as national parks, wildlife sanctuaries, wildlife reserves, controlled hunting areas, open hunting areas, and community conservation areas that are established to protect some of the well known and threatened biota in Ethiopia. EWCA is an overseer of the wildlife in the country, and is institutionally developing its capacity to implement effective conservation plans. There are also over 50 forest priority areas in Ethiopia that are being supplemented with broad-scale reforestation programs. The Institute of Biodiversity Conservation (Addis Ababa) is involved in recognizing, promoting and conserving Ethiopia's biological diversity (IBC, 2005). The Department of Life Sciences (Addis Ababa University, AAU) and several other universities and colleges educate young Ethiopians and conduct research on topics related to the value of the country's biodiversity. The Zoological Natural History Museum (ZNHM, AAU), as the only natural history museum in the country, holds only a very small amount of the diverse fauna found in the wild. Environment-related knowledge and issues are taught, monitored, controlled, developed, and publicized by various local bodies such as Environmental Protection Authority, Environmental Science Program of AAU, and Forum for Environment. Many other national and international non-governmental organizations operate in Ethiopia to save what is remaining of the living natural resources.

In particular, there are various recent research findings and ongoing works dealing with the diversity, distribution and conservation of different taxa of amphibians in Ethiopia. These efforts are an answer to the gap of knowledge we had until recently. Most of these projects focus on systematics and distribution of different species and genera in the country. Of particular interest are works on the genera *Phrynobatrachus* (Zimkus, 2008; Zimkus and Blackburn, 2008; Zimkus and Schick, 2010; Zimkus et al., 2010), *Xenopus* (Evans et al., 2011) the broad phylogeographic assessment of *Leptopelis* (Chapter 2) and *Ptychadena* (Chapter 3, and ongoing work by Xenia Freilich), and ongoing work of Simon Loader on some other taxa. Assessment of status of populations of a few highland endemic frogs (*Spinophrynoides osgoodi, Ericabatrachus baleensis, Balebreviceps hillmani, and Altiphrynoides malcolmi*) indicates that these species are threatened due to severely degraded habitats in the Bale Mountains (Gower et al., in press).

There are indigenous practices and knowledge that directly or indirectly contribute to conserve amphibians in Ethiopia. To our knowledge, amphibians are not used as sources of food in Ethiopia; in some places it is traditionally thought that killing a frog would bring bad luck. With regard to agriculture, enset cultivation, with thick undergrowth, is a potentially useful means to facilitate co-existence of humans and some arboreal species of *Leptopelis* as well as other ground-dwelling frogs (e.g., *Paracassina kounhiensis* and *Hemisus microscaphus*). In Indonesia, landscape-level cacao agroforestry with well maintained leaf litter has been found to be useful to support disturbance-tolerant species of amphibians (Wanger et al., 2009).

Well-kept grazing fields and healthy animal watering areas could also enable to keep grassland populations of *Leptopelis*, *Ptychadena* and other genera in good condition. For instance, at a stream at the type locality of *P. nana* in Dida'a plateau (Arsi), we have encountered other coexisting species of frogs on which leeches were attached (Fig. 4.7).

Fig. 4.7. Parasitic leech (unidentified species, Hirudinae) sucking blood from the vent area of Angola River Frog (*Amietia* cf. *angolensis*) (AM 110) co-existing with *Ptychadena nana*, Dida'a Plateau (Arsi-Robe). Photo credit: Abebe A. Mengistu 2010.

There are reports of predation and infestation of amphibians by leeches (Hirudinea, Annelida) in Europe and North America (Merilä and Sterner, 2002; Ayres and Comesaña, 2010; Stead, 2010; McCallum et al., 2011). Leeches are also known to predate on eggs and other developmental stages of amphibians (Romano and Cerbo, 2007), or serve as vectors to transmit amphibian pathogens (Raffel et al., 2006). There are other cases of helminth (McAllister et al., 2010) or blood parasites (Readel and Goldberg, 2010) of amphibians in Africa. Anti-helminthic treatment of a stream where a threatened population of an endemic species of *Ptychadena* lives (as in Arsi area of Ethiopia) can relieve both the frogs and domestic cattle from exo-parasitic infestation. However, experimental studies should precede such treatments to avoid unexpected deleterious effects on the population of amphibians and other aquatic fauna.

C) Mitigation

The aforementioned opportunities need be wisely exploited to better understand, share knowledge and apply feasible actions for amphibian conservation in Ethiopia; and the extent of the threats is plenty enough to justify the need for conservation.

There are scientific approaches used to prioritize species for conservation. For example, the status of populations of four monotypic endemic genera of amphibians (*Spinophrynoides osgoodi*, *Altiphrynoides malcolmi, Balebreviceps hillmani, Ericabatrachus baleensis*) in the Ethiopian Highlands has been assessed using quantitative records over decades (Gower et al., in press). Recent conservation strategies to protect the highly diverse amphibians of Madagascar include identifying priority areas for threatened species or overall species diversity, and including them in nature reserves (Ganzhorn et al., 1997). Virtually, protected areas have to be practically free of negative effects of synthetic chemicals, pollutants, global change, alien species and pathogens (Collins and Storfer, 2003). Defining protected areas or critical core habitat for adult amphibians does not suffice conservation plans (Porej et al., 2004), rather at least juvenile dispersal and habitat connectivity need to be included (Carr and Fahrig, 2001). Some species known as “gap species” are those residing outside protected areas, and need particular concern (Rodrigues et al., 2004). Cushman (2006) recommends species-specific characterization of habitats to evaluate the effects of habitat loss on amphibians.

Existence of extensive habitat degradation does not necessarily mean there is no hope for conservation of remaining populations. For instance, highly degraded habitats and deforestation have not yet resulted in clear extinctions of amphibian species in Madagascar (Andreone et al., 2005) and Costa Rica (Santos-Barrera et al., 2008). Persisting patches of forests could provide suitable habitats for some species (Bell and Donnelly, 2006). Artificial construction of ponds and wetlands also could enhance breeding for amphibians with affected original habitats (Cushman, 2006).

The fragmented distribution patterns of *Leptopelis* and *Ptychadena* in the Ethiopian highlands requires population-level and landscape-level conservation plans, incorporating the most threatened species as core targets. The use of multiple- rather than a single species of amphibian is recommended for meaningful understanding and usage of amphibians as biodiversity indicator (Sewell and Griffiths, 2009). The problems are complex, the geographic areas remote, and the inputs required for the solution immense. These are beyond the capacity of the country’s current level of economic and educational development. Therefore, mitigation of habitat degradation and other related problems of the crisis on amphibians and their habitat should involve a number of actors, including the government, NGOs, international donors, research institutions, or community-based organizations (USAID, 2008).

V) CONCLUSION

Many known or undescribed endemic highland species of *Leptopelis* (three out of seven) and *Ptychadena* (six out of nine) have a threatened status, and additional data are needed to check clearer trends and status of populations. The IUCN listing of the status of Ethiopian species of *Leptopelis* and *Ptychadena* requires revision following the revised taxonomic status and description of the newly revealed cryptic species (Chapters 2 and 3), and the results of the current assessment of geographic ranges, habitats and threats.

We suggest the elevation-based estimation of extent of occurrence, and its combined use with land cover and population data as a currently practical tool to understand status of species of amphibians in Ethiopia and prioritize them for conservation. Endemic *Leptopelis* and *Ptychadena* in the Ethiopian Highlands appear to have preference for a constant temperature range (10-25^{o}C). This demonstrates that climate change - increasing temperature (by about 0.8-1.8^{o}C in ten years) and locally reduced precipitation - resulting from natural patterns and/or human-induced factors could easily negatively affect these amphibians.

Community-level public awareness creation programs and improved basic education at grass root levels would be very helpful if the biology, values and conservation needs of threatened amphibian species are included in existing curricula. Broad-scale training at undergraduate and graduate level studies coupled with basic (taxonomy, biogeography, behavior, natural history) and problem-oriented research (conservation, utilization) on amphibians will improve local and regional knowledge. Communities should then be able to benefit from the values extracted through sustained development.

Ongoing decreases in species abundance and uncertain opportunities for recovery of extant species (McCallum, 2007) blight our hopes to save amphibians from extinction. It also seems less attention is given to amphibians than to other vertebrates in Ethiopia. Despite this, we are optimistic, me perhaps, to see improving concern and efforts in the near future. The preconditions are ethical: good will, responsible administration, professionalism, and love and respect for nature and future generations.

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REFERENCES

Abebe, Y.D. and Geheb, K. (eds.) (2003). Proceedings of a Seminar on the Resources and Status of Ethiopia's Wetlands, IUCN, vi + 116pp.

Alford, R.A., Dixon, P.M. and Pechmann, J.H.K. (2001). Global amphibian population declines. *Nature* 414: 449-500.

Andreone, F., Cadle, J.E., Cox, N., Glaw, F., Nussbaum, R.A., Raxworthy, C.J., Stuart, S.N., Vallan, D. and Vences, M. (2005). Species review of amphibian extinction risks in Madagascar: Conclusions from the Global Amphibian Assessment. *Conservation Biology* 19(6): 1790-1892.

Andreone, F., Carpenter, A.I., Cox, N., du Preez, L., Freeman, K., Furrer, S., Garcia, G., Glaw, F., Glos, J., Knox, D., Köhler, J., Mendelson III, J.R., Mercurio, V., Mittermeier, R.A., Moore, R.D., Rabibisoa, N.H.C., Randriamahazo, H., Randrianasolo, H., Raminosoa, N.R., Ramilijaona, O.R., Raxworthy, C.J., Vallan, D., Vences, M., Vieites, D.R. and Weldon, C. (2008). The Challenge of Conserving Amphibian Megadiversity in Madagascar. *PLoS Biol*. 6(5): e118. doi:10.1371/journal.pbio.0060118.

Ayres, C. and Comesaña, J. (2010). Leech prevalence in *Rana iberica* populations from northwestern Spain. *North-Western Journal of Zoology* 6(1): 118-121.

Behra, O. and Raxworthy, C.J. (1991). Exportations des amphibiens de Madagascar. Interet, danger et perspectives d'avenir. *Circalytes* 5: 45-49 (in French).

Bell, K.E. and Donnelly, M.A. (2006). Influence of forest fragmentation on community structure of frogs and lizards in Northeastern Costa Rica. *Conservation Biology* 20: 1750-1760.

Bowne, D.R. and Bowers, M.A. 2004. Interpatch movements in spatially structured populations: a literature review. *Landscape Ecology* 19(1): 1-20.

Brooks, T.M., Mittermeier, R.A., Mittermeier, C.G., da Fonsenca, G.A.B., Rylands, A.B., Konstant, W.R., Flick, P., Pilgrims, J., Oldfield, S., Magin, G. and Hilton-Taylor, C. (2002). Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology* 16: 1523-1739.

Carey, C. and Alexander, M.A. (2003). Climate change and amphibian declines: is there a link? *Diversity and Distributions* 9: 111-121.

Carr, L.W. and Fahrig, L. (2001). Effect of road traffic on two amphibian species of different vagility. *Conservation Biology* 15(4): 1071-1078.

CI (Conservation International, Content Partner) and McGinley, M. (Topic Editor) (2009). "Biological diversity in the Eastern Afromontane." In: Encyclopedia of Earth (Cleveland, C.J., ed. (Washington, D.C.: Environmental Information Coalition, National Council for Science and the Environment). [First published in the Encyclopedia of Earth October 13, 2008; Last revised June 25, 2009; Retrieved February 16, 2010].

CIESIN (2000). The Center for International Earth Science Information Network, Earth Institute, Columbia University. http://www.ciesin.org/. Accessed on 08 Aug. 2011.

Collins, J.P. and Storfer, A. (2003). Global amphibian declines: sorting the hypotheses. *Diversity and Distributions* 9: 89-98.

Cushman, S.A. 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation* 128: 231-240.

Elshamy, M.E., Seierstad, I.A. and Sorteberg, A. (2008). Impacts of climate change on Blue Nile flows using bias-corrected GCM scenarios. *Hydrol. Earth Syst. Sci. Discuss.* 5: 1407-1439.

Evans, B., Bliss, S.M., Mendel, S.A. and Tinsley, R.C. (2011). The Rift Valley is a major barrier to dispersal of African clawed frogs (*Xenopus*) in Ethiopia. *Molecular Ecology* 20(20): 4216-4230. DOI: 10.1111/j.1365-294X.2011.05262.x

Ganzhorn, J.U., Rakotosamimanana, B., Hannah, L., Hough, J., Iyer, L., Olivieri, S., Rajaobelina, S., Rodostrom, C. and Tilkin, G. (1997). Priorities for biodiversity conservation in Madagascar. *Primate Report* 48: 1-81.

Gascon, C., Collins, J. P., Moore, R. D., Church, D. R., McKay, J. E. and Mendelson, J. R. III (eds). (2007). Amphibian Conservation Action Plan. IUCN/SSC Amphibian Specialist Group. Gland, Switzerland and Cambridge, UK. 64p.

Goebel, W. and Odenyo, V. (1984). Ethiopia. Agroclimatic Resources Inventory for Land-use Planning. Ministry of Agriculture, Land Use Planning and Regulatory Department, UNDP, FAO. Technical Report DP/ETH/78/003, vol. 1, p. 208, vol. 2. P. 95.

Gower, D.J., Aberra, R.K., Schwaller, S., Largen, M.J., Collen, B., Spawls, S., Menegon, M., Zimkus, B.M, de Sá, R., Mengistu, A.A., Gebresenbet, F., Moore, R.D., Saber, S.A. and Loader, S.P. (in press). Long-term data for endemic frog genera reveal potential conservation crisis in the Bale Mountains, Ethiopia. *Oryx*.

Gower, D.J., Doherty-Bone, T.M., Aberra, R.K., Mengistu, A.A., Schwaller, S., Menegon, M., de Sá, R., Saber, S.A., Cunningham, A.A. and Loader, S.P. (2012). High prevalence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) across multiple taxa and localities in the highlands of Ethiopia. *Herpetological Journal* 22: 225–233, 2012.

Hailemariam, K. (1999). Impact of climate change on the water resources of Awash River Basin, Ethiopia. *Climate Research* 12: 91-96.

IBC (2005). National Biodiversity Strategy and Action Plan, Government of Ethiopia. Institute of Biodiversity Conservation. Addis Ababa, 103p.

IUCN (2011). IUCN Red List of Threatened Species. Version 2010.4. <www.iucnredlist.org>. Downloaded on 18 March 2011.

IUCN (2001). *IUCN Red List Categories and Criteria: Version 3.1.* IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK. ii + 30 p.

Lannoo, M.J., Lang, K., Waltz, T. and Phillips, G.S. (1994). An altered amphibian assemblage: Dickinson County, Iowa, 70 years after Frank Blanchard's survey. *American Midland Naturalist* 131: 311-319.

Lips, K.R. (1998). Decline of a tropical montane amphibian fauna. *Conservation Biology* 12: 106-112.

Madison, D.M. (1997). The immigration of radio-implanted Spotted Salamanders, Ambystoma maculatum. *Journal of Herpetology* 31: 542-551.

McCallum, M.L. (2007). Amphibian decline or extinction? Current declines dwarf background extinction rate. *Journal of Herpetology* 41(3): 483-491.

McAllister, C., Bursey, C.R. and Freed, P.S. (2010). Helminth parasites (Cestoidea, Nematoda, Pentastomida) of selected herpetofauna from Cameroon, West Africa. *Acta Parasitologica* 55(1): 90–93. DOI: 10.2478/s11686-010-0014-6

McCallum, M.L., Moser, W.E., Wheeler, B.A. and Trauth, S.E. (2011). Amphibian infestation and host size preference by the leech *Placobdella picta* (Verrill, 1872) (Hirudinida: Rhynchobdellida: Glossiphoniidae) from the Eastern Ozarks, USA. *Herpetology Notes* 4: 147-151 (published online on 19 April 2011).

Mengistu, A.A. (2004). The effect of Birbira, *Milletia ferruginea*, (Hochst.) Baker on some *Barbus* spp. in Gumara River (Lake Tana), Ethiopia. Unpublished MSc Thesis, School of Graduate Studies, Addis Ababa University. Addis Ababa.

Merilä, J. and Sterner, M. (2002). Medicinal leeches (*Hirudo medicinalis*) attacking and killing adult amphibians. *Ann. Zool. Fennici* 39: 343-346.

Nyseen, J., Vandenreyken, H., Poesen, J., Moeyersons, J., Deckers, J., Haile, M., Salles, C. and Govers, G. (2005). Rainfall erosivity and variability in the Northern Ethiopian Highlands. *Journal of Hydrology* 311: 172-187.

Osman, M. and Sauerborn, P. (2002). A preliminary assessment of characteristics and long-term variability of rainfall in Ethiopia – Basis for sustainable land use and resource management. Conference on International Agricultural Research for Development. Deutscher Tropentag, Witzenhausen, October 9-11, 2002.

Porej, D., Micacchion, M. and Hetherington, T.E. (2004). Core terrestrial habitat for conservation of local populations of salamanders and wood frogs in agricultural landscapes. *Biological Conservation* 120: 399-409.

Raffel, T.R., Dillard, J.R. and Hudson, P.J. (2006). Field evidence for leech-borne transmission of amphibian *Ichthyophonus* sp. *J. Parasitol.* 92(6): 1256-1264.

Rahmato, D. (2011). Land to Investors: Large-Scale Land Transfers in Ethiopia. Forum for Social Studies, Addis Ababa. 37p.

Randrianantoandro, C. and Hobinjatovo, T. (2011). New observations of Blanc's dwarf gecko (*Lygodactylus blancae* Pasteur, 1995) from the central highlands of Madagascar. *Herpetology Notes* 4: 233-237.

Readel, A.M. and Goldberg, T.L. (2010). Blood Parasites of Frogs From an Equatorial African Montane Forest in Western Uganda. *J. Parasitol.* 96(2): 448–450.

Regan, H.M., Lupia, R., Drinnan, A.N. and Burgman, M.A. (2001). The currency and tempo of extinction. *American Naturalist* 157: 1-10.

Rodrigues, A.S., Andelman, S.J., Bakarr, M.I., Boitani, L., Brooks, T.M., Cowling, R.M., Fishpool, L.D., Da Fonseca, G.A., Gaston, K.J., Hoffmann, M., Long, J.S., Marquet, P.A., Pilgrim, J.D., Pressey, R.L., Schipper, J., Sechrest, W., Stuart, S.N., Underhill, L.G., Waller, R.W., Watts, M.E. and Yan, X. (2004). Effectiveness of the global protected area network in representing species diversity. *Nature* 428: 640-642.

Romano, A. and Di Cerbo, A.R. (2007). Leech predation on amphibian eggs. *Acta Zoologica Sinica* 53(4): 750-754.

