At the crossroads of two biodiversity hotspots; the biogeographic patterns of Shimba Hills, Kenya

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Introduction

Biogeography

Until Wallace's pivotal contribution in 1876, our understanding of animal and plant species distribution was generally based on non-scientific principals. With Wallace (1876), the distribution of organisms could be understood from a historical perspective and this contribution heralded the birth of the science of biogeography. Since its original conception, biogeography has broadened its understanding from a purely historical science to incorporate current determinants of the patterns of species distribution. Biogeography seeks to answer the questions of why species are distributed where they are or put simply, why some areas have more species than others. Patterns of diversity distributions are determined by a number of factors, both current as well as historical. For example, environmental and geological history of an area (Crowe & Crowe, 1982; Fjeldsaå & Lovett, 1997; Ricklef, 2003; Dornelas, et al., 2006; Dimitrov et al., 2012), individual species ecology and physiology determines the ranges and abundance of species in an area (Duellman & Trueb, 1986; Hamilton, 1982; Hugget, 2004). Understanding patterns of species diversity also include taking into consideration dispersal ability and adaptability of species to past changes in the environment and how this influences the distribution of species through time (Farrel et al., 1992; Latham & Ricklefs, 1993). Therefore, historical and ecological processes both contribute to our understanding of biogeographic patterns.

The biogeographic field that focuses on historical causes of biodiversity patterns is known as historical biogeography. It is concerned with evolutionary processes spanning millions of years back in time. More recent historical determinants of biodiversity patterns at the intra-specific level can also be investigated, and this is called phylogeography (Avise et al., 1987). Phylogeography is a branch of historical biogeography that deals with the analysis of the relationship between population genetic structure and geography (see also Avise, 2000; Arbogast & Kenagey, 2001; Avise, 2004). Phylogeographic studies aim to characterise the roles played by recent environmental and historical factors that shaped the present diversity patterns (Zink, 2002; Lomolino et al., 2004). Such studies employ the use of molecular markers to examine both recent and deeper phylogeographic history of a species or an area (Avise, 1987, Avise, 2000; Zink, 2007). Phylogeographic studies were previously based on mitochondrial molecular markers as these genes are rapidly evolving and hence suitable for examining events in the recent past (Avise, 1987). However latest advances in the discipline of molecular biology has seen a rise in the use of other markers, from partial sequences such as chloroplast from plants and nuclear genes which are slow evolving and better suited for deeper phylogeographic history (Janzen et al., 2002), to genome wide comparisons (Davey & Blaxter, 2010; Macher et al., 2015). Phylogeographic studies may be conducted on single wide ranging species to understand how genetic diversity is distributed within its range (Zink, 2000) while the study of genetic diversity of several wide ranged co-occurring species constitute comparative phylogeography (Bermingham & Moritz, 1998). Comparative phylogeography investigates if members of a community have responded in concert to historical biogeographic factors and therefore if present genetic patterns can be explained by particular geographic processes (Zink, 1996; Avise, 2004). Further, the availability of information on the evolution rates of various molecular markers has made it even possible to estimate dates of population separations, thus through comparative phylogeography, it is possible to reconstruct the recent biogeographic history of an area (Bermingham & Moritz, 1998).

For a long time phylogeography has been the main method through which genetic patterns within species has been investigated. However advancements in other related fields such as bioinformatics and molecular biology has seen the incorporation of other tools such as spatial data in phylogeographic analysis. The advancements in the field of Geographical Information System (GIS) for example have seen the incorporation of spatial information in various fields of studies where previously this was not possible. One such area is the application of Species Distribution Modelling (SDM) in phylogeographic interpretations (Carstens & Richards, 2006; Chan, et al., 2011). Species distribution models also known as bioclimatic models, estimate potential species distributions by deriving environmental envelopes from distributions and projecting into an interpolated potential climate of an area (Pearson, 2007; Waltari & Guralnick, 2009). These models are based on the assumption that the ecological niche of a species determines its distribution (Nogués-Bravo, 2009). Species distribution models are produced by combining current environmental parameters and known occurrence data of a species fitted to a model to predict current distributions (Hugall, et al., 2002; Elith & Leathwick, 2009). When projected to past climates, SDM can also be used to generate potential suitable habitats in past climatic conditions, i.e., the paleo-distributions of species (Hugall, et al., 2002; Carstens & Richards, 2007). Paleo-distribution modelling have proved useful as alternative ways of establishing historical factors determining the current genetic structuring in species (Elith & Leathwick, 2009). This is true especially in taxa that lack good fossil representation like amphibians. Paleo-distribution modelling has been used extensively to provide *a priori* hypotheses or validate results from phylogeographic analysis. Paleo-distribution models shed light on the effects of past climatic conditions on the current patterns of species distribution therefore providing independent means to understand the current phylogeographic patterns of a species or an area (see Carstens & Richards, 2007; Waltari et al., 2007; Buckley et al., 2010; Ahmadzadeh et al., 2013). In addition, for studies involving co-distributed species, concordance in phylogeographic structures are often interpreted to mean a concerted response to a similar vicariance events with the assumption that the species must have also been co-distributed in the past and therefore SDM provides ways to test such assumptions (Guissan & Thuiller, 2005; Miller, 2010).

Amphibians as exemplar taxa for understanding phylogeographic history

Amphibians are favourable candidates for phylogoegraphic studies because of a number of physiological and ecological reasons. They are less vagile and have high affinity/philopatry to their breeding sites leading to populations with highly structured genetics over short geographical distances (Avise, 2004; Zeisset & Beebee, 2008). Amphibians are sensitive to small changes in the climate which may be attributed to divergence within some species (Graham et al., 2004; Buckley & Jets, 2007) and have diverse physiological adaptations (Duellman & Trueb, 1986) that enable them to respond idiosyncratically to environmental and geologic processes. Additionally amphibians are relatively common and easily sampled in breeding sites during the wet periods (Duellman & Trueb, 1986). Moreover amphibian phylogeography has been demonstrated as suitable for understanding historical aspects of species distribution (Zeisset & Beebee, 2008). Specifically for this study amphibians were selected due to the presence of wide spread species in our study site and adjacent areas which are important in establishing the historical genetic exchange among the sites or areas. In addition the apparently mixed assemblages of amphibians recently reported in Shimba Hills of Kenya-SHK (Bwong et al., *in press*) make them good model taxon for understanding the biogeographic history of Shimba Hills.

The Shimba Hills

The Shimba Hills of Kenya (here after SHK) is geographically located at the cross roads of two major biodiversity hotspots; the Coastal Forests of Eastern Africa (hereafter CFEA) and the Eastern Afromontane Biodiversity Region (here after EABR) specifically the neighbouring Eastern Arc Mountains (here after EAM) (Myers et al., 2000; Mittermier et al., 2004; Bwong et al., 2014) (Figure 1). SHK biodiversity has been associated with both the coastal forests (Azeria, et al., 2007; Burgess & Clarke, 2000) and also the Eastern Arc Mountains by some authors (see Lovett, 1998; Blackburn & Measey, 2009), while others have confirmed lack of any clear cut boundaries (Bwong et al., *in press*). Results from old and recent collections of its flora and fauna indicate that SHK harbours species associated with both EAM and CFEA as well as taxa that have affinity with west African Guineo-Congolian forest (Burgess & Clarke, 2000 and references therein; Malonza & Measey, 2005; Bwong et al., *in press*). Furthermore a detailed plant checklist of Shimba Hills by Luke (2005) pointed out the high diversity of flora in this area. Luke (2005) hypothesized that close proximity of SHK to the Usambara Mountains (part of the EABR) through similar climatic history and altitude range could be responsible for its high floral diversity. However the link between SHK to the Usambara Mountains has never been appropriately tested using phylogenetic approaches.

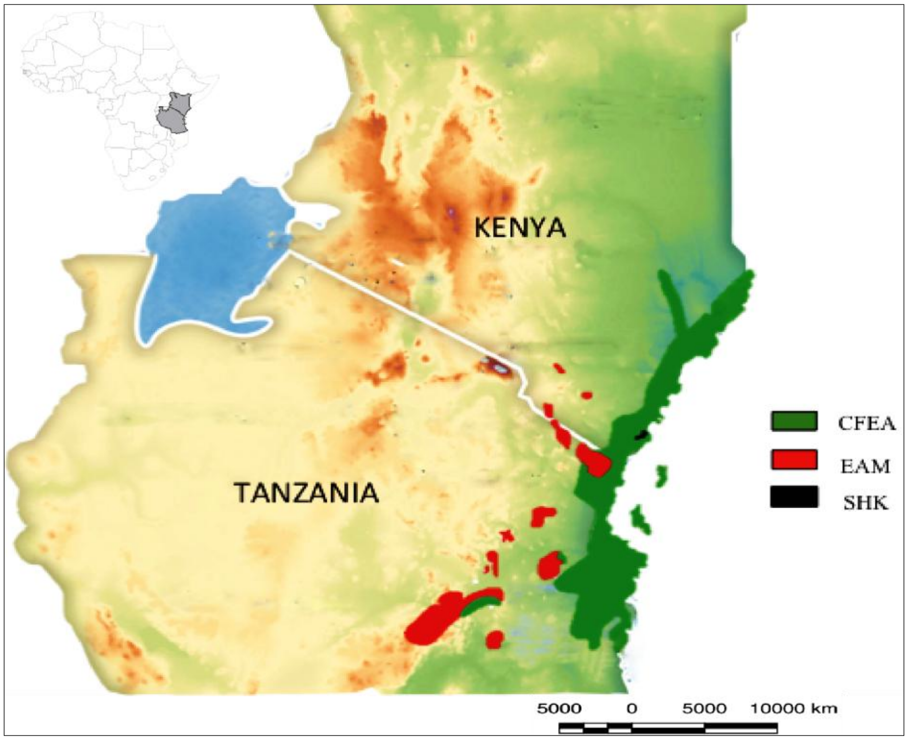

Fig. 1: Showing the cross road position of the Shimba Hills in between the Coastal Forests of Eastern Africa (CFEA) and the Eastern Arc Mountains (EAM). Map modified from https://www.travcoa.com.

Bwong et al. (*in press*) recently provided a comprehensive list of the amphibian fauna of the Shimba Hills National Reserve and discussed the biogeographic questions concerning the area. Based on the mixture of assemblages (Eastern Afromontane, Coastal forest and widespread faunas) and relative proportions of these species, the biogeographic history was speculated to be complex. It is unclear whether the area is composed of mainly new or old divergences due to the lack of phylogenetic data.

Bwong et al. (*in press*) stated this uncertainty “Do all the species (in the Shimba Hills National Reserve) show recent patterns of colonization to this area – or have some or all habitats existed for some time, favouring conditions that might have produced the stability to harbour endemic species.” For amphibians, with only one true endemic species (*Hyperolius rubrovermiculatus* Schiøtz, 1975) known (Bwong et al., *in press*) the patterns indicate a more recent history but this has yet to be tested across all species using appropriate phylogenetic and spatial data. Several questions remain to be answered with regard to the biogeographic history of SHK. Most importantly, it is unclear whether SHK is special as a repository of diversity or not or whether it has been stable for all the taxa currently found inhabiting the area.

Objectives

Biogeographic studies in the tropics are fewer compared to other regions despite the fact that diversity is higher in the tropics than elsewhere (Hewitt, 2004; Mittelbach et al., 2007). Thus the tropics provide opportunities for cross-taxonomic studies especially in understanding the history of its great diversity. African tropical biodiversity patterns in particular remain poorly understood and in some areas remain almost completely unknown (Hewitt, 2004; Duminil et al., 2013). One such area is Shimba Hills in coastal Kenya. The cross roads position of SHK between two biodiversity hotspots and the mixed assemblage of taxa present therein makes it an interesting area for a better understanding of the patterns of biodiversity distribution across the two hotspots. To date no study had been conducted to establish the biogeographical affiliation of SHK and its relationship to the two hotspots. Biogeographic studies incorporating historical approaches are not known from the entire coastal forests of Kenya including the SHK. Understanding the biogeographic history of SHK would be beneficial for current and future conservation activities especially in the wake of biodiversity conservation challenges such as climate change. It is against this background that the current study was undertaken to investigate patterns and timings of genetic exchanges between SHK and adjacent CFEA and EAM.

Chapter overview

Chapter 1: Amphibian diversity in Shimba Hills National Reserve, Kenya: A comprehensive list of specimens and species.

Authors: **Beryl A. Bwong**, Joash O. Nyamache, Patrick K. Malonza, Dominick V. Wasonga, Jacob M. Ngwava, Christopher D. Barratt, Peter Nagel & Simon P. Loader.

Status: Manuscript accepted for publication (Journal of East African Natural History).

Shimba Hills National reserve is a well known conservation area along the Kenyan coast. However despite several herpetological surveys in the area, no publication exists that consolidates the known amphibian biodiversity. We used both fieldwork as well as secondary data to compile an authoritative species list, the distribution of these species within the reserve as well as the habitat where they occur.

Chapter 2: Genetic, morphological and ecological variation in the congeners *Hyperolius mitchelli* Loveridge, 1953 and *Hyperolius rubrovermiculatus* Schiøtz, 1975 from East Africa.

Authors**: Beryl A. Bwong**, Lucinda P. Lawson, Christopher D. Barratt, Joash O. Nyamache, Michele Menegon, Daniel M. Portik, Patrick K. Malonza, Hendrik Müller, Peter Nagel & Simon P. Loader.

Status: Manuscript in preparation for resubmission (Acta Herpetologica).

The taxonomic status of *Hyperolius rubrovermiculatus* Schiøtz, 1975, the only amphibian endemic to the Shimba Hills, has been in question since the time of its description. The species was thought to be a subspecies of *H. mitchelli* Loveridge, 1953 (Channning & Howell, 2006) a wide ranging reed frog from northern Tanzania to Mozambique and Zimbabwe. We used integrated taxonomic methods including, morphological, molecular, acoustics and species distribution modelling to affirm the taxonomic status of *H. rubrovermiculatus.* In addition we propose description of a new species from the neighbouring Usambara, Nguu and Nguru Mountains in Tanzania.

Chapter 3: Three new species of *Callulina* (Amphibia: Anura: Brevicepitidae) from East Africa with conservation and biogeographical considerations for the whole genus.

Authors: **Beryl A. Bwong**, Alan Channing, Michele Menegon, Joash Nyamache Patrick K. Malonza, Christopher D. Barratt, Gabriela B. Bittencourt-Silva, Elena Tonelli, Peter Nagel & Simon P. Loader.

Status: Drafted Manuscript (Target Journal: Zootaxa).

A number of Eastern Arc endemic species have been recorded in the SHK. One of these is the Brevicipitid frog called *Callulina*. The only known *Callulina* record in SHK prior to this thesis was a single specimen collected in 1961 held at the American Museum of Natural History. Based on its morphological features this species was speculated to be either a *Callulina kisiwamsitu* or *C. stanleyi* based on preliminary morphometrics analysis (Loader et al., 2010). Two specimens were rediscovered during the current study and compared with congeneric species across the Eastern Arc Mountains. We used morphological and molecular methods to confirm the taxonomic status of SHK *Callulina* and in addition we propose description of three new *Callulina* species from the Eastern Arc Mountains in Tanzania.

Chapter 4: Phylogeography of amphibians of Shimba Hills, Kenya.

Authors: **Beryl A. Bwong**, Christopher D. Barratt, Patrick K. Malonza, Joash Nyamache, Peter Nagel & Simon P. Loader.

Status: Drafted manuscript (Target Journal: Molecular Phylogenetics and Evolution).

This chapter addresses the main research questions this thesis seeks to answer. A combination of molecular and spatial analysis were employed in order to understand phylogeographic patterns of SHK amphibians in relation to the adjacent Coastal Forest of East Africa and Afromontane Biodiversity Region and the factors that helped to shape the observed patterns.

Additional outputs

Peer Reviewed

Barratt, C.D., **Bwong, B.A**., Ostein, R.E., Rosauer, D.F., Doggart N., Nagel, P., Kissling, W.D & Loader, S.P. 2017. Environmental correlates of phylogenetic endemism in amphibians and conservation of refugia in the Coastal Forests of Eastern Africa. *Diversity and distributions* **23:**875-887.

Non- peer reviewed

Bwong, B.A., Malonza, P.K, Wasonga, D.V., Nagel, P., Nyamache, J.O. & Loader, S.P. 2014. At a biogeographical crossroads: Amphibian paradise in Shimba Hills of Kenya. *Froglog* **22**: 72–73.

References

Ahmadzadeh, F., Carretero, M.A., Rödder, D., Harris, D.J., Freitas, S.N., Perera, A. & Böhme, W. 2013. Inferring the effects of past climate fluctuations on the distribution pattern of Iranolacerta (Reptilia, Lacertidae): Evidence from mitochondrial DNA and species distribution models. *Zoologischer Anzeiger* **252**: 141–148. http://doi.org/10.1016/j.jcz.2012.05.002.

Arbogast, B.S. & Kenagy, G.J. 2001. Comparative phylogeography as an integrative approach to historical biogeography. *Journal of Biogeography* **28**: 819-825.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigell, C.A. et al. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* **18**: 489-522.

Avise, J.C. 2000. *Phylogeography: The history and formation of species*. Harvard University Press, Cambridge, MA.

Avise, J.C. 2004. *Molecular markers, natural history and evolution*. 2nd ed. Sinauer Associates, Inc. Sunderland, MA.

Azeria, E.T., Sanmartín, I., As, S., Carlson, A. & Burgess, N. 2007. Biogeographic patterns of the East African coastal forest vertebrate fauna. *Biodiversity and Conservation* **16**: 883–912. http://doi.org/10.1007/s10531-006-9022-0.

Bermingham, E. & Moritz, C. 1998. Comparative phylogeography: concepts and applications. *Molecular Ecology* **7**: 367–369. http://doi.org/10.1046/j.1365-294x.1998.00424.x.

Blackburn, D.C. & Measey, G.J. 2009. Dispersal to or from an African biodiversity hotspot? *Molecular Ecology* **18**: 1904–1915. http://doi.org/10.1111/j.1365-294X.2009.04156.x.

Burgess, N.D. & Clarke, G.P. (eds) 2000. *Coastal forests of Eastern Africa,* Xiii +443pp. IUCN, Gland, Switzerland and Cambridge, UK.

Bwong , B.A., Nyamache, J.O., Malonza, P.K., Wasonga, D.V., Ngwava, J,M., Barratt, C.D., Nagel, P. & Loader, S.P. (*in press).* Amphibina diversity of Shimba Hills National Reserve, Kenya; a comprehensive list of specimens and species. *Journal of East African Natural History*.

Bwong, B.A., Malonza, P.K., Wasonga, V.D., Nyamache, J.O., Nagel, P. & Loader, S.L. 2014. At a biogeographical crossroads: Amphibian paradise in Shimba Hills of Kenya. *Froglog* **22**: 72-73.

Buckley, L.B. & Jetz, W. 2007. Environmental and historical constraints on global patterns of amphibian richness. *Proceedings of the Royal Society of London B: Biological Sciences* **274**: 1167-1173.

Buckley, T.R., Marske, K. & Attanayake, D. 2010. Phylogeography and ecological niche modelling of the New Zealand stick insect *Citarchus hookeri* (white) support survival in multiple coastal refugia. *Journal of Biogeography* **37**: 682–695. http://doi.org/10.1111/j.1365-2699.2009.02239.x.

Carstens, B.C. & Richards, C.L. 2007. Integrating coalescent and ecological niche modeling in comparative phylogeography. *Evolution* **61**: 1439–1454. http://doi.org/10.1111/j.1558-5646.2007.00117.x.

Chan, L.M., Brown, J.L. & Yoder, A.D. 2011. Integrating statistical genetic and geospatial methods brings new power to phylogeography. *Molecular Phylogenetics and Evolution*, **59**: 523-537.

Channing, A. & Howell, K.M. 2006. *Amphibians of East Africa*. Cornell University Press Ithaca, New York.

Crowe, T.M. & Crowe, A.A. 1982. Patterns of distribution, diversity and endemism in Afrotropical birds. *Journal of Zoology*, *London* **198**: 417–442.

Davey, J.W. & Blaxter, M.L. 2010. RADSeq: next-generation population genetics. *Briefings in functional genomics* **9**: 416-423.

Dimitrov, D., Nogués-Bravo, D. & Scharff, N. 2012. Why do tropical mountains support exceptionally high biodiversity? The Eastern Arc Mountains and the drivers of *Saintpaulia* diversity. *PLoS ONE* **7**(11). http://doi.org/10.1371/journal.pone.0048908.

Dornelas, M., Connolly, S.R. & Hughes, T.P. 2006. Coral reef diversity refutes the neutral theory of biodiversity. *Nature* **440**: 80-82.

Duellman, W.E. & Trueb, L. 1986. *Biology of Amphibians.* Mc-Graw Hill Inc. New York.

Duminil, J., Brown, R.P., Ewédjè, E.E.B., Mardulyn, P., Doucet, J.L. & Hardy, O.J. 2013. Large-scale pattern of genetic differentiation within African rainforest trees: insights on the roles of ecological gradients and past climate changes on the evolution of *Erythrophleum* spp (Fabaceae). *BMC Evolutionary Biology* **13**: 195.

Elith, J. & Leathwick, J.R. 2009. Species distribution models: Ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution and Systematics* **40**: 677–697. http://doi.org/10.1146/annurev.ecolsys.110308.120159.

Farrell, B.D., Mitter, C. & Futuyma, D.J. 1992. Diversification at the insect-plant interface. *BioScience* **42**: 34-42.

Fjeldsaå, J. & Lovett, J.C. 1997. Geographical patterns of old and young species in African forest biota: the significance of specific montane areas as evolutionary centres. *Biodiversity and Conservation* **6**: 325–346. http://doi.org/10.1023/a:1018356506390.

Graham, C.H., Ron, S.R., Santos, J.C., Schneider, C.J. & Moritz, C. 2004. Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. *Evolution* **58**: 1781-1793.

Guisan, A. & Thuiller, W. 2005. Predicting species distribution: Offering more than simple habitat models. *Ecology letters* **8**: 993-1009.

Hamilton, A.C.1982. *Environmental history of Eastern Africa; a study of the Quaternary*. Academic press Inc (London) Ltd, London NW1.

Hewitt, G.M. 2004. The structure of biodiversity – insights from molecular phylogeography. *Frontiers in Zoology*, **1**: 4. http://doi.org/10.1186/1742-9994-1-4.

Hugall, A., Moritz, C., Moussalli, A. & Stanisic, J. 2002. Reconciling paleodistribution models and comparative phylogeography in the Wet Tropics rainforest land snail *Gnarosophia bellendenkerensis* (Brazier 1875). *Proceedings of the National Academy of Sciences of the United States of America* **99**: 6112–6117. http://doi.org/10.1073/pnas.092538699.

Hugget, R.J. 2004. *Fundamentals of biogeography.* Routledge Fundamentals of

Physical Geography, New York.

Janzen, F.J., Krenz, J.G., Haselkorn,T.S. & Brodie, E.D. 2002. Molecular phylogeography of common garter snakes (*Thamnophis sirtalis*) in western North America . *Molecular Ecology* **11**: 1739–1751.

Latham, R.E. & Ricklefs, R.E. 1993. Global patterns of tree species richness in moist forests: Energy-diversity theory does not account for variation in species richness. *Oikos* (325-333).

Loader, S.P., Gower, D.J., Ngalason, W. & Menegon, M. 2010. Three new species of *Callulina* (Amphibia: Anura: Brevicipitidae) highlight local endemism and conservation plight of Africa's Eastern Arc forests. *Zoological Journal of the Linnean Society* **160**: 496–514. http://doi.org/10.1111/j.1096-3642.2010.00652.x.

Lomolino, M.V., Sax D.F. & Brown J.H. 2004. *Foundations of biogeography classic papers with commentaries*. University of Chicago Press, Chicago, Illinois.

Lovett, J.C. 1998. Importance of the Eastern Arc Mountains for vascular plants. *Journal of East African Natural History* **87**: 59–74. http://doi.org/10.2982/0012-8317(1998)87[59:IOTEAM]2.0.CO;2.

Luke, Q. 2005. Annotated checklist of the plants of the Shimba Hills, Kwale District, Kenya. *Journal of East African Natural History* **94**: 5–120. http://doi.org/10.2982/0012-8317(2005)94[5:ACOTPO]2.0.CO;2.

Macher, J.-N., Rozenberg, A., Pauls, S.U., Tollrian R., Wagner, R. & Leese, F. 2015. Assessing the phylogeographic history of the montane caddisfly *Thremma gallicum* using mitochondrial and restriction-site-associated DNA (RAD) markers. *Ecology and evolution* **5**: 648-662.

Malonza, P.K. & Measey, G.J. 2005. Preliminary survey results on the status of amphibians and reptiles of Shimba Hills National Reserve, Kenya. Unpublished report submitted to the Kenya Wildlife Service and National Museums of Kenya 22pp.

Miller, J. 2010. Species distribution modeling. *Geography Compass* **4**: 490-509.

Mittelbach, G.G., Schemske, D.W., Cornell, H.V., Allen, A.P., Brown, J.M., Bush, M.B., Harrison, S.P., Hurlbert A.H., Knowlton N., Lessios, H.A., McCain, C.M., McCune, A.R., McDade, L.A., McPeek, M.A., Near, T.J., Price, T.D., Ricklefs, R.E., Roy, K., Sax, D.F., Schluter, D., Sobel, J.M., & Turelli M. 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecology letters* **10:** 315-331.

Mittermier, R.A., Gill, P.R., Hoffman, M., Pilgrim, J., Brooks, T., Mittermeier, C.G., Lamoreux, J. & da Fonesca, G.A.B. 2004. Hotspots revisited: Earth's biologically richest and most threatened terrestrial ecoregions. Connex, Mexico City.

Myers, N., Mittermeier, R.A., da Fonseca, G.A.B. & Kent, J. 2002. Biodiversity hotspots for conservation priorities. *Nature* **403**: 853–58. doi:10.1038/35002501.

Nogués-Bravo, D. 2009. Predicting the past distribution of species climatic niches. *Global Ecology and Biogeography* **18**: 521–531. http://doi.org/10.1111/j.1466-8238.2009.00476.x.

Pearson, R.G., Raxworthy, C.J., Nakamura, M. & Townsend, P.A. 2007. Predicting species distributions from small numbers of occurrence records: A test case using cryptic geckos in Madagascar. *Journal of Biogeography* **34**: 102–117. http://doi.org/10.1111/j.1365-2699.2006.01594.x.

Ricklefs, R.E. 2004. A comprehensive framework for global patterns in biodiversity. *Ecology letters* **7**: 1-15.

Wallace, A.R. 1876. *The geographical distribution of animals*. Harper & Brothers publishers, New York, Franklin Square.

Waltari, E. & Guralnick, R.P. 2009. Ecological niche modelling of montane mammals in the Great Basin, North America: Examining past and present connectivity of species across basins and ranges. *Journal of Biogeography* **36**: 148–161. http://doi.org/10.1111/j.1365-2699.2008.01959.x.

Waltari, E., Hijmans, R.J., Peterson, A.T., Nyári, Á.S., Perkins, S.L. & Guralnick, R.P. 2007. XLocating pleistocene refugia: Comparing phylogeographic and ecological niche model predictions. *PLoS ONE,* **2**(7). http://doi.org/10.1371/journal.pone.0000563.

Zeisset, I. & Beebee, T.J.C. 2008. Amphibian phylogeography: A model for understanding historical aspects of species distributions. *Heredity* **101**: 109–119. http://doi.org/10.1038/hdy.2008.30.

Zink, R.M. 1996. Comparative phylogeography in North American birds. *Evolution* **50**: 308-317.

Zink, R.M., Barrowclough, G.F., Atwood, J.L. & Blackwell-Rago, R.C. 2000. Genetics, taxonomy and conservation of the threatened California gnatcatcher. *Conservation Biology* **14**5: 1394-1405.

Zink, R.M. 2002. Methods in comparative phylogeography and their application to studying evolution in the north american aridlands. *Integrative and Comparative Biology* **42**: 953–959. http://doi.org/10.1093/icb/42.5.953.

Zink, R.M. 2007. Ecological exchangeability versus neutral molecular markers: the case of the great tit. *Animal Conservation* **10**: 369-373.

Chapter 1

Amphibian diversity in Shimba Hills National Reserve, Kenya: A comprehensive list of specimens and species.

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Manuscript *in press* (Journal of East African Natural History).

Amphibian diversity in Shimba Hills National Reserve, Kenya: A comprehensive list of specimens and species.

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Abstract

We present the first annotated amphibian checklist of Shimba Hills National Reserve (SHNR). The list comprises of 30 currently known amphibians (28 anurans and two caecilians), which includes 11 families and 15 genera. In addition, individual records per species, distribution in the reserve and brief remarks about the species are presented. The checklist is based on information from museum collections, field guides, unpublished reports and newly collected field data. We are able to confirm the presence of two Eastern Afromontane species in the SHNR: *Scolecomorphus* cf. *vittatus* and *Callulina* cf. *kreffti*. The latter has not been recorded since the original collection of a single specimen over 50 years ago. SHNR contains the highest number of amphibian species of any known locality in Kenya (about 30% of the country's total number); therefore it is of national conservation importance. Finally, we briefly discuss the biogeography of the SHNR and its connections to nearby biogeographic regions.

Keywords: coastal forests, checklist, zoogeography, amphibians, Shimba Hills

Introduction

The coastal forests of Kenya are part of the Coastal Forests of Eastern Africa biodiversity hotspot famed for its high species diversity and endemism (Burgess *et al.*, 1998; Myers, 2000) (see figure 1). Despite the apparent importance of the coastal forests, an assessment of the biological diversity has not been evenly conducted for all taxa across all areas. Some pivotal contributions have attempted to synthesize known information, *e.g.* Burgess & Clarke's monumental book (Burgess & Clarke, 2000) and a review of coastal forests (Burgess *et al.,* 1998) but these treatments all indicate the paucity of knowledge and the need to expand our understanding of the Coastal Forests of Eastern Africa hotspot. The lack of information is particularly true for specific countries in Eastern Africa such as Kenya and Mozambique. In Kenya, some taxonomic groups have attracted attention *e.g.* mammals (Hoft & Hoft, 1995; Oguge *et al.*, 2004; McDonald & Hamilton, 2010), butterflies (Rogo & Odulaja, 2001; Lemann & Kioko, 2005), dragonflies (Clausnitzer, 2003) and plants (Schmidt, 1991; Luke, 2005) but most other groups have been largely ignored (*e.g.* non-flying insects, reptiles and amphibians). Furthermore, geographic sampling has been concentrated at only a few specific places *e.g.* Arabuko-Sokoke Forest, with other areas such as the Shimba Hills, being largely ignored.

Relatively few amphibian studies have been conducted in the coastal forests of Kenya, despite the fact that research was first initiated over 80 years ago (Loveridge, 1935; Howell, 1993). Loveridge's expedition of 1934 concentrated mainly on the northern coastal forest elements (*e.g.* Tana River and Witu), and a few areas further south such as Arabuko-Sokoke Forest (Loveridge, 1935). The oldest comprehensive reports of amphibians of any coastal Kenya forest, after Loveridge (1935), were prepared by Drewes (1992) and Chira (1993) both of which were focused on Arabuko-Sokoke and Gedi Forests. Over ten years later Malonza *et al.* (2006) reported on the biogeography of amphibians and reptiles of the Tana River Primate National Reserve, a gallery forest along the Tana River. These two more recent studies are also based on the northern coastal forests with little comprehensive sampling in southern coastal Kenyan forests. Some preliminary surveys and new species descriptions alerted herpetologists to the potential value of southern Kenyan coastal forests (Schiøtz, 1975; Malonza & Measey, 2005), however, basic information is lacking on amphibians across Kenya. This lack of comprehensive studies on amphibians, in a region characterised by high levels of single locality endemism (Myers *et al.*, 2000) is of high concern, particularly given the alarming rate at which natural habitats are being modified due to human pressure (Tabor *et al.*, 2010). Increasing the knowledge of biodiversity in this area is a priority and of major importance to conservation efforts.

Figure 1. Map of the historical coverage of the Coastal Forests of Eastern African showing the location of Shimba Hills National Reserve.

Shimba Hills National Reserve (SHNR), located on the south coast, is the second largest coastal forest in Kenya (figure 2). The area is a mixture of different forest types (Schmidt, 1991, Bennun & Njoroge, 1999; Luke, 2005) and savanna habitats (Burgess *et al.*, 2004). The area is particularly interesting because it is located between two biodiversity hotspots, the Coastal Forests of Eastern Africa and the Eastern Afromontane biodiversity hotspot, specifically the Eastern Arc Mountains (see figure 1). Amphibian collecting in SHNR began in the 1960's by Alex Duff-Mackay, Ronalda Keith and Arne Schiøtz. These authors were mainly interested in "tree frogs" of the families Hyperoliidae and Arthroleptidae (genus *Leptopelis*). The herpetological collection of the National Museums of Kenya (NMK) indicates that several short period collections had been made in the reserve since then (P. K. Malonza, pers. comm.) but these efforts have not been consolidated into a comprehensive understanding of the amphibian fauna (Malonza & Measey, 2005). Some publications have made reference to SHNR amphibians but these are mainly selective based on the taxa of interest. Schiøtz (1974) revised the genus *Afrixalus* and described *Afrixalus sylvaticus* while Schiøtz, 1975 focused on ‘‘tree frogs’’ including the description of *Hyperolius rubrovermiculatus*. Loader *et al*. (2010) detailed the presence of a potentially undescribed brevicipitid, *Callulina* sp. from SHNR collected in 1961 by Ronalda Keith, the only known specimen.

The main objective of this paper is to consolidate all the amphibian records from SHNR throughout the years and present these in a single publication, which we hope will promote knowledge of the area. We use records from 1968–2015 from the NMK herpetological reference collection and other relevant natural history museums, including new data from field research conducted between 2012–2015. New sampling in 2013–2015 conducted by the authors of this study aimed to sample new sites or poorly surveyed places, in particular forested areas. We give an updated species list of SHNR amphibians and descriptions of new records. Confirmations of our identifications are made on the basis of morphological diagnoses and are complemented by molecular analysis (Bwong, unpublished data).

Material and methods

Description of study area

The Shimba Hills are a dissected plateau located between 4º09′–4º21′S and 39º17′–39º30′E in Kwale County on the Kenyan coast (see figure 2). The hills are located about 30 km southwest of Mombasa city.

The Shimba Hills were gazetted as forest reserve in 1903 (Bennun & Njoroge, 1999; Luke, 2005) and in 1956 the area was expanded and re-gazetted as a National Reserve (Davis, 1993). The hills rise from the coastal plain to form a table plateau between 120 and 450 m above sea level, and the underlying rock consists of upper Triassic Shimba grits and Pliocene Magarini sands (Davis, 1993; Bennun & Njoroge, 1999). The climate is hot and moist with a mean annual temperature of 24.2˚C (Blackett, 1994). Rainfall ranges from 855–1682 mm per annum with a bimodal pattern from April–June and October–December (Schmidt, 1991). The vegetation is a mix of grassland, scrubland and exotic plantations and forests. Six major forest types occur within the reserve; *Milicia* forests at Makadara and Longomwagandi forests and the western escarpment; *Afzelia - Erythrophleum* forests are found on the eastern and southern flanks of the escarpment; *Paramacrolobium* forests are found on the steep scarp slopes to the east and the west on the Makadara cliffs, Buffalo ridge and Upper Kivumoni and *Manilkara-Combretum* forests are found on the lower western side of the plateau (Davis, 1993; Luke, 2005).

Field methods

The results presented here are based on field research, analysis of literature and museum collections. In total, 751 specimens were evaluated. New specimens were obtained from fieldwork in and around the SHNR conducted in January 2012, December 2013, April and December 2014 and April–May 2015 (see table 1 for major sampling sites). Time-limited searches and Visual Encounter Surveys (VES) were conducted. Bucket pitfall traps with drift fences were also used. For each pitfall trap set, five buckets were used in an "X" shaped pattern where each bucket was placed at a distance of 5 m from each other, a modified array pattern derived from Heyer *et al*. (1994) and Rödel & Ernst (2004). The drift fence was made of transparent plastic sheeting 0.5 m high. Representative samples of all species recorded were euthanized using Tricaine mesalyte (TM MS-222) solution, then fixed in 10% formalin and later preserved in 70% ethanol. All the newly collected material is deposited at the National Museums of Kenya herpetology collection. Specimen identification was made using standard references (*e.g.* Schiøtz, 1999; Channing & Howell, 2006; Harper *et al*., 2010). Taxonomy in the checklist follows Frost *et al*. (2006) and updates from Frost (2016). Museum abbreviations given in the text are for the following:

AMNH	American Museum of Natural History, New York, USA
BMNH	Natural History Museum, London, United Kindom
CAS	California Academy of Sciences, San Francisco, USA

LACM	Natural History Museum of Los Angeles County, Los Angeles, USA
MVZ	Museum of Vertebrate Zoology, Berkeley, USA
NMK	National Museums of Kenya, Nairobi, Kenya
ZMUC	Zoological Museum - University of Copenhagen, Denmark

Table 1. Major sampling sites within SHNR.

Locality	Coordinates	Altitude (m)
Kivumoni Gate swamp	4°13′S,39°29′E	159
Longomwagandi Forest	4°13′S,39°25′E	398
Makadara Forest	4°14′S,39°23′E	426
Marere Head works	4°12′S,39°23′E	206
Marere Hill	4°13′S,39°24′E	383
Mkongani West	4°20′S,39°18′E	359
Mwadabara swamp	4°10′S,39°25′E	159
Mwele Forest	4°17′S,39°21′E	334
Pengo Hill	4°14′S,39°23′E	455
Reserve compound	4°10′S,39°26′E	323
Risley Forest	4°14′S,39°25′E	342
Sable Bandas	4°13′S,39°27′E	352
Shimba Lodge	4°11′S,39°25′E	290
Sheldrick Falls	4°16′S,39°23′E	146

Secondary data acquisition

In addition to the data from the field work, information on SHNR amphibians was obtained from unpublished field reports (Malonza & Measey, 2005), the herpetological collection at the NMK, BMNH, CAS, ZMUC, HerpNet (www.herpnet.org) as well as field guides (Channing & Howell, 2006; Spawls *et al*., 2006; Harper *et al*., 2010). All specimens from museums outside Kenya with questionable labels (*e.g.*

sp., cf.) and/or vague locality data were omitted from this list. This was mainly because we could not confirm their identification, especially given the often-confusing taxonomy of certain species and genera (*e.g.* Zimkus & Blackburn, 2008). All NMK specimens from SHNR collected prior to 2012 were examined by BAB and PKM to confirm their identity. Furthermore, we assembled data on sampling intensity in the SHNR based on the period of time visited by collectors from the specimens examined; these dates assume collections were carried out continuously.

Results

The list comprises 30 currently known amphibian species of SHNR (28 anurans and two caecilians), representing 11 families and 15 genera (see appendix 1 for all specimen records). Table 2 provides a summary of the amphibian collection efforts in SHNR and the number of species documented per sampling event. The table indicates in which year authors observed species. The current study recovered most of the species previously reported in the reserve and also added new records. We confirmed a new record of *Scolecomorphus* cf. *vittatus*, for Kenya and also recovered *Callulina* cf. *kreffti* last collected in the reserve in 1961 by Ronalda Keith. SHNR species available in other museums outside Kenya include 26 specimens at BMNH, 144 specimens at CAS, and about 50 specimens at ZMUC, (see table 2 for collector information and figure 2 for the spatial distribution of the common sampling in the SHNR).

Table 2. A list showing amphibian species sampling effort in SHNR from 1968-2015.

Year	Date	Collector names	No. species recorded
1968	2 Apr	A. Williams	2
1968	19–20 May	Alex Duff-Mackay & Arne Schiøtz	6
1977	No date	Alex Duff-Mackay	1
1977	Apr	L. P. Lounibos	1
1981	12 Apr	S. Reilly	6
1981	5–18 Jul	M. Tandy	10
1982	6 May	Alice Grandison	3
1984	Feb	Ryan	5
1998	5 Jun	Dan R. Buchholz et al	5
1998	3 Jul	A. Wise, Weatherby, C. & Ross, K.	3
2005	28–30 Sep	P.K. Malonza & J.G. Measey	12
2006	22–23 Apr	J.G. Measey, B. Bwong & Venu	4
2006	13–16 Sep	Jos Kielgast	11
2010	17–18 Dec	Miloslav Jirku	5
2012	2–10 Apr	V. Wasonga & J. Nyamache	8
2012	19–23 Jun	V. Wasonga & J. Nyamache	7
2012	12–16 Nov	J. Mueti & C. Ofori	2
2013	17–23 Dec	J. Nyamache & P. Mwasi	10
2014	30 Apr–4 May	J. Nyamache & P. Mwasi	13
2014	12–20 Jun	V. Wasonga, J. Ochong	12
2014	2 Sep	J. Nyamache	7
2015	27 Apr–1 May	B. Bwong & J. Nyamache	18
2015	12-14 May	J. Nyamache	14
2015	23–25 May	P.K Malonza & J. Nyamache	5

The checklist

The checklist entries consist of four parts. Records: accession numbers for all individual records per species ever collected in SHNR (see appendix 1 for all specimens from SHNR together with their museum numbers, collection date, collector name and locality). Distribution: mentions the exact locality within SHNR where the species has been recorded. Habitat: describes the general habitat in which the species occurs. Remarks: mentions any other relevant information, including taxonomic status, IUCN red list status if not Least Concern and endemism where applicable.

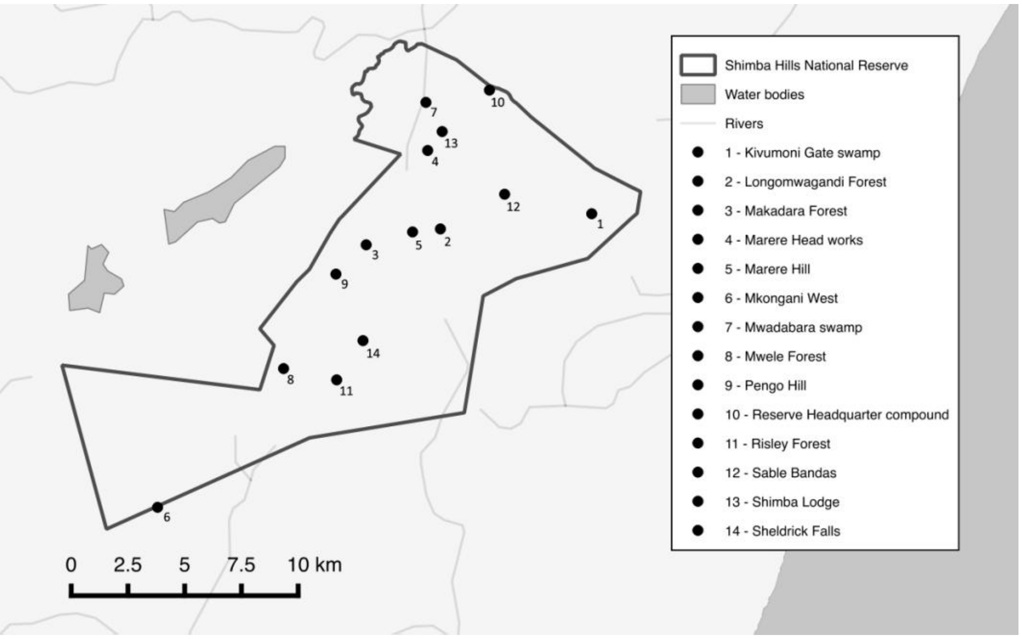

Figure 2. Map of Shimba Hills National Reserve showing major sampling sites.

Anura

Arthroleptidae

Arthroleptis stenodactylus Pfeffer, 1893

Records: NMK A4401/1–6; NMK A4460/1–3; NMK A4613; NMK A4654/1–2; NMK A5256; NMK A5459/1–2; NMK A5501; NMK A5502; NMK A5505; NMK A5516; NMK A5912; NMK A5913; NMK A5815; NMK A5806; NMK A5849; NMK A5852; NMK A5853/1–3; NMK A58971–2; NMK A6040; NMK A6045; NMK A6048; NMK A6111; CAS 155671–77.

Distribution: Longomwagandi Forest, Makadara Forest, Mwele Forest, Pengo Forest, Sheldrick Falls, Shimba Lodge Swamp.

Habitat: forest, savanna and degraded habitats.

Remarks: the taxonomy of this species is confusing given the likelihood that this taxon consists of more than one species. Pickersgill (2007) named a montane form (*Arthroleptis lonnbergi* Nieden, 1915) as different from *A. stenodactylus*, a presumably more widespread form. The specific relationship of the SHNR population to these units awaits formal clarification.

Arthroleptis xenodactyloides Hewitt, 1933

Records: NMK A4448/1–6; NMK A4459/1–8; NMK A4653/1–2; NMK A5515; NMK A5631/1–2; NMK A5805/1–4; NMK A5809/1–3; NMK A5816; NMK A5820/1–3; NMK A5851/1–7; NMK A5902/1–2; NMK A6019/1–3; NMK A6031; NMK A6037/1–2; NMK A6041/1–3; NMK A6042; NMK A6049; NMK A6059/1–2; NMK A6070/1–2; NMK A6079/1–2; NMK A6114; CAS 155604.

Distribution: Kaya Forest, Longomwagandi Forest, Makadara Forest. Marere Hill, Pengo Hill, Risley Forest, Sheldrick Falls.

Habitat: submontane forest, swamp, woodland and wet grassland.

Remarks: first recorded in SHNR as *A. adolfifriederici* Nieden, 1911 but the name later changed to *A. xenodactyloides* (see Blackburn, 2009). As with *A. stenodactylus*, the particular taxonomic name ascribed to the Shimba population is uncertain given the recognition of *A. stridens* Pickersgill, 2007, a similar form to *A. xenodactyloides*. Formal clarification will be required before this population can be assigned definitively to one of these species.

Leptopelis concolor Ahl, 1929

Records: NMK A4699/1–7; NMK A5845/1–12; NMK A5888/1–3; NMK A5089; NMK A6016/1–3; NMK A6051: NMK A6075; NMK A6084/1–2.

Distribution: Kivumoni Gate Swamp, Mwadabara Swamp, Shimba Lodge Swamp, Sheldrick Falls.

Habitat: coastal savanna woodland and grassland.

Remarks: Channing & Howell, 2006 consider this a junior synonym of *L. argenteus.*

Leptopelis flavomaculatus (Günther, 1864)

Records: NMK A787; A5844/1–5; NMK A6022/1–4; NMK A6044; CAS 153633–40; CAS 155630–31.

Distribution: Kivumoni Swamp, Shimba Lodge Swamp, Makadara Forest, Marere head works, Mwadabara Swamp, Sheldrick Falls.

Habitat: forest in both Coastal East Africa and Eastern Afromontane region.

Brevicipitidae

Callulina cf. *kreffti* Nieden, 1911

Records: AMNH 72724; NMK A6060; NMK A6113.

Distribution: Makadara Forest about 10 m from the picnic site.

Habitat: only known from forest.

Remarks: the first record of *Callulina* cf. *kreffti* in SHNR was by Ronalda Keith in 1961. She collected the specimen in Makadara Forest.This specimen is deposited at the AMNH. The presence of this frog in SHNR, however, only came to light recently (Loader *et al.*, 2010). Two individuals were collected during the current study in April and May 2015. With the addition of new specimens, the population is currently undergoing taxonomic evaluation.

Bufonidae

Sclerophrys gutturalis (Power, 1927)

Records: NMK A5855/1–4; BMNH 1982.842.

Distribution: National Reserve Headquarters compound.

Habitat: savanna, grassland and agricultural area.

Remarks: The genus name was originally *Bufo* Laurenti, 1768 which later changed to *Amietophrynus* Frost *et al.* 2006 and recently to *Sclerophrys* Tschudi, 1938 (see Ohler & Dubois, 2016).

Sclerophrys pusilla (Mertens, 1937)

Records: NMK A5507; NMK A5917/1–4.

Distribution: Sheldrick Falls area, Shimba Lodge Swamp.

Habitat: forest edge and humid savanna.

Remarks: recently recognized as being distinct from *S. maculatus* Hallowell, 1854. *S. pusilla* is found in Central, East and South Africa. Therefore all populations from these areas previously assigned to *S. maculatus* are currently assignable to *S. pusilla* (Poynton *et al*., 2016).

Sclerophrys steindachneri (Pfeffer, 1893)

Records: NMK A4452; NMK A5237; NMK A5366/1–5; NMK A5847.

Distribution: Kivumoni Gate Swamp, Sheldrick Falls, Shimba Lodge Swamp.

Habitat: humid grassland and woodland.

Mertensophryne micranotis (Loveridge, 1925)

Records: NMK A1150/1–9; NMK A5460; NMK A5464; NMK A5633; NMK A5911; NMK A5811; NMK A5819; NMK A5838/1–3; NMK A5898; NMK A6038/1–2; CAS 153698; BMNH 1980.195, BMNH 1980.197, BMNH 1982.395–396.

Distribution: Kaya Forest, Longomwagandi Forest, Makadara Forest, Sable bandas, Sheldrick Falls.

Habitat: lowland coastal forests and woodland.

Hyperoliidae

Afrixalus delicatus Pickersgill, 1984

Records: NMK A6054; NMK A6055/1–4; NMK A6068/1–4, ZMUC-R 73855; ZMUC-R 73948; ZMUC-R 73949; ZMUC-R 77457; ZMUC-R 77458.

Distribution: Mwadabara Swamp.

Habitat: savanna and grassland.

Afrixalus fornasini (Bianconi, 1849)

Records: NMK A4458/1–4; NMK A4611/1–5; NMK A4690/1–7; NMK A5252; NMK A5571; NMK A5810/1–2; NMK A5903; NMK A5954; NMK A6062/1–2; NMK A6085; CAS 157492.

Distribution: Kivumoni Gate Swamp, Mwadabara Swamp, Sheldrick Falls, Shimba Lodge Swamp.

Habitat: dense savanna and dry forest.

Afrixalus sylvaticus, Schiøtz, 1974

Records: NMK A3045/1–10; NMK A4440; NMK A4441/1–4; NMK A4703/1–6; NMK A5569/1–3; NMK A5814; NMK A5837; NMK A5902/1–3; NMK A5957/1–3; NMK A6028; NMK A6033/1–5; NMK A6043/1–4; CAS 155652–54; CAS 155947; MVZ 233824; MVZ 233825; BMNH 1982.857–859.

Distribution: Kivumoni Gate Swamp, Marere headworks, Sheldrick Falls, Shimba Lodge Swamp.

Habitat: lowland forest.

Remarks: this frog was first collected by Schiøtz in Kwale near SHNR in 1968. It was initially thought to be endemic to the type locality but has since been recorded in other coastal forest patches (Poynton, 2006). It is listed as vulnerable on the IUCN Red List of threatened species.

Hyperolius cf. *friedemanni* Channing *et al*., 2013

Records: NMK A3012/1–24; ZMUC-R 73916-937; ZMUC-R 77483.

Distribution: Shimba Lodge Swamp.

Habitat: humid and dense savanna.

Remarks: this species belongs to the original *H. nasutus* super species. Initial molecular analysis (Bwong, unpublished data) shows that it is closest to *H. friedemanni* (0.9% pairwise divergence) only known from the shores of Lake Malawi (Channing *et al*., 2013). Further investigations need to be done to confirm its taxonomic status.

Hyperolius argus Peters, 1854

Records: NMK A3041/1–2; NMK A4619/1–7; NMK A4700/1–6; NMK A4745/1–6; NMK A5508; NMK A5513; NMK A5568; NMK A5812/1–6; NMK A5904/1–2; NMK A6023/1–7; NMK A6053; NMK A6065.

Distribution: Kivumoni Gate Swamp, Mwadabara Swamp, Shimba Lodge Swamp.

Habitat: dense coastal savanna.

Hyperolius mariae Barbour & Loveridge, 1928

Records: NMK A3096/1–39; NMK A3168; NMK A5899; NMK A6027/1–2; NMK A6056; NMK A6067/1–2; NMK A6076/1–2; NMK A6086; NMK A6110; CAS 157496–98.

Distribution: Kivumoni Gate Swamp, Mwadabara Swamp, Shimba Lodge Swamp.

Habitat: bushland, savanna and grassland.

Hyperolius parkeri Loveridge, 1933

Records: MVZ 233910; MVZ 233909.

Distribution: Mwadabara Swamp.

Habitat: coastal savanna.

Hyperolius pusillus (Cope, 1862)

Records: NMK A/4449.

Distribution: Kivumoni Gate Swamp.

Habitat: coastal lowland savanna and bushland.

Remarks: this species was recorded in 2005–2006 (Malonza & Measey, 2005) but was not recorded in recent studies (2012–2015).

Hyperolius rubrovermiculatus Schiøtz, 1975

Records: NMK A788; NMK A2076/1–10; NMK A3169; NMK A4445, NMK A4447/1–3; NMK A4623/1–2; NMK A4704; NMK A5268; NMK A5488; NMK A5506; NMK A5801/1–5; NMK A5848; NMK A5900/1–2; NMK A5909; NMK A5958/1–3; NMK A6024/1–9; NMK A6034; NMK A6034;

NMK A6050/1–2; NMK A6064/1; LACM 50633, MVZ 233935; CAS 155635–46; CAS 155932–46; BMNH 1982.860–887.

Distribution: Kivumoni Gate Swamp, Mwadabara Swamp, Marere Head works, Shimba lodge Swamp, Sheldrick Falls.

Habitat: dry forest, dense humid savannah and farm bush.

Remarks: the only known endemic amphibian to SHNR and Kwale area. This frog is currently listed as endangered on the IUCN Red List (Schiøtz & Drewes, 2004). It was abundant at the Shimba Lodge and Mwadabara swamps, both of which are within the reserve. However a population at the Kivumoni Gate Swamp is facing habitat destruction as the swamp is being drained for agricultural expansion.

Hyperolius tuberilinguis Smith, 1849

Records: NMK A4450/1–5; NMK A4601/1–6; NMK A5269; NMK A5514; NMK A5961/1–4; NMK A6030/1–4; NMK A6058/1–2; NMK A6063/1–9; NMK A6083/1-8; CAS 153709–11.