Rothermel, B.B. (2004). Migratory success of juveniles: a potential constraint on connectivity for pond-breeding amphibians. *Ecological Applications* 14(5): 1535-1546.

Santos-Barrera, G., Pacheco, J., Mendoza-Quijano, F., Bolaños, F., Cháves, G., Daily, G.C., Ehrlich, P.R. and Ceballos, G. (2008). Diversity, natural history and conservation of amphibians and reptiles from the San Vito Region, southwestern Costa Rica. *Int. J. Trop. Biol.* 56(2): 755-778.

Seleshi, Y. and Demarée, G.R. (1995). Rainfall variability in the Ethiopian and Eritrean highlands and its links with the Southern Oscillation Index. *Journal of Biogeography* 22: 945-952.

Sewell, D. and Griiffiths, R.A. (2009). Can a single species be a good biodiversity indicator? *Diversity* 1: 102-117.

Stead, J.E. (2010). Predatory leeches (Hirudinida) may contribute to amphibian declines in the Lassen region, California. *Northwestern Naturalist* 91: 30-39.

Stuart, S.N., Chanson J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L. and Walker, R.W. (2004). Status and trends of amphibian declines and extinction worldwide. *Science* 306: 1783-1786.

USAID (2008). Ethiopia Biodiversity and Tropical Forests, 118/119 Assessment. United States Agency for International Development. Addis Ababa. 88p.

Wanger, T.C., Saro, A., Iskandar, D.T., Brook, B.W., Sodhi, N.S., Clough, Y. and Tscharntke, T. (2009). Conservation value of cacao agroforestry for amphibians and reptiles in South-East Asia:

combining correlative models with follow-up field experiments. *Journal of Applied Ecology* 46: 823-832.

Yeshanew, A. and Apparo, G. (1989). Annual rain water potential and its variability in drought years over Ethiopia. In: Conference on Climate and Water. Helsinki, Finland. September 1989, vol. 1. Suomen Akatemian, Helsinki, pp. 219-235.

Young, B.E., Lips, K.R., Reaser, J.K., Ibáñez, R., Salas, A.W., Cedeño, J.R., Coloma, L.A., Ron, R., La Marca, E., Meyer, J.R., Muñoz, A., Bolaños, F., Chaves, G. and Romo, D. (2001). Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology* 15: 2013-2023.

Zimkus, B. (2008). *Phrynobatrachus bullans*: Geographic distribution (Tanzania, Kenya, and Ethiopia). *Herpet. Rev.* 39(2): 235.

Zimkus, B. and Blackburn, D.C. (2008). Distinguishing features of the sub-Saharan frog genera *Arthroleptis* and *Phrynobatrachus*: a short guide for field and museum researchers. *Breviora* 513: 1-12.

Zimkus, B. and Schick, S. (2010). Light at the end of the tunnel: insights into the molecular systematic of East African puddle frogs (Anura: Phrynobatrachidae). *System. Biodiv.* 8(1): 39-47.

Zimkus, B., Rodel, M-O. and Hillers, A. (2010). Complex patterns of continental speciation: Molecular phylogenetics and biogeography of sub-Saharan puddle frogs (*Phrynobatrachus*). *Molec. Phylogen. Evol.* 55: 883-900.

SUMMARY

Amphibian Diversity, Distribution and Conservation in the Ethiopian Highlands: Morphological, Molecular and Biogeographic Investigation on *Leptopelis* and *Ptychadena* (Anura).

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Little is known about the diversity, distribution and population status of Ethiopian amphibians in general, and most of the existing knowledge is based on field data recorded about three decades ago or earlier. There are almost no genetic data available for molecular systematics studies. Species of the Tree Frogs (*Leptopelis* Günther 1859) and Grassland Frogs (*Ptychadena* Boulenger 1917) are solely distributed in Africa (mainly sub-Saharan), with 52 and 53 known species, respectively. Six species of *Leptopelis* and thirteen species of *Ptychadena* were recorded in Ethiopia. Together these two genera comprise 30% of all known species of amphibians in Ethiopia. Many of these species (five *Leptopelis* and five *Ptychadena*) are said to be endemic to the Ethiopian Highlands that are part of the Eastern Afromontane Biodiversity Hotspot. Geographically, the Ethiopian Highlands are bisected by the Rift Valley, and further fragmented by valleys and gorges, and comprise the highest concentration of elevated ground in Africa. Earlier taxonomic studies were mainly based on morphological and ecological studies, making it difficult to identify most species in museum collections or during field work. In addition, limited sampling from the geographic areas of each species made it difficult to make good estimates of the ranges of species, and to prioritize them for conservation.

The main objectives of this study are, using *Leptopelis* and *Ptychadena* as model taxa, 1) to explore the diversity and phylogeography of some amphibians in the Ethiopian Highlands and parts of the Rift Valley; 2) to preliminarily explain the geo-climatic events that are associated with the evolutionary history of these taxa; and 3) to re-evaluate the conservation status of species and their habitats by associating phylogenetic, biogeographic and ecological information. We tested hypotheses pertaining to correspondence of traditional morphological taxonomy versus molecular phylogeny, biogeographic distinctness of distribution patterns, evolutionary history of diversification, and evaluation of conservation status of species and populations in the highlands.

The field sampling conducted between 2006 and 2010 from several localities across and within the Ethiopian Rift Valley was substantial, but not enough, to fully assess the phylogeography of the above genera. This assessment, conducted for the first time for

Ethiopian *Leptopelis* and *Ptychadena*, was done using partial mitochondrial DNA sequences of the 12S and 16S genes. Estimation of phylogenetic relationships and divergence times was made using Maximum Likelihood and Maximum Parsimony methods. Morphological observations were made on fresh collections, holotypes and non-type museum materials. Biogeographic patterns were assessed using geographic distribution data. Geographic and spatial data were combined with new phylogenetic groupings to assess geographic ranges and habitat status of populations and species. We followed a new approach of 'elevation-based' extent of occurrence (instead of the simple polygons applied in the IUCN Red List maps) to estimate geographic ranges. Results were compared and evaluated with previous reports and online database.

Despite the wide taxonomic variation between the studied genera (*Leptopelis* and *Ptychaena*), we found similarities in some of their major taxonomic problems, as well as evolutionary, biogeographic and conservation aspects. The similarities are in: overlaps in many morphological characters (conservatism) among different species; misleading variation in some physical features within a population/species (homoplasy); monophyly of highland endemic species; cryptic phylo-groups embedded within known clades; relatively low genetic distance between species and recent evolutionary divergence times; habitat status and threats for survival of populations.

In both genera, the reconstructed phylogenetic relationships showed that the highland endemic species form well-supported monophyletic groups: the 'Ethiopian Highland *Leptopelis* species group', and the '*Ptychadena cooperi* species group'. Cryptic diversity of highland endemics was revealed, highlighting the possibility of having six candidate species (at least two *Leptopelis* and four *Ptychadena*) awaiting description. Two presumed lowland endemic species of *Ptychadena* (*P. filwoha* and *P. harenna*) were genetically found to be conspecific with other widely distributed (non-endemic) lowland species (*P. mascareniensis* and *P. anchietae*, respectively).

Wide overlaps in several morphological features made identification of some closely related species populations difficult. New diagnostic features were identified to characterize some of the most difficult groups (e.g., presence of spicules in males of *P. neumanni* contrasted with absence in *P. erlangeri* or *P. nana*).

As compared with some other African species for which molecular data are available, relatively small evolutionary distances were found among the highland species within each genus, explained by a possibly very recent radiation as estimated in the corresponding divergence times. Diversification of the highland endemics was probably associated with formation of the Ethiopian Highlands by volcanic activities and uplifting, and accompanying climatic changes between 30 and 6 Mya.

We observed congruence of phylogenetic groups with clear patterns of geographic distribution, allowing us to identify distinct biogeographic categories that can potentially

serve as units for conservation of Ethiopian amphibians. The Rift Valley and major river gorges appear important horizontal barriers delimiting geographic ranges of most species. There is no considerable vertical (altitudinal) segregation of the highland populations in the two genera.

The highland *Leptopelis* and *Ptychadena* comprise more diverse endemic species (16) than known before (10), contributing substantially to the biodiversity value of the Ethiopian Highlands. While range extension is revealed for some species in the current study, there is unfortunately a substantial reduction for others from what has been proposed previously. Land cover data and our field observation of many parts of the country for about two decades indicate that large parts of the suitable highland habitats for most species of *Leptopelis* and *Ptychadena* are severely degraded and fragmented by human activities. Lakes are drying, levels of rivers are decreasing, and the wooded and grassland vegetation of the highlands and the Rift Valley is being lost. Our revised assessment of populations showed that nine of the 16 studied species appear to be threatened at different levels. This implies an urgent need for revision of existing conservation status of these taxa to protect them in rapidly changing environments. Further work is needed in the areas of phylogeography, taxonomy, and natural history of populations and species in areas that are not covered in this study.

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የዶክትሬት ዲግሪ መመረቂያ ጽሑፍ፣ የባዝል ዩኒቨርሲቲ፣ ስዊዘርላንድ፡፡ ባዝል፣ 2004 ዓ.ም. ኢሜይል፡ abbefish@yahoo.com

በአጠቃላይ ስለ ኢትዮጵያ እንቁራሪቶች ብዝሀ-ህይወት፣ ስርጭትና ስብጥር ሁኔታ የሚታወቀው ጥቂት ሲሆን፣ በአብዛኛው አሁን ያለው ግንዛቤ ከሠላሳ ዓመታትና ከዚያም በፊት በተመዘገቡ የመስክ መረጃዎች ላይ የተመሰረተ ነው፡፡ የሞለኪዩላር ስነ-ህይወታዊ ዝምድናና ክፍፍል ጥናት ለማድረግ የሚውሉ የዘረ-መል መረጃዎች ምንም የሉም፡፡ *ሌፕቶፔሊስ* የሚባሉት የዛፍ እንቁራሪቶችና *ቲካዲና* የሚባሉት የሣር እንቁራሪቶች ስርጭታቸው ሙሉ በሙሉ በአፍሪካ (በዋናነት ከሰሀራ በታች) ሲሆን፣ እያንዳንዱ ምድብ በቅደም ተከተል 52 እና 53 የሚታወቁ ዝርያዎች አሉት፡፡ በኢትዮጵያ ውስጥ ስድስት የ*ሌፕቶፔሊስ* እና አስራ ሶስት የ*ቲካዲና* ዝርያዎች ተመዝግበዋል፡፡ እነዚህ ሁለት ምድቦች በአንድነት ሆነው ከሀገሪቱ እንቁራሪት ዝርያዎች 30 ከመቶ ይሸፍናሉ፡፡ ከእነዚህም ውስጥ ብዙዎቹ (አምስት የ*ሌፕቶፔሊስ* እና አምስት የ*ቲካዲና*) ዝርያዎች የምሥራቃዊ አፍሪካ አፍሮሞንቴን የብዝሀ-ህይወት ዋነኛ ማዕከል አንድ አካል በሆኑት በኢትዮጵያ ከፍታማ ቦታዎች ብቻ የሚገኙ የዚያ-ብቻ ናቸው፡፡ ከመልክአ-ምድር አንፃር የኢትዮጵያ ከፍታማ ቦታዎች በስምጥ ሸለቆ ለሁለት የተገመሱና ብሎም በሌሎች ዋና ዋና ሸለቆዎች የተፈረካከሱ ሲሆን፣ ከአፍሪካ አብላጫውን የከፍታማ ቦታዎች ክምችት ይዘዋል፡፡ ቀደም ያሉ የስነ-ዝርያ አወቃቀር ጥናቶች በዋናነት መሰረት ያደረጉት የውጫዊ ገፀ-አካላትና ስነ-አካባቢ ጥናት ላይ ስለነበር፣ አብዛኞቹን በቤተ-መዘክር የተከማቹና ከመስክ የሚሰበሰቡ አዳዲስ ናሙናዎችን ዝርያዎች መለየት አስቸጋሪ አድርጎታል፡፡ በተጨማሪም ከእያንዳንዱ ዝርያ መልክአ-ምድራዊ ይዞታ ውስን ናሙናዎች ብቻ መሰብሰባቸው የዝርያዎችን ስርጭት በአግባቡ ለማስላትና የእንክብካቤ አስፈላጊነትን ለመመዘን አስቸጋሪ አድርጎታል፡፡

የዚህ ጥናት ዋና ዋና ዓላማዎች፣ *ሌፕቶፔሊስ* እና *ቲካዲና* እንቁራሪቶችን በማሳያነት በመጠቀም፣ 1) በኢትዮጵያ ከፍታማ ቦታዎችና በስምጥ ሸለቆ ውስጥ የሚገኙ አንዳንድ እንቁራሪቶችን ብዝሀ-ህይወት፣ የዘረ-መል ትስስርና መልክአ-ምድራዊ ይዞታ መፈተሽ፤ 2) ከእነዚህ ዝርያዎች የአዝጋሚ-ለውጥ ታሪክ ጋር ቁርኝት ያላቸውን መልክአ-ምድራዊና የአየር ንብረት ክስተቶች ለማብራራት፤ እንዲሁም 3) የዘረ-መል ትስስር፣ ህይወታዊ-መልክአ-ምድርና ስነ-ምህዳራዊ መረጃዎችን አጣምሮ የዝርያዎችንና የምቹ መኖሪያ ቦታዎቻቸውን የእንክብካቤ ሁኔታ እንደገና ለመመዘን ነው፡፡ በዚህ ጥናት የተፈተሹት መላ ምቶች የሚያተኩሩት የተለመደው በውጫዊ ገፀ-አካል መረጃ ላይ የተመሰረተ የስነ-ዝርያ አወቃቀር ከሞለኪዩላር ስነ-ህይወታዊ ዝምድናና ክፍፍል ጋር ያለውን ተስማሚነት፣ የስርጭት ሥርዓቶችን ህይወታዊ-መልክአ-ምድር ግልፅነት፣ በዝርያዎች አበዛዝ አዝጋሚ-ለውጥ ታሪክ፣ እና የከፍታማ ቦታዎቹን የዝርያዎችና ስብስቦች የእንክብካቤ ሁኔታ በመመዘን ዙሪያ ነው፡፡

በበርካታ የኢትዮጵያ ከፍታማ ቦታዎችና በስምጥ ሸለቆ ውስጥ ከ1998 ዓ.ም. እስከ 2002 ዓ.ም. ድረስ የተከናወነው ከመስክ ናሙና የማሰባሰብ ሥራ ከላይ የተጠቀሱትን ዝርያዎች የዘረ-መል ትስስርና መልክአ-ምድራዊ ይዞታ ሙሉ በሙሉ ለመፈተሽ የሚያስችል ነበር፣ ነገር ግን ያለቀለት አይደለም፡፡ ይህ በኢትዮጵያ *ሌፕቶፔሊስ* እና *ቲካዲና* የእንቁራሪት ዝርያዎች ላይ ለመጀመሪያ ጊዜ የተደረገ ጥናት የተከናወነው 12ኤስ እና 16ኤስ የሚባሉ ከፊል የማይቶኮንድሪያል ዲ.ኤን.ኤ. ድርድሮችን በመጠቀም ነው፡፡ የዘረ-መል ትስስር እና የዝርያዎች ክፍፍል ጊዜ ስሌት የተሠራው ማክሲመም ላይክሊሁድ እና ማክሲመም ፓርሲሞኒ ዘዴዎችን በመጠቀም ነው፡፡ የውጫዊ አካል ክፍሎች ጥናት የተካሄደው በአዳዲስ ናሙናዎች፣ በዓይነቴዎችና ሌሎችም የቤተ-መዘክር ስብስቦች ላይ ነው፡፡ የስነ-ህይወታዊ-መልክአ-ምድር ሥርዓት ዳሰሳ የተሠራው የመልክአ-ምድራዊ ሥርጭት መረጃ በመጠቀም ነው፡፡ የመልክአ-ምድራዊና ተዛማጅ የህዋ-ምስላዊ መረጃዎችን ከአዳዲስ የዘረ-መል ምድቦች ጋር በማጣመር የዝርያዎችን መልክአ-ምድራዊ ይዞታና ተስማሚ መኖሪያ ቦታዎች ማጥናት ተችሏል፡፡ ይህንንም ለማድረግ ዓለም-አቀፍ የተፈጥሮ እንክብካቤ ኅብረት (IUCN) ከሚጠቀምበት ለመጥፋት የተቃረቡ ዝርያዎች ግርድፍ የካርታ ሥራ ዘዴ ይልቅ የየቦታዎቹን መልክአ-ምድራዊ ከፍታ

መጠን መሰረት ያደረገ የመገኛ ቦታ መጠን ስሌትን በአዲስ መልክ አቅርበናል፡፡ የተገኙት ውጤቶችም ከቀደምት ዘገባዎችና የድረ-ገፅ ዝርዝሮች ጋር ተገምግመው ተገናዝበዋል፡፡

በዚህ ጥናት የተዳሰሱት (*ሌፕቶፔሊስ* እና *ቲካዲና*) በስፋት የተለያየ የስነ-ዝርያ አወቃቀር ቢኖራቸውም፣ በዐበይት የስነ-ዝርያ አወቃቀር ችግሮቻቸው፣ እንዲሁም የአዝጋሚ-ለውጥ፣ ስነ-ህይወታዊ-መልክአ-ምድር እና እንክብካቤ ሁኔታዎች ተመሳሳይነት አላቸው፡፡ ተመሳሳይነታቸውም፡- በየዝርያዎቹ መካከል ባለው የበርካታ ገፀ-አካላት ልኬት መጠን መመሳሰል፣ በየዝርያዎቹ አንዳንድ ገፀ-አካላት ላይ ያለው አሳሳች ልዩነት፣ በከፍታማ ቦታዎች የሚገኙት የዚያ-ብቻ ዝርያዎች ከአንድ የጋራ ጥንታዊ የዘር ግንድ መገኘት፣ የድብቅ ዝርያዎች በሚታወቁ የዘር ቅርንጫፎች ውስጥ መወሸቅ፣ በየዝርያዎቹ መካከል ያለው አንፃራዊ ዝቅተኛ የዘረ-መል ልዩነትና ቅርብ የሆነ የአዝጋሚ-ለውጥ ክፍፍል ጊዜ፣ የመኖሪያ አካባቢ ሁኔታና ለእንስሳቱ ክምችት ህልውና አስጊ የሆኑት ነገሮች ናቸው፡፡

በሁለቱም ክፍለ-ምድብ በኩል፣ የተዘጋጁት የዘረ-መል ትስስሮች ያመለከቱት የከፍታማ ቦታዎች የዚያ-ብቻ ዝርያዎች በደንብ የተደገፈ አንድ የጋራ የዘር ሀረግ እንዳላቸው ነው፤ እነዚህም የኢትዮጵያ ከፍታማ ቦታዎች *ሌፕቶፔሊስ* ዝርያ ምድብ እና የ*ቲካዲና ኩፐሪ* ዝርያ ምድብ ናቸው፡፡ በከፍታማ ቦታዎች የሚገኙ የዚያ-ብቻ ድብቅ ብዝሀ-ህይወት ተገልጦ መታየት በዝርዝር መገለፅ ያለባቸው ስድስት (ቢያንስ ሁለት የ*ሌፕቶፔሊስ* እና አራት የ*ቲካዲና*) እጩ ዝርያዎች ሊኖሩ እንደሚችሉ አመላክቷል፡፡ ከዚህ ቀደም ራሳቸውን ችለው የተፈረጁ ሁለት በዝቅተኛ ቦታዎች ብቻ የሚገኙ ዝርያዎች (*ቲካዲና ፍልውሀ* እና *ቲካዲና ሀረና*) ከሌሎች በስፋት ከሚገኙ (የዚያ-ብቻ ያልሆኑ) የዝቅተኛ ቦታዎች ዝርያዎች (በቅደም ተከተል *ቲካዲና ማስካረኒየንሲስ* እና *ቲካዲና አንኪየቴ*) ጋር በዘረ-መል አወቃቀራቸው አምሳያዎች ሆነው ተገኝተዋል፡፡

የቅርብ ትስስር ባለቸው ዝርያዎች መካከል የሚታየው የበርካታ ገፀ-አካላት ልኬት መጠን በስፋት መመሳሰል ዝርያዎቹን የመለየት ሥራን አስቸጋሪ አድርጎታል፡፡ አንዳንድ ለመለየት እጅግ በጣም አስቸጋሪ ምድቦችን መግለጫ የሚሆኑ አዳዲስ መለያ ገፅታዎች ተገኝተዋል፤ ለምሳሌ፡- የጥቃቅን አሸዋ-መሰል መለያ የአካል ክፍሎች በወንድ *ቲካዲና ኒውማኒ* ዝርያ ላይ መኖርና በአንፃሩ በወንድ *ቲካዲና ኢርላንገሪ* ወይም *ቲካዲና ናና* ዝርያዎች ላይ አለመኖር ይጠቀሳል፡፡

የዘረ-መል መረጃ ከተሰበሰበላቸው የተወሰኑ ሌሎች ተዛማጅ የአፍሪካ ዝርያዎች ጋር ሲወዳደሩ፣ በእያንዳንዱ ክፍለ-ምድብ ውስጥ ባሉት የኢትዮጵያ ከፍታማ ቦታዎች ዝርያዎች መካከል አንፃራዊ አነስተኛ የአዝጋሚ ለውጥ ልዩነት ተገኝቷል፤ ይህም ሊብራራ የሚችለው ከቅርብ ጊዜ በፊት በተከሰተ የዝርያዎች ዓይነት መስፋፋት ነው፡፡ የከፍታማ ቦታዎች ብቻ የሆኑ ዝርያዎች መስፋፋት ከ30-6 ሚሊዮን ዓመታት በፊት በእሳተ-ገሞራና በምድር ወደላይ የመነሳት ሂደት ከኢትዮጵያ ከፍታማ ቦታዎች መመስረት ጋር እና ከተዛማጅ የአየር ንብረት ለውጥ ጋር ግንኙነት እንደነበረው ይገመታል፡፡

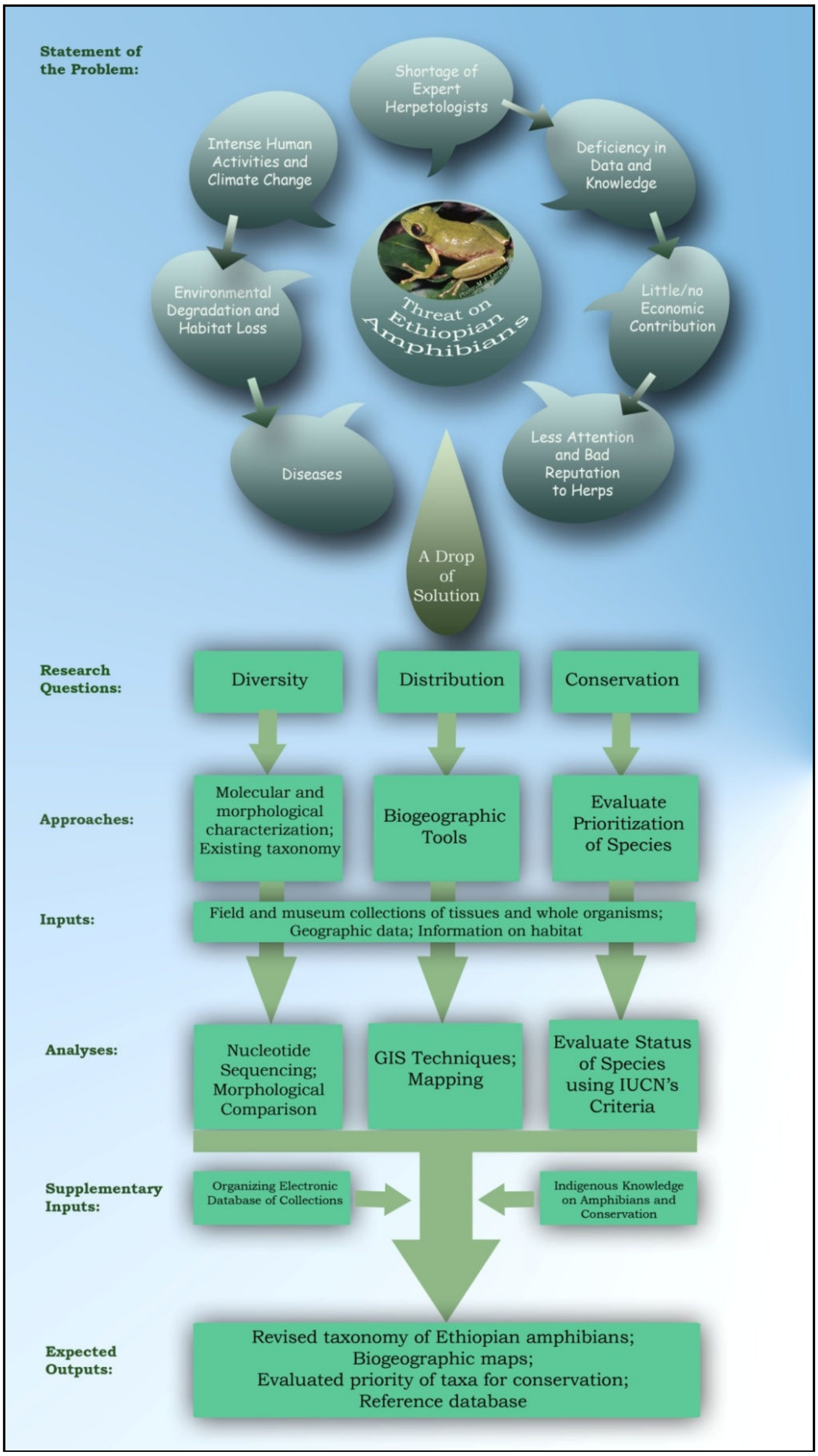
የዘረ-መል ምድቦች ከመልክአ-ምድራዊ ስርጭታቸው የጠራ አወቃቀር ጋር የሚገጣጠም መሆኑ፣ የኢትዮጵያን እንቁራሪት ዝርያዎች ለመንከባከብ ሊያገለግሉ የሚችሉ የህይወታዊ-መልክአ-ምድር ክፍፍሎችን ለመለየት አስችሏል፡፡ የአብዛኞዎቹን ዝርያዎች መልክአ-ምድራዊ ይዞታ የሚወስኑት የአግድሞሽ ማገጃዎች የስምጥ ሸለቆና ዐበይት የወንዝ ሸለቆዎች ናቸው፡፡ በሁለቱ የከፍታማ ቦታዎች ክፍለ-ምድብ ውስጥ በመልክአ-ምድራዊ ከፍታ ላይ የተመሰረተ ክፍፍል ጎልቶ አይታይም፡፡

የከፍታማ ቦታዎቹ *ሌፕቶፔሊስ* እና *ቲካዲና* ከዚህ በፊት ይታወቅ ከነበረው (10) የበለጠ ሰፊ (16) የኢትዮጵያ-ብቻ ዝርያዎች ሲኖሯቸው፣ ይህም የኢትዮጵያ ከፍታማ ቦታዎች ላላቸው የብዝሀ-ህይወት ዋጋ በእጅጉ አስተዋፅዖ አለው፡፡ በአሁኑ ጥናት በአንዳንድ ዝርያዎች በኩል የመገኛ ቦታ መስፋፋት የታየ ሲሆን፣ በሌሎች በኩል ደግሞ በፊት ከሚታወቀው የጎላ መቀነስ ታይቷል፡፡ የመሬት ሽፋን መረጃና በሀገሪቱ በርካታ ክፍሎች ለሀያ ዓመታት ያደረግነው የመስክ ምልከታ እንደሚያሳየው፣ በበርካታ ከፍታማ ቦታዎች የሚገኙ የአብዛኞዎቹ የ*ሌፕቶፔሊስ* እና *ቲካዲና* ዝርያዎች ምቹ የመኖሪያ ቦታዎች በሰው-ሰራሽ እንቅስቃሴዎች ምክንያት በአስከፊ ሁኔታ እየተመናመኑና እየተበጣጠሱ ናቸው፡፡ ሀይቆች በመድረቅ ላይ ናቸው፤ የወንዞች መጠነ-ልክ እየቀነሰ ነው፤ እንዲሁም የከፍታማ ቦታዎችና የስምጥ ሸለቆ የዛፍና የሣር ሽፋን በመጥፋት ላይ ነው፡፡ አሁን የተደረገው የዝርያዎች እንክብካቤ ሁኔታ ዳሰሳ እንደሚያሳየው በጥናቱ ከተገለፁት 16 ዝርያዎች ውስጥ ዘጠኙ በተለያየ ደረጃ ለመጥፋት በአስጊ ሁኔታ ውስጥ ያሉ ናቸው፡፡ ይህም የሚያመለክተው አሁን ያለውን የዝርያዎች እንክብካቤ ሁኔታ በመከለስ በፍጥነት በሚለዋወጡ አካባቢዎች ውስጥ ጥበቃ ማድረግ በአስቸኳይ እንደሚያስፈልግ ነው፡፡ በዚህ ጥናት ውስጥ ባልተካተቱ አካባቢዎች በስነ-ዘረመል-ትስስርና መልክአ-ምድር፣ ስነ-ዝርያ አወቃቀር እና በዝርያዎች የተፈጥሮ ታሪክ ላይ ቀጣይ ሥራ ያስፈልጋል፡፡

Summary in Pictures

APPENDIXES

Appendix 1.1. Flowchart summarizing the current research questions and possible answers outlined in the project concept on amphibian diversity, distribution and conservation in Ethiopia (extracted from poster presented in Luxembourg in 2008 (Mengistu et al., 2008).



Statement of the Problem:

Shortage of Expert Herpetologists

Deficiency in Data and Knowledge

Little/no Economic Contribution

Less Attention and Bad Reputation to Herps

Diseases

Environmental Degradation and Habitat Loss

Intense Human Activities and Climate Change

Threat on Ethiopian Amphibians

A Drop of Solution

Research Questions: Diversity; Distribution; Conservation

Approaches: Molecular and morphological characterization; Existing taxonomy; Biogeographic Tools; Evaluate Prioritization of Species

Inputs: Field and museum collections of tissues and whole organisms; Geographic data; Information on habitat

Analyses: Nucleotide Sequencing; Morphological Comparison; GIS Techniques; Mapping; Evaluate Status of Species using IUCN's Criteria

Supplementary Inputs: Organizing Electronic Database of Collections; Indigenous Knowledge on Amphibians and Conservation

Expected Outputs:

Revised taxonomy of Ethiopian amphibians;
Biogeographic maps;
Evaluated priority of taxa for conservation;
Reference database

Appendix 2.1. List of Ethiopian specimens and localities used to assess molecular phylogeny of *Leptopelis* in the current study.

DNA tag no. Univ. of Basel	Voucher Field no. (ZNHM-AAU no.)	Phylogenetic grouping	Category of Highland Segment	Name of locality	Latitude (N)	Longitude (E)	Altitude (m)	GenBank Accession no. (12S; 16S)
T827	MW-6344	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Katcha, Bale	6.7156	39.7239	2371	JX464970; JX464901
T907	SL-013	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Rira, Bale	6.7591	39.7214	2811	JX464973; JX464904
T909	SL-023	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Rira, Bale	6.7591	39.7214	2811	JX464974; JX464905
T917	SL-038	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Harenna, Bale	6.7162	39.7255	2364	JX464976; JX464907
T921	SL-050	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Katcha, Bale	6.7156	39.7239	2371	JX464977; JX464908
T923	SL-048	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Harenna, Bale	6.7559	39.7263	2813	JX464978; JX464909
T931	SL-082	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Harenna, Bale	6.7530	39.7190	2788	JX464980; JX464911
T938	SL-096	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Rira, Bale	6.7713	39.7245	2922	JX464982; JX464913
T939	SL-098	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Rira, Bale	6.7713	39.7245	2922	JX464983; JX464914
T941	SL-102	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Rira, Bale	6.7713	39.7245	2922	JX464985; JX464916
T943	SL-106	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Rira, Bale	6.7713	39.7245	2922	JX464986; JX464917
T944	SL-108	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Rira, Bale	6.7713	39.7245	2922	JX464987; JX464918
T1010	Z-37666 (A2008-090)	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	Southern	Bore, Sidamo	6.3338	38.6464	2628	JX464993; JX464924
T1011	Z-37667 (A2008-091)	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	Southern	Bore, Sidamo	6.3338	38.6464	2628	JX464994; JX464925
T1012	Z-37668 (A2008-092)	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	Southern	Bore, Sidamo	6.3338	38.6464	2628	JX464995; JX464926
T1099	AK-2065	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Shawe R., Bale	6.6386	39.7339	1907	JX465018; JX464949
T1100	AK-2077	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Shawe R., Bale	6.6386	39.7339	1907	JX465019; JX464950
T1196	SL-172	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Wendo Genet	7.0987	38.6404	1966	JX465020; JX464951
T1197	SL-174	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Wendo Genet	7.0987	38.6404	1966	JX465021; JX464952
T1207	AK-1984	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Rira, Bale	6.7591	39.7214	2811	JX465024; JX464955
T1212	AK-2015	*L.* cf. *ragazzii* (Arsi-Bale-Bore)	South-central	Katcha, Bale	6.7155	39.7242	2373	JX465027; JX464958
T815	MW-6301	*L. gramineus* (Arsi- Bale-Bore)		(Unknown?)	(Unknown?)	(Unknown?)	(Unknown?)	JX464968; JX464899
T821	MW-6322	*L. gramineus* (Arsi- Bale-Bore)	South-central	Rira, Bale	6.7591	39.7214	2811	JX464969; JX464900
T831	MW-6356	*L. gramineus* (Arsi- Bale-Bore)	South-central	Dinshu, Bale	(Unknown?)	(Unknown?)	(Unknown?)	JX464971; JX464902
T996	Z-37626 (A2008-050)	*L. gramineus* (Arsi- Bale-Bore)	Southern	Bore, Sidamo	6.3895	38.6145	2594	JX464988; JX464919
T997	Z-37627 (A2008-051)	*L. gramineus* (Arsi- Bale-Bore)	Southern	Bore, Sidamo	6.3895	38.6145	2594	JX464989; JX464920
T998	Z-37629 (A2008-053)	*L. gramineus* (Arsi- Bale-Bore)	Southern	Bore, Sidamo	6.3692	38.6060	2672	JX464990; JX464921
T1002	Z-37650 (A2008-074)	*L. gramineus* (Arsi- Bale-Bore)	Southern	Bore, Sidamo	6.0899	38.7712	2203	JX464991; JX464922
T1009	Z-37662 (A2008-086)	*L. gramineus* (Arsi- Bale-Bore)	Southern	Bore, Sidamo	6.2333	38.7082	2555	JX464992; JX464923
T1279	AM-102	*L. gramineus* (Arsi- Bale-Bore)	South-central	Dida'a town, Arsi	7.8689	39.6272	2444	JX465033; JX464964
T905	SL-011	*L. gramineus* (Bale)	South-central	Rira, Bale	6.7591	39.7214	2811	JX464972; JX464903

DNA tag no. Univ. of Basel	**Voucher Field no. (ZNHM-AAU no.)**	**Phylogenetic grouping**	**Category of Highland Segment**	**Name of locality**	**Latitude (N)**	**Longitude (E)**	**Altitude (m)**	**GenBank Accession no. (12S; 16S)**
T910	SL-025	*L. gramineus* (Bale)	South-central	Rira, Bale	6.7591	39.7214	2811	JX464975; JX464906
T927	SL-066	*L. gramineus* (Bale)	South-central	Rira, Bale	6.7713	39.7245	2922	JX464979; JX464910
T937	SL-094	*L. gramineus* (Bale)	South-central	Rira, Bale	6.7713	39.7245	2922	JX464981; JX464912
T940	SL-100	*L. gramineus* (Bale)	South-central	Rira, Bale	6.7713	39.7245	2922	JX464984; JX464915
T1210	AK-2007	*L. gramineus* (Bale)	South-central	Katcha, Bale	6.7155	39.7242	2373	JX465025; JX464956
T1211	AK-2009	*L. gramineus* (Bale)	South-central	Katcha, Bale	6.7155	39.7242	2373	JX465026; JX464957
T1280	AM-125	*L. gramineus* (Shoa)	Central	Hora village, Shoa	8.7858	37.8620	3081	JX465034; JX464965
T1281	AM-126	*L. gramineus* (Shoa)	Central	Hora village, Shoa	8.7858	37.8620	3081	JX465035; JX464966
T1282	AM-127	*L. gramineus* (Shoa)	Central	Hora village, Shoa	8.7858	37.8620	3081	JX465036; JX464967
T1071	SL-343	*L. susanae* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2138	37.5850	2270	JX465000; JX464931
T1199	SL-349	*L. susanae* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2138	37.5850	2270	JX465022; JX464953
T1202	AK-1854	*L. susanae* (Gughe-Kaffa)	Southwestern	Saja, Kaffa	7.5019	36.0918	1956	JX465023; JX464954
T1067	SL-330	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2031	37.5605	2515	JX464996; JX464927
T1068	SL-332	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2031	37.5605	2515	JX464997; JX464928
T1069	SL-339	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2138	37.5850	2270	JX464998; JX464929
T1070	SL-341	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2138	37.5850	2270	JX464999; JX464930
T1072	SL-345	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2138	37.5850	2270	JX465001; JX464932
T1073	SL-367	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2138	37.5850	2270	JX465002; JX464933
T1074	SL-369	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2138	37.5850	2270	JX465003; JX464934
T1075	SL-371	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2138	37.5850	2270	JX465004; JX464935
T1077	SL-375	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2138	37.5850	2270	JX465005; JX464936
T1078	SL-377	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2138	37.5850	2270	JX465006; JX464937
T1079	SL-379	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2138	37.5850	2270	JX465007; JX464938
T1080	SL-381	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2138	37.5850	2270	JX465008; JX464939
T1081	SL-383	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2138	37.5850	2270	JX465009; JX464940
T1082	SL-385	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Gughe Mountain	6.2138	37.5850	2270	JX465010; JX464941
T1089	AK-1766	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Mankira, Kaffa	7.1956	36.2844	2647	JX465011; JX464942
T1090	AK-1774	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Mankira, Kaffa	7.1956	36.2844	2647	JX465012; JX464943
T1092	AK-1792	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Koma, Kaffa	7.3180	36.0782	1889	JX465013; JX464944
T1093	AK-1794	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Koma, Kaffa	7.3180	36.0782	1889	JX465014; JX464945
T1096	AK-1839	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Saja, Kaffa	7.5019	36.0918	1956	JX465015; JX464946
T1097	AK-1844	*L. vannutellii* (Gughe-Kaffa)	Southwestern	Saja, Kaffa	7.5019	36.0918	1956	JX465016; JX464947
T1274	AM-021	*L. yaldeni* (Gojam)	Northwestern	Debre Markos	10.3211	37.7291	2405	JX465028; JX464959
T1275	AM-022	*L. yaldeni* (Gojam)	Northwestern	Debre Markos	10.3211	37.7291	2405	JX465029; JX464960