Distribution: Kivumoni Gate Swamp, Mwadabara Swamp, Sheldrick Falls, Shimba Lodge Swamp.

Habitat: coastal savanna, woodland, bushland, grassland and thicket.

Kassina maculata (Duméril, 1853)

Records: NMK A739/1–9; NMK A3003/1–5; NMK A4455/1–2; NMK A4697/1–4; NMK A5736/1–4; NMK A5960; NMK A6057.

Distribution: Sheldrick Falls, Reserve compound, Mwadabara Swamp, Shimba Lodge Swamp.

Habitat: savanna, bushland, grassland and farmbush.

Kassina senegalensis (Duméril and Bibron, 1841)

Records: NMK A/4696; CAS 153695.

Distribution: Kivumoni Gate Swamp.

Habitat: savanna.

Remarks: this species was last collected in 2006 but has not been recorded since, though one specimen was collected in 2014 just outside the reserve in a pit fall trap in Mukurumudzi dam.

Rhacophoridae

Chiromantis xerampelina Peters, 1854

Records: NMK A4705/1–5; NMK A5451; NMK A5462; NMK A5841; NMK A5956; NMK A6021.

Distribution: Mkongani West Forest, Mwadabara Swamp, Sable Bandas, Shimba Lodge Swamp, Sheldrick Falls.

Habitat: savanna, shrubland, disturbed forest and agricultural land.

Hemisotidae

Hemisus marmoratus (Peters, 1854)

Records: NMK A5453/1–2; NMK A5511; NMK A5570.

Distribution: Mkongani West Forest, Sheldrick Falls.

Habitat: savanna and gallery forest.

Phrynobatrachidae

Phrynobatrachus acridoides (Cope, 1867)

Records: NMK A5808; NMK A5813/1–7; NMK A5804/1–2; NMK A5843; NMK A5846; NMK A5906/1–2; NMK A6029/1–5; NMK A6035/1–4; NMK A6046/1–4; NMK A6052/1–3; NMK A6069/1–2; NMK A6071; CAS 155621–23, CAS 155632–34; CAS 157494–95.

Distribution: Kivumoni Gate Swamp, Marere head works, Mwadabara Swamp, National Reserve compound, Shimba Lodge Swamp, Sheldrick Falls.

Habitat: dry and humid savanna, shrubland, grassland and coastal habitat.

Remarks: first collected in 2005. In 2006 a specimen identified as *P. natalensis* Smith, 1849 was later re-identified as *P. acridoides* by PKM. This species displays diverse dorsal colour patterns with males having a bright green or brown mid-dorsal band, while females lack the bands.

Ptychadenidae

Ptychadena anchietae (Bocage, 1868)

Records: NMK A3550/1–7; NMK A4443/1–5; NMK A4686/1–3; NMK A5241; NMK A5243; NMK A5452; NMK A5461; NMK A5463; NMK A5818/1–4; NMK A5807/1–5; NMK A5834; NMK A5835; NMK A5896/1–5; NMK A5953/1–2; NMK A6025; NMK A6026/1–4; NMK A6032; NMK A6074; CAS 153697; CAS 155624; CAS 157491.

Distribution: Buffalo River, Kivumoni Gate Swamp, Marere circuit, Mkongani West Forest, National Reserve compound, Sheldrick Falls, Shimba Lodge Swamp.

Habitat: woodland, savanna, residential and agricultural areas.

Ptychadena oxyrhynchus (Smith, 1849)

Records: NMK A6073; NMK A6108.

Distribution: Mwadabara Swamp, Shimba Lodge Swamp, Kivumoni Gate Swamp.

Habitat: degraded forest, humid savanna, woodlands and farmland.

Ptychadena sp.

Records: NMK A73/1-3; NMK A5800.

Distribution: Shimba Lodge Swamp.

Habitat: moist grassland, savanna.

Remarks: the taxonomic status of this frog is currently unknown. The dorsal colour pattern resembles *P. mascareniensis* but preliminary molecular analysis (Bwong, unpublished data) places it closer to *P. porosissima.* Further study on this taxon is required to reveal its true identity.

Pipidae

Xenopus muelleri (Peters, 1844)

Records: NMK A737/1–2; NMK A3553/1–6; NMK A4442; NMK A4693/1–4; NMK A4694; NMK A4698/1–5; NMK A5572/1–5; NMK A5840; NMK A5842; CAS 153694, CAS 155626–29, CAS 155668–69.

Distribution: Kivumoni Gate Swamp, Marere head works, Shimba Lodge Swamp, National reserve compound.

Habitat: aquatic habitat in dry savanna and humid savanna and forest.

Gymnophiona

Herpelidae

Boulengerula changamwensis Loveridge, 1932

Records: NMK A4395/1–11; NMK A4750; NMK A5465; NMK A5504; NMK A5510; NMK A5918/1–3; NMK A5803/1–2; NMK A5817/1–2; NMK A5850; NMK A5908/1–2; NMK A6020; NMK A6039/1–6; NMK A6047/1–2; NMK A6061/1–2; NMK A6078; NMK A6080/1–2; NMK A6112/1–2; NMK A6061/1–2; NMK L/1887 (see Nussbaum and Hinkel, 1994).

Distribution: Longomwagandi, Makadara Forest, Pengo Forest, Kivumoni Forest, Mwele Forest, Marere Hill, Sheldrick Falls.

Habitat: lowland moist forest and plantation.

Remarks: IUCN Endangered, (IUCN, 2013a) with the only protected population in the Buda Forest and SHNR. Nussbaum & Hinkel (1994) first noted the presence of this species in the Shimba Hills on the basis of a dried misidentified amphisbaenid held in NMK.

Scolecomorphidae

Scolecomorphus cf. *vittatus* Boulenger, 1895

Records: NMK A5458, BMNH 1909.6.5.6 (?) see comment below.

Distribution: Makadara Forest.

Habitat: montane, submontane and lowland forest also in cultivated land.

Remarks: the single specimen (NMK A5458) was collected in May 2014 under a decaying log. The 15 cm long individual was coloured black dorsally with a yellow pinkish lateral and ventral side. The single specimen represents the first *bona fide* record for Kenya. Previously it was only known from the Eastern Arc Mountains (Nussbaum, 1985; IUCN, 2015) but Nussbaum (1985) noted a single specimen from Mombasa (BM 1909.6.5.6) collected by Hinde in 1895. Nussbaum questioned the precise provenance of this specimen (see figure 10; p.46 in Nussbaum, 1985). The wider distribution of this species in Kenya will need to be evaluated by more extensive sampling.

Discussion

The thirty amphibian species of SHNR presented in this checklist is more than double the number that was reported in the preliminary study of Malonza & Measey (2005). The increase is clearly linked to the relative paucity of sampling in the area previously, following a classic pattern of increasing species discovery over time. In terms of numbers of species, the SHNR shows a comparatively elevated level of diversity to surrounding areas. For example, Arabuko-Sokoke Forest, the largest coastal forest in East Africa, has 26 recorded species (Drewes, 1992), Taita Hills, the only Eastern Arc Mountain in Kenya, also has 26 species (Malonza, *et al.*, 2010). Such comparisons show, based on the current sampling, that the SHNR has the highest amphibian diversity in Kenya. Neighbouring areas in Tanzania, such as the West Usambara and Pare Mountains are also comparable (see table 1 in Loader *et al.*, 2011). This differs from areas further south such as the East Usambara, Nguru and Uluguru Mountains, which show substantially higher species diversity (Poynton *et al.*, 2007; Menegon *et al.*, 2008).

The high diversity in SHNR compared to other Kenyan localities may be attributed to a number of factors, but direct comparisons are hindered by the relatively different sizes of areas and intensities of sampling conducted in each area. However, one key aspect appears to be the heterogeneous habitats in the SHNR, the area consists of six forest types, woodland and grassland habitats within the reserve (Davis, 1993; Luke, 2005) allowing for a variety of species from different biogeographic zones. The amphibian fauna of SHNR consists of a combination of species from the Eastern Afromontane Region and Coastal Forests of Eastern Africa, in addition to the numerous widespread species occurring in varying types of savanna habitats. Within the SHNR, we therefore have a broad representation of all possible habitats found across Kenya unlike other comparable regions.

There are a few amphibian species of particular note to be found in the Shimba Hills. One species appears to be endemic to the reserve, *Hyperolius rubrovermiculatus,* although the taxonomy of this taxon is currently unresolved (see Channing & Howell, 2006). Furthermore, one taxon, *Scolecomorphus* cf. *vittatus*, might be recognized as being distinct from other Eastern Arc populations. This level of endemism (2–3 species) may be considered low when compared to the East Usambara Mountains where eight amphibian species (Poynton *et al*., 2007) are endemic. However, as far as vertebrate fauna is concerned, this may be considered relatively high, as no endemic bird or mammal species have been recorded in the reserve to date (cca.kws.go.ke/shimbaHills.html; Bennun & Njoroge, 1999). Only 20% of the amphibians in SHNR belong to the Coastal Forest ecoregion, including species such as *Mertensophryne micranotis*, *Afrixalus sylvaticus* and *Hyperolius rubrovermiculatus* (Poynton, 1999; Schiøtz, 1999; Burgess & Clarke, 2000). Eastern Afromontane species are represented by *Scolecomorphus* cf. *vittatus* and *Callulina* cf. *kreffti* indicating some association of SHNR with this region. However, the majority of the SHNR amphibian fauna belong to the widespread fauna found in savanna regions forming a mosaic of fragmented habitats intermixing with coastal forest. These extend inland into drier areas, stretching along the coast from southern Somalia through Kenya, Tanzania and Mozambique to the eastern coast of South Africa. These include savanna living species as well as those confined to the dry semi-deciduous forest (bushland savanna) (Schiøtz, 1999). About 23 species (76%) occur here including *Afrixalus fornasini, Hyperolius parkeri, H. pusillus, H. tuberilinguis, H. argus, Leptopelis concolor, Kassina maculata* and *Xenopus muelleri*. Even further, wide-ranging species are represented by *Hemisus marmoratus, Kassina senegalensis, Phrynobatrachus acridoides*, *Ptychadena anchietae*, and *P. oxyrhynchus.* However, it should be noted that taxonomy of many of these species is poorly known and might reveal more taxonomic units and further divisions to their currently rather large distributions.

This checklist contains all the amphibians of SHNR as currently known. This does not preclude the possibility that new discoveries will not be made in the future. The following species were expected from the reserve given that they have been recorded very close to the reserve or their IUCN red list presumed range includes SHNR: *Phrynobatrachus mababiensis* FitzSimons, 1932; *Phrynomantis bifasciatus* Smith, 1847; *Pyxicephalus angusticeps* Parry, 1982; *Ptychadena mossambica* Peters, 1854; *Ptychadena schillukorum* Werner, 1908 (Channing & Howell, 2006; Harper *et al.*, 2010, IUCN, 2013b). Further sampling across the area is required to understand if these species occur. Furthermore, as can be seen from figure 2, surveys have been relatively concentrated in some parts and large areas await sampling.

These areas include both higher elevation forest areas, which might produce more specimens of typical Eastern Afromontane amphibians such as those already collected, and potentially new undescribed species. Further sampling of such areas are required if a complete list of the area is to be made.

Conclusion

SHNR has the highest amphibian diversity in Kenya, accounting for about 30% of the country's amphibians. Fortunately, the area is relatively well protected being a National Reserve and is frequently visited by tourists, who provide solid economic revenue. These features suggest its long-term future is relatively well secured. The area could provide an important basis for understanding amphibians in Kenya more broadly and promote their conservation. The amphibians in the reserve represent a mix of both Eastern Afromontane, widespread Coastal Forest species and pan African species, potentially therefore making it an important area for further expanding our knowledge on various biological questions including phylogeography, behaviour and community ecology.

One major biological question will be interpreting the biogeographic history of SHNR given the various species that can be found within the reserve. At present, it remains unclear whether the observed diversity and endemism is the result of habitat stability within coastal forest or recent colonization from other areas such as the Eastern Arc Mountains. To address these questions a more detailed understanding of the historical biogeography of all lineages in SHNR and other neighbouring coastal forests is required. Key to any kind of understanding of such questions though is the establishment of baseline data as outlined in this publication.

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Suplementary Material

Appendix S1: A list of all known amphibian records from Shimba Hills National Reserve indicating museum number, collector name, date and locality. Records with asterisks were obtained from the HerpNet.

References

Blackburn, D.C. (2009). Description and phylogenetic relationships of two new species of miniature *Arthroleptis* (Anura: Arthroleptidae) from the Eastern Arc Mountains of Tanzania. *Breviora* **517** (517)**:** 1–17. doi:10.2992/0097-4463-77.1.211.

Blackett, H.L. (1994). Forest Inventory Report No. 4 Shimba Hills, Mkongani North and Mkongani West Forest. KIFCON, Karura Forest station Nairobi.

Bennun, L. & P. Njoroge (1999). *Important Bird Areas*. East Africa Natural History Society, Nairobi. Pp. 116–120.

Burgess, N.D. & G.P. Clarke (eds) (2000). *Coastal Forests of Eastern Africa*. IUCN, Gland, Switzerland and Cambridge, UK.

Burgess, N.D., G.P Clarke & W.A. Rodgers (1998). Coastal forests of Eastern Africa: Status, endemism patterns and their potential causes. *Biological Journal of the Linnean Society* **64:** 333–367.

Burgess, N., J.D. Hales, E. Underwood, E. Dinerstein, D. Olson, I. Itoua, J. Schipper, T. Ricketts & K. Newman (2004). *Terrestrial Ecoregions of Africa and Madagascar: a conservation assessment*. Island Press, Washington D.C.

Channing, A., A. Hillers, S. Lötters, M.O. Rödel, S. Schick, W. Conradie, D. Rödder, V. Mercurio, P. Wagner, J.M. Dehling, L.H. Du Preez, J. Kielgast, & M. Burger (2013). Taxonomy of the super-cryptic *Hyperolius nasutus* group of long reed frogs of Africa (Anura: Hyperoliidae), with descriptions of six new species. *Zootaxa* **3620**(3)**:** 301–50. doi:10.11646/zootaxa.3620.3.1.

Channing, A. & K.M. Howell (2006). *Amphibians of East Africa*. Cornell University Press, Ithaca, New York.

Chira, M. (1993). Ecological study of herpetofauna in the Arabuko-Sokoke and Gede coastal forests of Kenya. Unpublished Msc thesis, College of Biological and Physical sciences, University of Nairobi, Kenya 88pp.

Clausnitzer, V. (2003). Dragonfly communities in coastal habitats of Kenya: indication of biotope quality and the need of conservation measures. *Biodiversity and Conservation* **12**(2)**:** 333–356.

Davis, G. (1993). Shimba Hills, Mkongani and Mwaluganje Forest Biodiversity Overview. KIFCON, Karura Forest station, Nairobi.

Drewes, R.C. (1992). *Amphibian species of the Gedi and Arabuko-Sokoke Forests, Kenya.* California Academy of Sciences, California, USA 32pp.

Frost, D.R. (2016). Amphibian species of the world: An online reference. Version 6.0. [accessed on 15 March 2016]. Electronic database accessible at http://research.amnh.org /herpetology/amphibia/index.html. American Museum of Natural History, New York, USA.

Frost, D.R., T. Grant, J. Faivovich, R.H. Bain, A. Haas, C.F.B. Haddad, R.O. De Sa, A. Channing, M. Wilkinson, S.C. Donnellan & C.J. Raxworthy, (2006). The amphibian tree of Life. *Bulletin of the American Museum of Natural History* **297:** 1–129.

Harper, E.B., G.J. Measey, D.A. Patrick, M. Menegon & J.R. Vonesh (2010). *Field Guide to Amphibians of the Eastern Arc Mountains and Coastal Forests of Tanzania and Kenya.* Camerapix Publishers International, Nairobi.

Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.-A. Hayek & M.S. Foster (1994).

Measuring and monitoring biological diversity. Standard methods for amphibians. Smithsonian Institute, Washington & London.

Hoft, R. & M. Hoft (1995). The differential effects of elephants on rain forest communities in the Shimba Hills, Kenya. *Biological Conservation* **73**(1)**:** 67–79. doi:10.1016/0006-3207(94)00105-Y.

Howell, K.M. (1993). Herpetofauna of the East African Forests. In J.C. Lovett & S.K. Wasser (eds.), *Biogeography of the Rain Forests of Eastern Africa*. Cambridge University Press, Cambridge. Pp. 173–201.

IUCN SSC Amphibian Specialist Group (2013a). *Boulengerula changamwensis*. The IUCN Red List of Threatened Species 2013: e.T59495A16943655. http://dx.doi.org/10.2305/IUCN.UK.2013-2.RLTS.T59495A16943655.en [accessed 14 March 2016].

IUCN SSC Amphibian Specialist Group (2013b). *Ptychadena schillukorum*. The IUCN Red List of Threatened Species 2013: e.T58523A18401331. http://dx.doi.org/10.2305/IUCN.UK.2013-2.RLTS.T58523A18401331.en. [accessed 12 March 2016].

IUCN (2015). IUCN Red List of Threatened Species. Version 2015. <www.iucnredlist.org>. [accessed 2 December 2015].

Lehman, I. & E. Kioko (2005). Lepidoptera diversity, floristic composition and structure of three Kaya Forests on the South Coast of Kenya. *Journal of East African Natural History* **94**(1)**:** 121–63. doi:10.2982/0012-8317(2005)94 [121:LDFCAS]2.0.CO;2.

Loader, S.P., D.J. Gower, W. Ngalason & M. Menegon (2010). Three new species of *Callulina* (Amphibia: Anura: Brevicipitidae) highlight local endemism and conservation plight of Africa's Eastern Arc Forests. *Zoological Journal of the Linnean Society* **160**(3)**:** 496–514. doi:10.1111/j.1096-3642.2010.00652.x.

Loader, S.P., J.C. Poynton, L.P. Lawson, D.C. Blackburn & M. Menegon (2011). Herpetofauna of montane areas of Tanzania. 3. Amphibian diversity in the northwestern Eastern Arc Mountains, with the description of a new species of *Arthroleptis* (Anura: Arthroleptidae). *Fieldiana Life and Earth Sciences* **4**(4)**:** 90–102. doi:10.3158/2158-5520-4.1.90.

Loveridge, A. (1935). Scientific results of an expedition to the rain forests regions in Eastern Africa I. New reptiles and amphibians from East Africa. *Bulletin of the Museum of Comparative Zoology at Harvard* **79:** 3–19.

Luke, Q. (2005). Annotated checklist of the plants of the Shimba Hills, Kwale District, Kenya. *Journal of East African Natural History* **94**(1)**:** 5–120. doi:10.2982/0012-8317(2005)94[5:ACOTPO]2.0.CO;2.

Malonza, P.K. & G.J. Measey (2005). Preliminary survey results on the status of amphibians and reptiles of Shimba Hills National Reserve, Kenya. Unpublished report submitted to the Kenya Wildlife Service and National Museums of Kenya 22pp.

Malonza, P.K., S. Lötters & G.J. Measey (2010). The montane forest-associated amphibian species of the Taita Hills, Kenya. *Journal of East African Natural History* **99**(1)**:** 47–63. doi:10.2982/028.099.0103.

Malonza, P.K., V.D. Wasonga, V. Muchai, D. Rotich, B.A. Bwong & A.M. Bauer (2006). Diversity and biogeography of herpetofauna of the Tana River Primate National Reserve, Kenya. *Journal of East African Natural History* **95**(2)**:** 95–109. doi:10.2982/0012-8317(2006)95[95:DABOHO]2.0.CO;2.

McDonald, M.M. & H. Hamilton (2010). Phylogeography of the Angolan black and white colobus monkey, *Colobus angolensis palliatus*, in Kenya and Tanzania. *American Journal of Primatology* **72**(8)**:** 715–24. doi:10.1002/ajp.20828.

Menegon, M., N. Doggart & N. Owen (2008). The Nguru Mountains of Tanzania, an outstanding hotspot of herpetofaunal diversity. *Acta Herpetologica* **3**(2)**:** 107–27.

Myers, N., R.A. Mittermeier, G.A.B. Fonseca & J. Kent (2000). Biodiversity hotspots for conservation priorities. *Nature* **403**(6772)**:** 853–58. doi:10.1038/35002501.

Nussbaum, R.A. (1985). Systematics of caecilians (Amphibia: Gymnophiona) of the family Scolecomorphidae. *Ocassional Paper of the Museum of Zoology University of Michigan* **713:** 1–49.

Nussbaum, R.A. & H. Hinkel (1994). Revision of East African caecilians of the genera *Afrocaecilia* Taylor and *Boulengerula* Tornier (Amphibia: Gymnophiona: Caeciliidae). *Copeia* **1994:** 750–760.

Oguge, N., R. Hutterer, R. Odhiambo & W. Verheyen (2004). Diversity and structure of shrew communities in montane forests of Southeast Kenya. *Mammalian Biology-Zeitschrift für Saugetierkunde* **69**(5)**:** 289–301.

Ohler, A. & A. Dubois (2016). The identity of the South African toad *Sclerophrys capensis* Tschudi, 1838 (Amphibia, Anura). *PeerJ* **4**, p.e 1553. doi 10.7717/peerj.1553.

Pickersgill, M. (2007). *Frog Search. Results of Expedition to Southern and Eastern Africa*. Edition Chimaira, Frankfurt am Main.

Poynton, J.C. (1999). Distribution of amphibians in Sub-Saharan Africa, Madagascar and Seychelles. In W.E. Duellman (ed.), *Patterns of distribution of amphibians: A global perspective*. John Hopkins University Press, Baltimore & London.

Poynton, J.C. (2006). On dwarf spiny reedfrogs in Tanzanian Eastern lowlands (Anura: *Afrixalus*). *African Journal of Herpetology* **55**(2)**:** 167–69.

Poynton, J.C., S.P. Loader, E. Sherratt & B.T. Clarke (2007). Amphibian diversity in East African biodiversity hotspots: altitudinal and latitudinal patterns. *Biodiversity and Conservation* **16**(4)**:** 1103–18. doi:10.1007/s10531-006-9074-1.

Poynton, J.C, S.P. Loader, W. Conradie, M-O. Rödel & H.C. Liedtke (2016). Designation and description of a neotype of *Sclerophrys maculatus* (Hallowell, 1854) and reinstatment of *S. pusilla* (Mertens, 1937) (Amphibia: Anura: Bufonidae). *Zootaxa* **4098**(1)**:** 73–94. doi.org/10.11646/zootaxa.0000.0.0.

Rödel, M-O. & R. Ernst (2004). Measuring and monitoring amphibian diversity in tropical forests. I. an evaluation of methods with recommendations for standardization. *Ecotropica* **10**(1)**:** 1–14.

Rogo, L. & A. Odulaja (2001). Butterfly populations in two forest fragments at the Kenya Coast. *African Journal of Ecology* **39**(3)**:** 266–75. doi:10.1046/j.1365-2028.2001.00313.x.

Schiøtz, A. (1974). Revision of the genus *Afrixalus* (Anura) in eastern Africa. *Videnskabelige meddelelser fra Dansk naturhistorisk forening* **137:** 9–18.

Schiøtz, A. (1975). *The Treefrogs of Eastern Africa*. Steenstrupia, Copenhagen.

Schiøtz, A. (1999). *The Treefrogs of Africa*. Ed. Chimaira. Frankfurt am Main.

Schiøtz, A. & R. Drewes (2004). *Hyperolius rubrovermiculatus*. The IUCN Red List of Threatened Species 2004: e.T56200A11435701. http://dx.doi.org/10.2305/IUCN.UK.2004.RLTS.T56200A11435701.en. [accessed 12 March 2016].

Schmidt, R. (1991). *Ecology of a tropical lowland rain forest. Plant communities, soil characteristics, and nutrients relations of the forests in the Shimba Hills National Reserve*. Dissertationes Botanicae Band 179. J. Cramer. Berlin. Stuttgart.

Spawls, S., K. Howell & R.C. Drewes (2006). *Reptiles and amphibians of East Africa.* A&C Black Publishers Ltd., London.

Tabor, K., N.D Burgess, B.P. Mbilinyi, J. Kashaigili & M.K. Steiniger (2010). Forest and wood cover and change in Coastal Tanzania and Kenya, 1990 to 2000. *Journal of East African Natural History* **99**(1)**:** 19–45.

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

CHAPTER II

Genetic, morphological and ecological variation in the congeners *Hyperolius mitchelli* Loveridge, 1953 and *Hyperolius rubrovermiculatus* Schiøtz, 1975 from East Africa.

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Genetic, morphological and ecological variation in the congeners *Hyperolius mitchelli* Loveridge, 1953 and *Hyperolius rubrovermiculatus* Schiøtz, 1975 from East Africa

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Running title: Systematics of *Hyperolius mitchelli* and *Hyperolius rubrovermiculatus*.

Abstract

The taxonomic validity of *Hyperolius rubrovermiculatus* has been questioned in the literature with respect to *H. mitchelli* due to morphological similarity between the two and high genetic diversity within *H. mitchelli*. To date no study has ever investigated the relationship between these two lineages. To assess the species status of the two congeners, *H. mitchelli* and *H. rubrovermiculatus,* we use molecular, morphological, bioacoustic and species distribution modelling analyses. We report on the paraphyly of *H. mitchelli* with respect to *H. rubrovermiculatus,* with the former showing considerable genetic differentiation among geographically structured populations. To resolve the paraphyletic status of *H. mitchelli* in our analysis we propose the description of a new species. The primary features distinguishing these species are dorsal colour pattern (between the closely related *Hyperolius new sp*. and *H. rubrovermiculatus*), skin texture (between the females of *H. rubrovermiculatus* versus those of *H. new sp*. and *H. mitchelli*) and high levels of genetic divergence (distinguishing *H. mitchelli* from the clade containing *H. new sp*. and *H. rubrovermiculatus*). Morphometric differentiation was low, as was call variation among groups. The proposed new species, *Hyperolius new sp*. resolves the paraphyletic relationship of *H. mitchelli* to *H. rubrovermiculatus.*

Keywords: coastal forests, cryptic diversity, Eastern Arc Mountains, *Hyperolius,* phylogeography.

Introduction

Hyperolius Rapp, 1842 is the most species-rich amphibian genus in Africa, with over 143 described species (Frost et al., 2016) distributed across many habitat types. Although many members of the genus can be diagnosed using a combination of morphological characters aided by habitat, call or distributional information (Schiøtz, 1975; Channing and Howell, 2006), many species are morphologically very similar and intraspecific variation is high. As a result, the genus *Hyperolius* has proven to be a difficult group taxonomically and resolving the numerous species complexes within *Hyperolius* remains a daunting challenge (Schiøtz 1975; Poynton and Broadley, 1987; Frost et al., 2016).