DNA tag no. Univ. of Basel	Voucher Field no. (ZNHM-AAU no.)	Phylogenetic grouping	Category of Highland Segment	Name of locality	Latitude (N)	Longitude (E)	Altitude (m)	GenBank Accession no. (12S; 16S)
T1276	AM-023	*L. yaldeni* (Gojam)	Northwestern	Debre Markos	10.3211	37.7291	2405	JX465030; JX464961
T1277	AM-024	*L. yaldeni* (Gojam)	Northwestern	Debre Markos	10.3211	37.7291	2405	JX465031; JX464962
T1098	AK-1850	*Leptopelis* sp. (Saja)	Southwestern	Saja, Kaffa	7.5019	36.0918	1956	JX465017; JX464948
T1278	AM-050	*Leptopelis* sp. (Soddo)	Northwestern	Soddo, Wolayita	6.8319	37.7184	1839	JX465032; JX464963

Appendix 2.2. Descriptive summary (top six rows) and student's t-test results (bottom eleven rows) for pairwise comparison of means of ratios of nine morphometric characters assessed in the current study for *Leptopelis* of Ethiopia. The statistical test does not include *Leptopelis* sp. (Saja) because of only one sample size. The type of character is shown on the top-left corner of each table. p-values in bold fonts represent significantly different pairs of means at a significance level of 0.05.

A) Head width

HW:SVL	*gramineus* Arsi-Bale-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
n	9	14	3	6	37	7	1	20	57	25
mean	**38**	**38**	**42**	**36**	**38**	**38**	**40**	**37**	**39**	**38**
min	34	35	38	34	33	36	40	35	35	34
max	41	42	45	37	43	40	40	41	45	44
sd	2.2	2.0	4.0	1.3	2.4	1.6	N/A	1.6	2.0	2.4
t-test (p-values)	*gramineus* Arsi-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
gramineus Arsi-Bale-Bore	1.0000									
gramineus Bale	0.7591	1.0000								
gramineus Gamo Gofa	0.1962	0.2199	1.0000							
gramineus Shoa	0.1159	**0.0323**	0.1215	1.0000						
ragazzii East	0.9818	0.6974	0.2024	**0.0410**	1.0000					
ragazzii West	0.4747	0.6182	0.2551	**0.0192**	0.3683	1.0000				
sp. Saja	N/A	N/A	N/A	N/A	N/A	N/A	N/A			
susanae	0.6220	0.2988	0.1756	0.1198	0.4302	0.1465	N/A	1.0000		
vannutellii	**0.0437**	**0.0219**	0.3881	**0.0007**	**0.0005**	0.1354	N/A	**0.0000**	1.0000	
yaldeni	0.5722	0.7612	0.2390	**0.0142**	0.4436	0.8115	N/A	0.1423	**0.0279**	1.0000

B) Femur length

FmL:SVL	*gramineus* Arsi-Bale-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
n	9	14	3	6	37	7	1	20	57	25
mean	**42**	**42**	**46**	**39**	**49**	**50**	**54**	**49**	**50**	**50**
min	39	35	44	34	38	46	54	45	31	43
max	49	48	50	44	55	53	54	52	58	58
sd	3.1	4.3	3.6	3.9	4.0	2.3	N/A	1.7	4.4	3.5
t-test (p-values)	*gramineus* Arsi-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
gramineus Arsi-Bale-Bore	1.0000									
gramineus Bale	0.8728	1.0000								
gramineus Gamo Gofa	0.1437	0.1272	1.0000							
gramineus Shoa	0.1392	0.1812	**0.0389**	1.0000						
ragazzii East	**0.0000**	**0.0000**	0.3256	**0.0006**	1.0000					
ragazzii West	**0.0000**	**0.0000**	0.1967	**0.0003**	0.3065	1.0000				
sp. Saja	N/A	N/A	N/A	N/A	N/A	N/A	N/A			
susanae	**0.0001**	**0.0000**	0.3603	**0.0011**	0.7623	0.1772	N/A	1.0000		
vannutellii	**0.0000**	**0.0000**	0.2015	**0.0004**	0.1995	0.9882	N/A	0.0506	1.0000	
yaldeni	**0.0000**	**0.0000**	0.2554	**0.0004**	0.5686	0.5974	N/A	0.3303	0.5230	1.0000

C) Tibia length

TbL:SVL	*gramineus* Arsi-Bale-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
n	9	14	3	6	37	7	1	20	57	25
mean	**38**	**38**	**41**	**35**	**48**	**47**	**53**	**46**	**50**	**48**
min	33	29	39	33	39	43	53	41	38	45
max	41	45	42	37	56	50	53	50	55	55
sd	2.4	4.0	1.5	1.6	3.4	2.1	N/A	2.5	3.1	2.2
t-test (p-values)	*gramineus* Arsi-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
gramineus Arsi-Bale-Bore	1.0000									
gramineus Bale	0.7750	1.0000								
gramineus Gamo Gofa	**0.0430**	0.0798	1.0000							
gramineus Shoa	**0.0403**	**0.0397**	**0.0063**	1.0000						
ragazzii East	**0.0000**	**0.0000**	**0.0024**	**0.0000**	1.0000					
ragazzii West	**0.0000**	**0.0000**	**0.0026**	**0.0000**	0.2410	1.0000				
sp. Saja	N/A	N/A	N/A	N/A	N/A	N/A	N/A			
susanae	**0.0000**	**0.0000**	**0.0095**	**0.0000**	**0.0026**	0.2094	N/A	1.0000		
vannutellii	**0.0000**	**0.0000**	**0.0027**	**0.0000**	**0.0197**	**0.0112**	N/A	**0.0000**	1.0000	
yaldeni	**0.0000**	**0.0000**	**0.0043**	**0.0000**	0.8792	0.1835	N/A	**0.0007**	**0.0124**	1.0000

D) Foot length

FtL:SVL	*gramineus* Arsi-Bale-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
n	9	14	3	6	37	7	1	20	55	25
mean	**63**	**64**	**68**	**56**	**72**	**72**	**78**	**69**	**72**	**74**
min	57	47	63	51	55	68	78	57	62	61
max	70	71	74	61	85	77	78	77	82	81
sd	4.3	5.9	5.6	3.8	6.4	3.1	N/A	4.3	3.9	4.9
t-test (p-values)	*gramineus* Arsi-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
gramineus Arsi-Bale-Bore	1.0000									
gramineus Bale	0.7732	1.0000								
gramineus Gamo Gofa	0.3138	0.3799	1.0000							
gramineus Shoa	**0.0036**	**0.0020**	**0.0435**	1.0000						
ragazzii East	**0.0002**	**0.0004**	0.3210	**0.0000**	1.0000					
ragazzii West	**0.0004**	**0.0008**	0.3171	**0.0000**	0.9745	1.0000				
sp. Saja	N/A	N/A	N/A	N/A	N/A	N/A	N/A			
susanae	**0.0028**	**0.0068**	0.6260	**0.0000**	0.1087	0.1411	N/A	1.0000		
vannutellii	**0.0002**	**0.0002**	0.2913	**0.0000**	0.7601	0.8169	N/A	**0.0198**	1.0000	
yaldeni	**0.0000**	**0.0000**	0.1889	**0.0000**	0.1836	0.2393	N/A	**0.0034**	0.1656	1.0000

E) Tibia length to femur length ratio

TbL:FmL	*gramineus* Arsi-Bale-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
n	9	14	3	6	37	7	1	20	57	25
mean	**0.9**	**0.9**	**0.9**	**0.9**	**1.0**	**0.9**	**1.0**	**0.9**	**1.0**	**1.0**
min	0.8	0.8	0.8	0.8	0.8	0.9	1.0	0.9	0.7	0.9
max	1.0	1.0	0.9	1.0	1.2	1.0	1.0	1.0	1.5	1.1
sd	0.1	0.1	0.1	0.1	0.1	0.0	N/A	0.0	0.1	0.1
t-test (p-values)	*gramineus* Arsi-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
gramineus Arsi-Bale-Bore	1.0000									
gramineus Bale	0.6017	1.0000								
gramineus Gamo Gofa	0.6466	0.4381	1.0000							
gramineus Shoa	0.7090	0.9305	0.4878	1.0000						
ragazzii East	**0.0030**	**0.0027**	0.0833	**0.0236**	1.0000					
ragazzii West	0.1577	0.3362	0.2427	0.3875	**0.0082**	1.0000				
sp. Saja	N/A	N/A	N/A	N/A	N/A	N/A	N/A			
susanae	0.1426	0.3114	0.2490	0.3805	**0.0007**	0.9617	N/A	1.0000		
vannutellii	**0.0010**	**0.0007**	0.0618	**0.0107**	0.4062	**0.0017**	N/A	**0.0001**	1.0000	
yaldeni	**0.0062**	**0.0073**	0.1000	**0.0399**	0.5421	**0.0251**	N/A	**0.0044**	0.1625	1.0000

F) Inner meta-tarsal tubercle length

IMTL:SVL	*gramineus* Arsi-Bale-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
n	9	14	3	6	37	7	1	20	57	25
mean	**7**	**7**	**7**	**8**	**5**	**5**	**5**	**5**	**5**	**5**
min	5	4	6	7	3	4	5	4	3	4
max	9	9	8	10	8	6	5	6	6	7
sd	1.2	1.2	0.8	0.9	1.0	0.6	N/A	0.5	0.6	0.7
t-test (p-values)	*gramineus* Arsi-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
gramineus Arsi-Bale-Bore	1.0000									
gramineus Bale	0.9223	1.0000								
gramineus Gamo Gofa	0.6073	0.6417	1.0000							
gramineus Shoa	**0.0095**	**0.0060**	0.0772	1.0000						
ragazzii East	**0.0008**	**0.0000**	**0.0256**	**0.0000**	1.0000					
ragazzii West	**0.0025**	**0.0002**	**0.0265**	**0.0000**	0.3690	1.0000				
sp. Saja	N/A	N/A	N/A	N/A	N/A	N/A	N/A			
susanae	**0.0043**	**0.0003**	**0.0454**	**0.0001**	**0.0333**	0.5003	N/A	1.0000		
vannutellii	**0.0010**	**0.0000**	**0.0327**	**0.0001**	0.9626	0.3019	N/A	**0.0040**	1.0000	
yaldeni	**0.0029**	**0.0001**	**0.0389**	**0.0001**	0.1179	0.7757	N/A	0.5792	**0.0383**	1.0000

G) Inner meta-tarsal tubercle length

IMTL:T1L	*gramineus* Arsi-Bale-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
n	8	14	3	6	35	7	1	20	54	25
mean	**40**	**35**	**36**	**51**	**27**	**29**	**29**	**29**	**28**	**29**
min	29	29	34	39	18	25	29	23	21	25
max	78	45	39	72	40	32	29	41	36	43
sd	16.3	4.7	2.7	11.7	5.2	2.1	N/A	3.5	3.1	4.2
t-test (p-values)	*gramineus* Arsi-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
gramineus Arsi-Bale-Bore	1.0000									
gramineus Bale	0.4233	1.0000								
gramineus Gamo Gofa	0.5149	0.6602	1.0000							
gramineus Shoa	0.1884	**0.0221**	**0.0282**	1.0000						
ragazzii East	0.0588	**0.0000**	**0.0099**	**0.0040**	1.0000					
ragazzii West	0.0922	**0.0004**	**0.0233**	**0.0058**	0.1490	1.0000				
sp. Saja	N/A	N/A	N/A	N/A	N/A	N/A	N/A			
susanae	0.1045	**0.0007**	**0.0273**	**0.0063**	0.0630	0.6644	N/A	1.0000		
vannutellii	0.0717	**0.0000**	**0.0263**	**0.0050**	0.4170	0.3069	N/A	0.1150	1.0000	
yaldeni	0.0967	**0.0004**	**0.0225**	**0.0059**	0.1177	0.8778	N/A	0.7888	0.2350	1.0000

H) Disc of toe-IV diameter

DT4D:T1L	*gramineus* Arsi-Bale-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
n	8	14	3	6	35	7	1	20	54	25
mean	**16**	**14**	**13**	**15**	**25**	**30**	**24**	**27**	**28**	**26**
min	9	0	11	11	15	27	24	19	17	19
max	23	20	15	18	36	33	24	35	36	32
sd	4.6	4.8	2.0	2.6	4.9	2.3	N/A	3.6	4.3	3.5
t-test (p-values)	*gramineus* Arsi-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
gramineus Arsi-Bale-Bore	1.0000									
gramineus Bale	0.4627	1.0000								
gramineus Gamo Gofa	0.2205	0.5453	1.0000							
gramineus Shoa	0.7783	0.5514	0.2356	1.0000						
ragazzii East	**0.0002**	**0.0000**	**0.0006**	**0.0000**	1.0000					
ragazzii West	**0.0000**	**0.0000**	**0.0002**	**0.0000**	**0.0009**	1.0000				
sp. Saja	N/A	N/A	N/A	N/A	N/A	N/A	N/A			
susanae	**0.0001**	**0.0000**	**0.0004**	**0.0000**	0.1532	**0.0214**	N/A	1.0000		
vannutellii	**0.0001**	**0.0000**	**0.0012**	**0.0000**	**0.0248**	**0.0417**	N/A	0.5248	1.0000	
yaldeni	**0.0002**	**0.0000**	**0.0009**	**0.0000**	0.4446	**0.0034**	N/A	0.4321	0.1060	1.0000

I) Tympanum diameter to eye diameter ratio

TyD:ED	*gramineus* Arsi-Bale-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
n	8	13	3	6	31	7	1	20	53	25
mean	**53**	**51**	**60**	**55**	**54**	**50**	**41**	**49**	**52**	**48**
min	44	39	44	46	42	47	41	38	36	38
max	70	73	80	69	72	56	41	59	72	63
sd	8.5	8.6	18.4	8.8	7.4	3.0	N/A	5.8	6.7	6.3
t-test (p-values)	*gramineus* Arsi-Bore	*gramineus* Bale	*gramineus* Gamo Gofa	*gramineus* Shoa	*ragazzii* East	*ragazzii* West	sp. Saja	*susanae*	*vannutellii*	*yaldeni*
gramineus Arsi-Bale-Bore	1.0000									
gramineus Bale	0.6117	1.0000								
gramineus Gamo Gofa	0.5684	0.4765	1.0000							
gramineus Shoa	0.6115	0.3277	0.7008	1.0000						
ragazzii East	0.6848	0.2318	0.6347	0.7879	1.0000					
ragazzii West	0.5127	0.9383	0.4664	0.2632	0.0540	1.0000				
sp. Saja	N/A	N/A	N/A	N/A	N/A	N/A	N/A			
susanae	0.3201	0.6002	0.4192	0.1709	**0.0129**	0.4844	N/A	1.0000		
vannutellii	0.7664	0.6936	0.5188	0.3936	0.1532	0.4162	N/A	0.1283	1.0000	
yaldeni	0.1943	0.3530	0.3808	0.1126	**0.0021**	0.1822	N/A	0.5394	**0.0255**	1.0000

Appendix 3.1. List of criteria, diagnostic characters and attributes used to identify Ethiopian species of *Ptychadena*, summarized from Largen (2001).

1. Distribution: localities and broader region
2. Altitude range: in meters
3. Habitat: savanna, grassland, marshes, forest clearings or edges, herbaceous vegetation
4. Webbing of foot: extensive, moderate, brief
5. Number of phalanges free of webbing on Toe IV: 1.5-2.0, 2.0-3.25, 2.5-3.5
6. Number of phalanges free of webbing on Toe V: 0-0.75, 0.25-1.5, 1.25-2.5
7. Dorsal skin folds: fragmented, 4 complete pairs, 5 complete pairs
8. Supernumerary tubercles: present, absent
9. Foot sole color: uniform, bicolor
10. Inner meta-tarsal tubercle: moderate less compressed, large compressed
11. Outer meta-tarsal tubercle: present, absent, large elongate, small round
12. Pale triangle on snout: present, absent
13. Dorsum markings: dark marking present, absent
14. Pale vertebral and/or tibial line: present, absent
15. Thigh posterior markings: bold longitudinal pale and dark stripes, fine-beaded lines on dark background, feeble dark streaks on pale background, pale spots on dark background
16. Size: larger, smaller, smallest
17. Snout-vent length: in mm
18. Hind limb: long, moderate, brief
19. Tibia length / snout-vent length: 51-64%, 43-62%, 40-50%
20. Gular slits: inferior, semi-inferior, superior
21. Snout-vent length / foot length: <0.5, >0.5
22. Ventrum color and patterns: immaculate, pale grey to dark pattern

Appendix 3.2. List of specimens and localities used to assess molecular phylogeny of Ethiopian species of *Ptychadena* in the current study.

DNA tag no. (Univ. Basel)	**Voucher Field no. (ZNHM-AAU no.)**	**Phylogenetic grouping**	**Category of Highland Segment**	**Name of locality**	**Latitude (N)**	**Longitude (E)**	**Altitude (m)**	**GenBank Accession no. (12S; 16S)**
T1066	SL365	*P. anchietae* (Bale-EthRV)	Lowland-Rift Valley	L. Abaya	(Unknown)	(Unknown)	(Unknown)	JX465062; JX464853
T1290	AM-031	*P. anchietae* (EthLow)	Lowland-Rift Valley	Soddo, Wolayita	6.8293	37.7534	1800	JX465087; JX464878
T1291	AM-032	*P. anchietae* (EthLow)	Lowland-Rift Valley	Soddo, Wolayita	6.8293	37.7534	1800	JX465088; JX464879
T1293	AM-038	*P. anchietae* (EthLow)	Lowland-Rift Valley	Soddo, Wolayita	6.8293	37.7534	1800	JX465090; JX464881
T1294	AM-048	*P. anchietae* (EthLow)	Lowland-SW	Sawla, Gemu Gofa	6.3350	36.9296	1260	JX465091; JX464882
A15512	A15512	*P. anchietae* (EthLow)	South-central	Yadot River, Bale	6.4222	39.8537	1237	Unaccessioned
A15513	A15513	*P. anchietae* (EthLow)	South-central	Yadot River, Bale	6.4222	39.8537	1237	Unaccessioned
A15516	A15516	*P. anchietae* (EthLow)	South-central	Chiri, Bale	6.4823	39.7500	1496	Unaccessioned
A15517	A15517	*P. anchietae* (EthLow)	South-central	Chiri, Bale	6.4823	39.7500	1496	Unaccessioned
A15546	A15546	*P. anchietae* (EthLow)	Lowland-SC	Web R., Sof Omar, Bale	6.9058	40.8468	1199	Unaccessioned
T947	SL388	*P.* cf. *cooperi* (Arsi-Bale)	South-central	Dinshu, Bale	7.0958	39.7900	3166	JX465047; JX464838
T948	SL390	*P.* cf. *cooperi* (Arsi-Bale)	South-central	Dinshu, Bale	7.0958	39.7900	3166	JX465048; JX464839
T1305	AM-112	*P.* cf. *cooperi* (Arsi-Bale)	South-central	Dida'a (Chefe), Arsi	7.8579	39.5783	2412	JX465100; JX464891
T1306	AM-118	*P.* cf. *cooperi* (Arsi-Bale)	South-central	Dida'a (Jida-Robe), Arsi	7.9205	39.6074	2452	JX465101; JX464892
Z37703	Z37703	*P.* cf. *cooperi* (Arsi-Bale)	South-central	Gaysay, Dinshu, Bale	7.1104	39.7468	3055	Unaccessioned
Z37704	Z37704	*P.* cf. *cooperi* (Arsi-Bale)	South-central	Gaysay, Dinshu, Bale	7.1104	39.7468	3055	Unaccessioned
Z37705	Z37705	*P.* cf. *cooperi* (Arsi-Bale)	South-central	Gaysay, Dinshu, Bale	7.1104	39.7468	3055	Unaccessioned
Z37706	Z37706	*P.* cf. *cooperi* (Arsi-Bale)	South-central	Gaysay, Dinshu, Bale	7.1104	39.7468	3055	Unaccessioned
T1003	Z-37652	*P. erlangeri* (Bore1-Bale)	Southern	Koti Balcha F., Bore	6.0899	38.7712	2203	JX465052; JX464843
T1004	Z-37653	*P. erlangeri* (Bore1-Bale)	Southern	Mea Boko R., Bore	6.1071	38.7592	2232	JX465053; JX464844
T1215	AK2029	*P. erlangeri* (Bore1-Bale)	South-central	Shawe River, Bale	6.6386	39.7339	1907	JX465080; JX464871
A15515	A15515	*P. erlangeri* (Bore1-Bale)	South-central	Chiri, Bale	6.4823	39.7500	1496	Unaccessioned
A15524	A15524	*P. erlangeri* (Bore1-Bale)	South-central	S of Katcha, Bale	6.5759	39.7219	1751	Unaccessioned
A15525	A15525	*P. erlangeri* (Bore1-Bale)	South-central	S of Katcha, Bale	6.5759	39.7219	1751	Unaccessioned
Z37651	Z37651	*P. erlangeri* (Bore1-Bale)	Southern	Koti Balcha Forest, Bore	6.0899	38.7712	2203	Unaccessioned
T1292	AM-035	*P. mascareniensis* (NE Afr)	Lowland-Rift Valley	Soddo, Wolayita	6.8293	37.7534	1800	JX465089; JX464880

DNA tag no. (Univ. Basel)	**Voucher Field no. (ZNHM-AAU no.)**	**Phylogenetic grouping**	**Category of Highland Segment**	**Name of locality**	**Latitude (N)**	**Longitude (E)**	**Altitude (m)**	**GenBank Accession no. (12S; 16S)**
T1295	AM-056	*P. mascareniensis* (NE Afr)	Lowland-Rift Valley	SW shore of L. Langano	7.5228	38.7710	1600	JX465092; JX464883
T1296	AM-058	*P. mascareniensis* (NE Afr)	Lowland-Rift Valley	W shore of L. Ziway	7.9243	38.5572	1645	JX465093; JX464884
T1297	AM-059	*P. mascareniensis* (NE Afr)	Lowland-Rift Valley	W shore of L. Ziway	7.9243	38.5572	1645	JX465094; JX464885
T1298	AM-061	*P. mascareniensis* (NE Afr)	Lowland-Rift Valley	W shore of L. Ziway	7.9243	38.5572	1645	JX465095; JX464886
T1299	AM-062	*P. mascareniensis* (NE Afr)	Lowland-Rift Valley	W shore of L. Ziway	7.9243	38.5572	1645	JX465096; JX464887
A15576	A15576	*P. mascareniensis* (NE Afr)	Lowland - Rift Valley	Filwoha, Awash NP	9.0549	40.0694	857	Unaccessioned
A15578	A15578	*P. mascareniensis* (NE Afr)	Lowland - Rift Valley	Filwoha, Awash NP	9.0549	40.0694	857	Unaccessioned
Z37589	Z37589	*P. mascareniensis* (NE Afr)	Lowland - Rift Valley	S. shore of L. Langano	7.5403	38.8031	1545	Unaccessioned
Z37592	Z37592	*P. mascareniensis* (NE Afr)	Lowland - Rift Valley	E. shore of L. Chitu	7.4070	38.4258	1563	Unaccessioned
T819	MW6314	*P. nana* (Arsi-Bale)	South-central	Rira, Bale	6.7713	39.7245	2992	JX465037; JX464828
T820	MW6316	*P. nana* (Arsi-Bale)	South-central	Rira, Bale	6.7713	39.7245	2992	JX465038; JX464829
T823	MW6326	*P. nana* (Arsi-Bale)	South-central	Katcha, Bale	6.7135	39.7247	2828	JX465039; JX464830
T850*	MW6313	*P. nana* (Arsi-Bale)	South-central	Dinshu, Bale	(Unknown)	(Unknown)	(Unknown)	Unaccessioned
T928	SL068	*P. nana* (Arsi-Bale)	South-central	Rira, Bale	6.7733	39.7254	2910	JX465041; JX464832
T929	SL070	*P. nana* (Arsi-Bale)	South-central	Rira, Bale	6.7733	39.7254	2910	JX465042; JX464833
T933	SL086	*P. nana* (Arsi-Bale)	South-central	Harenna Forest, Bale	6.7733	39.7254	2910	JX465043; JX464834
T934	SL088	*P. nana* (Arsi-Bale)	South-central	Harenna Forest, Bale	6.7732	39.7252	2908	JX465044; JX464835
T935	SL090	*P. nana* (Arsi-Bale)	South-central	Harenna Forest, Bale	6.7676	39.7222	2873	JX465045; JX464836
T936	SL092	*P. nana* (Arsi-Bale)	South-central	Harenna Forest, Bale	6.7530	39.7190	2788	JX465046; JX464837
T1208	AK1999	*P. nana* (Arsi-Bale)	South-central	WWF camp, Rira, Bale	6.7591	39.7214	2811	JX465076; JX464867
T1209	AK2001	*P. nana* (Arsi-Bale)	South-central	WWF camp, Rira, Bale	6.7591	39.7214	2811	JX465077; JX464868
T1213	AK2025	*P. nana* (Arsi-Bale)	South-central	Katcha swamp, Bale	5.8697	39.0066	1687	JX465078; JX464869
T1214	AK2027	*P. nana* (Arsi-Bale)	South-central	Katcha swamp, Bale	5.8697	39.0066	1687	JX465079; JX464870
T1300	AM-103	*P. nana* (Arsi-Bale)	South-central	Dida’a town, Arsi	7.8689	39.6272	2444	JX465097; JX464888
T1301	AM-104	*P. nana* (Arsi-Bale)	South-central	Dida’a town, Arsi	7.8689	39.6272	2444	JX465098; JX464889
T1302	AM-107	*P. nana* (Arsi-Bale)	South-central	Dida’a (Chefe), Arsi	7.8579	39.5783	2412	JX465099; JX464890
A15527	A15527	*P. nana* (Arsi-Bale)	South-central	S of Katcha, Bale	6.7151	39.7241	2378	Unaccessioned
A15556	A15556	*P. nana* (Arsi-Bale)	South-central	Kela R., Dinshu, Bale	7.0724	39.7785	3222	Unaccessioned
Z37711	Z37711	*P. nana* (Arsi-Bale)	South-central	Rira, Bale	6.7590	39.7214	2801	Unaccessioned

DNA tag no. (Univ. Basel)	Voucher Field no. (ZNHM-AAU no.)	Phylogenetic grouping	Category of Highland Segment	Name of locality	Latitude (N)	Longitude (E)	Altitude (m)	GenBank Accession no. (12S; 16S)
T1059	SL314	*P. neumanni* (Gughe-Kaffa)	Southwestern	Doshka Forest, Gughe Mts.	6.2031	37.5605	2515	JX465056; JX464847
T1060	SL316	*P. neumanni* (Gughe-Kaffa)	Southwestern	Doshka Forest, Gughe Mts.	6.2031	37.5605	2515	JX465057; JX464848
T1061	SL318	*P. neumanni* (Gughe-Kaffa)	Southwestern	Doshka Forest, Gughe Mts.	6.2031	37.5605	2515	JX465058; JX464849
T1062	SL320	*P. neumanni* (Gughe-Kaffa)	Southwestern	Doshka Forest, Gughe Mts.	6.2031	37.5605	2515	JX465059; JX464850
T1063	SL322	*P. neumanni* (Gughe-Kaffa)	Southwestern	Doshka Forest, Gughe Mts.	6.2031	37.5605	2515	JX465060; JX464851
T1064	SL324	*P. neumanni* (Gughe-Kaffa)	Southwestern	Doshka Forest, Gughe Mts.	6.2031	37.5605	2515	JX465061; JX464852
T1101	AK1715	*P. neumanni* (Gughe-Kaffa)	Southwestern	Bonga, Kaffa	7.2672	36.2590	1789	JX465063; JX464854
T1102	AK1717	*P. neumanni* (Gughe-Kaffa)	Southwestern	Bonga, Kaffa	7.2672	36.2590	1789	JX465064; JX464855
T1103	AK1719	*P. neumanni* (Gughe-Kaffa)	Southwestern	Bonga, Kaffa	7.2672	36.2590	1789	JX465065; JX464856
T1104	AK1730	*P. neumanni* (Gughe-Kaffa)	Southwestern	Bonga, Kaffa	7.2500	36.2500	1900	JX465066; JX464857
T1105	AK1745	*P. neumanni* (Gughe-Kaffa)	Southwestern	Berta River, Bonga, Kaffa	7.2720	36.2600	1727	JX465067; JX464858
T1106	AK1747	*P. neumanni* (Gughe-Kaffa)	Southwestern	Bonga, Kaffa	7.2720	36.2603	1754	JX465068; JX464859
T1107	AK1770	*P. neumanni* (Gughe-Kaffa)	Southwestern	Mankira Forest, Kaffa	7.2173	36.2655	1626	JX465069; JX464860
T1108	AK1803	*P. neumanni* (Gughe-Kaffa)	Southwestern	Koma swamp, Kaffa	7.3101	36.1205	1895	JX465070; JX464861
T1109	AK1864	*P. neumanni* (Gughe-Kaffa)	Southwestern	Saja Forest, Kaffa	7.4871	36.0940	2027	JX465071; JX464862
T1110	AK1866	*P. neumanni* (Gughe-Kaffa)	Southwestern	Saja Forest, Kaffa	7.4871	36.0940	2027	JX465072; JX464863
T1111	AK1868	*P. neumanni* (Gughe-Kaffa)	Southwestern	Saja Forest, Kaffa	7.4871	36.0940	2027	JX465073; JX464864
T1198	SL336	*P. neumanni* (Gughe-Kaffa)	Southwestern	Angle Forest, Gughe Mts.	6.2138	37.5850	2270	JX465074; JX464865
A15511	A15511	*P. neumanni* (Gughe-Kaffa)	South-central	Yadot River, Bale	6.4222	39.8537	1237	Unaccessioned
T834	MW6366	*P.* sp. (Beda Buna – Akaki)	Southwestern	Bada Buna, Kaffa	(Unknown)	(Unknown)	(Unknown)	JX465040; JX464831
A15587	A15587	*P.* sp. (Beda Buna – Akaki)	Central	Akaki, S of Addis Ababa	8.8355	38.7997	2038	Unaccessioned
T994	Z-37624	*P.* sp. (Bore2-Wenchi)	Southern	Gutu Forest, Bore	6.3895	38.6145	2594	JX465049; JX464840
T995	Z-37625	*P.* sp. (Bore2-Wenchi)	Southern	Gutu Forest, Bore	6.3895	38.6145	2594	JX465050; JX464841
T999	Z-37630	*P.* sp. (Bore2-Wenchi)	Southern	Bidika Forest, Bore	6.3692	38.6060	2672	JX465051; JX464842
T1005*	Z-37658	*P.* sp. (Bore2-Wenchi)	Southern	Erba Muda Bulliyo, Bore	6.2333	38.7082	2555	Unaccessioned
T1006*	Z-37659	*P.* sp. (Bore2-Wenchi)	Southern	Erba Muda Bulliyo, Bore	6.2333	38.7082	2555	Unaccessioned
T1013	Z-37675	*P.* sp. (Bore2-Wenchi)	Southern	Melka Alati R., Bore	6.3338	38.6464	2628	JX465054; JX464845
T1014	Z-37676	*P.* sp. (Bore2-Wenchi)	Southern	Melka Alati R., Bore	6.3338	38.6464	2628	JX465055; JX464846
T1203	AK1918	*P.* sp. (Bore2-Wenchi)	Southern	Alayu, Bore	6.3365	38.6484	2676	JX465075; JX464866
T1307	AM-119	*P.* sp. (Bore2-Wenchi)	Central	Hora village, L. Wenchi	8.7858	37.8620	3081	JX465102; JX464893
T1308	AM-120	*P.* sp. (Bore2-Wenchi)	Central	Hora village, L. Wenchi	8.7858	37.8620	3081	JX465103; JX464894

DNA tag no. (Univ. Basel)	**Voucher Field no. (ZNHM-AAU no.)**	**Phylogenetic grouping**	**Category of Highland Segment**	**Name of locality**	**Latitude (N)**	**Longitude (E)**	**Altitude (m)**	**GenBank Accession no. (12S; 16S)**
T1309	AM-121	*P*. sp. (Bore2-Wenchi)	Central	Hora village, L. Wenchi	8.7858	37.8620	3081	JX465104; JX464895
T1310	AM-122	*P*. sp. (Bore2-Wenchi)	Central	Hora village, L. Wenchi	8.7858	37.8620	3081	JX465105; JX464896
T1311	AM-123	*P*. sp. (Bore2-Wenchi)	Central	Hora village, L. Wenchi	8.7858	37.8620	3081	JX465106; JX464897
T1312	AM-124	*P*. sp. (Bore2-Wenchi)	Central	Hora village, L. Wenchi	8.7858	37.8620	3081	JX465107; JX464898
T1313*	AM-128	*P*. sp. (Bore2-Wenchi)	Central	Hora village, L. Wenchi	8.7858	37.8620	3081	Unaccessioned
Z37616	Z37616	*P*. sp. (Bore-Wenchi)	Southern	Gutu Forest, Bore	6.3895	38.6145	2594	Unaccessioned
Z37693	Z37693	*P*. sp. (Bore-Wenchi)	Southern	Melka Alati R., Bore	6.3338	38.6464	2628	Unaccessioned
T1285	AM-025	*P*. sp. (Debre Markos)	Northwestern	Debre Markos, Gojam	10.3193	37.7305	2400	JX465083; JX464874
T1286	AM-026	*P*. sp. (Debre Markos)	Northwestern	Debre Markos, Gojam	10.3193	37.7305	2400	JX465084; JX464875
T1287	AM-027	*P*. sp. (Debre Markos)	Northwestern	Debre Markos, Gojam	10.3193	37.7305	2400	JX465085; JX464876
T1288	AM-028	*P*. sp. (Debre Markos)	Northwestern	Debre Markos, Gojam	10.3193	37.7305	2400	JX465086; JX464877
T1289*	AM-029	*P*. sp. (Debre Markos)	Northwestern	Debre Markos, Gojam	10.3193	37.7305	2400	Unaccessioned
A15552	A15552	*P*. sp. (Goba)	South-central	N of Bele, Goba, Bale	6.9201	40.3155	2112	Unaccessioned
Z37757	Z37757	*P*. sp. (Goba)	South-central	Bamo R., Goba, Bale	7.0138	39.9647	2711	Unaccessioned
T1283	AM-016	*P. wadei* (Andasa)	Northwestern	Andassa, Gojam	11.5122	37.4921	1721	JX465081; JX464872
T1284	AM-019	*P. wadei* (Andasa)	Northwestern	Andassa, Gojam	11.5122	37.4921	1721	JX465082; JX464873

* Eggs or tadpoles

Appendix 3.3. Summary of qualitative morphological data for current and historic museum specimens of *Ptychadena* from Ethiopia. Features in columns 2, 4, and 7 were used (together with some morphometric characters) to compare our results with a previous report by Largen (1997). The remaining columns are additional features assessed in the current study.