There are over twenty *Hyperolius* species known from the Eastern Arc Mountains and coastal forests of Kenya and Tanzania, (Schiøtz, 1975; Channing and Howell, 2006; Harper et al., 2010; Channing et al., 2013; Loader et al., 2015; Barratt et al., 2017). Some of these are widespread species complexes that are thought to harbour several cryptic species. For example, the *Hyperolius nasutus* Günther, 1864 complex, previously thought to contain at least eight valid species, now has 16 (Channing et al., 2013; Frost et al., 2016) and the recently revised *Hyperolius spinigularis* Stevens, 1971 complex originally consisted of three species but now has seven recognized species (Loader, et al., 2015; Barratt, et al., 2017). Considering that the Eastern Arc Mountains and coastal forests of Kenya and Tanzania fall into two biodiversity hotspots, (the Eastern Afromontane and the Coastal Forests of Eastern Africa) that are characterized by high levels of single-site endemism (Myers et al., 2000), detailed studies of allegedly wide-ranging species might reveal cryptic diversity with multiple range-restricted species. Such findings are valuable from a taxonomic point of view, but are also important for evaluating biogeographic patterns and species conservation. For example, the splitting of a formerly widespread species into discrete taxonomic units often means the range of the resulting new species is more limited and this may increase their susceptibility to extinction and priority for conservation (Bickford et al., 2007; Vieites et al., 2009; Oliver et al., 2013).

Hyperolius mitchelli Loveridge, 1953 is recognized as having a wide-ranging distribution from northeastern Tanzania through Malawi and Mozambique and large molecular variation amongst populations (Schiøtz, 1975; Lawson, 2010). Though not strictly considered a species complex, the delimitation of *H. mitchelli* has been historically problematic. At the time of the original description of *H. mitchelli* (from Malawi), it was considered a subspecies of *H. substriatus* (See Pickersgill 2007), erroneously called *Hyperolius puncticulatus* Pfeffer, 1893 by Loveridge, 1953 at the time. *Hyperolius* *mitchelli* is easily recognized from *Hyperolius substriatus* due to acoustic call differences and colour (juvenile phase (Ph J), adult phase (PhF) and a light spot on the heels in *H. mitchelli*) (Schiøtz 1975, 1999). From a molecular perspective, phylogenetic investigations support *H. substriatus* and *H. mitchelli* as being distinctive species (Lawson, 2010).

Apart from the association with *H. substriatus*, *H. mitchelli* has also been affiliated with *Hyperolius rubrovermiculatus* Schiøtz, 1975, which is endemic to the Shimba Hills in southeastern Kenya. Schiøtz (1975) recognized *H. rubrovermiculatus* as a distinctive species based primarily on the colour and skin texture of females. Under systematical remarks on *H. rubrovermiculatus* sp. nov., Schiøtz (1975, p. 156) commented on the similarity between *H. mitchelli* and *H. rubrovermiculatus* but continued to describe *H. rubrovermiculatus* based on the fact that "*H. mitchelli* is otherwise a very constant species showing practically no variation throughout its great range". Channing and Howell (2006) synonymized *H. rubrovermiculatus*, commenting on *H. mitchelli* (p.171) that "one of the colour morphs was previously known as *H. rubrovermiculatus*" and the distribution of *H. mitchelli* is given as from southeastern Kenya, and northern eastern Tanzania through Malawi to Mozambique. However six years later, Channing et al. (2012) considered *H. mitchelli* and *H. rubrovermiculatus* as separate species and Kenya was no longer included in the range for *H. mitchelli*. Besides *H. substriatus* and *H. rubrovermiculatus, H. mitchelli* is a very distinctive species and not similar to any other known described Hyperoliid species.

To date no study has established the ecological, molecular and morphological variation of both *H. mitchelli* and *H. rubrovermiculatus* across their ranges in order to better understand the taxonomic status of these species. We sample across the ranges of both species and assess their taxonomic distinctiveness using genetic, morphological, acoustic and distribution data. From these analyses we propose the description of a new species and evaluate the biogeographic and conservation implications of our results.

Methods

Sampling

Fieldwork in Malawi, Tanzania and Kenya was conducted by the authors and colleagues between 1998 and 2015. These surveys have contributed to the collection of voucher specimens of *H. mitchelli* and *H. rubrovermiculatus* from numerous populations (Fig. 1).

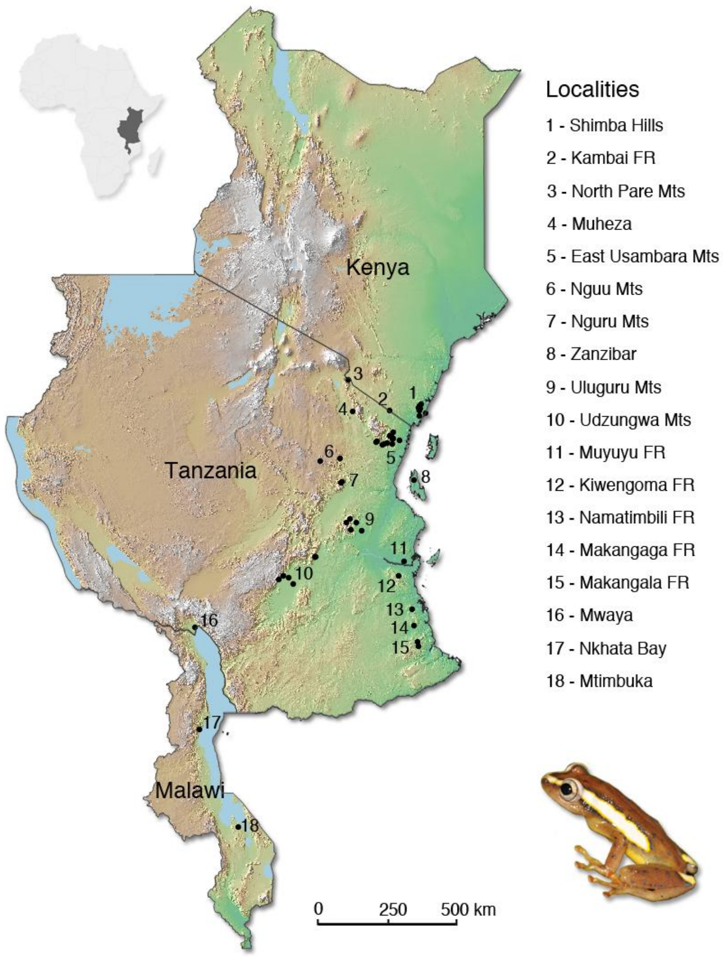

Fig. 1. Map showing sampling localities of *H. mitchellli* and *H. rubrovermiculatus* specimens used in the study.

Part of this material has been documented in previous studies (e.g. Lawson, 2010). For *H. rubrovermiculatus*, fresh material was collected between December 2013 and May 2014 in Shimba Hills, Kenya as part of an ongoing project in this area (Bwong et al., 2014). Additional sampling of *H. mitchelli* in the Tanzanian coastal forest was also made. Specimens collected in these projects were sampled through the opportunistic search method of Heyer et al. (1994). Liver, thigh muscle and/or toe clips were preserved in absolute ethanol for DNA extraction. All tissue samples from the freshly collected material for this study are deposited at the Institute of Biogeography, Department of Environmental Sciences, University of Basel, Switzerland. Specimens were euthanized using Tricaine mesalyte (TM MS-222) and fixed in 10% formaldehyde (formalin). They were later stored in 70% ethanol and deposited at the Field Museum of Natural History, Chicago, USA; National Museums of Kenya, Nairobi, Kenya; Natural

History Museum, London, UK; Science Museum of Trento, Italy, and the University of Dar es Salaam, Tanzania. Additional samples were added from various institutions for morphological analysis (see Appendix I). These included the holotype and paratypes of *H. rubrovermiculatus* from the Zoological Museum of the University Copenhagen, Denmark, and the holotype and paratypes of *H. mitchelli* from the Museum of Comparative Zoology, Harvard, USA. Further specimens were obtained from the California Academy of Sciences, USA, Field Museum of Natural History, Chicago, USA, Natural History Museum, London, UK, and the Science Museum of Trento, Italy.

Genetics

Total DNA was extracted from preserved tissue samples using the DNeasy blood and tissue kit (Qiagen, Valencia, CA). Extraction, amplification and sequencing followed standard protocols (see Loader et al., 2010). Each of the newly collected individuals was barcoded to verify its identity using the 16S mitochondrial gene. Sequences were aligned in Geneious v6.1.2 (http:www.geneious.com, Kearse et al., 2012) using MAFFT v7.017 (Katoh et al., 2002) with default settings. For a subset of individuals with quantitatively sufficient DNA, we also amplified an additional mitochondrial gene (NADH dehydrogenase subunit 2 - ND2) and two nuclear loci (Cellular myelocytomatosis - C-myc, and Proopiomelanocortin - POMC) following Lawson, (2010). Appendix II provides details on voucher specimens used in this study, their origin, available genes and associated GenBank numbers where applicable.

We used two alignments to reconstruct the genetic relationships of *H. mitchelli* and *H. rubrovermiculatus*; the first alignment included all available barcoded samples (partial ca. 600bp 16S rRNA fragment) of *H. mitchelli* and *H. rubrovermiculatus.* The second alignment consisted of all major geographical areas represented by a multi-locus dataset (ND2, C-myc, POMC) complementing previous analyses (Lawson, 2010). All populations were represented by at least a single specimen apart from the North Pare population for which only 16S data was available, and the Zanzibar population of *H. mitchelli* for which no sequences were available. We also investigated single gene trees to examine resolution in the reconstructed phylogenetic relationships.

The evolutionary relationships of the species based on the barcode (mtDNA) alignment were reconstructed using Bayesian (MrBayes v3.2; Ronquist et al., 2012) and Maximum Likelihood (RAxML v8.0.0; Stamatakis, 2014) methods with a single outgroup species (*H. substriatus*) chosen due to its close relationship with *H. mitchelli* recovered from previous phylogenetic analyses (Lawson, 2010). In the

MrBayes analyses, four simultaneous Markov chains were run for 20 million generations and sampled every 1000 generations; discarding the first two million generations as burn-in. We set the substitution type to mixed rates to allow the Markov chains to sample over space of all possible reversible substitution models. RAxML analysis used the rapid hill-climbing-algorithm and the GTRGAMMA model, and node support was evaluated by non-parametric bootstrapping with 1000 replicates. To further examine population variation within our extensive geographic sampling, we employed a haplotype-network analysis using the program PopART (www://popart.otago.ac.nz). We used TCS (Templeton, 1992) networks to reconstruct the relationships among lineages.

For the second multi-locus alignment, we conducted two different analyses involving different sets of samples and genes. Our first analysis consisted of all four genes for all samples, which included many individuals with missing data (56 samples). The second analysis was conducted on a subset of 30 samples in order to maximize complete coverage of samples that had sequence data for all genes. All analyses on these two multi-locus datasets were conducted using MrBayes and RAxML using a single outgroup (*H. substriatus*) and substitution models (Table 1) determined using PartitionFinder v1.1.1 (Lanfear et al., 2012). The first concatenated alignment included six partitions for all four genes (Supplementary Table 1). Both sets of partitioned analysis were run using MrBayes with parallel runs of four simultaneous Markov chains for 30 and 10 million generations respectively, sampling every 1000 generations from the chain and discarding the first 10% of each as burn-in. Support for groupings was evaluated using posterior probabilities (Ronquist et al., 2012). Maximum Likelihood analysis was conducted with RAxML using the rapid hill climbing algorithm and the GTRGAMMA substitution model partitioned by gene and codon according to PartitionFinder (Supplementary Table 1).

We used BEAST v2.1.3 (Bouckaert et al., 2014) to estimate the divergence times between clades and within subclades on the multi-locus dataset. The rate-calibrated tree was reconstructed without an outgroup for improved precision of branch length estimates. All coding regions (exons in C-myc, POMC; coding region in ND2) were analyzed with the SRD06 model, while non-coding regions were assigned the highest probability models based on jModelTest v2.1.6 (Darriba et al., 2012). BEAST was run for 15 million generations with unlinked loci, independent mutation rates (specified below), strict molecular clocks, and a coalescent, constant size tree-prior. The maximum-clade-credibility tree was calculated using TreeAnnotator in BEAST. Locus substitution rates were taken from previous amphibian studies: 16S: 0.00277/lineage/mya (Lemmon et al., 2007); ND2: 0.00957/lineage/mya (Crawford, 2003); C-myc: 0.0006334/lineage/my (Lawson, 2010); and POMC: 0.000721/lineage/my (Lawson, 2010).

In order to estimate the number of species in the group, we conducted a Bayesian version of the General Mixed Yule-Coalescent analysis (bGMYC). The analysis was run using the bGMYC package (Reid and Carstens, 2012) in R v. 3.2.1 (R Development Core Team, 2015) and 100 random trees from BEAST analysis with a cut-off point of 0.5 where all individuals having a posterior probability of conspecificity greater than 0.5 are lumped into returned species (Reid and Carstens, 2012). Results were projected on the output maximum-clade-credibility tree from BEAST analysis.

Species trees were assessed with *BEAST. Models of molecular evolution and settings were the same in both BEAST and *BEAST analyses except for the use of a Yule tree prior for the latter analysis and unlinked trees between genes (mtDNA linked). Analysis in *BEAST used individuals from the same mountain block as discrete units (after confirmation of monophyly from individual-based tree constructions). Stability for BEAST and *BEAST runs were evaluated visually and through effective sample size (ESS) scores above 200 estimated in TRACER v1.6 (Rambaut et al., 2014).

Genetic distances were calculated using Geneious software (v6.1.2) and the Species Delimitation plugin v1.04 for Geneious Pro (Masters et al., 2011) was used to evaluate the taxonomic units in *H. mitchelli*.

Lastly, to address alternative phylogenetic hypotheses we enforced topological constraints on our RAxML trees and performed AU, KH and SH topology tests in CONSEL v0.2 (Shimodaira and Hasegawa, 2001). To test whether the monophyly of *H. mitchelli* (the current morphological hypothesis of this group) could be statistically rejected we conducted a topology test using 16S data (the most geographically extensively sampled data). In this analysis we constrained all *H. mitchelli* northern populations (subclades IV and VI) to the southern clade (subclades I–III), Constraint 1, reflecting the current taxonomy. We also ran this constraint without the North Pare specimen constrained as part of the same group (Constraint 2). Finally, we ran topology tests using the same constraints based on the multi-locus dataset with 30 samples (ND2, C-myc, POMC).

Morphology

Fifteen standard body measurements were taken per specimen. These include distance from the tip of the snout to urostyle (SUL), head width (HW) at the broadest, head length (HLD) from the tip of the snout diagonal to the corner of the mouth, head length (HLJ) from the tip of the snout diagonal to the jaw bone end, nostril to snout length (NS) measured from the centre of the nostril to the tip of the snout, inter-narial distance (IN) measured from the centre of each nose, eye to snout distance (EN) measured from the front part of the eye to the centre of the nose, horizontal eye distance (EE), inter-orbital distance (IO), measured from the front part of each eye, tibia fibular length (TL) measured from the knee to the ankle, thigh length (THL), tibiale fibulare length (TFL) measured from the ankle to the base of the foot, foot length (FL) measured from the base of the foot to the tip of the fourth toe, Fore-limb length (FLL) measured from the base of hand to the elbow and hand length (HL) measured from the base of the hand to the tip of the third finger. Measurements were taken to the nearest 0.1 mm using vernier calipers under a LEICA MZ 8 light microscope. A total of 213 specimens both recently collected by us and from museum collections were measured. All variables were first regressed against snout to urostyle length to remove the effect of size. After which the resulting residuals were used in Principal Component Analysis (PCA) using STATISTICA software (STATSOFT, 2007). Box plot was used to determine how SUL varied among populations. In addition, qualitative body characters used to describe the holotypes for *H. mitchelli* and *H. rubrovermiculatus* were recorded. These include; presence or absence of white patch on the heel; presence or absence of dark spots on the dorsum; presence or absence of light cantho-lateral bands which run almost to groin area (Loveridge, 1953; Schiøtz, 1975; Poynton and Broadley, 1987); presence or absence of a black border on the edge of the white canthus-lateral band. Male and females were differentiated based on the presence (males) and absence (females) of vocal sacs. Since both females and immature males lack vocal sacs we omitted from the analysis all specimens that lacked vocal sacs and were less than 21 mm in SUL.

Bioacoustics

Calls were recorded for *H. rubrovermiculatus* from Shimba Hills and from populations of *H. mitchelli* in Pemba, Nguru Mountains, Uluguru mountains, the lowlands of the Udzungwa mountains (Mang'ula) and Coastal Forests (Makangala forest). The calls were recorded using OLYMPUS digital recorder, DS-30 and were analysed using the seewave package (Sueur et al., 2008) in R. Call parameters per individual assessed included mean call duration, mean pause duration and mean dominant frequency. None of the specimens from which these calls were recorded was barcoded.

Species distribution modelling

We used species distribution models (SDM; Peterson, 2001; Elith and Leathwick, 2009), as a proxy for the abiotic environmental requirements of each lineage. As each lineage was entirely allopatric, and distribution of *H. rubrovermiculatus* is limited to Shimba Hills, assessing similarity of niche requirements is tentative due to potential spatial autocorrelation as opposed to local adaptation. Nevertheless, SDMs still remain a valuable tool for investigating differences in ecology.

Presence data were obtained from all localities sampled for this study as well as verified coordinates based on museum collections. The modelling of species followed two strategies: (1) our taxonomic conclusions based on genetics, morphology and call, and (2) the units defined by the genetic species delimitation approaches. For the latter approach we took the clades that were recognized by bGMYC (0.5 cutoff) and then recognized only clades that were >2% divergent from the nearest sister group in 16S. This approach was used to combat the known tendency of bGMYC to over-split lineages (Satler et al., 2013). In both sets of analyses, the *H. mitchelli* population from North Pare Mountains was not included in modelling approaches as the number of localities were so few (<5) and the geographic resolution was limited (1 km^2). Furthermore, the Zanzibar sample (for which genetic data were not available) was not included in the analysis given the uncertainty of its phylogenetic position.

All geo-referenced localities were validated for coordinate errors. SDMs for each lineage were created using the maximum entropy algorithm implemented in Maxent v3.3.3k (Phillips et al., 2006). Maxent is a machine-learning algorithm, popular for predicting species and habitat distributions using presence only data. All models were generated for an area limited to southern Kenya through central Malawi. Climatic data consisted of the 19 bioclimatic variables available in the WorldClim database with a 30-arc-second resolution (Hijmans et al., 2005) describing aspects of temperature and rainfall. As these 19 variables are highly correlated, we also evaluated a subset of variables with Pearson's correlation coefficients below 0.7: using ENMTools (Warren et al., 2010): Mean diurnal range, temperature seasonality, temperature annual range, mean temperature of coldest quarter, precipitation of wettest month, precipitation seasonality, precipitation of driest quarter, precipitation of warmest quarter, precipitation of coldest quarter. Due to the similarity of results, the model with all 19 variables was used for ENM modelling and further analyses. Distribution surfaces were created as the mean of 100 iterations. Model performance was evaluated using Area under Receiver Operating Characteristic curve (AUC) statistics with AUC > 0.5, indicating a better than random model prediction (Elith et al., 2006).

Climatic similarity of all species was assessed by Principal Component Analysis (PCA) using bioclim variables associated with GPS coordinates in the MASS (Venables and Ripley, 2002) and ggbiplot (Wickham, 2009) packages in R (95% confidence ellipse probability threshold) for all lineages. PCA analysis allows us to evaluate similarity even for species with limited distribution which cannot be modeled in Maxent. This method can help to elucidate habitat similarity for range-restricted taxa.

Museum abbreviations

Natural History Museum, London, UK (BMNH).

California Academy of Sciences, San Francisco, USA (CAS).

Field Museum of Natural History, Chicago, USA (FMNH).

Museum of Comparative Zoology, Harvard, Massachusetts, USA (MCZ).

National Museums of Kenya, Nairobi (NMK).

Science Museum of Trento, Italy (MTSN).

Zoological Museum University of Copenhagen, Denmark (ZMUC).

Results

16S alignment

The first alignment, using partial mitochondrial 16S gene sequences of the two species, revealed two deeply divergent clades with high support (Fig. 2A). *Hyperolius mitchelli* is shown to be paraphyletic, with populations of *H. mitchelli* from central-southern Tanzania and Malawi (hereafter southern clade) being separated from a clade that includes *H. mitchelli* from East and West Usambara, Nguu, Nguru and Pare mountains (hereafter northern clade) along with *H. rubrovermiculatus*.

Within the southern clade, populations from Lindi and coastal areas including Makangala Forest Reserve, Muyuyu Forest Reserve and Noto plateau group together (subclade I). This subclade forms a weakly supported grouping with subclade II, which consists of populations from lowland Udzungwa Mountains/Kilombero valley. Subclade III consists of populations from Uluguru Mountains, coastal forests (Makangaga and Namatimbili forest reserves) in Tanzania and Luwawa in Malawi (the region of the type locality of *H. mitchelli*). Though subclade III is weakly supported, each geographical area forms a well-supported group.

The northern clade, consisting of *H. rubrovermiculatus* and northern population of *H. mitchelli*, is composed of three well-supported distinct subclades (IV–VI). A single sample from the North Pare (subclade IV) forms a sister lineage to a clade containing populations of *H. mitchelli* from Usambara (East and West), Nguu and Nguru Mountains (subclade VI) and *H. rubrovermiculatus* (subclade V). Maximum Likelihood reconstructions of the 16S data using RAxML inferred the same topology as the Bayesian analysis.

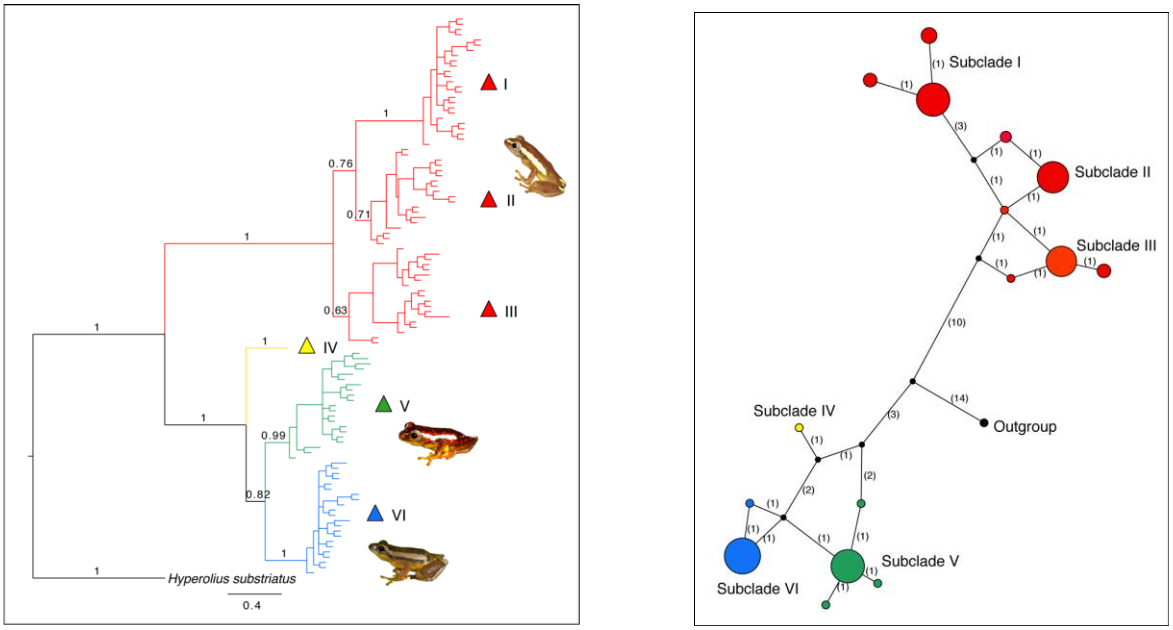

Fig. 2. (A). MrBayes topology of 16S alignment and photos of *H. mitchelli* northern and southern clades and *H. rubrovermiculatus*. (B) TCS haplotype network based on the 16S alignment. The haplotype size is proportional to the number of samples it represents, the colour codes represents, red = *H. mitchelli* subclade I-III, blue = *H. Mitchelli* subclade VI, yellow = *H. mitchelli* subclade IV and green = *H. rubrovermiculatus.*

The TCS haplotype network for the 16S gene revealed 16 haplotypes for the *H. mitchelli* and *H. rubrovermiculatus* group (Fig. 2B). These haplotypes were geographically structured, with no haplotypes being shared between clades or even between subclades. Three haplotype groups were found within the southern clade reflecting the 16S tree topology. *Hyperolius rubrovermiculatus* (subclade V) had four haplotypes while *H. mitchelli* from Usambara, Nguu and Nguru (subclade VI) shared two haplotypes (Fig. 2B).

Multi-locus alignments

The results from the first set of multi-locus analyses (all individuals, large amounts of missing data) resulted in a topology congruent with the 16S gene tree. Support values for some nodes were low, however, presumably due to missing data. In the second analysis of 30 individuals with complete representation of all genes, the topology was similar to that shown by 16S data, with strong levels of support in both MrBayes and RAxML approaches (Fig. 3).

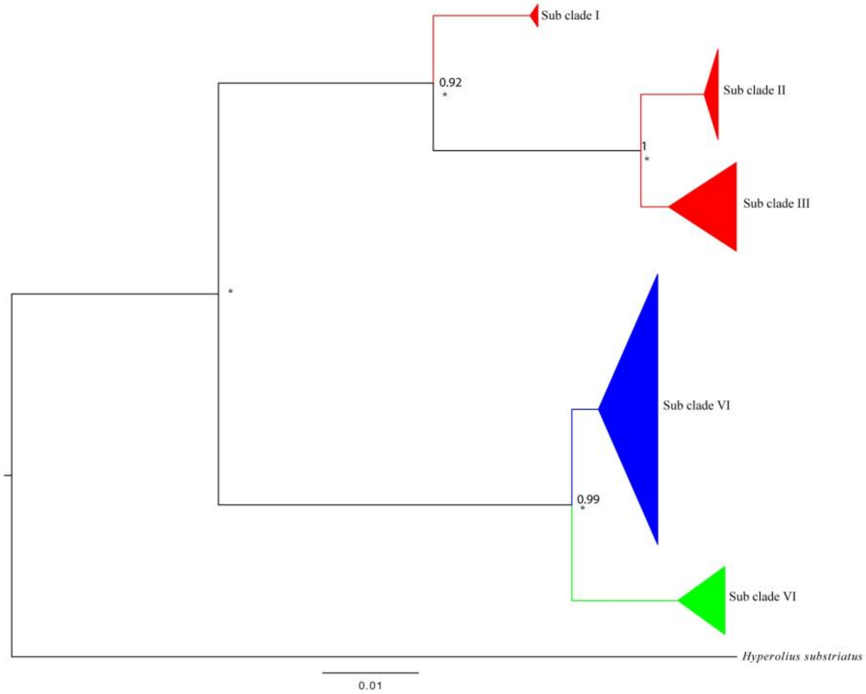

Fig. 3. Maximum Likelihood topology based on the second multi-locus dataset with 30 samples with * showing nodes with full posterior probaity support.

The ultrametric tree from BEAST analysis indicates that the separation between the northern clade and southern clade took place around 13.2 million years ago (mya) (11.1–15.5 95% Highest Posterior Density (HPD)) (Fig. 4). Divergence between subclade II and subclade III occurred around 5.4 mya (4.5–6.4 95% HPD), while the split between subclade V and VI occurred around 2.9 mya (2.3–3.7 95% HPD). Most of the divergences within the northern clade and southern clade occurred recently, ca. 2.5 mya onwards.

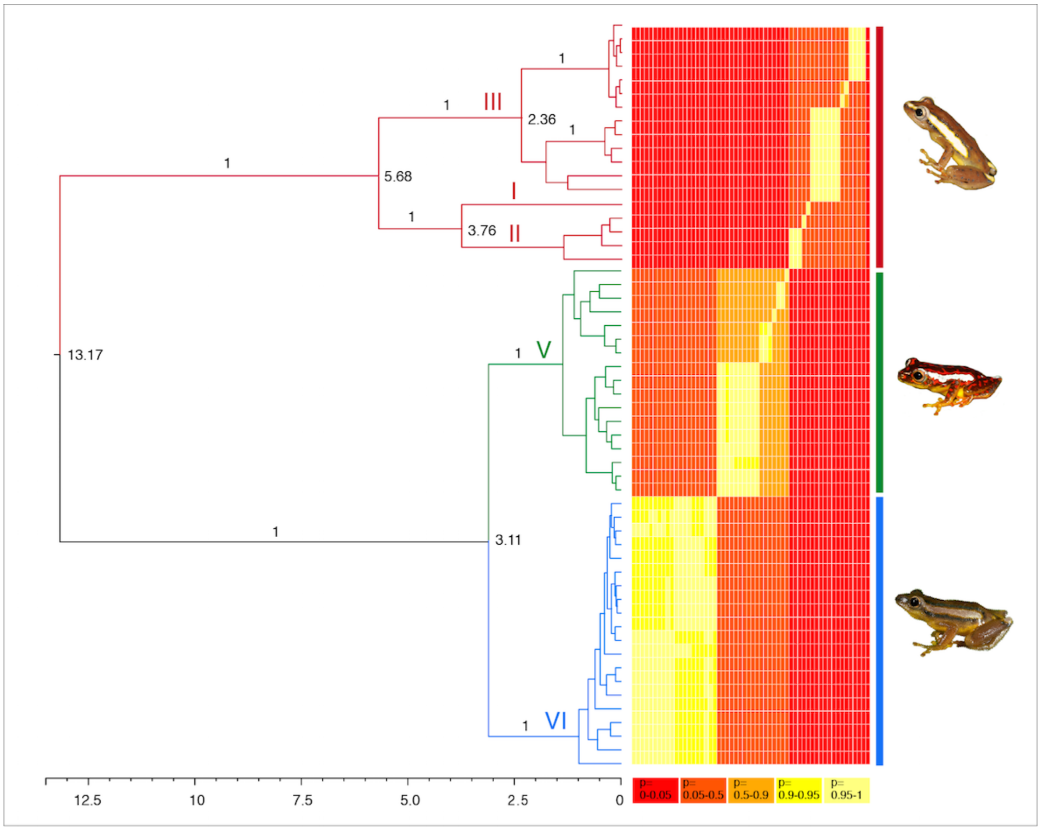

Fig. 4. Left BEAST topology based on the multi-locus alignment showing the divergence time estimates between the two clades and within subclades. Right, results from bGMYC analysis based on the 0.5 cut off point showing putative species (yellow).

Genetic distances using the 16S gene showed considerable diversity among clades. The two major clades showed 7.4% average divergence, while the average divergence between subclades I and II is 2%, and between subclade II and III 2.1% (see Table 1). The genetic differences are potentially indicative of lineages being distinct species. Species estimates in bGMYC using the standard 0.5 cut-off identified two main lineages in the northern clade (Fig. 4). For the southern clade, six putative species were identified. However, taking into consideration 16S divergence patterns where lineages ca. >2% are considered distinct, a stronger weight of evidence for three units were shown, including (1) subclade I; (2) subclade II and (3) subclade III (Fig. 4). The species tree from *BEAST analysis recovered the two major clades with maximum probability support (Fig. 5). However the subclades within both major clades received less support compared to other trees. The *BEAST tree differed from the 16S and multi-locus trees by shifting the position of subclade I to the northern clade and making it a sister taxon to *H.*

rubrovermiculatus. However this relationship was weakly resolved and the uncertainty of placement is likely due to the missing data for the sole individual representing this population. Overall, despite some variability in results between analyses and alignments, all analyses provided strong support for the paraphyly of *H. mitchelli*, with *H. rubrovermiculatus* forming a sister clade to *H. mitchelli* populations from northeastern Tanzania.

Table 1. Species delimitation results for *Hyperolius mitchelli* and *Hyperolius rubrovermiculatus* using the Species Delimitation Plugin for Geneious Pro with the 16S Bayesian phylogeny from Fig. 1. Intra-dist shows intra-specific genetic distance between samples within each species (values of 0 indicate a single representative per species), Inter-dist shows inter-specific genetic distance to the closest relative. (Roman numerals I-VI represents the subclades identified using 16S data.

Species	Closest species	Monophyletic?	Intra dist	Inter dist - closest	Intra/inter
H. substriatus	*H. rubrovermiculatus*	Yes	0.00E+00	0.096	0.00E+00
H. mitchelli (I-III)	*H. rubrovermiculatus*	Yes	0.017	0.074	0.23
H. rubrovermiculatus	*H. mitchelli* (I-III)	Yes	0.012	0.074	0.16
H. mitchelli (IV)	*H. mitchelli* (VI)	Yes	0.00E+00	0.022	0.00E+00
H. rubrovermiculatus	*H. mitchelli* (VI)	Yes	0.004	0.018	0.21
H. mitchelli (I)	*H. mitchelli* (II)	Yes	0.002	0.020	0.02
H. mitchelli (II)	*H. mitchelli* (III)	Yes	0.009	0.021	0.4

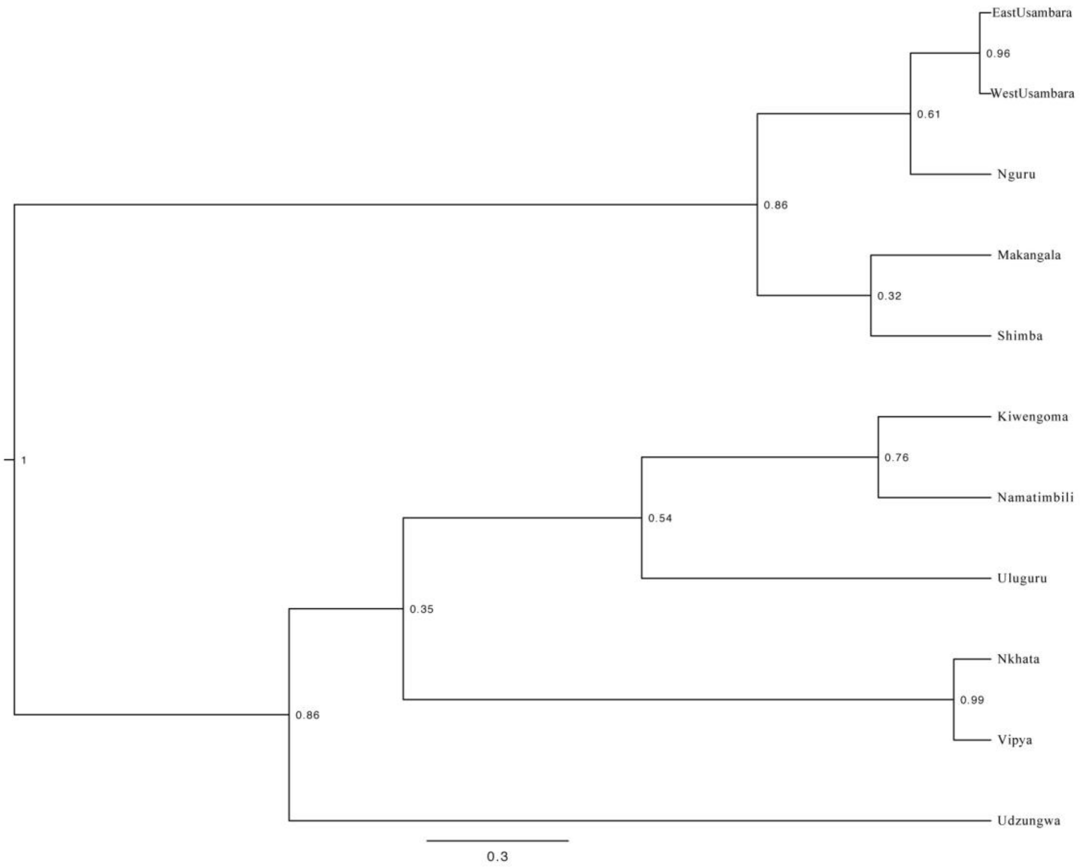

Fig. 5. Species tree from *BEAST based on a single sample per locality.

Topology tests of the alternative relationships (monophyly of *H. mitchelli* northern and southern clades, sister to *H. rubrovermiculatus*) were significantly suboptimal in all tests of both Constraints 1 and 2. Tests of the monophyly of *H. mitchelli* using multi-locus alignment was also strongly rejected (Supplementary Table 2).

Morphology

Following molecular results, *H. mitchelli* was split into two groups (northern clade subclade VI, and southern clade, subclades I–III); hence, all the descriptive and multivariate analyses were conducted based on three groups; the northern and southern *H. mitchelli* subclades and *H. rubrovermiculatus*. Northern *H. mitchelli* subclade IV only had a single specimen and was therefore excluded from morphological analysis. When all the specimens were analyzed together by sex, females had bigger SUL than the males (ANOVA n = 204; df =1, F = 76.48; P = 0.000). There was no significant difference in the

SUL among the males (ANOVA n = 148; df = 2; F = 0.41; P = 0.67) or the females (N = 56; df = 2; F = 3.12; P = 0.05) of the three groups (Supplementary Fig.1A and B). The minimum and maximum SUL measurements recorded in the three subclades are (in mm): Northern *H. mitchelli* subclade VI, male (n = 51) 22.2 and 28.8 female (n = 20) 20 and 32.3; southern *H. mitchelli* subclades I–III, male (n = 43) 21 and 28.5, female (n = 14) 22.9 and 30.5; *H. rubrovermiculatus*, male (n = 54) 21.3 and 29.6 females (n = 22) 24 and 32.1). PCA could not distinguish males or females of the three groups (Supplementary Fig. 2A and B). When ratios of various body parts to SUL were compared between the sexes only three showed significant differences; TL to SUL and HL to SUL were larger in males while HTL to SUL were larger in females. In addition male *H. rubrovermiculatus* had larger HW, FL, EE, IN TFL to SUL ratios, northern *H. mitchelli* subclade VI had larger EN and IO to SUL ratios while southern *H. mitchelli* subclade I–III had larger THL to SUL ratio. In females however there was no significant difference among the groups based on body measurements to SUL ratios except for HLJ to SUL which was larger in *H. rubrovermiculatus* while TL was larger in northern *H. mitchelli* subclade VI.

In addition to the morphometric analysis, we examined qualitative characters of subclades. In terms of similarities, *H. rubrovermiculatus,* northern *H. mitchelli* subclade VI and southern *H. mitchelli* subclades I–III all (almost uniformly) have a broad white canthal and dorsolateral stripe as well as a white patch on the heel (Fig. 2A). The dorsal coloration, however, is markedly different between *H. rubrovermiculatus* and the two clades of *H. mitchelli*. *Hyperolius rubrovermiculatus* lacks a black border around the white canthal and dorsolateral stripes or even on the heel unlike in both *H. mitchelli* subclades (Fig. 2A). The females of *H. rubrovermiculatus* have diverse dorsal colour patterns in life ranging from tan to black with orange/red vermiculations (Fig 2A) while the males are mostly greyish in colour but may be golden, golden-brown or even green with blue throats. Both northern and southern *H. mitchelli* subclades have very similar colouration with no sexual dichromatism. The dorsal colour is mostly orange especially for northern subclade VI while the intensity of the orange colour reduces towards the south of its range; Malawi specimens are mostly brown. The presence of white heel spots was almost uniform in both *H. mitchelli* subclades, though some individuals lacked these. The dorsal skin in life of females for both subclades of *H. mitchelli* is rough while those of *H. rubrovermiculatus* have smooth skin.

The single male individual from North Pare (subclade IV) is very similar to *H. rubrovermiculatus* in dorsal colour pattern. The specimen had a tan dorsal colour pattern with a white dorsolateral stripe and a white heel not bordered with black (like those of *H. rubrovermiculatus*). The skin was rough and lacked any spots.

Bioaccoustics

We analysed calls from five individuals that were opportunistically collected; one from *H. rubrovermiculatus*, one from northern *H. mitchelli* subclade VI (Pemba, Nguru Mountains) and three from southern *H. mitchelli* subclades I–III (from Uluguru mountains, lowlands of Udzungwa mountains - Mang'ula and Makangala forest). The quality of the recordings was not optimal as some were obtained with background calls and/or noise. Due to this low recording quality and lack of data on prevailing weather conditions during call recordings, comparing the call properties was difficult. Oscillograms and sound spectrograms of the five call bouts are given in Supplementary Fig. 3. *Hyperolius rubrovermiculatus* had relatively lower values for the call parameters measured (Supplementary Table 3). All of the *H. mitchelli* subclades I–III calls had mean dominant frequency above 4 kHz. *Hyperolius mitchelli* subclade VI recorded the highest mean call duration of 0.09s, while *H. rubrovermiculatus* recorded the lowest at 0.03s. However, these call properties are based on single specimen per locality thus caution should be exercised when comparing with each other or other reported call properties for *H. mitchelli* and *H. rubrovermiculatus*. Additional call properties are shown in supplementary Table 3.

Species distribution modelling

Two ecological niche models were constructed based on the taxonomic conclusions made in this paper: 1) major phylogenetic groups; northern *H. mitchelli* subclade VI, southern subclades I–III and *H. rubrovermiculatus*, and 2) clades recognized by bGMYC clades which were supported by the 2% divergence criteria i.e. *H. mitchelli* subclade I, subclade II, subclade III, subclade VI and *H. rubrovermiculatus*, modelled independently (totalling 5 lineages). In the first analysis, ecological niche models for the southern *H. mitchelli* subclades I–III (14 points) and northern *H. mitchelli* subclade VI (19 points) showed that the three lineages occupy distinct ecological niches with little overlap between the three subclades (Fig. 6A and B). Furthermore, *H. rubrovermiculatus* range map does not overlap with predicted distribution of northern *H. mitchelli*'s subclade VI. Principle Component Analysis (PCA) of 19 bioclimatic variables largely separates the northern and southern *H. mitchelli* clades. Eleven variables were responsible for 61.8% of the total variation (Fig. 7A and B).

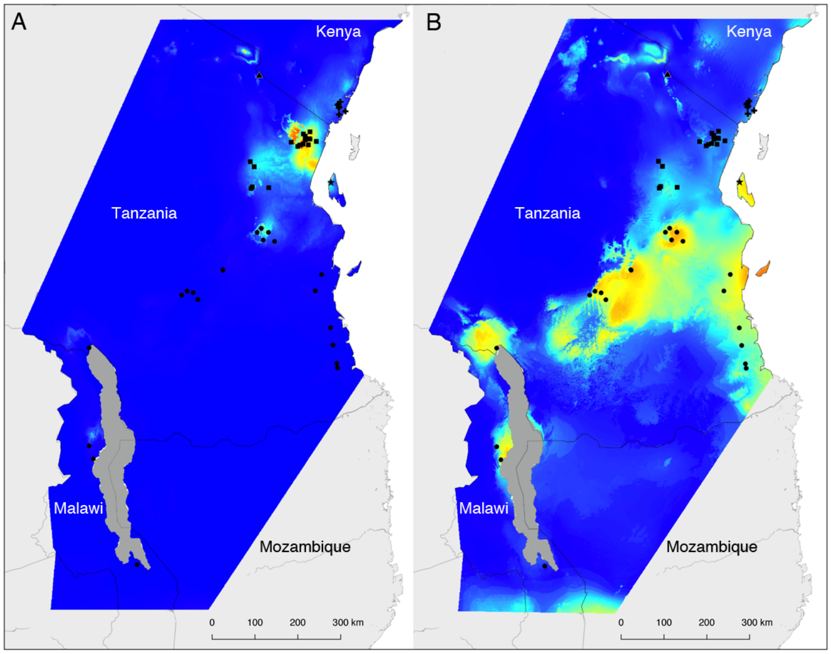

Fig. **6**. Maxent Niche Modelling for (A) *Hyperolius mitchelli* northern clade, (B) southern clade respectively.

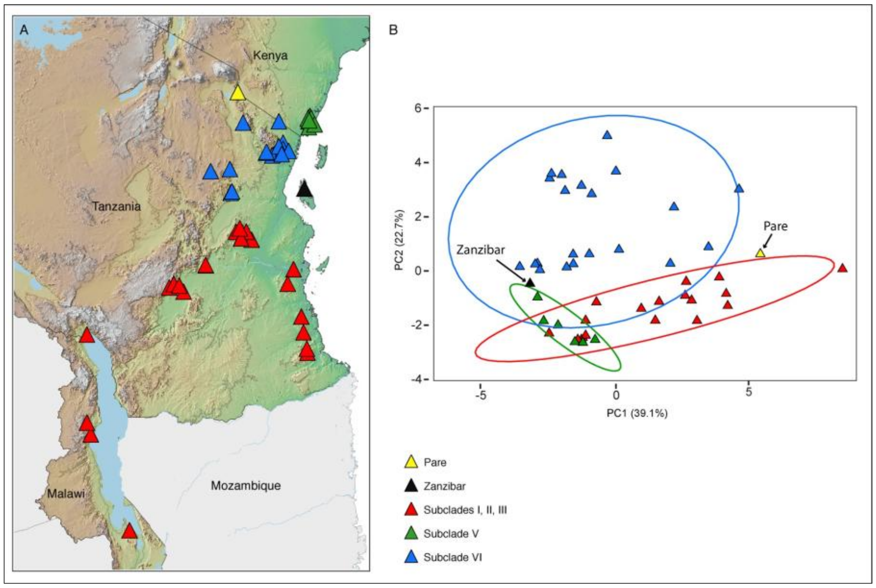

Fig. 7. (A) A map showing clade distributions and (B) Scatter plot of PCA of Bioclimatic data for *H. mitchelli* subclade I-III (red), *H. mitchelli* subclade VI (blue), *H. mitchelli* from Pare Mountains (yellow), *H. mitchelli* from Zanzibar (black) and *H. rubrovermiculatus* (green).

Variables contributing to the first axis (PC1) were bio1 (annual mean temperature), bio5 (max temperature of warmest month), bio6 (min temperature of coldest month), bio8 (mean temperature of wettest quarter), bio9 (mean temperature of driest quarter), bio10 (mean temperature of warmest quarter), bio11 (mean temperature of coldest quarter). PC2 was dominated by bio14 (precipitation of coldest month), bio15 (precipitation seasonality), bio17 (precipitation of driest quarter, and bio19 (precipitation of coldest quarter) (Table S4). In the second analysis with multiple southern clade lineages (*H. mitchelli* from subclade I, 3 points *H. mitchelli* from subclade II, 5 points and *H. mitchelli* from subclade III, 6 points), there was greater overlap in predicted distributions between climatic regions of southern clade and northern clade *H. mitchelli*. These results, however, were mainly due to the expanded range of *H. mitchelli* subclade III to northern areas. Despite the low number of points, AUC results indicated that the models performed better than random as all values were above 0.8. Overall, the results indicate that there may be ecological niche divergence exhibited among all clades, but further sampling of localities is required to fully evaluate this hypothesis and test its significance.

Taxonomy

The following proposed description of a new species is preliminary and await formal publication. The description is aimed to resolve the paraphyletic status of *H. mitchelli* with regard to *H. rubrovermiculatus*. We note that the single specimen from North Pare (subclade IV) remains taxonomically unresolved (grouping as sister group to *H. mitchelli* subclade VI and *H. rubrovermiculatus*) and this is likely to represent another new cryptic species. More material will be required to evaluate its morphological variability. Furthermore our analysis provides evidence that the southern clade includes more than one potential species given the comparable genetic differences, but this remains the subject of future extended research across this region – currently poorly understood and lacking thorough sampling and available specimens.

Hyperolius new sp.

Holotype — BMNH 2002.630 (KMH 23126), female (Fig. 8), collected on 15 January 2001 by Frontier Tanzania researchers (a group of volunteers doing biodiversity research in Tanzania) in Nilo Nature Reserve, East Usambara Mountains, Tanga Region, Tanzania.

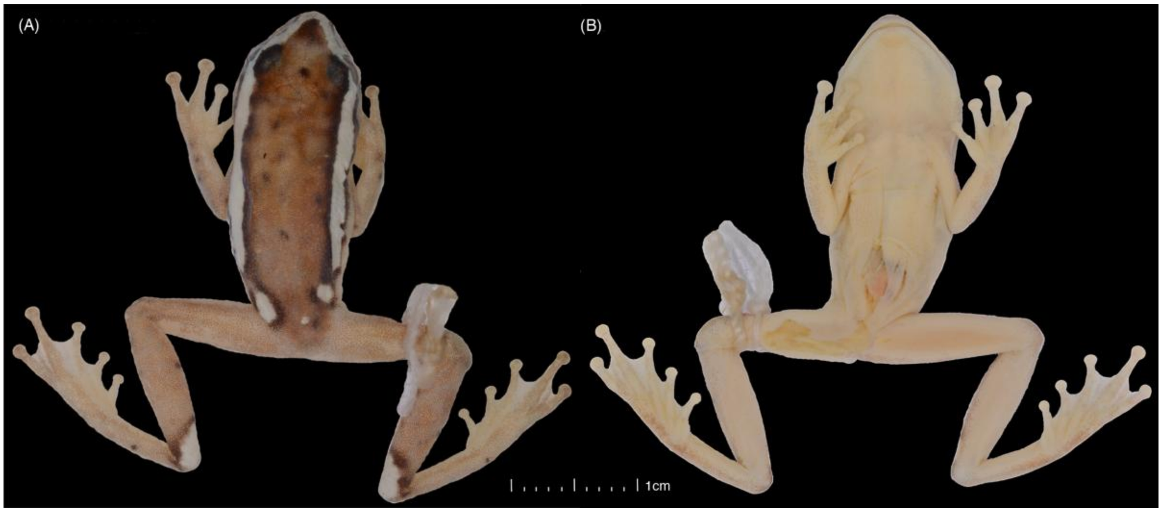

Fig. 8. (A) Dorsal view of *H. new sp*. showing the white lateral band and white spots on the heels and (B) the ventral view.

Paratypes — We restrict paratype material to localities within the East Usambara Mountains and surrounding lowlands on the basis that further detailed morphological/molecular analysis might uncover additional cryptic lineages. The paratypes are made up of two males from: Nilo Forest Reserve, East Usambara (BMNH 2002.628, BMNH 2002.629) and seven females from: Nilo Forest Reserve, East Usambara (BMNH 2002.631, BMNH 2002.632); Kambai Forest Reserve, East Usambara (BMNH 2000.758); Mtai Forest Reserve, East Usambara (BMNH 2002.411, BMNH 2002.412, BMNH 2002.413, BMNH 2002.514). All collected by Frontier Tanzania researchers on the same date as the holotype.

Referred material; 47 males from the following localities: East Usambara (ZMUC-R073872, ZMUC-R073873, ZMUC-R074180, ZMUC-R076814, ZMUC-R076820, ZMUC-R076821, ZMUC-R076822, ZMUC-R076823, ZMUC-R076825, ZMUC-R076826, ZMUC-R076827, ZMUC-R076828, ZMUC-R076829, ZMUC-R076830, ZMUC-R076831, ZMUC-R076832, ZMUC-R076833, ZMUC-R076834, ZMUC-R076835, ZMUC-R77588, ZMUC-R077646, ZMUC-R77647, ZMUC-R077816, ZMUC-R079371, ZMUC-R079372, ZMUC-R079373, ZMUC-R771485, ZMUC-R0771486, ZMUC-R771487, FMNH274303, FMNH274307, FMNH274329, FMNH274330, FMNH274411, MTSN9523, MTSN9549); West Usambara (FMNH275027, FMNH275028), Tanga (SL1952, SL1953), Nguu Mountains (MTSN5159), Lutindi, (MCZ A149045, MCZ A149046), Nguru Mountains, (MW7203, MW7205, MW7208, MTSN8277) and 12 females from East Usambara (CAS 173002, R076824, R077586, R077587, R079263, FMNH274328, ZMUC-R076819), Nguu Mountains (MTSN5161, MTSN7518, MTSN7519, MTSN7520) and Nguru Mountains MW7210. These were collected by multiple people as follows;

FMNH – Lucinda P. Lawson between April 2006 and March 2007; MCZ – Joanna Larson in 2012; MW – Mark Wilkinson in 2008 deposited at the BMNH; ZMUC-R – Arne Schiøtz 1 March 1970 and E. Werdenkinch on 26 December 1975 and 1 December 1976; MTSN were collected by Michele Menegon in February 2002 while SL – were collected by Christopher D. Barratt in December 2013 and are deposited at the University of Dar es Salaam in Tanzania.

Diagnosis — The species is referred to *Hyperolius* due to the following characteristics: Pupil horizontal; vocal sac present in male, with the gular flap oval with free margins on lateral and posterior sides; terminal discs on fingers and toes expanded and rounded; tympanum hidden (Schiøtz, 1999; Channing and Howell, 2006). *Hyperolius new sp*. can be distinguished from other *Hyperolius* in East Africa (Schiøtz et al., 1999; Channing and Howell, 2006; Haper et al., 2010) by; throat without spines (spinose asperities on gular flap in males of *H. davenporti* Loader, Lawson, Portik and Menegon, 2015, *H. burgessi* Loader, Lawson, Portik and Menegon, 2015, *H. spinigularis* Stevens, 1971, *H. ukwiva* Loader, Lawson, Portik and Menegon, 2015), light heel spot usually present (always absent in all *Hyperolius* in the area except *H. mitchelli* and *H. rubrovermiculatus*); no translucent green belly skin (present in *H. nasutus* complex Gunther, 1864, *H. pusillus*, Cope, 1862); no sharply pointed snout (present in *H. parkeri*); generally rough and granulose skin in both sexes (smooth in most *Hyperolius* females except *H. mitchelli*). In the phylogenetic analysis (see Genetic results section), *H. new sp*. is sister to *H. rubrovermiculatus*, with an uncorrected avarage p-distance of 1.8% (1.6–2.0%) and is 7.4% divergent from *H. mitchelli*. *Hyperolius new sp*. differs from *H. rubrovermiculatus* with the latter having rough skin in males and a brightly coloured red and white/black dorsal patterning in adult females (see pictures of live specimens in Figure 2A). However, *H. new sp*. dorsal colour pattern is very similar to that of *H. mitchelli* except that the intensity of the orange hue reduces as one moves south where Malawi specimens are brown in colour. Both *H. new sp*. and *H. mitchelli* have a white dorsolateral band and heel bordered with black, unlike in *H. rubrovermiculatus* where the black border is missing. In addition, both males and females of *H. new sp*. and *H. mitchelli* have a rough dorsal skin unlike in *H. rubrovermiculatus* where only the males have a rough skin.