Column no. →	1	2	3	4	5	6	7	8	9	10	11	12
Species /populations/	**n**	**OMT (+/-)**	**Tymp. Marking**	**Mid-vert. Line**	**Snout Top Marking**	**Spicules**	**Dorsal Skin fold**	**Thigh Top**	**Tibia Top**	**Dorso-lateral Ridge**	**Tymp. Vs. DLR**	**Vocal Sac Color**
New samples 2006-2010 (n=90)												
P. nana (Arsi-Bale)	13	-	None	White Vt/Th/Br	Lined	None	Fragmented	None/ Faint	Thin/faint	White, fragm./ continuous	Separate/ Closer	Light /Dark
P. sp. (Bore2-Wenchi)	16	-	None	White/Dull Ye/Gr-ye	Lined/ Immaculate	None/ Slight	Fragmented	None/ Very thin	Thin/ Very thin	White/Orange Frag./Contin.	Closer/ Touch	Light/ Gre.-yel.
P. erlangeri (Bore1-Bale)	4	-	None	White Th/Br	Lined	None/ Brief	Fragmented	Thin/Faint None	Thin/ Very thin	White Frag./Contin.	Separate/ Closer	Dark
P. neumanni (Gughe-Kaffa)	23	-	Dull/White Broad/Thin	Dull/White Broad/V.Thin	Lined/ Immaculate	Moderate/ Extensive	Fragmented	Very thin/ None	Very thin/ None	White/Dull Frag.	Touch/ Closer	Dark
P. cf. *cooperi* (Arsi-Bale)	7	-	White/Dull Thin/Broad	White/Dull Br/Vt	Lined	None	Fragmented	Faint/ None	Thin/ Faint	White/Dull Frag.	Touch/ Closer	NA
P. sp. (Debre Markos)	4	-	White Long	White Br/Th	Immaculate/ Lined	Extensive /Very brief	Fragmented	Thin/ None	Thin	White/Dull Frag.	Touch	White
P. wadei (Andassa)	3	+	None	White/Dull Br	Immaculate	None	Long	None	Thin	White Contin.	Touch	Dark
P. anchietae (ETH)	5	-	None	None	Pale triangle	None	Long	None	None	White/Dull Frag./Contin.	Closer/ Touch	Dark
P. mascareniensis (ETH)	15	Abs.	White/Long	Faint/Vt	Immac.	None	Long	None	None	White/Contin.	Touch	NA
Historic museum collections (n=58)												
P. cooperi	14	-	Dull Thin	None/White V. thin	Lined/ Immaculate	None	Fragmented	Spotted No line	No line/ Very thin	Dull/Brown Frag./Contin.	Closer	Dull
P. erlangeri	8	-	Du/Wh Thin	None/Dull/White Thin	Lined/Patt/ Immaculate	None/ Extensive	Fragmented	Thin/ No line	Thin/ No line	White/Yellow/ Brown Fragm./Contin.	Closer/ Touch/ Separate	Dark
P. anchietae (harenna)	7	-	Dull Thin	None	Immaculate	Brief/ None	Fragmented	No line	No line	White Fragm.	Closer	Dark
P. mascareniensis	2	-	Dull Thin	Dull/White Thin/V. thin	Lined/ Immaculate	Very fine	Long 4 pairs	Spotted/ No line	Very thin/ Thin	Brown/Pale Contin.	Separate/ Closer	Dark
P. nana	1	-	None	None	Immaculate	None	Fragmented	None	None	Less clear	Separate	NA
P. neumanni	7	-	White Thin	White Broad	Immaculate	Very fine	Fragmented	None	None	White Fragm.	Separate	Dark
P. wadei	19	+	Dull/None Thin/V. thin	Dull/Dark Broad/Short	Immaculate	None	Long 4 or 3 pairs	None	Thin/ None	White Fragm/Contin.	Separate / Closer	Dark

Appendix 3.4. Descriptive summary (top six rows) and student's t-test results (bottom ten rows) for pairwise comparison of means of ratios of eleven morphometric characters assessed in the current study for *Ptychadena* of Ethiopia. The type of character is shown on the top-left corner of each table. p-values in bold fonts represent significantly different pairs of means at a significance level of 0.05.

A) Head width

HW:SVL	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
n	17	12	22	21	7	14	16	5	25
mean	32.8	33.0	32.5	34.8	32.2	33.6	32.7	30.1	33.1
min	28.5	30.6	28.0	32.0	27.3	29.8	27.3	28.5	30.4
max	35.8	35.0	35.3	37.8	34.7	36.7	36.8	31.6	36.4
sd	2.0	1.6	2.1	1.8	2.3	2.0	2.2	1.4	1.6
p-values (t-test)	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
mascareniensis	1.0000								
anchietae	0.7215	1.0000							
wadei	0.6867	0.4378	1.0000						
cooperi	**0.0021**	**0.0062**	**0.0003**	1.0000					
sp. Debre Markos	0.5603	0.4168	0.7381	**0.0219**	1.0000				
nana	0.2321	0.3795	0.1108	0.0875	0.1775	1.0000			
sp. Bore-Wenchi	0.9376	0.6847	0.7738	**0.0041**	0.6111	0.2364	1.0000		
erlangeri	**0.0101**	**0.0066**	**0.0163**	**0.0004**	0.0942	**0.0019**	**0.0126**	1.0000	
neumanni	0.5791	0.8849	0.2879	**0.0015**	0.3512	0.3877	0.5591	**0.0061**	1.0000

B) Femur length

FmL:SVL	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
n	17	12	22	19	7	14	16	5	25
mean	46	52	41	50	47	49	49	53	54
min	43	48	33	44	41	39	43	52	48
max	52	55	50	58	55	55	58	58	62
sd	2.8	2.3	5.0	4.0	4.2	5.5	4.7	2.4	3.5
p-values (t-test)	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
mascareniensis	1.0000								
anchietae	**0.0000**	1.0000							
wadei	**0.0005**	**0.0000**	1.0000						
cooperi	**0.0046**	**0.0335**	**0.0000**	1.0000					
sp. Debre Markos	0.8725	**0.0112**	**0.0201**	0.1157	1.0000				
nana	0.1282	**0.0485**	**0.0004**	0.6099	0.3097	1.0000			
sp. Bore-Wenchi	**0.0367**	**0.0419**	**0.0000**	0.7775	0.1928	0.8076	1.0000		
erlangeri	**0.0006**	0.3612	**0.0000**	**0.0240**	**0.0054**	**0.0220**	**0.0216**	1.0000	
neumanni	**0.0000**	0.1940	**0.0000**	**0.0023**	**0.0037**	**0.0096**	**0.0055**	0.9753	1.0000

C) Tibia length

TbL:SVL	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
n	17	12	22	19	7	14	16	5	25
mean	49	60	50	54	54	53	56	62	60
min	45	55	42	48	46	42	50	58	53
max	58	65	56	60	64	60	68	64	69
sd	3.4	3.8	4.2	3.9	5.9	6.3	5.3	2.8	3.5
p-values (t-test)	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
mascareniensis	1.0000								
anchietae	**0.0000**	1.0000							
wadei	0.6396	**0.0000**	1.0000						
cooperi	**0.0012**	**0.0001**	**0.0055**	1.0000					
sp. Debre Markos	0.0803	**0.0415**	0.1178	0.8407	1.0000				
nana	0.0514	**0.0025**	0.0974	0.8280	0.7480	1.0000			
sp. Bore-Wenchi	**0.0004**	**0.0163**	**0.0012**	0.2138	0.5693	0.2625	1.0000		
erlangeri	**0.0000**	0.4281	**0.0000**	**0.0008**	**0.0192**	**0.0012**	**0.0070**	1.0000	
neumanni	**0.0000**	0.6167	**0.0000**	**0.0000**	0.0583	**0.0032**	**0.0194**	0.2002	1.0000

D) Foot length

FtL:SVL	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
n	17	12	11	18	4	14	16	5	25
mean	74	80	71	79	69	79	79	83	83
min	66	75	60	66	63	58	69	80	77
max	86	87	77	87	71	88	93	88	100
sd	5.5	3.3	5.7	5.5	4.1	9.6	7.0	2.8	5.0
p-values (t-test)	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
mascareniensis	1.0000								
anchietae	**0.0017**	1.0000							
wadei	0.0997	**0.0002**	1.0000						
cooperi	**0.0202**	0.4690	**0.0010**	1.0000					
sp. Debre Markos	0.0635	**0.0060**	0.5100	**0.0066**	1.0000				
nana	0.1501	0.6156	**0.0160**	0.9412	**0.0102**	1.0000			
sp. Bore-Wenchi	**0.0334**	0.6823	**0.0017**	0.8695	**0.0046**	0.8543	1.0000		
erlangeri	**0.0003**	0.0899	**0.0000**	**0.0370**	**0.0018**	0.1405	0.0920	1.0000	
neumanni	**0.0000**	**0.0408**	**0.0000**	**0.0166**	**0.0022**	0.1327	0.0724	0.9612	1.0000

E) Hind limb length

SVL:HiLi	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
n	17	12	11	18	4	14	16	5	25
mean	59	52	60	55	61	56	55	50	51
min	51	50	55	49	59	49	46	49	44
max	65	55	68	61	67	72	61	53	55
sd	3.4	1.6	4.4	3.6	3.7	7.4	4.6	1.3	2.4
p-values (t-test)	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
mascareniensis	1.0000								
anchietae	**0.0000**	1.0000							
wadei	0.5914	**0.0001**	1.0000						
cooperi	**0.0018**	**0.0052**	**0.0064**	1.0000					
sp. Debre Markos	0.3177	**0.0125**	0.5534	**0.0320**	1.0000				
nana	0.1923	0.0639	0.1291	0.6101	0.0822	1.0000			
sp. Bore-Wenchi	**0.0048**	**0.0475**	**0.0073**	0.8191	**0.0244**	0.5339	1.0000		
erlangeri	**0.0000**	0.0730	**0.0000**	**0.0004**	**0.0070**	**0.0164**	**0.0048**	1.0000	
neumanni	**0.0000**	0.2013	**0.0000**	**0.0005**	**0.0092**	**0.0285**	**0.0110**	0.4280	1.0000

F) Tibia length to femur length ratio

TbL:FmL	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
n	17	12	22	19	7	14	16	5	25
mean	1.1	1.2	1.2	1.1	1.2	1.1	1.1	1.2	1.1
min	0.9	1.1	1.0	1.0	1.1	1.0	1.0	1.1	1.0
max	1.2	1.4	1.4	1.2	1.3	1.2	1.2	1.2	1.2
sd	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1
p-values (t-test)	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
mascareniensis	1.0000								
anchietae	**0.0094**	1.0000							
wadei	**0.0000**	0.0583	1.0000						
cooperi	0.5370	**0.0275**	**0.0000**	1.0000					
sp. Debre Markos	**0.0479**	0.7803	0.2829	0.0805	1.0000				
nana	0.2938	**0.0479**	**0.0000**	0.6877	0.1103	1.0000			
sp. Bore-Wenchi	**0.0026**	0.4510	**0.0007**	**0.0199**	0.4219	**0.0459**	1.0000		
erlangeri	0.0583	0.9793	0.1311	0.0996	0.7826	0.1391	0.5779	1.0000	
neumanni	**0.0286**	0.1805	**0.0001**	0.1375	0.2385	0.2662	0.3205	0.3167	1.0000

G) Tibia length to foot length ratio

TbL:FtL	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
n	17	12	11	18	4	14	16	5	25
mean	67	75	75	68	73	68	70	74	72
min	61	69	68	64	72	64	66	71	63
max	72	87	80	76	75	72	73	78	79
sd	3.3	6.5	3.5	2.8	1.6	2.4	2.2	3.0	3.8
p-values (t-test)	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
mascareniensis	1.0000								
anchietae	**0.0007**	1.0000							
wadei	**0.0000**	0.7765	1.0000						
cooperi	0.1498	**0.0030**	**0.0001**	1.0000					
sp. Debre Markos	**0.0001**	0.3266	0.2948	**0.0011**	1.0000				
nana	0.2339	**0.0023**	**0.0000**	0.7723	**0.0008**	1.0000			
sp. Bore-Wenchi	**0.0006**	**0.0257**	**0.0026**	**0.0142**	**0.0223**	**0.0072**	1.0000		
erlangeri	**0.0019**	0.5966	0.7230	**0.0071**	0.6128	**0.0058**	**0.0471**	1.0000	
neumanni	**0.0000**	0.1014	**0.0369**	**0.0008**	0.2062	**0.0004**	0.1439	0.1795	1.0000

H) Toe I length

T1L:FtL	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
n	17	12	11	18	4	14	16	5	25
mean	20	19	20	20	19	18	18	17	17
min	19	17	18	18	16	15	16	15	12
max	22	22	22	22	20	22	22	19	19
sd	0.9	1.6	1.3	1.2	1.7	2.1	2.0	1.6	1.6
p-values (t-test)	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
mascareniensis	1.0000								
anchietae	**0.0175**	1.0000							
wadei	0.2348	0.2181	1.0000						
cooperi	0.4872	0.0563	0.5278	1.0000					
sp. Debre Markos	0.2022	0.9572	0.4256	0.2779	1.0000				
nana	**0.0097**	0.5965	0.1036	**0.0273**	0.7574	1.0000			
sp. Bore-Wenchi	**0.0006**	0.2381	**0.0194**	**0.0025**	0.4777	0.5695	1.0000		
erlangeri	**0.0101**	0.0804	**0.0195**	**0.0127**	0.1885	0.1769	0.3331	1.0000	
neumanni	**0.0000**	**0.0048**	**0.0001**	**0.0000**	0.1381	**0.0429**	0.1169	0.9323	1.0000

I) Inner meta-tarsal tubercle length

IMTL:T1L	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
n	17	12	11	18	4	14	16	5	25
mean	22.7	27.8	28.1	23.2	30.3	29.6	32.6	27.4	27.0
min	17.7	17.6	18.6	19.6	27.1	22.5	19.2	24.2	20.4
max	29.5	42.1	38.7	29.3	35.1	46.1	50.0	30.7	42.4
sd	2.9	7.5	6.0	2.6	3.5	5.5	8.1	3.0	4.6
p-values (t-test)	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
mascareniensis	1.0000								
anchietae	**0.0425**	1.0000							
wadei	**0.0162**	0.9341	1.0000						
cooperi	0.5962	0.0619	**0.0254**	1.0000					
sp. Debre Markos	**0.0156**	0.3969	0.4001	**0.0210**	1.0000				
nana	**0.0005**	0.5026	0.5123	**0.0008**	0.7803	1.0000			
sp. Bore-Wenchi	**0.0002**	0.1170	0.1036	**0.0003**	0.3927	0.2366	1.0000		
erlangeri	**0.0196**	0.8640	0.7666	**0.0308**	0.2364	0.2806	**0.0435**	1.0000	
neumanni	**0.0006**	0.7443	0.6227	**0.0014**	0.1645	0.1527	**0.0199**	0.8394	1.0000

J) Inner meta-tarsal tubercle length

IMTL:SVL	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
n	17	12	14	19	7	14	16	5	25
mean	3.43	4.17	3.90	3.68	4.09	4.23	4.55	3.88	3.81
min	2.64	2.71	2.93	2.96	3.76	3.32	3.42	3.60	3.06
max	5.51	6.11	4.95	4.63	5.08	5.23	6.05	4.27	6.00
sd	0.7	1.1	0.5	0.4	0.5	0.6	0.7	0.3	0.6
p-values (t-test)	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
mascareniensis	1.0000								
anchietae	**0.0477**	1.0000							
wadei	**0.0421**	0.4282	1.0000						
cooperi	0.2197	0.1470	0.2147	1.0000					
sp. Debre Markos	**0.0143**	0.8113	0.4115	0.0659	1.0000				
nana	**0.0018**	0.8784	0.1359	**0.0078**	0.5625	1.0000			
sp. Bore-Wenchi	**0.0001**	0.2994	**0.0072**	**0.0002**	0.0765	0.1818	1.0000		
erlangeri	**0.0466**	0.3884	0.9179	0.2363	0.3496	0.1052	**0.0059**	1.0000	
neumanni	0.0830	0.2901	0.6427	0.4236	0.2169	0.0503	**0.0018**	0.7020	1.0000

K) Tympanum diameter to eye diameter ratio

TyD:ED	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
n	17	12	21	18	7	14	16	5	24
mean	70	78	68	67	77	68	65	70	76
min	54	62	46	47	64	52	56	59	63
max	86	94	128	81	91	85	81	80	89
sd	7.5	10.3	19.5	9.0	9.6	8.4	6.5	10.1	7.4
p-values (t-test)	*mascareniensis*	*anchietae*	*wadei*	*cooperi*	sp. Debre Markos	*nana*	sp. Bore-Wenchi	*erlangeri*	*neumanni*
mascareniensis	1.0000								
anchietae	**0.0451**	1.0000							
wadei	0.6217	0.0689	1.0000						
cooperi	0.2672	**0.0081**	0.8603	1.0000					
sp. Debre Markos	0.1423	0.8402	0.1291	**0.0431**	1.0000				
nana	0.5258	**0.0203**	0.9261	0.6783	0.0759	1.0000			
sp. Bore-Wenchi	0.0503	**0.0018**	0.5634	0.4999	**0.0188**	0.2722	1.0000		
erlangeri	0.9531	0.1914	0.7520	0.5896	0.2730	0.7682	0.3755	1.0000	
neumanni	**0.0218**	0.6014	0.0885	**0.0018**	0.8415	**0.0101**	**0.0000**	0.2660	1.0000

Appendix 4.1. Locality data and geographic categories for the studied Ethiopian amphibians. Shaded rows are type localities. Sources of data: Old records (pre-1975): MJL = Malcolm J. Largen (Largen, 2001); current and recent records (2006-2010): AAM = Abebe A. Mengistu, BMZ = Breda M. Zimkus, NGS = National Geographic Society, SPL = Simon P. Loader.