Hyperolius new sp. is equal in Snout to Urostlye Length (SUL) to *H. mitchelli* and *H. rubrovermiculatus*. Their males have an average size (SUL in mm) of 24.5 (n = 54) vs 23.9 (n = 48) and 24.4 (n = 54) while their females measure 28.2 (n = 20) vs 26.2 (n = 14) and 28.0 (n = 22) in *H. mitchelli* and *H. rubrovermiculatus* respectively.

In vocalisation *H. new sp*. has a 3.5 kHz average dominant frequency in advertisement call, compared to *H. mitchelli* (4.4 kHz) and *H. rubrovermiculatus* (3.2 kHz), and a 0.09 pulse call (0.04 ms *H. mitchelli*, 0.03 ms *H. rubrovermiculatus*).

Description of holotype — Moderate-sized hyperoliid. Pupil horizontal. Snout blunt, slightly rounded (Fig. 8). Canthus rostralis angular, slightly convex on the horizontal plane and slightly concave on the vertical plane. The body measurements are as follows; SUL = 25.4 mm, HW = 9.4 mm, HLD = 7.9 mm, HLJ = 8.4 mm, NS = 1.4 mm, IN = 2.3 mm, EN = 2.9 mm, EE = 2.9 mm, IO = 5.0 mm, TL = 13.1 mm, THL = 13 mm, TFL = 8.3 mm, FLL = 6.6 mm, HL = 7.3 mm, FL = 10.3 mm. Toes have expanded fleshy discs, webbing is moderate, almost reaching distal tubercle on the first and third toes and the middle tubercle of the fourth toe. The hands have expanded rounded fleshy discs. Webbing just reaching distal subarticular tubercle of the outer finger and slightly reduced on all other fingers. Dorsal skin surface is smooth while the ventral skin surface strongly granular.

Colouration in preservative — The holotype has a light brown dorsal colour, with darkly and thickly edged white dorsolateral stripes (width 1.3 mm at level of eye, thickening at mid-body to 1.7 mm), ending ¾ posteriorly on the dorsum. The stripes are followed posteriorly by an irregular blotch on either side near the leg insertion. A large spot on the heel (length 3 mm) white with a dark line. Small black chromatophores forming irregular spots on mid and anterior parts of dorsum (see Fig. 8). Forelimbs, hindlimbs are similar to dorsal colouration. The ventral side is cream-coloured.

Tadpoles have been described for this species (as *H. mitchelli*; see Channing and Crapon de Caprona, 1987).

Paratypes — Head and body proportions are in close agreement with those of the holotype (Appendix I). The colour patterns of specimens is in general close agreement with that of the holotype with variations in the thickness of the lateral dorsal stripe (e.g. BMNH 2000.628, BMNH 2000.632), presence of irregular posterior blotches (e.g. BMNH 2000.631) or their absence (e.g. BMNH 2000.628). The heel spot is generally large (>2.5 mm) and conspicuous.

Colour patterns of adults in life — Head and dorsum are brown with a creamy white mottling on the back. In some individuals, the mottling extends along the side of the animal. The ventral side is generally white with the exception of the asperities in males, which are dark brown to black. Forelimbs and hindlimbs are mottled creamy white matching the dorsum, with flashes of orange on the thighs and feet.

Sexual dimorphism — Females (n = 20, x = 28.2 mm, SD = 3.1) attain larger SUL than the males (n = 54, x = 24.5 mm, SD = 1.8) (Supplementary Fig. 1A and B). Males are easily distinguished from females by the presence of a gular sac.

Advertisement call — A single call from Nguru mountains is a short scream similar to the call of *H. rubrovermiculatus*. The mean dominant frequency is 3.5 kHz, mean signal duration of 0.09 s and mean duration between notes of 0.61 s (see Supplementary Fig. 3).

Distribution and conservation — The species is found distributed in Nguru, Nguu, West and East Usambara Mountains (including East Usambara lowlands) (See Fig. 7A). The holotype and paratypes were collected in the transition zone at the edge of submontane forest (>800 m above sea level) with canopy height of less than10 m, ground vegetation layer cover of more than 50% and shrub layer cover less 10%. The new species has a restricted distribution and may qualify for vulnerable threat category of the IUCN Redlist.

Discussion

Molecular data based on both mitochondrial (16S, ND2) and a multi-locus alignment (16S, ND2, C-myc, POMC) show unambiguously that *H. mitchelli* is paraphyletic. Two major clades of *H. mitchelli* were recovered in all optimal topologies (one consisting of populations from north-eastern Tanzania and the other consisting of populations from central and southern Tanzania and Malawi). The northern clade of *H. mitchelli* clusters with the geographically adjacent population of *H. rubrovermiculatus* from Shimba Hills southeastern Kenya. Our new finding of paraphyly in *H. mitchelli* warrants a taxonomic solution.

To resolve the paraphyly of *H. mitchelli*, two options were available; 1) use the name *H. mitchelli* to describe all subclades including *H. rubrovermiculatus* and subclade VI of *H. mitchelli* (= *H. new sp*.), or, 2), retain both *H. mitchelli* and *H. rubrovermiculatus* given they exhibit substantial genetic and minor morphological variation and describe the subclade VI of *H. mitchelli* as a new species (*H. new sp*.). We here argue that option 2 is the optimal solution. Although option 1 retains monophyly of *H. mitchelli*, substantial and consistent genetic (>8%) and morphological variations (colour and skin texture) is shown between *H. mitchelli* and *H. rubrovermiculatus*. The later is an isolated and diagnosable evolutionary significant unit and therefore deserves taxonomic recognition. Furthermore, previous authors have generally supported the view that *H. rubrovermiculatus* is a distinct species with only one leading authority questioning its potential recognition (Channing and Howell, 2006) but later changing their mind (Channing et al., 2012).

We recognize that *H. new sp*. is only marginally genetically divergent from *H. rubrovermiculatus* (1.8%) but given consistent morphological, ecological, and acoustic differences, is a distinct and diagnosable species from *H. rubrovermiculatus*. Examples of species estimated to be “young” are evident in the taxonomic literature for amphibians (e.g. Portilo and Greenbaum, 2014). In contrast to the marginal genetic differences shown between *H. new sp*. and *H. rubrovermiculatus*, *H. new sp*. shows large genetic differences (>8%) from *H. mitchelli* however is less easily distinguished morphologically. Only niche differences and minor dorsal colour variation are able to distinguish these two species. Conclusively though, alternative phylogenetic hypotheses of *H. new* sp. grouping with *H. mitchelli,* (reflecting current taxonomy) resulted in a tree significantly suboptimal than our best tree.

Morphological data on the three species discussed in this paper did not reflect the genetic divergence between clades. We did not find significant differences in the various body measurements of the three species nor did PCA distinguish among them. However, there is a distinct dorsal colour variation between *H. new sp*. and its closest sister species *H. rubrovermiculatus*. Firstly, in *H. new sp*. there is no sexual dichromatism unlike in *H. rubrovermiculatus* where males and females have different dorsal colours/patterns. Secondly *H. new sp*. have a black border on the white dorsolateral band and the white heel which is absent in *H. rubrovermiculatus*. Finally, in *H. new sp*. both male and females have a rough dorsum unlike in *H. rubrovermiculatus* where only the males have a rough dorsum (Schiøtz, 1975; Schiøtz, 1999; Harper et al., 2010). *Hyperolius new* sp. is however very similar to *H. mitchelli* in terms of dorsal colouration. The base colour of the dorsum of *H. new sp*. is orange while *H. mitchelli* is brown (Fig. 2A). In the literature the dorsum colour of *H. mitchelli* has been described as varying from orange to brown but nowhere has this been associated with a particular region or population (see Schiøtz, 1975; Channing and Howell, 2006; Harper et al., 2010). Presence/absence of the white spots on the heel and black spots on the body are variable as was also noted by Poynton and Broadley (1987) who considered them unreliable for diagnosing *H. mitchelli*. It is not well understood why *H. mitchelli* sensu stricto and *H. new sp*. have maintained a similar colour pattern from Malawi through northern Tanzania, despite high levels of molecular divergence. This is in contrast to *H. new sp*. and *H. rubrovermiculatus* which are less than 200 km apart, less divergent and yet exhibit very different dorsal colour patterns. Patterns of colouration variability and cryptic species within amphibians are common, particularly so in *Hyperolius* (see Liedtke et al., 2014). Amphibians are known to be morphologically conservative (Cherry et al., 1978) and genetic studies have revealed many cryptic species in taxa that were once thought to be widespread and our study adds to this common pattern (Barratt et al., 2017). For example McLeod (2006) discovered 22 distinct evolutionary lineages in *Limnonectes kuhlii* Tschudi, 1838 historically thought to be a single species. Most of these new species descriptions were backed by additional taxonomic evidence, such as genetics, bioacoustics and or ecological niche modeling.

Evidence for the distinction among *H. new sp.*, *H. mitchelli* and *H. rubrovermiculatus* is further supported by modelling their distributions. Species distribution models provide evidence of a potential divergence of geographical distribution between *H. mitchelli* and *H. new sp*. The differences between the mainly submontane *H. new sp.* and the strictly lowland *H. rubrovermiculatus* coupled with colour differences could represent an example of peripatric speciation similar to that noted in the *H. spinigularis* complex (Lawson et al., 2015). An evaluation of population genetic patterns will be required to provide critical evidence towards such a hypothesis.

Preliminary inferences on acoustic differences among species were outlined in this paper. All the three species call from vegetation around water bodies (Schiøtz, 1999; Channing and Howell, 2006) with *H. new sp.*, *H. mitchelli* and *H. rubrovermiculatus* possible distinction based on their call properties. Schiøtz (1975; 1999) reported the dominant frequency and call duration of *H. mitchelli* from East Usambara (now *H. new sp.*) to be 3500 cps and 0.05 s respectively while Channing and Howell (2006) described the call of *H. mitchelli* (plus *H. rubrovermiculatus*) as having a dominant frequency of 3.6 kHz and about 0.1 s long (compare this study for *H. new sp*. 3.5 kHz and 0.09 s respectively). For *H. rubrovermiculatus,* Schiøtz (1975) reported dominant frequency and call duration 3000–3500 cps and 0.05 s compared to 3.16 kHz and 0.03 s in our study. Further, Rödder and Böhme (2009) reported the dominant frequency and call duration of *H. mitchelli* from Uluguru (Subclade III) 1.68–4.63 kHz and 0.21–0.42 s respectively. In this study, we recorded 4.48–5.14 kHz and 0.03–0.04 s for *H. mitchelli* subclades I–III which are comparable to their study. From these analyses, *H. mitchelli* seems to have higher dominant frequency while *H. rubrovermiculatus* has the lowest. Further, the call duration for *H. new sp.* appears to be longer than those of *H mitchelli* and *H. rubrovermiculatus*. Our call property results, however, should be interpreted with caution since they represent recordings from single specimens per locality and in addition data on prevailing weather conditions were not recorded (Giacoma and Castellano, 2001). Further studies of acoustic variation in these species across their geographical distributions are necessary.

Hyperolius mitchelli has been known as a wide-ranging frog from northeastern Tanzania all the way to Malawi, with little indication that cryptic species might exist. Beyond the proposed description of *H. new sp.*, *H. mitchelli* sensu stricto is also shown to have high geographic variability, with the average distance among populations over 2% (Table 1). This genetic difference is not reflected in our limited sampling of morphological variation among populations. The potential description of these species requires further sampling of these populations which allow a better estimation of morphological variation.

Based on molecular clock estimations, the divergence between the *H. mitchelli* clade and the *H. new sp./H. rubrovermiculatus* clade was during the Miocene 13.2 mya (11.1–15.5 95% HPD), separating central/southern Tanzania and Malawi populations from those of the northeastern Tanzania and Shimba Hills. Similar results have been recorded in other taxa covering these ranges, including amphibians (Blackburn and Measey, 2009; Lawson, 2010) and birds (Bowie et al., 2004). Numerous geographical changes have occurred in East Africa, including volcanism (Griffiths, 1993), habitat changes (deMenocal, 1995; Lovett, 1993) and riverine barrier changes (Griffiths, 1993), which could account for separating populations. Within *H. mitchelli* sensu stricto (southern clade), divergence between subclade I and subclade II (4.5–6.4 mya 95% HPD) is comparable with that between *H. new sp.* and *H. rubrovermiculatus* (2.3–3.7 mya 95% HPD). Most of divergences within the *H. new sp.*, *H. rubrovermiculatus* and *H. mitchelli* mainly occurred recently, from 2.5 mya onwards. All divergence occurred prior to the Pleistocene period commonly associated with many species divergences within the region (deMenocal, 1995; Bryja et al., 2014).

Hyperolius rubrovermiculatus is listed as Endangered (EN) by the IUCN Redlist of threatened species, while *H. mitchelli* is listed as of least concern (LC). With the proposed description of the subclade VI of the original *H. mitchelli* as a new species (*H. new sp.*), we emphasize the need to re-evaluate some of the wide-ranging species in this region. The newly described species may qualify for listing in IUCN Redlist threat categories and targeted conservation initiative for its conservation may be priority.

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Supplementary material

Fig. S1. (A) Box plot of snout to urostyle length (SUL) of males and (B) females samples of *H. mitchelli* subclades I-III, *H. mitchelli*, subclade VI and *H. rubrovermiculatus*.

Fig. S2 (A) PCA of males and (B) females of *H. mitchelli* subclades VI (blue), *H. mitchelli* subclade I-III (red) and *H. rubrovermiculatus* (green) showing lack of differentiation among the samples.

Fig. S3. Oscillograms and spectrograms showing call properties of *H. mitchelli* subclades I, II, III, VI and *H. rubrovermiculatus* (subclade V).

Table S1. Substitution models from jModelTest v2.1.6 used in the multi locus analysis 1 and 2 respectively.

Table S2. Topology test results of alternative phylogenetic relationships based (A) 16S and (B) Multi-locus alignment. 16S: Optimal – optimal tree, Constraint 1 – *H. mitchelli* subclades I-III + subclades IV and VI. Constraint 2 – subclades VI + subclades I-III. Multi-gene dataset (ND2, C-myc, POMC): Optimal – optimal tree, Constraint1 – subclade VI + subclades I-III. obs – the observed log-likelihood difference, bp – bootstrap probability, np – bootstrap probability calculated from multiscale bootstrap, pp = Bayesian posterior probability. AU – Approximately Unbiased test, KH, Kishino-Hasegawa test, SH – Shimodaira-Hasegawa test, WKH – Weighted Kishino-Hasegawa test, WSH – Weighted Shimodaira-Hasegawa test.

Table S3. Summary of call properties for *H. mitchelli* from subclade I = Makangala forest, subclade II = Udzungwa Mountains, subclade III = Uluguru Mountains, subclade VI from Nguru Mountains and *H. rubrovermiculatus* from Shimba Hills.

Table S4. Factor loadings and standard deviation of the first four principal components (PC) of the 19 bioclim variables used in SDM.

References

Barratt, C.D., Bwong, B.A., Onstein, R.E., Rosauer, D.F., Menegon, M., Doggart, N., Nagel, P., Kissling, W.D., Loader, S.P. (2017): Environmental correlates of phylogenetic endemism and the conservation of refugia in the coastal forests of Eastern Africa. Divers. Distrib. **23**:875-887 (doi: 10.1111/ddi.12582).

Barratt, C.D., Lawson, L.P., Bittencourt-Silva, G.B., Doggart, N., Morgan-Brown, T., Nagel, P., Loader, S.P. (2017): A new, narrowly distributed, and critically endangered species of spiny-throated reed frog (Anura: Hyperoliidae) from a highly threatened coastal forest reserve in Tanzania. Herpetol. J. **27**: 13-24.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K., Das, I. (2007): Cryptic species as a window on diversity and conservation. Trends Ecol. Evol. **22**: 148-155.

Blackburn, D.C., Measey, G.J. (2009): Dispersal to or from an African biodiversity hotspot? Mol. Ecol. **18**:1904–1915. http://doi.org/10.1111/j.1365-294X.2009.04156.x.

Bouckaert, R., Heled, J., Kühnert, D. Vaughan, T., Wu, C.-H., Xie, D. Suchard, M.A., Rambaut, A., Drummond, A.J. (2014): BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. **10**: e1003537. http://dx.doi.org/10.1371/journal. pcbi. 1003537.

Bowie, R.C.K., Fjeldså, J., Hackett, S.J., Crowe, T.M. (2004): Systematics and Biogeography of Double-Collared Sunbirds from the Eastern Arc Mountains , Tanzania. The Auk, **121**: 660–681. http://doi.org/10.2307/4090305.

Bryja, J., Mikula, O., Patzenhauerová, H., Oguge, N. O., Šumbera, R., Verheyen, E. (2014): The role of dispersal and vicariance in the Pleistocene history of an East African mountain rodent, Praomys delectorum. J. Biog. **41**: 196–208. http://doi.org/10.1111/jbi.12195.

Bwong, B.A, Malonza, P.K, Wasonga, D.V., Nyamache, J.O., Nagel, P. Loader, S.P. (2014): At a biogeographical crossroads: Amphibian paradise in Shimba Hills of Kenya. Froglog, **22**: 72–73.

Channing, A., Capron de Caprona, M.-D. (1987): The tadpole of Hyperolius mitchelli (Anura: Hyperoliidae). S. Afr. J. Zool. **22**: 235-237.

Channing, A., Howell, K.M. (2006): Amphibians of East Africa. Cornell University Press Ithaca, New York.

Channing, A., Rödel, M.O., Channing, J. (2012): Tadpoles of Africa: The biology and identification of all known tadpoles in sub-Saharan Africa. Edition Chimaira, Frankfurt am Main.

Channing, A., Hillers A., Lötters, S., Rödel, M., Schick, S., Conradie, W., Rödder, D., Mercurio V., Wagner, P., Dehling, J.M., Du Preez, L.H., Kielgast, J., Burger, M. (2013): Taxonomy of the super-cryptic *Hyperolius nasutus* group of long reed frogs of Africa (Anura: Hyperoliidae), with descriptions of six new species. Zootaxa**, 3620**: 301–350. http://doi.org/10.11646/zootaxa.3620.3.1.

Cherry, L.M., Case, S.M., Wilson, A.C. (1978): Frog perspective on morphological difference between Humans and Chimpanzees. Science **200**: 209-211.

Crawford, A.J. (2003): Relative rates of nucleotide substitution in frogs. J. Mol. Evol. **57**: 636-641.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D. (2012): JModelTest 2: more models, new heuristics and parallel computing. Nature Methods **9**: 772.

Elith, J., Graham, C., the NCEAS species distribution modelling group (2006): Novel methods improve prediction of species' distributions from occurrence data. Ecography **29**: 129-151.

Elith, J., Leathwick, J.R. (2009): Species Distribution Models: Ecological Explanation and Prediction Across Space and Time. Ann. Rev. Ecol., Evol.Syst. **40**: 677–697. http://doi.org/10.1146/annurev.ecolsys.110308.120159.

Frost, D.R. (2016): Amphibian Species of the World: an Online Reference Version 6.0 (accessed on 21st September 2016). Electronic database accessible at http://research.amnh.org/herpetology/amphibia/index.html. Am. Mus. Nat. Hist. New York, USA.

Giacoma, C., Castellano, S. (2001): Advertisement call variation and speciation in the *Bufo viridis* Complex, in Ryan, M.J. (ed), Anuran Communication. Smithsonian Institution Press. Washington and London.

Griffiths, C.J. (1993): The geological evolution of East Africa in Lovett, J.C., Wasser, S.K. (Eds.). Biogeography and ecology of the rain forests of Eastern Africa. Cambridge University Press.

Harper, E.B., Measey, G.J., Patrick, D.A., Menegon, M., Vonesh, J.R. (2010): Field guide to amphibians of the Eastern Arc Mountains and Coastal Forests of Tanzania and Kenya. Camerapix Publishers International, Nairobi, Kenya.

Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., Hayek, L.-A., Foster, M.S. (1994):

Measuring and monitoring biological diversity. Standard methods for amphibians. Washington and London.

Hijmans, R.J., Cameron, S.E., Para, J.L., Jones, P.G., Jarvis A. (2005): Very high resolutions interpolated climate surfaces for global land areas. Int. J. Climatol. **25**: 1965-1978.

Kato, K., Misawa, K., Kuma K. Miyata, T. (2002): MAFT: a novel method for rapid sequence alignment based on fast Fourier transform. Nucleic Acids Res. **30**: 3059-3066.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T. (2012): Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **28**: 1647-1649.

Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S. (2012): PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 2**9**: 1695-1701.

Lawson, L.P. (2010): The discordance of diversification: Evolution in the tropical-montane frogs of the Eastern Arc Mountains of Tanzania. Mol. Ecol., **19**: 4046–4060. http://doi.org/10.1111/j.1365-294X.2010.04788.x.

Lawson, L.P., Bates, J.M., Menegon, M., Loader, S.P. (2015): Divergence at the edges: peripatric isolation in the montane spiny throated reed frog complex. BMC Evol. Biol., **15**: 128. http://doi.org/10.1186/s12862-015-0384-3.

Lemmon, E.M., Lemmon, A.R., Cannatella, D.C. (2007): Geological and climatic forces driving speciation in the continentally distributed trilling chorus frogs (*Pseudacris*). Evolution 6**1**: 2086-2103.

Liedtke, H.C., Huegli, D., Dehling, J.M., Pupin, F., Menegon, M., Plumptre, A.J., Kujirakwinja, D., Loader, S.P. (2014): One or two species? On the case of *Hyperolius discodactylus* Ahl, 1931 and H. alticola Ahl, 1931 (Anura: Hyperoliidae). Zootaxa **3768**: 253–290. http://doi.org/10.11646/zootaxa.3768.3.2.

Loader, S.P., Gower, D.J., Ngalason, W., Menegon, M. (2010): Three new species of *Callulina* (Amphibia: Anura: Brevicipitidae) highlight local endemism and conservation plight of Africa's Eastern Arc forests. Zool. J. Linn. Soc., **160**: 496–514. http://doi.org/10.1111/j.1096-3642.2010.00652.x.

Loader, S.P., Lawson, L.P., Portik, D.M., Menegon, M. (2015): Three new species of spiny throated reed frogs (Anura: Hyperoliidae) from evergreen forests of Tanzania. BMC Res. Notes, **8**: http://doi.org/10.1186/s13104-015-1050-y.

Loveridge, A. (1953): Zoological results of a fifth Expedition to East Africa. IV. Amphibians from Nyasaland and Tete. Bull. Mus. Comp. Zool. Harvard. V.**110**: 325-406.

Lovett, J.C. (1993): Climatic history and forest distribution in eastern Africa in Lovett, J.C., Wasser, S.K. (Eds.). Biogeography and ecology of the rain forests of Eastern Africa. Cambridge University Press.

Masters, B.C., Fan, V., Ross, H.A. (2011): Species delimitation: Geneious plugin for the exploration of species boundaries. Mol. Ecol. Res. **11**: 154–157.

McLeod, D.S. (2010): Of least concern? Systematics of a cryptic species complex: *Limnonectes kuhlii* (Amphibia: Anura: Dicroglossidae). Mol. Phylogenet. Evol. **56**: 991–1000. http://doi.org/10.1016/j.ympev.2010.04.004.

Myers, N., Mittermeier, R.A., da Fonseca, G.A.B., Kent, J. (2000): Biodiversity hotspots for conservation priorities. Nature **403**: 853–58. doi:10.1038/35002501.

deMenocal, P.B. (1995): Plio-Pleistocene African climate. Science **270**: 53-59.

Oliver, L.A., Rittmeyer, E.N., Kraus, F., Richards, S.J., Austin, C.C. (2013): Phylogeny and phylogeography of *Mantophryne* (Anura: Microhylidae) reveals cryptic diversity in New Guinea. Mol. Phyl. Evol. **67**: 600–607. http://doi.org/10.1016/j.ympev.2013.02.023.

Peterson, A.T. (2001): Predicting species geographic distributions based on ecological niche modeling. The Condor **103:** 599–605. http://doi.org/10.1650/0010-5422(2001)103.

Phillips, S.J., Anderson, R.P., Schapire, R.E. (2006): Maximum entropy modeling of species distribution. Ecol. Model. **190**: 231-259.

Pickersgill, M. (2007): Frog search. Results of expedition to Southern and Eastern Africa. Edition Chimaira, Frankfurt am Main.

Portillo, F., Greenbaum, E. (2014): At the edge of a species boundary: A new and relatively

young species of *Leptopelis* (Anura: Arthroleptidae) from the Itombwe Plateau, Democratic Republic of the Congo. Herpetologica **70**: 100-119.

Poynton, J.C., Broadley, D.G. (1987): Amphibia Zambesiaca 3. Rhacophoridae and Hyperoliidae. Ann. Natal Mus., **28**: 161–229. Retrieved from http://content.ajarchive.org/cgibin/showfile.exe?CISOROOT=/03040798&CISOPTR=421%5Cnhttp://hdl.handle.net/10499/AJ401.

R Development Core Team (2015): R*:* A language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria. v3.2.1. Available from: <http://www.R-project.org>.

Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J. (2014): Tracer v1.6, Available from: <http://beast.bio.ed.ac.uk/Tracer>.

Reid, N.M., Carstens, B.C. (2012): Phylogenetic estimation error can decrease the accuracy of species delimitation: a Bayesian implementation of the general mixed Yule-coalescent model. BMC Evol. Biol. 12: 196.

Ronquist, F., Teslenko, M., Van der Ma**rk**, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P. (2012): MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. **61**: 539**-**542.

Rödder, D., Böhme, W. (2009): Who‘s who? Comparison of the advertisement calls of two East African sister species of *Hyperolius* (Anura: Hyperoliidae). Salamandra **45**: 180-185.

Satler, J.D., Carstens, B.C., Hedin, M. (2013): Multi-locus species delimitation in a complex of morphologically conserved trapdoor spiders (Mygalomorphae, Antrodiaetidae, Aliatypus). Syst. Biol. **62**: 805-823.

Schiøtz, A. (1975): The Treefrogs of Eastern Africa. Steenstrupia, Copenhagen.

Schiøtz, A. (1999): The Treefrogs of Africa. Edition Chimaira. Frankfurt am Main.

Shimodaira, H., Hasegawa, M. (2001): CONSEL: for assessing the confidence of phylogenetic tree selection. Bioinformatics **17**: 1246**-**1247.

Stamatakis, A. (2014): RAxML Version 8: a tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics **30**: 1312–1313.

Statsoft, Inc. (2007): STATISTICA (data analysis software system) version 8.0. www.statsoft.com.

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

Templeton, A.R, Crandall, K.A., Sing, C.F. (1992): A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics, **132**: 619–633.

Venables, W.N., Ripley, B.D. (2002): Modern applied statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0.

Vieites, D. R., Wollenberg, K.C., Andreone, F., Köhler, J., Glaw, F., Vences, M. (2009): Vast underestimation of Madagascar’s biodiversity evidenced by an integrative amphibian inventory. P. Natl. Acad. Sci. USA **106**: 8267–8272. http://doi.org/10.1073/pnas.0810821106.

Warren, D.L., Glor, R.E., Turelli, M. (2010): ENMTools: a toolbox for comparative studies of environmental niche models. Ecography **33**: 607-611.

Wickham, H. (2009): ggplot2 Elegant Graphics for Data Analysis. Springer-Verlag New York.

APPENDIX

Appendix I. List of specimens their museum numbers, locality and morphometric data in mm.

Species	ID	Lat	Long	Sex	SUL	HW	HLD	HLD	NS	IN	EN	EE	IO	TL	THL	TFL	FL	FLL	HL
H. new sp.	BM2002.628	-4.910	38.664	M	25.5	9.1	8	8.8	1.4	2.3	3	3	5.1	12.3	12.5	8	9.6	5.8	6.3
H. new sp.	BM2002.629	-4.910	38.664	M	23.7	8.4	7	8.3	1.3	2	2.6	2.9	4.9	13.1	12.9	8.5	6.4	6.7	6.5
H. new sp.	BM2002.630	-4.910	38.664	F	25.4	9.4	7.9	8.4	1.4	2.3	2.9	2.9	5	13.1	13	8.3	10.3	6.6	7.3
H. new sp.	BM2002.631	-4.910	38.664	F	21.8	7.8	7.2	7.8	1.4	2.2	2.3	2.9	4.8	10.4	10.5	6.8	8.4	5.4	5.8
H. new sp.	BM2002.632	-4.910	38.664	F	25.1	8.8	8.2	8.9	1.5	2.4	2.9	3.1	5.4	12.2	13.1	7.9	10.1	6.1	5.6
H. new sp.	BM2000.758	-4.300	38.683	F	24.9	8.3	7.5	8	1.4	2.3	2.6	2.6	5.1	13.2	12.7	7.5	10.4	6.1	6.6
H. new sp.	BM1994.606	-5.583	38.645	F	27.3	9.3	8	9	1.8	2.5	2.8	3.4	4.8	14.6	13.4	9.4	11.4	6.5	6.7
H. new sp.	BM2002.514	-4.983	38.788	F	24	8.7	7.7	8.6	1.3	2.2	2.5	2.8	5.1	12.3	11.8	7.4	9.5	5.5	6.9
H. new sp.	CAS 169301	-5.144	38.520	M	25.6	8.8	7.6	8.9	1	2.3	2.4	5	3.3	12	10.8	7.5	10.3	5.6	7
H. new sp.	CAS 173002	-4.826	38.788	F	26.2	8.7	8.1	9.6	1.1	2.3	2.2	5.1	3.5	12.5	12.5	8.4	10.5	6.1	6.7
H. new sp.	FMNH274303	-5.113	38.753	M	24.7	8.4	7.9	9.6	1.1	1.9	2.5	3.4	4.9	11.7	10.2	7.2	10.1	5.1	6.6
H. new sp.	FMNH274304	-5.113	38.753	M	23.2	7.1	6.9	7.8	1	1.9	2.2	2.5	4.1	11.2	9.5	6.4	9.3	4.4	6.7
H. new sp.	FMNH274305	-5.113	38.753	M	24.9	7.9	7.4	8.7	1	1.8	2.4	3.3	4.7	11.3	10.6	5.6	9.8	4.9	6
H. new sp.	FMNH274306	-5.113	38.753	F	30.2	10.3	8.5	9.7	1.6	2.2	2.8	3.8	5.7	13.3	12.4	8.3	11.9	5.7	7.7
H. new sp.	FMNH274307	-5.113	38.753	M	24.1	7.9	7.1	8.5	0.9	1.7	2.5	3.2	4.6	12.1	9.7	7.1	9.4	4.7	6.4

H. new sp.	FMNH274327	-4.826	38.788	F	28.5	9.7	8.7	9.8	1.3	2.2	2.9	3.7	5.5	12.8	11.3	7.8	11	5.4	7
H. new sp.	FMNH274328	-4.826	38.788	F	28.2	9.4	7.8	9.9	1.3	2.2	2.7	3.4	5.5	13.3	11.8	7.9	11.1	5.8	7.3
H. new sp.	FMNH274329	-4.826	38.788	M	23.3	9.1	7.8	8.6	1.1	1.9	2.6	3.7	5.2	12.2	11.9	7.3	10.8	4.2	7.5
H. new sp.	FMNH274330	-4.826	38.788	M	23.5	9.1	7.3	9.2	1.2	2.2	2.5	3.1	4.8	12.1	11.2	7.4	10.2	5.1	7.2
H. new sp.	FMNH274331	-4.826	38.788	M	24.2	8.4	7.2	8.6	1.1	2.1	2.5	3.5	4.6	11.8	9.6	7.6	9.6	4.8	6.4
H. new sp.	FMNH274411	-5.113	38.753	M	23.2	7.7	6.5	7.9	0.9	2.1	2.4	2.9	4.1	11.1	10.2	6.1	8.6	4	6.1
H. new sp.	FMNH275025	-5.054	38.378	M	26.2	8.7	7.1	8.8	0.9	2.3	2.9	3	5.2	11.9	10.5	6.4	9.4	5.4	6.3
H. new sp.	FMNH275026	-5.054	38.378	M	23.5	8.2	7.7	8.8	1.1	2.1	2.6	3	4.7	10.5	8.6	5.8	8.3	5.1	6.6
H. new sp.	FMNH275027	-5.054	38.378	M	26.9	8.4	7.3	9	1.3	2.3	2.5	3.6	5.1	11.3	10.2	6.6	9.2	5	6.4
H. new sp.	FMNH275028	-5.054	38.378	M	23.8	7.7	7.7	8.6	1	2.2	2.3	2.6	4.4	11.1	9.9	6.6	9.6	4.8	6.5
H. new sp.	FMNH275029	-5.054	38.378	M	23.5	7.2	6.7	8.3	0.9	1.8	2.1	2.9	4.1	9.8	8.5	4.9	7.7	4.8	5.1
H. new sp.	MTSN 9523	-4.981	38.758	M	26.1	8.5	7.1	7.7	1.3	1.6	2.6	3.1	4.5	13.2	12.7	7.9	11.2	6.6	6.6
H. new sp.	MTSN 9549	-4.976	38.762	M	24	8	7.1	7.6	1.4	1.6	2.2	3.1	4.6	12.5	12.4	7.3	7.3	6	7.3
H. new sp.	MTSN7520	-5.533	37.001	F	30.5	10.5	8.7	11	1.4	2.6	2.9	3.8	6.5	14.6	12.7	8.7	13.2	5.9	7.8
H. new sp.	MTSN5160	-5.480	37.475	F	30.1	9.6	8.8	10.1	1.4	2.4	2.9	3.7	5.8	13.5	11	7.2	10.6	5.8	7.4
H. new sp.	MTSN7519	-5.533	37.001	F	25.3	9	8.2	9.4	1.2	2.4	2.7	3	5.6	19.9	10.8	7.4	11.5	5.6	7.2
H. new sp.	MTSN5159	-5.480	37.475	M	25.1	7.9	7.5	8.8	1.3	2.2	2.5	3.2	5.1	11.8	10.3	6.4	10.5	5.4	6.5
H. new sp.	MTSN7518	-5.533	37.001	F	25	8.8	8.2	9.4	1.2	2.1	2.5	3.1	5.1	12.9	11	7.2	10.1	5.2	5.7
H. new sp.	MTSN8277	-6.030	37.526	M	25.5	8.5	7.9	9.7	1	2.2	2.3	3.4	5.2	12.7	10.2	6.4	9.5	4.5	6.3
H. new sp.	MTSN5161	-5.480	37.475	F	30.8	10.5	8.9	10.7	1.3	2.5	3.2	3.8	6.1	15.2	12.4	8.8	12.4	6.3	7.4

H. new sp.	MW7210	-6.066	37.498	F	28	9.6	8.4	9.8	1.4	2.6	2.7	2.9	5.6	13.7	12.8	8.5	10.5	6.1	7.1
H. new sp.	MW7208	-6.066	37.498	M	23.9	8	7.5	9.3	1.3	2.3	2.6	2.8	5	11.8	11.6	7.5	9.9	5.8	6.7
H. new sp.	MW7203	-6.066	37.498	M	22.8	7.8	7.3	8.6	1.2	2.3	2.6	2.4	4.9	11.8	10.9	7.6	8.8	5.6	6.1
H. new sp.	MW7205	-6.066	37.498	M	25.2	8.5	7.7	9	1.1	2.3	2.6	2.9	5.1	13.2	12	8.3	10.5	6	6.8
H. new sp.	ZMUCR077816	-4.333	37.8	M	22.7	7.6	6.7	7.9	1.2	2.1	2.4	3.3	4.8	10.7	9.1	5.5	8.8	4.4	5.7
H. new sp.	ZMUCR079263	-5.117	38.567	F	32.2	11.8	9.6	11.3	2.1	2.7	3.2	4.4	6.8	15.5	13.8	8.8	13.1	6.0	9.4
H. new sp.	ZMUCR077586	-5.117	38.567	F	26.6	8.8	8.5	9.3	1.1	2.7	2.6	3.7	4.8	12.7	11.4	6.4	9.7	5.3	7.1
H. new sp.	ZMUCR077587	-5.117	38.567	F	31.9	11.2	9.9	10.9	1.5	2.2	2.8	4.1	6.3	16.3	15	9.2	11.8	5.9	9
H. new sp.	ZMUCR079373	-5.117	38.567	M	24.4	8.1	7.2	8.5	1.1	2.1	2.7	3.5	4.9	12.1	9	6.6	10.2	5	6.9
H. new sp.	ZMUCR079372	-5.117	38.567	M	25.2	8.9	7.9	9.2	1.2	2.2	2.5	3.9	4.9	11.4	9.9	6.1	8.8	4.5	6.6
H. new sp.	ZMUCR079371	-5.117	38.567	M	26.6	9.2	7.7	9.2	1.4	2.2	2.6	3.6	5.5	13.2	10.7	7.9	10	5.5	7.5
H. new sp.	ZMUCR77588	-5.117	38.567	M	27.5	9.8	7.9	9.5	1.4	2.2	2.6	2.8	5.2	13.3	12.2	7.6	11.1	5.5	7.9
H. new sp.	ZMUCR077646	-5.117	38.567	M	28.8	10	9.4	10.4	1.1	2.3	2.4	3.9	6.3	13.9	12.3	8.1	10.8	5.6	7.3
H. new sp.	ZMUCR076822	-5.117	38.567	M	27.7	9	7.9	10.1	1.1	2.2	2.7	3.7	5.7	12.9	11.1	7.5	10.6	5.2	7.2
H. new sp.	ZMUCR771485	-5.117	38.567	M	24.6	8.1	7.1	9	1.2	2.1	2.2	3.4	4.9	12.1	10.5	7	10.2	4.9	7.1
H. new sp.	ZMUCR076827	-5.117	38.567	M	25.3	8.4	7.1	8.8	1	1.9	2.4	3.6	5.6	12.1	10.5	7.2	10.6	5.3	7.1
H. new sp.	ZMUCR076833	-5.117	38.567	M	23.4	7.7	6.7	8.5	1.1	2.1	2.5	3.4	5.1	11.4	9.8	6.5	9.1	4.5	6.5
H. new sp.	ZMUCR076824	-5.117	38.567	F	31.5	10.6	9.7	11.7	1.2	2.4	3.2	3.7	6.6	14.3	12.2	8.7	12.6	6.3	7.9
H. new sp.	ZMUCR076823	-5.117	38.567	M	26.1	9.2	8.2	9.4	1.1	2.2	3	3.5	5.6	11.9	10.7	7.2	9.6	4.9	6.6
H. new sp.	ZMUCR076831	-5.117	38.567	M	22.2	7.7	6.3	8.8	1	1.9	2.6	3.6	5.2	11.1	9.9	6.4	9.4	4.5	6.2

H. new sp.	ZMUCR076830	-5.117	38.567	M	25.9	8.3	7.5	9.3	1.2	2.3	2.7	3.7	5.2	12.9	11.1	7.9	10.5	4.7	7.5
H. new sp.	ZMUCR076828	-5.117	38.567	M	22.3	7.2	7.6	8.3	1	2.2	2.5	3.1	4.6	11	9.2	6.9	8.3	4.3	6.5
H. new sp.	ZMUCR076835	-5.117	38.567	M	24.4	8.4	7.3	8.9	1.1	2.1	2.5	3.5	5.1	12	9.5	7.1	9.9	5.1	6.6
H. new sp.	ZMUCR076826	-5.117	38.567	M	24.4	8.3	7.8	9	1	2.1	2.8	3.3	5.3	12	9.5	7.7	9.6	5.3	6.7
H. new sp.	ZMUCR076829	-5.117	38.567	M	24.4	8.3	7.6	9.2	1	2.2	2.8	3.5	5.6	12.5	10.4	7.7	10.3	5.6	7.3
H. new sp.	ZMUCR771487	-5.117	38.567	M	24.6	8.6	7.4	9	1.2	2.3	3	3.8	5.2	11.6	10.2	7.4	9	4.5	6.5
H. new sp.	ZMUCR076821	-5.117	38.567	M	25.5	8.9	7.6	9	1.1	2.2	2.5	3.5	5.4	12.6	10.8	7.5	10.6	5.4	6.6
H. new sp.	ZMUCR077647	-5.117	38.567	M	23.5	7.7	6.1	8.1	0.9	1.6	2.7	2.8	4.4	11.3	9.1	6.6	9.9	3.8	6.9
H. new sp.	ZMUCR076834	-5.117	38.567	M	20.5	7.5	6.1	7.4	0.9	1.8	2	2.7	4.7	9.9	8	5.6	8.2	4.1	6.5
H. new sp.	ZMUCR076825	-5.117	38.567	M	22.4	7.7	6.9	7.7	1.2	2.3	2.1	3.2	4.4	10.8	9.6	6.7	8.7	4.6	6.1
H. new sp.	ZMUCR771486	-5.117	38.567	M	23.6	7.7	6.9	8.7	1.1	1.7	2.3	3	4.7	12.1	10.5	7.2	9.3	4.9	6.6
H. new sp.	ZMUCR076814	-5.117	38.567	M	26.7	8.8	7.7	9.5	1.1	2.4	2.7	3.4	5.5	12.6	11.1	7.4	10.2	5.6	7.8
H. new sp.	ZMUCR076819	-5.117	38.567	F	32.3	10.6	10.3	12	1.2	2.6	3.2	4.7	7.2	14.9	12.7	8.4	13.4	6.9	8.3
H. new sp.	ZMUCR074180	-5.117	38.567	M	26.2	7.8	7.8	8.8	1.1	2.2	2.5	3.3	5.1	12.1	11.2	7.4	10.3	5	7.4
H. new sp.	ZMUCR076832	-5.117	38.567	M	22.3	7.9	6.9	8.5	0.7	1.9	2.7	3.2	4.9	11.3	10	6.2	9.4	4.6	6.1
H. new sp.	ZMUCR076820	-5.117	38.567	M	25.3	8.3	7.7	9	1.2	2.2	2.4	3.6	5.6	12.4	10.4	7.4	10.5	5.4	6.2
H. new sp.	ZMUCR073872	-5.117	38.567	M	23.7	7.9	7.7	9.5	1	2.2	2.4	3.1	5.3	11.6	10.1	7.5	9.4	4.8	7.4
H. new sp.	ZMUCR073873	-5.091	38.633	M	28.3	8.5	8.3	9.6	1.2	2.4	2.9	3.5	5.7	14.1	11.5	8.3	11.1	5.5	7.8
H. new sp.	SL1952	-5.034	38.924	M	20.4	7.8	6.8	7.2	1.1	1.4	1.7	3.2	3.4	10.3	10.3	6.2	8.4	3.6	5.7
H. new sp.	SL1953	-5.034	38.924	M	19.8	7.2	5.9	6.6	1	1.6	1.5	2.7	1.8	10.2	10.2	6.6	8	3.8	5.2

H. new sp.	MCZ A149046	-5.076	38.369	M	23.8	7.8	6.8	8.8	1.1	2.1	2.2	3.1	5.3	11.7	9.9	7	10.1	4.4	5
H. new sp.	MCZ A149045	-5.076	38.369	M	23.8	8.3	6.3	8.6	1.1	2.4	2.3	3.1	5.8	11.8	9.9	6.9	10.2	5.2	6.5
H. mitchelli	BMNH1980.183	-5.58	39.15	F	26.4	9.3	7.5	9.3	1.3	2.5	2.8	3.1	5.2	12.7	12.4	7.9	10.5	6.2	6.9
H. mitchelli	BMNH1980.184	-5.58	39.15	M	22.9	7.6	6.7	8.2	1.2	2.3	2.6	2.6	4.6	10.8	10.8	6.7	8.5	4.8	5.6
H. mitchelli	FMNH274979	-11.698	33.950	M	22.2	7.2	6.6	7.6	0.8	1.6	2.2	2.5	4.4	10.3	8.9	5.6	8.8	4.4	5.1
H. mitchelli	FMNH274982	-11.698	33.950	M	21.3	6.6	5.8	6.8	0.8	1.8	1.6	3.7	5.4	13.8	12.2	8.1	11.6	5.9	7.9
H. mitchelli	FMNH274980	-11.698	33.950	M	23.7	7.2	7.1	7.7	1.1	2.2	2.5	2.7	4.4	10.6	9.2	5.5	9.1	4.3	5.7
H. mitchelli	FMNH274985	-11.698	33.950	M	22.8	7.2	6.6	7.3	0.9	2	1.9	2.4	4.8	11.2	8.4	5.5	8.8	5.2	5.5
H. mitchelli	FMNH274995	-11.698	33.950	M	22.8	7.6	6.6	7.5	0.8	1.7	2.2	2.4	3.8	10.2	8.6	5.9	8.3	4.8	5.3
H. mitchelli	MCZ A27272	-14.30	35.000	F	27.4	8.2	8.3	10.4	1.5	2.4	3	3.8	6.2	12.2	11.3	7.8	11.3	4.9	7.2
H. mitchelli	MCZ A27273	-14.300	35.000	M	22.7	7.8	6.6	8.7	0.9	2.2	1.9	3.4	5.1	11.1	9.1	6.5	8.9	4.4	5.8
H. mitchelli	FMNH274989	-11.984	34.046	M	22.7	6.9	6.2	7.8	1	1.8	2.1	2.5	4.4	10.5	9.6	5.8	8.8	4.6	6
H. mitchelli	FMNH274990	-11.984	34.046	M	25.2	7.5	7.4	8.5	1	2.2	2.4	3.1	4.9	11.2	10.6	7.1	9.2	5.4	6.2
H. mitchelli	FMNH274992	-11.984	34.046	F	28.8	8.9	7.6	9.2	1.2	2.2	2.6	3.6	4.8	11.9	10.5	7.6	11.1	5.9	6.9
H. mitchelli	FMNH274993	-11.984	34.046	M	25.8	8.3	7.7	9.1	1.1	2.1	2.7	3.2	4.5	12.2	11.1	7.2	9.8	5.1	6.7
H. mitchelli	FMNH274996	-11.984	34.046	M	25.5	7.8	6.6	8.3	1	2.2	2.6	2.8	4.4	11.4	9.2	6.1	9.2	4.7	5.9
H. mitchelli	FMNH274391	-7.842	36.878	M	22.9	7.4	6.8	7.9	1	1.8	2.1	2.6	4.4	11	9.2	6.4	9.5	4.6	6.4
H. mitchelli	FMNH274392	-7.842	36.878	M	25.5	8.1	7.9	8.9	0.9	2.2	2.5	3.2	5.4	12.3	11.1	7.5	10.3	4.6	6.7
H. mitchelli	FMNH275039	-7.029	37.627	M	28.4	9.7	8.6	9.8	1.1	2.2	2.8	3.8	5.3	13.7	13.3	8.4	11.1	5.7	8
H. mitchelli	FMNH275030	-6.941	37.719	M	28.5	9.7	8.3	9.8	1.1	2.1	2.7	3.3	5.2	12.7	11.9	7.8	11.6	5.7	6.6

H. mitchelli	FMNH275032	-6.941	37.719	M	27.8	9.7	8.6	9.6	1.2	2.1	3	3.3	5.4	12.1	11.8	8.5	11.4	6.4	7.7
H. mitchelli	FMNH275034	-6.941	37.719	M	27.9	9.4	8.1	9.6	1.3	1.9	2.7	3.4	5.3	12.3	11.6	7.6	11.6	5.6	6.5
H. mitchelli	FMNH275038	-6.941	37.720	M	27.1	8.7	7.7	9.1	1.1	2.1	2.7	3.8	4.6	13.2	12.7	7.5	10.2	5.8	6.8
H. mitchelli	FMNH275040	-7.029	37.627	M	26.6	9.2	7.9	9.8	1.1	2.2	2.7	3.2	5.4	13	11.7	7.2	11.4	5.4	7.8
H. mitchelli	FMNH275044	-6.941	37.719	M	27.2	8.8	7.8	9.6	1.3	2.1	2.6	3.1	5.2	12.5	11.5	7.8	10.6	5.5	6.9
H. mitchelli	FMNH275041	-7.029	37.627	M	26.7	9.1	8.4	9.4	1.1	2.2	2.7	3.4	5.1	12.1	11.3	6.8	9.4	5	7.4
H. mitchelli	MCZ A17162	-9.550	33.950	F	23.9	7.8	7	8.8	1.4	2.2	2.3	2.8	4.9	11.5	10.2	6.9	8.8	5.4	5.8
H. mitchelli	MTSN 7675	-7.223	38.013	F	29.1	9.7	8	9	1.2	2.2	2	3.6	4.9	14.6	14.6	9.1	11.2	6	7.3
H. mitchelli	MTSN 7676	-7.223	38.013	M	28.1	8.7	8.3	9	1.3	2	2.3	3.7	4.6	13.2	13.4	8.3	10.9	5.8	7.9
H. mitchelli	MTSN 7682	-7.223	38.013	M	27.4	9.1	8.2	8.8	1.1	2	2.3	3.4	4.8	14	14	9	11.6	6.3	7.8
H. mitchelli	MTSN 7683	-7.223	38.013	M	24.6	8.2	7.4	7.9	1.3	1.7	2.1	3.3	4.6	13.8	14	8.3	11.4	6.7	7.1
H. mitchelli	MTSN 7707	-7.025	37.880	M	19	4.9	4	4.6	0.8	1.9	1.5	2.2	2.1	7.6	7.5	5	7.2	3.9	4.4
H. mitchelli	MTSN 7709	-7.025	37.880	M	26	8.3	7.8	9.1	1.3	1.9	2.3	3.6	4.4	12.2	12.3	8	10.8	5.6	6.5
H. mitchelli	MTSN5756	-8.398	35.979	F	26.6	8.7	8.1	10.1	1.1	2.4	2.9	3.1	5.6	12.6	10.2	8.3	10.6	5.4	7.6
H. mitchelli	MTSN5764	-8.398	35.979	M	26.3	8.4	7.9	9.5	1.3	2.4	2.9	3.6	5.5	12.4	10.1	6.7	9.6	5.2	6.9
H. mitchelli	ZMUCR077155	-8.5	36.333	F	24.6	8	7.5	8.8	1.2	2.2	2.7	3.2	4.8	11.6	9.7	6.7	9.9	4.6	6.9
H. mitchelli	ZMUCR77157	-8.5	36.333	M	23.5	7.9	6.9	9.1	1.1	1.8	2.4	3.2	4.1	11.3	9.7	6.1	9.4	4.4	6.1
H. mitchelli	ZMUCR77159	-8.5	36.333	F	26.7	9.1	7.5	9.8	1.5	2.1	2.7	3.3	5.5	12.6	10.7	7.2	10.6	5.1	6.6
H. mitchelli	ZMUCR77160	-8.5	36.333	M	21.6	7.4	6.9	7.9	1.2	2.1	2.2	2.7	4.6	11	9.4	5.9	8.5	4.5	6.6
H. mitchelli	ZMUCR77171	-8.5	36.333	M	20.7	7.2	6.8	8.1	1.2	2.1	2.2	2.7	4.5	10.2	9.2	5.3	8.1	4.1	5.5

H. mitchelli	ZMUCR77172	-8.5	36.333	M	21.4	6.7	6.7	7.5	0.8	1.8	2	2.9	4.6	10.5	9.6	5.4	8.3	4.4	5.6
H. mitchelli	ZMUCR77176	-8.5	36.333	M	22.2	7	7.1	8.3	0.8	1.8	2	3.2	4.2	10.3	9.2	6.2	6.8	4.2	5.8
H. mitchelli	ZMUCR77166	-8.5	36.333	M	24.9	8.6	7.6	8.8	1.3	2.1	2.5	3.1	4.9	11.9	10.5	6.7	9.8	4.8	6.5
H. mitchelli	SL1522	-9.994	39.388	M	21.8	7.9	7.5	8	1.3	1.9	2	2.6	3.8	9.9	10.2	6	8.7	5.3	5.6
H. mitchelli	SL1523	-9.994	39.388	F	23.8	9.2	7.3	8.7	1.5	1.7	2.4	2.9	4.3	11.2	11	6.6	9.9	4.3	5.5
H. mitchelli	SL1554	-9.994	39.388	F	23.2	8.4	7.2	8.2	1.3	2	2.9	3.7	4.7	11.8	11.6	7.2	9.3	5.2	6.5
H. mitchelli	SL1555	-9.994	39.388	F	22.9	8.8	7.4	8.8	1.3	2	2.3	3.3	4.4	12	11.9	7	9.5	5.1	5.9
H. mitchelli	SL1560	-9.994	39.388	M	19.9	7.4	5.9	7.3	1	1.8	2.2	2.8	4	9.9	9.8	6.3	7.8	4.2	5.3
H. mitchelli	SL1562	-9.994	39.388	M	19.2	7.3	6.1	7.9	1.3	1.6	1.9	3.2	4.1	10.6	10.5	7	9	5	6.4
H. mitchelli	SL1588	-9.895	39.374	F	23.6	9.6	6.6	7.6	1.4	1.4	2	3	4.8	10.6	10.8	7	8.2	5.6	6.2
H. mitchelli	SL1589	-9.895	39.374	M	24.2	8.8	7.2	7.8	1.4	2	2.1	3.4	4	12	12.3	7.7	10.5	6.8	7.3
H. mitchelli	SL1601	-9.895	39.374	M	23	9.4	6.8	7.6	1.3	1.8	2.2	3	4.3	10.3	10.5	6.6	8.5	5.7	6.6
H. mitchelli	SL1602	-9.895	39.374	M	23.8	8.9	6.7	8	1.3	1.6	1.9	2.7	3.9	11.6	11.4	7.4	8.4	5	6.3
H. mitchelli	SL1628	-9.111	39.238	M	21	8.1	7.2	7.5	1.2	2	2.2	2.7	3.9	12.2	12	7.4	8.6	5	6.3
H. mitchelli	SL1635	-9.495	39.292	M	25	9.3	7	7.8	1.6	2.5	2.3	3.3	4.5	12.4	12.3	8	10.3	6.2	6.4
H. mitchelli	SL1636	-9.495	39.292	M	23.2	8.6	7.2	8.1	1.3	1.9	1.7	3.5	3.3	11.8	11.9	7	8.3	5.3	6.6
H. mitchelli	SL1638	-9.495	39.292	M	22.1	7.5	6.9	7.3	1.2	1.6	2	2.7	3.2	10.8	10.6	6.6	6.9	4.8	5.7
H. mitchelli	SL1674	-8.304	38.903	M	24.8	8.6	7.5	8.2	1.4	1.8	2.3	3	4.1	13	13	8.3	10.2	6	7
H. mitchelli	SL1675	-8.304	38.903	M	21.9	8.7	7.6	8.2	1.4	1.5	2	3.3	4.6	12.7	12.7	7.9	10.1	5.6	6.5
H. mitchelli	SL1765	-8.349	36.228	M	20.7	7.3	6.1	7	1.1	1.2	1.8	2.7	3.9	10.4	10.3	6	8.4	3.4	5.6