A) *Leptopelis gramineus* (Shoa-Arsi-Bale-Bore-Southwest highlands)

Ser. No.	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	5.9167-6.3000	37.9167-36.2667	~2000	Southwestern	Badditu and Dime (between)	MJL 2001
2	5.8000	39.2000	1900	Southern	Kebre Mengist	MJL 2001
3	5.8697	39.0066	1687	South-central	Katcha, Bale	NGS Team 2009
4	6.0333	38.9500	2500	Southern	Kebre Mengist	MJL 2001
5	6.0899	38.7712	2203	Southern	Bore, Sidamo	BMZ 2006
6	6.1000	37.3667	3000-3200	Southwestern	Bonke Valley	MJL 2001
7	6.2333	38.7082	2555	Southern	Bore, Sidamo	BMZ 2006
8	6.2667	37.6667	2600	Southwestern	Dorse	MJL 2001
9	6.2833	37.6667	2600	Southwestern	Chencha	MJL 2001
10	6.3333	37.6667	2700	Southwestern	Chencha	MJL 2001
11	6.3692	38.6060	2672	Southern	Bore, Sidamo	BMZ 2006
12	6.3833	38.5833	2650	Southern	Kebre Mengist	MJL 2001
13	6.3895	38.6145	2594	Southern	Bore, Sidamo	BMZ 2006
14	6.4500	38.5833	2750	Southern	Agereselam	MJL 2001
15	6.5000	38.5833	2900	Southern	Wando	MJL 2001
16	6.6667	39.7333	1950	South-central	Shawe River	MJL 2001
17	6.7000	39.7333	2400	South-central	Katcha	MJL 2001
18	6.7591	39.7214	2811	South-central	Rira, Bale	SPL&AAM 2008
19	6.7713	39.7245	2922	South-central	Rira, Bale	SPL&AAM 2008
20	6.8500	40.0500	3900	South-central	Goba	MJL 2001
21	6.9167	39.8333	3000	South-central	Tagona River	MJL 2001
22	6.9667	39.7500	3600	South-central	Little Batu	MJL 2001
23	6.9833	40.0167	3100-3200	South-central	Goba	MJL 2001
24	7.0500	38.8333	2600	South-central	Webi Shebeli bridge	MJL 2001
25	7.0667	39.6667	3550	South-central	Dinshu	MJL 2001
26	7.1000	39.7833	3100	South-central	Dinshu	MJL 2001
27	7.1167	39.7167	3000	South-central	Dinshu	MJL 2001
28	7.5500	36.5667	2200	South-central	Belete Forest	MJL 2001
29	7.7500	39.3000	2700-3000	South-central	Arsi Mountains	MJL 2001
30	7.8333	39.5000	NA	South-central	Didda Plateau	MJL 2001
31	7.8689	39.6272	2444	South-central	Dida'a town, Arsi	AAM 2010
32	8.7858	37.8620	3081	Central	Hora village, Shoa	AAM 2010
33	8.8000	37.9333	3100	Central	Wonchi	MJL 2001
34	9.0333	37.8333	2750	Central	Wouramboulchi	MJL 2001
35	9.0333	38.7500	2400-2700	Central	Addis Ababa	MJL 2001
36	9.0500	38.3833	2500	Central	Addis Alam	MJL 2001
37	9.0500	38.6333	2585	Central	Gefersa Reservoir	MJL 2001
38	9.0667	36.5500	2150	Central	Nakamti (= Lekempti)	MJL 2001
39	9.1667	38.2500	2500	Central	Meta	MJL 2001
40	9.6500	39.7667	3000	Central	Let Marefia	MJL 2001
41	9.8333	39.7333	3300	Central	Debre Sina	MJL 2001

B) *Leptopelis ragazzii* (Shoa-Arsi-Bale-Bore)

Ser. No.	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	5.8697	39.0066	1687	South-central	Katcha, Bale	NGS Team 2009
2	6.2500	38.7500	2700	Southern	Irba Moda	MJL 2001
3	6.3338	38.6464	2628	Southern	Bore, Sidamo	BMZ 2006
4	6.3500	38.6333	2700	Southern	Bore	MJL 2001
5	6.3833	38.5833	2650	Southern	Kebre Mengist	MJL 2001
6	6.6000	38.4167	1930	Southern	Wando	MJL 2001
7	6.6386	39.7339	1907	South-central	Shawe, Bale	NGS Team 2009
8	6.7000	39.7333	2400	South-central	Katcha	MJL 2001
9	6.7156	39.7239	2371	South-central	Katcha, Bale	SPL&AAM 2008
10	6.7162	39.7255	2364	South-central	Harenna, Bale	SPL&AAM 2008
11	6.7500	39.7333	2830	South-central	Katcha	MJL 2001
12	6.7530	39.7190	2788	South-central	Harenna, Bale	SPL&AAM 2008
13	6.7559	39.7263	2813	South-central	Harenna, Bale	SPL&AAM 2008
14	6.7591	39.7214	2811	South-central	Rira, Bale	SPL&AAM 2008
15	6.7713	39.7245	2922	South-central	Rira, Bale	SPL&AAM 2008
16	6.9833	40.0167	3100-3200	South-central	Goba	MJL 2001
17	7.0000	40.0167	2800	South-central	Micha River	MJL 2001
18	7.0987	38.6404	1966	South-central	Wendo Genet	SPL&AAM 2008
19	7.1000	39.7833	3100	South-central	Dinshu	MJL 2001
20	7.1667	38.6667	2250	South-central	Shashamane	MJL 2001
21	7.7500	39.3000	2700-3000	South-central	Arsi Mountains	MJL 2001
22	8.8000	37.9333	3100	Central	Wonchi	MJL 2001
23	9.0000	37.4500	2500	Central	Ghedo	MJL 2001
24	9.0333	37.8333	2750	Central	Shoa, Wouramboulchi	MJL 2001

C) *Leptopelis* sp. (Saja)

Ser. No.	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	7.5019	36.0918	1956	Southwestern	Saja, Kaffa	NGS Team 2009

D) *Leptopelis* sp. (Soddo)

Ser. No.	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	6.8319	37.7184	1839	Northwestern	Soddo, Ethiopia	AAM 2010

E) *Leptopelis susanae* (Southwest highlands)

Ser. No.	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	5.9667	37.3000	2700	Southwestern	Bulta	MJL 2001
2	6.1000	37.3667	3000-3200	Southwestern	Bonke	MJL 2001
3	6.2138	37.5850	2270	Southwestern	Gughe Mountain	SPL&AAM 2008
4	6.2667	37.6667	2600	Southwestern	Dorse	MJL 2001
5	6.3333	37.6667	2700	Southwestern	Chencha	MJL 2001
6	7.5019	36.0918	1956	Southwestern	Saja, Kaffa	NGS Team 2009

F) *Leptopelis vannutellii* (Southwest highlands)

Ser. No.	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	5.9167-6.3000	37.9167-36.2667	~2000	Southwestern	B/n Badditu and Dime	MJL 2001
2	5.9667	37.3000	2700	Southwestern	Bulta	MJL 2001
3	6.1000	37.3667	3000-3200	Southwestern	Bonke	MJL 2001
4	6.2031	37.5605	2515	Southwestern	Gughe Mountain	SPL&AAM 2008
5	6.2138	37.5850	2270	Southwestern	Gughe Mountain	SPL&AAM 2008
6	6.2167	37.6667	2400	Southwestern	Dorse	MJL 2001
7	6.9667	35.4333	1500	Southwestern	Mizan Teferi	MJL 2001
8	7.1167	35.7500	2000	Southwestern	Shishinda	MJL 2001
9	7.1956	36.2844	2647	Southwestern	Mankira, Kaffa	NGS Team 2009
10	7.2333	35.9667	1800	Southwestern	Bonga	MJL 2001
11	7.3180	36.0782	1889	Southwestern	Koma, Kaffa	NGS Team 2009
12	7.3333	36.2167	1750	Southwestern	Bonga	MJL 2001
13	7.5019	36.0918	1956	Southwestern	Saja, Kaffa	NGS Team 2009
14	7.5500	36.5667	2200	Southwestern	Beleta Forest	MJL 2001
15	7.6667	36.8833	1840	Southwestern	Badda Buna Forest	MJL 2001
16	8.2833	36.0833	2000	Southwestern	Gabba River bridge	MJL 2001
17	8.3667	35.8167	1500	Southwestern	Scecchi River bridge	MJL 2001
18	9.7500	35.5500	NA	Western	Didesa Valley	MJL 2001

G) *Leptopelis yaldeni* (Gojam)

Ser. No.	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	10.2333	38.0333	2500	Northwestern	Debre Marcos	MJL 2001
2	10.3211	37.7291	2405	Northwestern	Debre-Markos	AAM 2010
3	10.3500	37.7333	2500	Northwestern	Debre Marcos	MJL 2001
4	10.8667	37.0667	2500	Northwestern	Enjiabara	MJL 2001
5	10.9333	37.0000	2500	Northwestern	Enjiabara	MJL 2001
6	11.0500	36.9667	2700	Northwestern	Enjiabara	MJL 2001
7	11.3167	36.9500	2000	Northwestern	Dangila	MJL 2001
8	11.3833	37.0000	2000	Northwestern	Dangila	MJL 2001

H) *Ptychadena cooperi* (Shoa-Arsi-Bale-Bore)

Ser. No.	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	6.3500	38.6333	2700	South	Bore	MJL 2001
2	6.3833	38.5833	2650	South	Kebre Mengist	MJL 2001
3	6.5500	38.4667	2500	South Central	Allata	MJL 2001
4	7.0000	39.2667	3000	South Central	Ghedira River, Bale	MJL 2001
5	7.0000	40.0167	2800	South Central	Micha River, E of Goba	MJL 2001
6	7.0162	39.7225	3446	South Central	Dinshu	BMZ2006
7	7.0958	39.7900	3166	South Central	Dinshu HQ, Bale Mountains	SPL&AAM 2008
8	7.1000	39.7500	3100	South Central	Dinshu	MJL 2001
9	7.1000	39.7667	3000	South Central	Dinshu	MJL 2001
10	7.1000	39.8167	3100	South Central	Dinshu	MJL 2001
11	7.1104	39.7468	3055	South Central	Gaysay	BMZ2006
12	7.1333	39.7500	3200	South Central	Gaysay	MJL 2001
13	7.2667	39.2833	2500	South Central	Dodola and Aselle	MJL 2001
14	7.8579	39.5783	2412	South Central	Dida'a (Chefe), Ethiopia	AAM 2010
15	7.9205	39.6074	2452	South Central	Dida'a (Jida-Robe), Arsi	AAM 2010
16	8.8000	37.9333	3100	Central	Lake Wonchi	MJL 2001
17	9.0333	37.8333	2750	Central	Wouramboulchi	MJL 2001
18	9.0500	38.6333	2585	Central	Addis Ababa	MJL 2001
19	9.8333	38.4167	2500	Central	Tullu Milchi	MJL 2001

I) *Ptychadena erlangeri* (Bore-Bale)

Ser. No.	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	6.0899	38.7712	2203	South	Bore, Ethiopia	BMZ 2006
2	6.1071	38.7592	2232	South	Bore, Ethiopia	BMZ 2006
3	6.2500	37.9167	1300	South	Abaya Lake, E. of	MJL 2001
4	6.4823	39.7500	1496	Lowland-S	Chiri	BMZ2006
5	6.5759	39.7219	1751	South Central	Harenna	BMZ2006
6	6.6386	39.7339	1907	South Central	Shawe River, Harenna Forest, Bale Mountains	NGS Team 2009
7	6.7151	39.7241	2378	South Central	Harenna	BMZ2006

J) *Ptychadena nana* (Arsi-Bale)

Ser. No.	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	5.8697	39.0066	1687	South Central	Katcha Swamp, Harenna Forest, Bale Mountains	NGS Team 2009
2	6.7135	39.7247	2828	South Central	Katcha rivulet	SPL 2006
3	6.7151	39.7241	2378	South Central	Katcha	BMZ2006
4	6.7530	39.7190	2788	South Central	Harenna, Bale Mountains	SPL&AAM 2008
5	6.7590	39.7214	2801	South Central	Rira	BMZ2006
6	6.7591	39.7214	2811	South Central	nr. To WWF building, Harenna Forest, Bale Mountains	NGS Team 2009
7	6.7676	39.7222	2873	South Central	Harenna, Bale Mountains	SPL&AAM 2008
8	6.7732	39.7252	2908	South Central	Harenna, Bale Mountains	SPL&AAM 2008
9	6.7733	39.7254	2910	South Central	Rira area, Bale Mountains	SPL&AAM 2008
10	6.7750	39.7263	2881	South Central	Rira	SPL 2006
11	7.0526	39.7609	3280	South Central	Dinshu	BMZ2006
12	7.0724	39.7785	3222	South Central	Dinshu	BMZ2006
13	7.0958	39.7900	3166	South Central	Dinsho	SPL 2006
14	7.8333	39.5000	2000-3000	South-Central	Didda Plateau	MJL 2001
15	7.8579	39.5783	2412	South Central	Dida’a (Chefe), Ethiopia	AAM 2010
16	7.8689	39.6272	2444	South Central	Dida’a town, Ethiopia	AAM 2010

K) *Ptychadena neumanni* (Southwest highlands)

Ser. No.	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	6.2031	37.5605	2515	Southwest	Near Dorse, Doshka Forest, Gughe Mountains	SPL&AAM 2008
2	6.2138	37.5850	2270	Southwest	Near Chencha, Angle Forest, Gughe Mountains	SPL&AAM 2008
3	6.3333	36.8333	2000	Southwest	Gadat, Gofa	MJL 2001
4	6.4222	39.8522	1237	Lowland-S	Dolo Mena	BMZ2006
5	7.2173	36.2655	1626	Southwest	Near to Mankira, SW Forest	NGS Team 2009
6	7.2500	36.2500	1900	Southwest	Bonga Town, SW Forest	NGS Team 2009
7	7.2672	36.2590	1789	Southwest	Bonga Swamp, SW Forest	NGS Team 2009
8	7.2720	36.2600	1727	Southwest	Barta Stream, SW Forest	NGS Team 2009
9	7.2720	36.2603	1755	Southwest	Stream - Bonga, SW Forest	NGS Team 2009
10	7.3101	36.1205	1895	Southwest	Koma Swamp, SW forest	NGS Team 2009
11	7.4871	36.0940	2027	Southwest	Saja Forest Road, SW Forest	NGS Team 2009

L) *Ptychadena* sp. (Bada Buna – Akaki)

Ser. No.	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	7.6608	36.8901	1693	Southwest	Bada Buna	SPL 2006
2	8.8355	38.7997	2038	Central	Akaki	BMZ2006
3	8.8464	38.4570	2048	Central	Tefki, Awash River	BMZ2006

M) *Ptychadena* sp. (Goba)

Ser. No.	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	6.9201	40.3155	2112	South Central	Bele	BMZ2006
2	7.0138	39.9647	2711	South Central	Goba	BMZ2006

N) *Ptychadena* sp. (Debre Markos)

Ser. No.	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	10.3193	37.7305	2400	Northwest	Debre-Markos, Ethiopia	AAM 2010

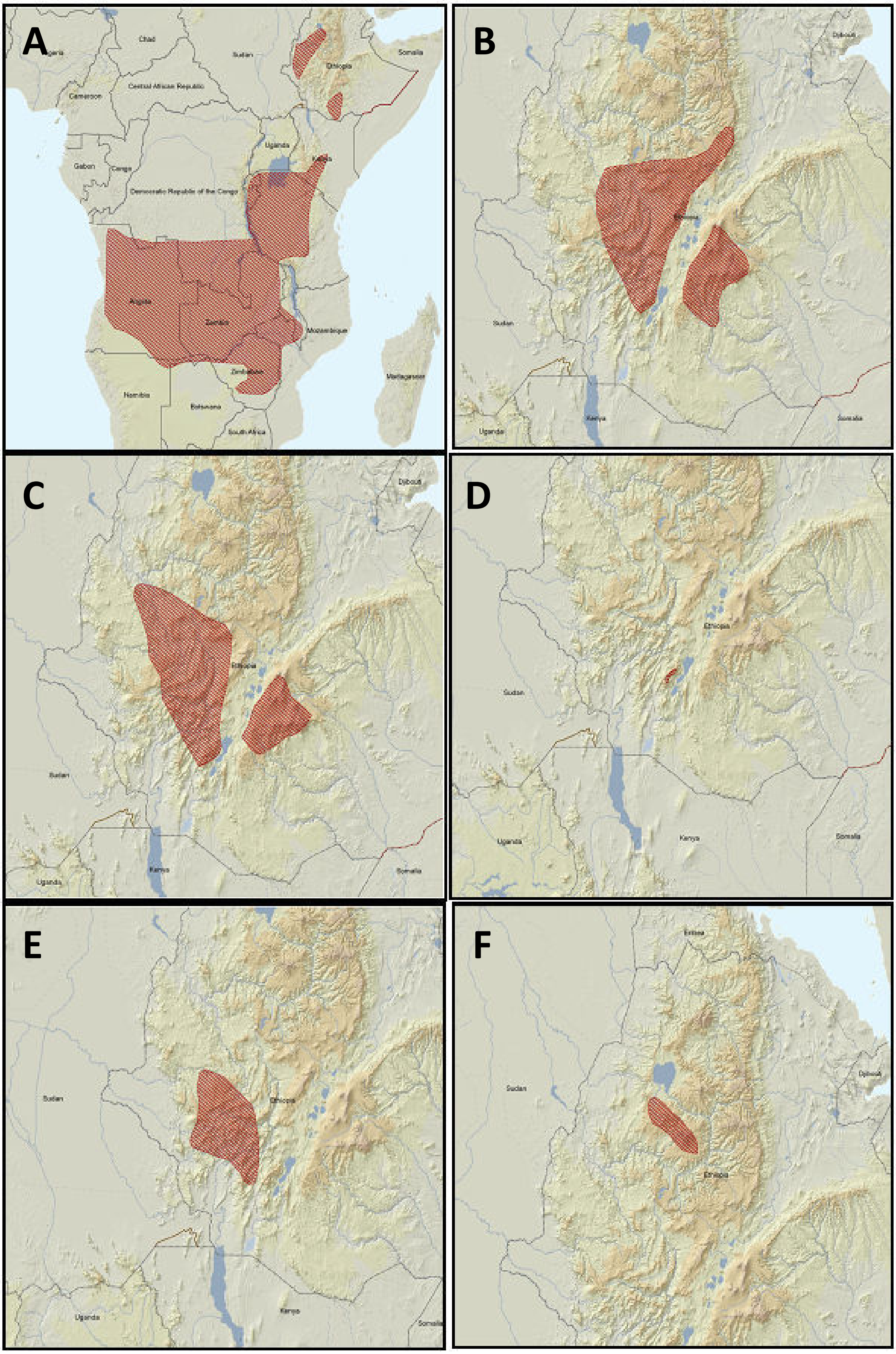
O) *Ptychadena* sp. (Bore-Wenchi)

Ser. No.	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	6.0899	38.7712	2203	South	Bore	BMZ2006
2	6.2333	38.7082	2555	South	Bore, Ethiopia	BMZ 2006
3	6.3338	38.6464	2628	South	Bore, Ethiopia	BMZ 2006
4	6.3692	38.6060	2672	South	Bore, Ethiopia	BMZ 2006
5	6.3895	38.6145	2594	South	Bore, Ethiopia	BMZ 2006
6	8.7858	37.8620	3081	Central	Hora village, Ethiopia	AAM 2010

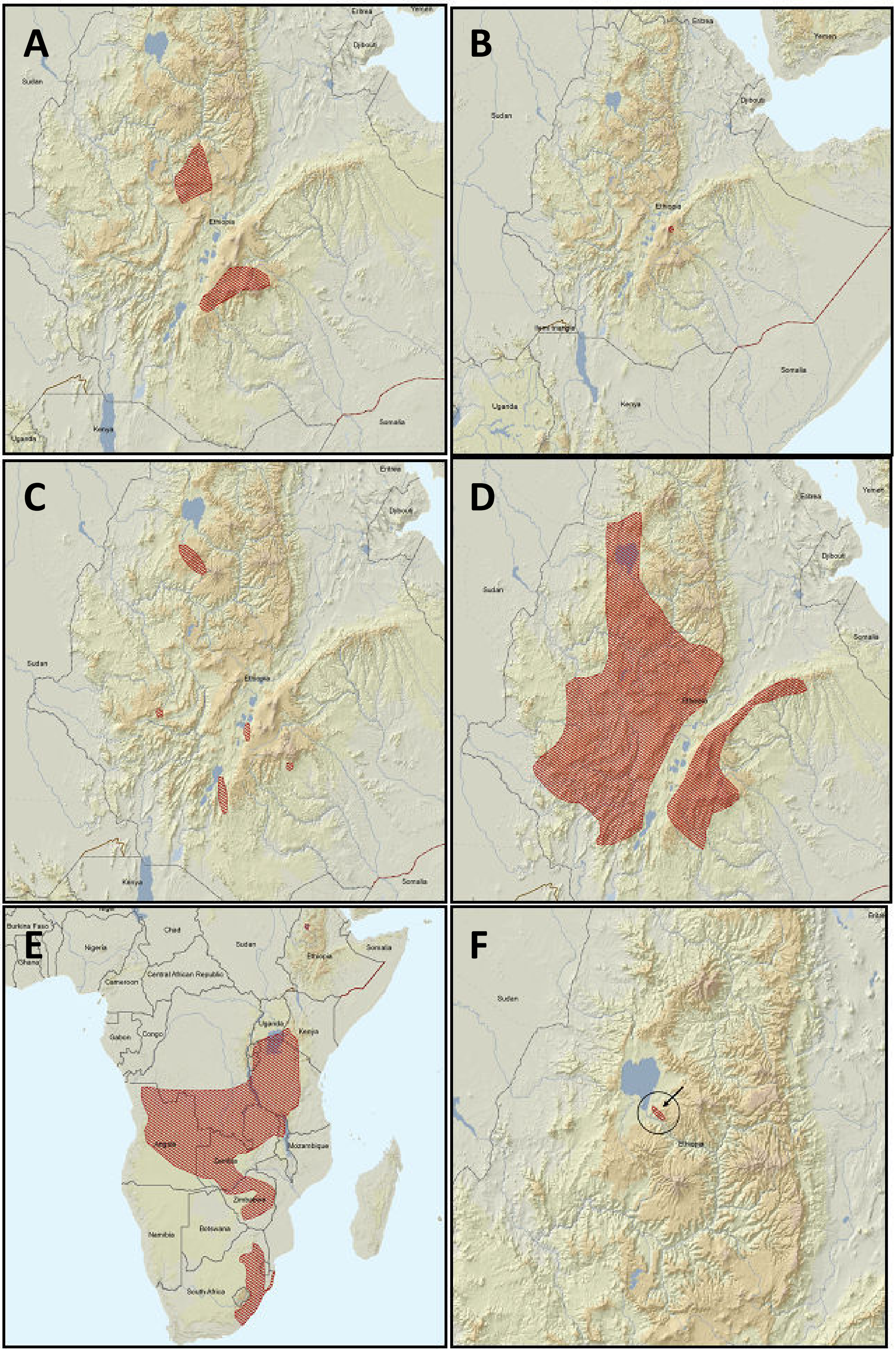
P) *Ptychadena wadei* and *P.* cf. *porosissima* (Gojam)

Ser. No.	Species	Northing (deg.)	Easting (deg.)	Elevation (m)	Geographic Category	Locality name	Source
1	*P. wadei*	11.4667	37.6333	1800	Northwest	Tisisat Falls	MJL 2001
2	*P. wadei*	11.5122	37.4921	1721	Northwest	Andassa, Ethiopia	AAM 2010
3	*P.* cf. *porosissima*	11.5833	37.4167	1850	Northwest	Tsana (= Tana)	MJL 2001
4	*P. wadei*	11.5833	37.4167	1850	Northwest	Bahar Dar	MJL 2001

Appendix 4.2. Maps of previously known distribution of species of *Leptopelis* in Ethiopia: A) *L. bocagii*, B) *L. gramineus*, C) *L. ragazzii*, D) *L. susanae*, E) *L. vannutellii*, F) *L. yaldeni*. (Source: IUCN, 2011).



Appendix 4.3. Maps of previously known distribution of the studied species of *Ptychadena* in Ethiopia: A) *P. cooperi*, B) *P. nana*, C) *P. erlangeri*, D) *P. neumanni*, E) *P. porosissima*, F) P. wadei, G) *P. anchietae*, H) *P. harenna*, I) *P. mascareniensis*, J) *P. filwoha* (Source: IUCN, 2011).



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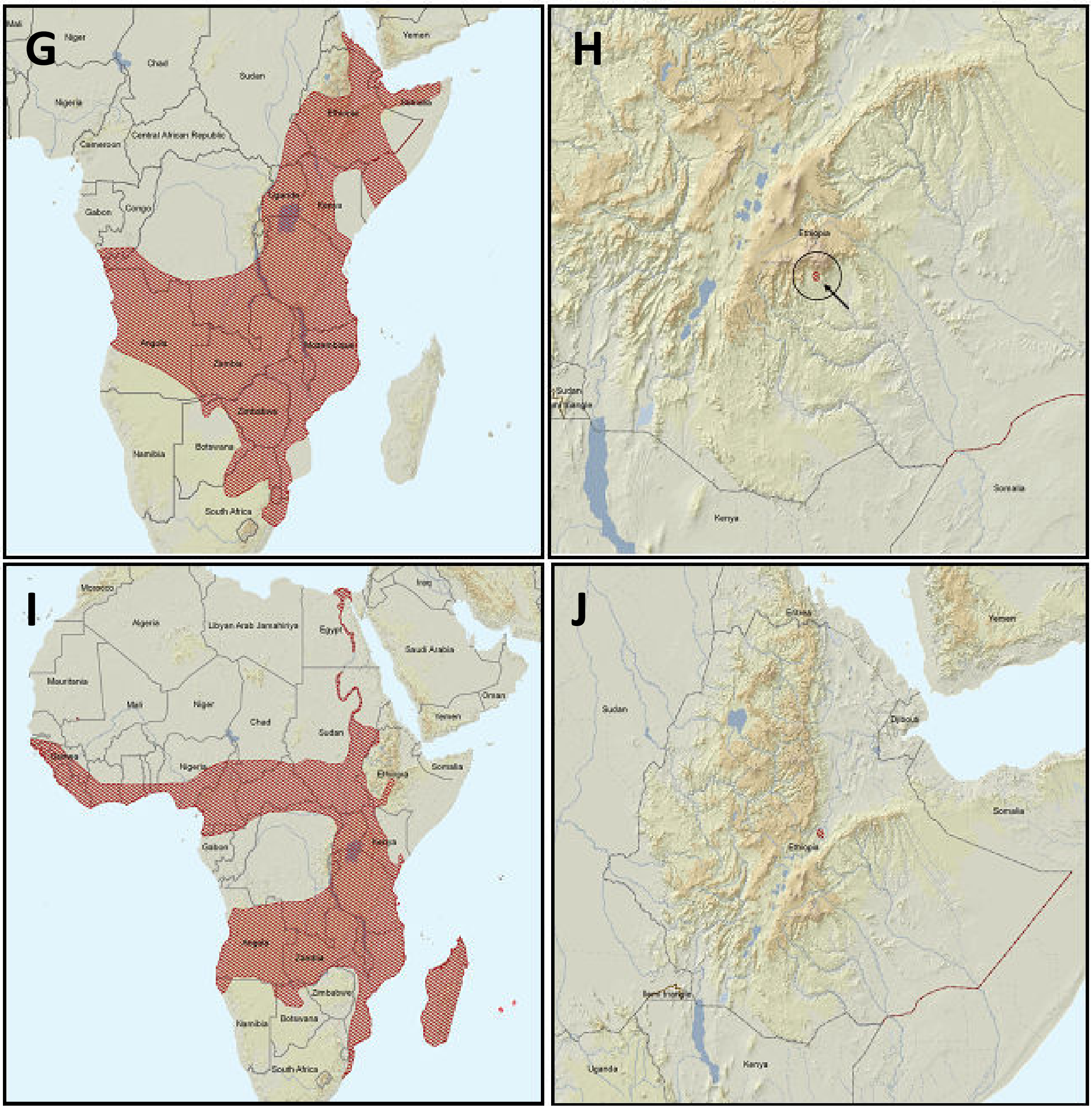

CURRICULUM VITAE

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PhD thesis: Amphibian Diversity, Distribution and Conservation in the Ethiopian Highlands: Morphological, Molecular and Biogeographic Investigation on *Leptopelis* and *Ptychadena* (Anura)

MSc: School of Graduate Studies, Addis Ababa University. Addis Ababa, 2004.

MSc thesis: The effect of Birbira, *Milletia ferruginea*, (Hochst.) Baker on some *Barbus* spp. in Gumara River (Lake Tana), Ethiopia

BSc: Department of Marine Biology, Asmara University. Asmara, 1992

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Systematics and biogeography of amphibians and fishes in Ethiopia, with focus on tree frogs (*Leptopelis*), ridged frogs (*Ptychadena*) and cyprinid fishes (*Varicorhinus*).

Assessment and conservation of faunal biodiversity, in particular threatened endemic amphibians, fishes, as well as aquatic and forest habitats.

Work Experience:

Previous positions:

2006 – 2008: **Collections Manager,** Zoological Natural History Museum, Addis Ababa University.

2003 – 2006: **Program Coordinator**, The Biological Society of Ethiopia; and **Editorial Assistant**, *Ethiopian Journal of Biological Sciences*.

1999 – 2001: **Senior Fisheries Biologist**, Fisheries Research and Training Center (FRTC), Bahir Dar, Ethiopia.

1992 – 1999: **Fisheries Expert**, Fogera Woreda Agricultural Office (South Gondar, Ethiopia).

Courses taught:

Vertebrate Zoology: Department of Biology Education, Addis Ababa University (April to July 2008).

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Fisheries Biology: Woreta Agricultural Training Center (South Gondar, Ethiopia), in two round programs.

Workshops Organized:

IVth International Conference of the Pan African Fish and Fisheries Association (PAFFA), Addis Ababa, Ethiopia, 2008.

Inaugural Conference of the Ethiopian Fisheries and Aquatic Sciences Association (EFASA), Addis Ababa, 2007.

16tth Annual Conference and Workshop of the Biological Society of Ethiopia on Biology Education and Research in Ethiopia: Status and beneficiaries, Addis Ababa, 2006.

“Sensitized Girls = Healthy Generation”: Sensitization and Mobilization Workshops on HIV/AIDS for Female Students from Addis Ababa University, Kotebe College of Teachers’ Education and Private Higher Education Institutions in Addis Ababa, The Biological Society of Ethiopia, Addis Ababa, 2004-2005.

15th Annual Conference and Workshop of the Biological Society of Ethiopia on HIV/AIDS and Associated Diseases, Addis Ababa, 2005.

14th Annual Conference and Workshop of the Biological Society of Ethiopia on Modernizing Agriculture: A way out of food insecurity?, Addis Ababa, 2004.

Membership:

The Biological Society of Ethiopia (BSE): Regular member, February 1999 to date.

Ethiopian Fisheries and Aquatic Sciences Association (EFASA): Founding member, 2007 to date.

Ethiopian Wildlife and Natural History Society (EWNHS). Member, 2007 to date.

Swiss Systematics Society (SSS). Member, 2010 to date.

Society for the Study of Amphibians and Reptiles (SSAR). Member, 2011 to date.

IVth International Conference of the Pan-African Fish and Fisheries Association (PAFFA): Member of the Local Organizing Committee, Addis Ababa, September 2008.

Participation:

SSS Day 2010. Annual Conference of the Swiss Systematics Society. November 2010, Lugano.

Symposium on Biodiversity Hotspots: Evolution and Conservation. March 2009, Luxembourg.

Attended on various research review meetings, seminars, workshops and TV talk-shows held in Ethiopia during the period 1992 to date.

Publications:

Abebe Ameha Mengistu (2011). Amphibian Diversity, Distribution and Conservation in the Ethiopian Highlands: Morphological, Molecular and Biogeographic Investigation on *Leptopelis* and *Ptychadena* (Anura). Unpublished PhD thesis, Section of Biogeography, Department of Environmental Sciences, University of Basel, Basel.

D.J. Gower, T.M. Doherty-Bone, R.K. Aberra, **A. A. Mengistu**, S. Schwaller, M. Menegon, R. de Sá, S.A. Saber, A.A. Cunningham and S.P. Loader (in press). High prevalence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) across multiple taxa and localities in the highlands of Ethiopia.

David J. Gower, Roman Kassahun Aberra, Silvia Schwaller, Malcolm J. Largen, Ben Collen, Steven Spawls, Michele Menegon, Breda M. Zimkus, Rafael de Sá, **Abebe A. Mengistu**, Robin Moore, Samy Saber and Simon P. Loader (in press). Long-term data for endemic frog genera reveal potential conservation crisis in the Bale Mountains, Ethiopia. *Oryx*.

Abebe A. Mengistu, P. Nagel, A. Getahun, S. Saber and S. Loader (submitted). Update on Amphibian Diversity, Distribution and Conservation in the Ethiopian Highlands: with emphasis on *Leptopelis* and *Ptychadena* (Anura).

Abebe A. Mengistu, Alemu Assefa and Desta Kassa (submitted). Abundance, length-weight relationship and breeding season of *Varicorhinus beso* in Lake Tana, Ethiopia.

Frank Weinsheimer, **Abebe A. Mengistu** and Dennis Rödder (2010). Potential distribution of threatened *Leptopelis* spp. (Anura, Arthroleptidae) in Ethiopia derived from climate and land-cover data. *Endangered Species Research* 9: 117–124.

Simon Loader, **Abebe Mengistu**, Silvia Schwaller, David Gower, Peter Nagel, Abebe Getahun, Samy Saber and Roman Kassahun (2009). Are Ethiopian Highlands changing? Amphibians as ecosystem indicators. Mountain Forum Bulletin, Vol. 9 Issue 2, July 2009. Pages 5-6.

Abebe A. Mengistu, S. Loader, A. Getahun, S. Saber and P. Nagel (2009). Amphibian Diversity and Conservation in the Ethiopian Highlands. Abstract and Poster. Symposium on Biodiversity Hotspots: Evolution and Conservation. March 2009, Luxembourg.

Abebe Ameha Mengistu (2007). Report on Faunal Biodiversity. In: Rapid Biodiversity Assessment of Saja, Mankira and Boqa (Adiyo) Forests in Kaffa, Ethiopia. *Ethiopian Wildlife and Natural History Society and FAO.* Addis Ababa, October 2007.

Abebe Getahun and **Abebe A. Mengistu** (Eds.) (2006). Field and Laboratory Techniques for Zoological Museum Collections: Manual on Methods of Collection, Preservation and Management. The Zoological Natural History Museum, Addis Ababa University, Addis Ababa.

Abebe Ameha, Belay Abdissa and Teferi Mekonnen (2006). Abundance, length-weight relationship and breeding season of *Clarias gariepinus* in Lake Tana, Ethiopia. *SINET: Ethiopian Journal of Science* 29(2): 171-176.

Abebe Ameha and Alemu Assefa (2002). The fate of the barbs of Gumara River, Ethiopia. *SINET, Ethiopian Journal of Science* 25(1): 1-18. Abstract published in the *Proceedings of the Third International Conference of the Pan African Fish and Fisheries Association (PAFFA)*, Cotonou (Benin), November 2003.

Abebe A. Mengistu (2001). Fishery Resources in Lake Tana. In: *Proceedings of the Wetland Awareness Creation and Activity Identification Workshop in Amhara National Regional State. EWNRA.* Bahir Dar.

Abebe Ameha and Adugna Beyene (1996). The Importance of the Fisheries on Gumara River: Focus on the barbs. In: *Lake Fisheries Development Project Working Paper No. 21,* Addis Ababa, (National Fisheries Seminar, November 1995, Ziway).

Edited and designed publications:

Abebe Getahun and Eshete Dejen (in press). Fishes of Lake Tana: A Field Guide. Nile Basin Initiative, Addis Ababa.

FFE (2006). The 2006 Flood Disaster vis-à-vis Environmental Degradation in Ethiopia: Report on Studies for Selected Sites. Forum for Environment, Addis Ababa.

FFE (2006). Public Meetings on Non-timber Forest Products of Ethiopia. Proceedings, Forum for Environment, Addis Ababa.

FFE (2005). Public Meetings on Science and Technology to Save our Trees: Deliberations on Salient (Renewable) Energy Issues in Ethiopia. Proceedings, Forum for Environment, Addis Ababa.

ALF (2006). *A Decade of ALF*, December 2006; Poster on the 10th Aklilu Lemma Fund for Scholarships and Research. Addis Ababa University.

EJBS (March 2005). *Eth. J. Biol. Sci.*, Vol. 4 No. 1.

EJBS (September 2004). *Eth. J. Biol. Sci.*, Vol. 3 No. 2.

EJBS (March 2004). *Eth. J. Biol. Sci.*, Vol. 3 No. 1.

BSE (2005). Sensitization and Mobilization on HIV/AIDS. Proceedings, The Biological Society of Ethiopia, Addis Ababa.

FFE (2004). Public Meetings on Integrated Forest Policy Development in Ethiopia. Proceedings, Forum for Environment, Addis Ababa.

BSE (2004). Modernizing Agriculture: A way out of food insecurity? Proceedings, The Biological Society of Ethiopia, Addis Ababa.

Post-Graduates of Biology: Class of 2004 (with Abstracts of M.Sc. Theses), Bulletin, Department of Biology, Addis Ababa University, 2004.

BSE (2003). Wetlands and Aquatic Resources of Ethiopia. Proceedings, The Biological Society of Ethiopia, Addis Ababa.

Grants Received:

PhD dissertation grant: Freiwilligen Akademischen Gesellschaft, Basel (October-November 2011).

Travel grant: SYNTHESYS: Synthesis of Systematic Resources, European Union-funded Integrated Activities Grant (May 2010).

Travel grant: Ernst Mayr Travel Grants, Museum of Comparative Zoology, Harvard University (January 2010).

Field research grant: National Geographic Society (July-August 2009).

PhD scholarship: University of Basel, and Stipendiencommission für Nachwuchskräfte aus Entwicklungsländern (Basel-Stadt, Switzerland) (September 2008 – November 2011).

MSc thesis research grant: School of Graduate Studies, Addis Ababa University (July 2002 – June 2003).

Research grant: Ethiopian Science and Technology Commission (September 2000 – August 2001).

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