H. mitchelli	BM1920.5.10.14	-6.941	37.719	F	30.5	10	9	10.4	1.9	2.5	3.3	3.1	5.6	13.9	14.3	9	11.2	6.4	7.4
H. mitchelli	BM1920.5.10.15	-6.941	37.719	F	28.8	9.6	8.7	9.4	1.8	2.7	3	3	5.2	13.4	13.4	8.5	12	6.7	7.9
H. rubrovermiculatus	NMK A5762/1	-4.375	39.563	M	22.5	8.3	6.3	8.5	1.2	2	2.2	3.1	4.2	12	9.4	7.5	8	4.9	5.8
H. rubrovermiculatus	NMK A5762/3	-4.375	39.563	M	24.3	8.8	6.9	10.3	1.1	1.9	2.2	2.7	4.2	11.6	10.7	6.8	8	4.6	5.2
H. rubrovermiculatus	NMK A5801/5	-4.238	39.396	F	29.2	10.6	8.5	9.9	1	1.9	2.7	3	4.2	13.1	10.2	8.8	8.8	6.9	6.1
H. rubrovermiculatus	NMK A5801/5	-4.238	39.396	M	22.7	8.4	7	8.3	11	1.7	2.2	3.6	4.4	11.6	10.4	6.6	8.2	4.2	5.7
H. rubrovermiculatus	NMK A5801/4	-4.238	39.396	M	29.6	11.3	8.1	9.8	1.3	2.8	2.5	3.3	5.9	16.1	14.4	13.4	12.7	6.5	5.5
H. rubrovermiculatus	NMK A5801/2	-4.238	39.396	M	23.7	8.8	6.1	8.6	0.8	2	2.4	3	5	12.9	10.2	7.5	8.6	6	4.9
H. rubrovermiculatus	NMK A5801/1	-4.238	39.396	F	30.4	11.2	9.1	11.7	1.2	2.2	3	3.3	5.6	15.7	13.4	9.1	9.8	7.1	7.4
H. rubrovermiculatus	NMK A5848	-4.238	39.396	F	31.2	10.8	7.3	11.2	1.6	2.2	2.7	3.4	5.4	15.1	12.0	9.2	9.5	6.6	7.0
H. rubrovermiculatus	NMK A5801/3	-4.238	39.396	F	30.7	10.9	9.2	10.5	1.5	2.7	3.4	3.7	6.0	15.3	13.0	8.6	10.9	6.2	7.2
H. rubrovermiculatus	NMK A5900/1	-4.238	39.396	M	22.9	8.3	7.7	8.1	0.9	2	2.2	2.6	4.9	12.5	10.8	6.5	9.1	5.1	6.2
H. rubrovermiculatus	NMK A5900/2	-4.238	39.396	M	23.9	8.3	8	8.3	1.3	2.2	2.6	3.4	5	11.7	9.4	7.1	8.8	5.2	6.1
H. rubrovermiculatus	NMK A5909	-4.217	39.483	M	21.3	6.9	6.6	7.1	0.7	1.6	2.3	3.5	4.2	10.2	8.2	6.2	8.2	4.6	5.5
H. rubrovermiculatus	NMK A5958/1	-4.276	39.431	M	23.1	8.3	7.3	8	1.4	1.7	2.1	2.9	4.5	11.2	9	5.8	9.4	4.6	6.2
H. rubrovermiculatus	NMK A5958/2	-4.276	39.431	M	24.4	8.5	6.5	10.1	1.2	1.7	2.6	3.6	4.4	12	9.9	7.5	9.4	5	6.9
H. rubrovermiculatus	NMK A5958/3	-4.276	39.431	M	23.3	9.1	7.1	8.3	1.1	1.8	2.2	2.7	4.6	11.5	9.9	7.5	10	5.2	6.2
H. rubrovermiculatus	NMK A5959	-4.276	39.431	M	25.6	8.3	7.4	8.9	0.9	2.1	1.9	3.6	5	12.2	10.7	7.4	8.8	5	5.4
H. rubrovermiculatus	NMK A5961/1	-4.276	39.431	M	22.3	8.6	7.7	9	0.9	2.2	2.5	3.6	4.9	12.7	10.4	7.9	10	5.3	6.9
H. rubrovermiculatus	NMK A5961/2	-4.276	39.431	M	24.5	8.7	8.1	9.7	1	2.5	2.9	3.2	5.6	11.9	10.7	7.4	10.5	4.7	6

H. rubrovermiculatus	NMK A5961/3	-4.276	39.431	M	24.7	8.8	7.4	8.6	1.2	2	2.7	3.7	5.2	12.5	10.3	7.2	10.7	5.5	5.9
H. rubrovermiculatus	NMK A5961/4	-4.276	39.431	M	22.2	7.7	6.9	8.2	0.9	2	2.5	3.6	5	12.3	10.3	6.6	9.5	5	6.5
H. rubrovermiculatus	ZMUCR077434	-4.167	39.451	M	23.4	8.1	6.9	8.3	1.2	2.2	2.4	3.5	5.1	11.1	9.2	6.6	9.4	5.1	5.7
H. rubrovermiculatus	ZMUCR073967	-4.167	39.451	M	24.5	9	6.1	8.4	1.1	2.4	2.6	3.3	5.3	11.9	10.2	7.4	10	4.7	6.6
H. rubrovermiculatus	ZMUCR073863	-4.167	39.451	M	25.6	8.9	7.9	9.6	1	2.1	2.5	3.7	5.6	12.4	9.7	7.5	10.5	4.6	6.7
H. rubrovermiculatus	ZMUCR078099	-4.167	39.451	M	25.9	8.8	7.9	9.7	1.3	2.6	2.7	3.5	5.8	13.1	11.2	8.2	10.5	5.2	7.1
H. rubrovermiculatus	ZMUCR078098	-4.167	39.451	M	23.8	8.5	7.3	8.8	0.9	2.1	2.6	3.6	5.7	12.3	10.5	7.5	8.7	5.7	6.5
H. rubrovermiculatus	ZMUCR078103	-4.167	39.451	M	24.6	8.9	7.7	8.8	1	2.3	2.7	3.2	5.6	12.4	11	7	10.2	5	6.8
H. rubrovermiculatus	ZMUCR078095	-4.167	39.451	M	24	8.3	7.1	8.4	0.8	2.4	2.3	3.5	5.3	11.7	10.5	6.9	10.1	4.5	6.4
H. rubrovermiculatus	ZMUCR078094	-4.167	39.451	M	25.2	8.8	7.6	9.4	1.3	2.3	2.6	3.2	5.4	11.8	10.2	7.1	9.8	5	6.3
H. rubrovermiculatus	ZMUCR078100	-4.167	39.451	M	24.4	8.7	7.6	9.3	1.2	2.2	2.9	3.5	5.8	12.3	10.8	7.7	10.2	4.8	6.4
H. rubrovermiculatus	ZMUCR078102	-4.167	39.451	M	23.5	7.9	7.4	8.8	1	2.1	1.8	3.5	5.1	11.9	10.1	7.2	8.8	5	6.8
H. rubrovermiculatus	ZMUCR078096	-4.167	39.451	M	25.4	8.5	7.9	9.1	1	2.2	2.8	3.2	5.3	12.8	11.7	7.6	10.6	5.6	7.2
H. rubrovermiculatus	ZMUCR078101	-4.167	39.451	M	23.3	8.4	6.8	8.3	1	2.1	2.3	3.5	5.5	11.5	9.6	7.2	10.5	5	7.6
H. rubrovermiculatus	ZMUCR078097	-4.167	39.451	M	25.8	9.2	7.6	9.1	1.2	2.2	2.3	3.9	5.2	12.5	11.8	7.4	10.7	5.3	7.3
H. rubrovermiculatus	ZMUCR073865	-4.167	39.451	M	23.9	9.2	6.9	8.6	1.1	1.8	2.2	3.4	5	12.6	9.6	6.9	9.1	5.5	6.2
H. rubrovermiculatus	ZMUCR073951	-4.167	39.450	M	22.7	8.5	6.6	8.2	1	2.1	2.5	3.3	4.9	10.9	9.5	6.2	8.8	3.7	5.6
H. rubrovermiculatus	ZMUCR073864	-4.167	39.450	M	25	9.1	7.7	9.6	1.5	2.2	2.5	3.7	5.5	12.3	11.3	7.3	10.6	5.2	6.4
H. rubrovermiculatus	ZMUCR073972	-4.167	39.450	M	26.1	9.9	7.6	9.7	1.4	2.2	2.7	3.7	5.1	13.1	11.9	7.7	10.2	5.8	7.1
H. rubrovermiculatus	ZMUCR078093	-4.167	39.450	M	25.9	8.9	7.6	9.5	1.2	2.2	2.7	3.8	5.3	13	11.6	7.2	11.7	5.2	6.7

H. rubrovermiculatus	ZMUCR078092	-4.167	39.45	M	25.5	9.4	6.9	9.2	1.4	1.8	2.4	3.6	5.3	12.2	11.2	6.9	9.9	4.7	6.9
H. rubrovermiculatus	ZMUCR073854	-4.217	39.417	M	29.6	10	8.6	9.8	1.8	2.3	3.1	4.4	6	13.9	12.4	8.2	12.6	5.8	7.1
H. rubrovermiculatus	CAS 155636	-4.200	39.417	M	25.3	8.7	7.2	8.8	1.2	2.4	2.2	4.8	3.1	11.6	9.7	7.6	11.1	5	7.1
H. rubrovermiculatus	CAS 155637	-4.200	39.417	M	23	7.8	6.9	7.8	1	2	2.3	4.1	2.7	11.4	10.2	7.1	10.4	4.8	7.2
H. rubrovermiculatus	CAS 155638	-4.200	39.417	M	24.5	8.8	7.1	9.2	1.2	2.3	2.6	4.7	3.1	11.8	9.8	8.1	10	5.7	7
H. rubrovermiculatus	CAS 155639	-4.200	39.417	M	26.3	8.7	7.1	8.7	1.1	2.1	2.4	5.2	3.2	12.8	11.3	8.7	11.5	6	7.6
H. rubrovermiculatus	CAS 155640	-4.200	39.417	M	24.6	8.2	7.4	8	1.1	2.2	2.1	4.9	2.9	11.8	10.3	7.6	10.3	5.5	7.4
H. rubrovermiculatus	CAS 155643	-4.200	39.417	F	28.3	10.5	8.7	9.9	1.5	2.6	2.6	5.5	3.7	12.4	10.8	8.2	11.8	6	7.9
H. rubrovermiculatus	CAS 155644	-4.200	39.417	M	23.7	8.2	6.8	7.3	1.1	2.1	2.2	4.8	3	10.9	9.7	7.3	9.6	4.9	6.1
H. rubrovermiculatus	CAS 155646	-4.200	39.417	M	23.2	7.6	6.6	8.3	1	2	2.2	4.6	2.9	10.7	10.2	7.2	9.1	4.8	6.8
H. rubrovermiculatus	CAS 155932	-4.300	39.417	M	26	8.5	7.4	8.7	0.9	2	2.4	5	3.2	12.4	12.2	8.1	11.1	6	7.3
H. rubrovermiculatus	CAS 155933	-4.300	39.417	M	23.1	8	6.8	8.1	1.2	2.2	2.1	4.7	2.9	11.5	10.3	7.4	9.2	5.1	7.1
H. rubrovermiculatus	CAS 155934	-4.300	39.417	M	26.2	9.3	7.3	8.8	1.2	2.2	2.2	4.3	2.8	12.3	12	7.8	12.1	5.8	7
H. rubrovermiculatus	CAS 155935	-4.300	39.417	M	25.7	8	7.2	8.5	1.3	2.4	2.5	5.1	3.2	11.6	10.1	8	10.6	4.8	7.1
H. rubrovermiculatus	CAS 155937	-4.300	39.417	M	24.1	8.3	6.8	8.1	1.1	2.1	2.5	5	3.3	11.7	11	7.7	10	4.6	6.7
H. rubrovermiculatus	CAS 155938	-4.300	39.417	M	21.7	7.6	7.1	8.3	1	2	2.2	4.7	2.7	10.2	9.7	6.5	8.4	4.6	5.6
H. rubrovermiculatus	CAS 155940	-4.300	39.417	M	23.4	8	6.9	8.5	1	2	2.3	5.1	3.3	12.1	10	7.6	10.1	5.2	7.1
H. rubrovermiculatus	CAS 155942	-4.300	39.417	M	25.3	8.9	7.3	9.2	1.2	2.4	2.5	4.7	3.4	11.7	11.2	7.8	10.9	4.6	7.1
H. rubrovermiculatus	CAS 155943	-4.300	39.417	M	24.9	8.5	7.1	8.2	1.2	2.4	2.3	5	3	11.5	11.1	8	11.2	5.6	7.1
H. rubrovermiculatus	CAS 155944	-4.300	39.417	F	32.1	10.6	9	11.03	1.4	2.7	2.9	6	3.6	13.7	14.4	9.3	12.8	6.8	8.6

H. rubrovermiculatus	CAS 155945	-4.300	39.417	M	26.2	8.3	6.8	8.1	1.2	2.3	2.5	5.1	3.2	12.1	11.1	8.4	10.5	4.7	7.2
H. rubrovermiculatus	CAS 155946	-4.300	39.417	M	23.2	8	6.4	8	1.2	2.2	2.3	4.8	2.9	11	10.3	7.2	9.7	4.6	6.8
H. rubrovermiculatus	NMK A3169	-4.300	39.417	F	24	9	7.1	9.5	1.6	2.9	2.2	3.8	6.4	12.3	12.6	8	10.1	5.3	5.8
H. rubrovermiculatus	NMK A5488/1	-4.158	39.417	F	24.03	8.3	7.5	9.2	1.4	2.3	2.4	2.9	4.3	10.3	12.3	7.5	9.5	4.2	5.8
H. rubrovermiculatus	NMK A5550/4	-4.375	39.563	F	27.9	9.4	8.6	10.8	1.5	2.3	2.5	3.6	6.6	12.7	13.9	9.3	10.6	5.9	8
H. rubrovermiculatus	NMK A5980/4	-4.375	39.563	F	25.1	9.2	7.9	9.2	1.2	2	2.4	3.3	5.6	12.2	13.3	7.3	11.2	5.9	6.4
H. rubrovermiculatus	NMK A5980/1	-4.375	39.563	F	27.6	9.2	8.3	10.5	1.2	2	2.9	4	5.3	11.7	13.7	8	11.8	5.6	7.4
H. rubrovermiculatus	NMK A5980/2	-4.375	39.563	F	26.9	9.32	8.2	10.2	2	2.6	2.8	3.2	5.1	12.7	13.4	8.4	10.6	5.8	6.6
H. rubrovermiculatus	NMK A5980/3	-4.375	39.563	F	28.6	9.8	8.8	11.2	1.3	2.4	3.3	3.3	5.2	12.7	14.6	8.2	12	6.7	6.9
H. rubrovermiculatus	NMK A5506/2	-4.238	39.396	F	29.7	9.8	8.9	10.5	1.3	2.2	2.8	3.6	5.5	13	14.5	9.3	12.2	6.9	6.7
H. rubrovermiculatus	NMK A5558	-4.375	39.563	F	27	9.7	7.9	10.8	1.3	1.7	2.7	3.7	4.8	10.9	12.6	8.9	10.5	5.4	5.2
H. rubrovermiculatus	NMK A4623/1	-4.217	39.483	F	24	7.2	6.7	8.5	1.3	1.5	2.5	2.8	4.5	9.8	11.5	7.3	9.3	5.1	5.4
H. rubrovermiculatus	NMK A4623/2	-4.217	39.483	F	28.2	9.6	8	9.6	1.4	1.9	2.7	3.9	4.8	12.5	13.5	8.4	11.3	5.2	7.8
H. rubrovermiculatus	NMK A6178/1	-4.238	39.396	F	28.1	9.8	9.4	10.9	1.3	2	2.9	3.7	5.1	12.3	13.8	8.5	11.8	5.4	7.8
H. rubrovermiculatus	NMK A6178/2	-4.238	39.396	F	27.2	9.3	9.5	10.5	1.2	2.2	2.9	3.6	4.9	12.8	14	8.7	11.2	5.5	7.2
H. rubrovermiculatus	NMK A5417	-4.238	39.473	F	27.3	9.7	8.5	10.6	1.2	1.9	2.8	3.7	5	13.2	13.8	8.3	11.3	6	7.4
H. rubrovermiculatus	NMK A5268/3	-4.238	39.396	F	30.2	9.8	9.2	12.2	1.4	2	2.8	3.8	5.1	13.7	14.1	8.4	11.2	5.9	6.9
H. rubrovermiculatus	NMK A5268/4	-4.238	39.396	F	29.3	8.9	7.7	10.9	1.1	2.2	2.8	3.7	4.2	13.6	14	8.3	10.2	5.4	6

Appendix II. List of specimens, locality and available genes and GenBank Accession numbers; 1 represent unaccessioned sequences while 0 represent missing genes repectively.

ID	Species	Locality	16S	C-myc	ND2	POMC
FMNH274327	*H. new sp*.	East Usambara	1	HM772380.1-HM772381.1	HM772448.1	HM772312.1 HM772313.1
FMNH274328	*H. new sp*.	East Usambara	0	HM772382.1-HM772383.1	HM772449.1	HM772314.1-HM772315.1
FMNH274329	*H. new sp*.	East Usambara	0	HM772384.1-HM772385.1	HM772450.1	HM772316.1 HM772317.1
FMNH274330	*H. new sp*.	East Usambara	0	HM772386.1-HM772387.1	HM772451.1	HM772318.1-HM772319.1
FMNH274331	*H. new sp*.	East Usambara	0	HM772388.1-HM772389.1	HM772452.1	HM772320.1-HM772321
FMNH274406	*H. new sp*.	East Usambara	0	0	HM772453.1	0
FMNH274407	*H. new sp*.	East Usambara	0	0	HM772454.1	0
FMNH274408	*H. new sp*.	East Usambara	0	0	HM772455.1	0
FMNH274409	*H. new sp*.	East Usambara	0	0	HM772456.1	HM772312.1 HM772313.1
FMNH274410	*H. new sp*.	East Usambara	0	0	HM772457.1	HM772324.1-HM772325.1
FMNH274332	*H. new sp*.	Magoroto	1	HM772390.1-HM772391.1	HM772458.1	HM772326.1-HM772327.1
FMNH274411	*H. new sp*.	East Usambara	0	0	HM772459.1	0
FMNH274303	*H. new sp*.	East Usambara	0	HM772392.1-HM772393.1	HM772460.1	HM772328.1-HM772329.1
FMNH274304	*H. new sp*.	East Usambara	0	HM772394.1-HM772395.1	HM772461.1	HM772330.1-HM772331.1
FMNH274305	*H. new sp*.	East Usambara	0	HM772396.1-HM772397.1	HM772462.1	HM772332.1-HM772333.1
FMNH274306	*H. new sp*.	East Usambara	0	HM772398.1-HM772399.1	HM772463.1	HM772334.1-HM772335.1
FMNH274307	*H. new sp*.	East Usambara	0	HM772400.1-HM772401.1	HM772464.1	HM772336.1-HM772337.1

FMNH274413	*H. new sp*.	East Usambara	KX953901	0	HM772465.1	0
FMNH274414	*H. new sp*.	East Usambara	0	HM772402.1-HM772403.1	HM772466.1	0
FMNH274415	*H. new sp*.	East Usambara	0	HM772404.1-HM772405.1	HM772467.1	HM772338.1-HM772339.1
FMNH274416	*H. new sp*.	East Usambara	0	HM772406.1-HM772407.1	HM772468.1	HM772340.1-HM772341.1
FMNH274417	*H. new sp*.	East Usambara	0	HM772408.1-HM772409.1	HM772469.1	0
FMNH274532	*H. new sp*.	East Usambara	0	HM772414.1-HM772415.1	HM772476.1	HM772302.1-HM772303.1
FMNH274533	*H. new sp*.	East Usambara	0	HM772416.1-HM772417.1	HM772477.1	HM772304.1-HM772305.1
FMNH274534	*H. new sp*.	East Usambara	0	HM772418.1-HM772419.1	HM772478.1	HM772306.1-HM772307.1
FMNH274535	*H. new sp*.	East Usambara	0	HM772420.1-HM772421.1	HM772479.1	HM772308.1-HM772309.1
FMNH274536	*H. new sp*.	East Usambara	0	0	HM772480.1	HM772310.1-HM772311.1
FMNH274271	*H. new sp*.	Nguru	KX953899	HM772432.1-HM772433.1	0	HM772310.1-HM772311.1
MW07204	*H. new sp*.	Nguru	0	HM772444.1-HM772445.1	HM772474.1	HM772342.1-HM772343.1
MTSN5159	*H. new sp*.	Nguru	KX954004	0	0	1
MTSN5160	*H. new sp*.	Nguru	KX954005	0	HM772481.1	0
MTSN7518	*H. new sp*.	Nguru	KX954006	0	0	0
MTSN7519	*H. new sp*.	Nguru	KX954007	0	0	0
MTSN 9523	*H. new sp*.	Segoma Forest	KX953979	0	0	1
MTSN 9549	*H. new sp*.	Segoma Forest	KX953980	0	0	0
CB 13.806	*H. new sp*.	Mbayani bwawa	KX953958	0	0	0
CB 13.807	*H. new sp*.	Mbayani bwawa	KX953959	0	0	0

CB 13.808	*H. new sp*.	Mbayani bwawa	KX953960	0	0	0
CB 13.809	*H. new sp*.	Mbayani bwawa	KX953961	0	0	0
CB 13.810	*H. new sp*.	Mbayani bwawa	KX953962	0	0	0
CB 13.811	*H. new sp*.	Mbayani bwawa	KX953963	0	0	0
CB 13.812	*H. new sp*.	Mbayani bwawa	KX953964	0	0	0
CB 13.813	*H. new sp*.	Mbayani bwawa	KX953965	0	0	0
CB 13.831	*H. new sp*.	Mbayani bwawa	KX953966	0	0	0
CB 13.832	*H. new sp*.	Mbayani bwawa	KX953967	1	1	1
BM 2002.628	*H. new sp*.	Nilo FR	KX953968	0	0	0
BM 2002.629	*H. new sp*.	Nilo FR	KX953969	0	0	0
BM 2002.631	*H. new sp*.	Nilo FR	KX953970	0	0	0
BM 2002.632	*H. new sp*.	Nilo FR	KX953971	0	0	0
CB 13.028	*H. mitchelli*	Makangala	KX953909	1	1	1
CB 13.029	*H. mitchelli*	Makangala	KX953910	0	0	0
CB 13.045	*H. mitchelli*	Makangala	KX953911	0	0	0
CB 13.046	*H. mitchelli*	Makangala	KX953912	0	0	0
CB 13.011	*H. mitchelli*	Makangala	KX953913	0	0	0
CB 13.012	*H. mitchelli*	Makangala	KX953914	0	0	0
CB 13.027	*H. mitchelli*	Makangala	KX953915	0	0	0
CB 13.133	*H. mitchelli*	Makangala	KX953916	0	0	0

CB 13.134	*H. mitchelli*	Makangala	KX953917	0	0	0
CB 13.135	*H. mitchelli*	Makangala	KX953918	0	0	0
CB 13.144	*H. mitchelli*	Makangala	KX953919	0	0	0
CB 13.145	*H. mitchelli*	Makangala	KX953920	0	0	0
CB 13.149	*H. mitchelli*	Makangala	KX953921	0	0	0
CB 13.150	*H. mitchelli*	Makangala	KX953922	0	0	0
CB 13.151	*H. mitchelli*	Makangala	KX953923	0	0	0
CB 13.152	*H. mitchelli*	Makangala	KX953924	0	0	0
CB 13.153	*H. mitchelli*	Makangala	KX953925	0	0	0
CB 13.156	*H. mitchelli*	Makangala	KX953926	0	0	0
CB 13.158	*H. mitchelli*	Makangala	KX953927	0	0	0
CB 13.159	*H. mitchelli*	Makangala	KX953928	0	0	0
CB 13.228	*H. mitchelli*	Noto	KX953929	0	0	0
CB 13.229	*H. mitchelli*	Noto	KX953930	0	0	0
CB 13.241	*H. mitchelli*	Noto	KX953931	0	0	0
CB 13.242	*H. mitchelli*	Noto	KX953932	0	1	0
CB 13.413	*H. mitchelli*	Muyuyu	KX953944	0	0	0
CB 13.563	*H. mitchelli*	Kabasira	KX953945	0	0	0
CB 13.564	*H. mitchelli*	Kabasira	KX953946	0	0	0
CB 13.565	*H. mitchelli*	Kabasira	KX953947	0	0	0

CB 13.566	*H. mitchelli*	Kabasira	KX953948	0	0	0
CB 13.567	*H. mitchelli*	Kabasira	KX953949	0	0	0
CB 13.568	*H. mitchelli*	Kabasira	KX953950	0	0	0
CB 13.569	*H. mitchelli*	Kabasira	KX953951	0	0	0
CB 13.570	*H. mitchelli*	Kabasira	KX953952	0	0	0
CB 13.571	*H. mitchelli*	Kabasira	KX953953	0	0	0
CB 13.572	*H. mitchelli*	Kabasira	KX953954	0	0	0
CB 13.573	*H. mitchelli*	Kabasira	KX953955	0	0	0
CB 13.595	*H. mitchelli*	Kabasira	KX953956	0	0	0
CB 13.598	*H. mitchelli*	Kabasira	KX953957	1	1	1
FMNH275040	*H. mitchelli*	Morogoro	0	HM772422.1-HM772423.1	HM772483.1	1
MCZ A-32199	*H. mitchelli*	Hongohondo	KX953981	0	1	1
MUSE 11051	*H. mitchelli*	Mgeta	KX953982	0	0	0
MUSE 11060	*H. mitchelli*	Mgeta	KX953983	0	0	0
MUSE 11061	*H. mitchelli*	Mgeta	KX953984	0	0	0
MUSE 11062	*H. mitchelli*	Mgeta	KX953985	0	0	0
FMNH274390	*H. mitchelli*	Udzungwa	0	HM772410.1-HM772411.1	HM772491.1	HM772364.1-HM772365.1
FMNH274391	*H. mitchelli*	Udzungwa	KX953900	HM772412.1-HM772413.1	HM772492.1	HM772366.1-HM772367.1
FMNH274392	*H. mitchelli*	Udzungwa	0	0	HM772493.1	HM772368.1-HM772369.1
SL3012	*H. mitchelli*	Udzungwa	1	0	0	0

CB 13.289	*H. mitchelli*	Namatimbili	KX953933	1	1	1
CB 13.304	*H. mitchelli*	Makangaga	KX953934	0	0	0
CB 13.305	*H. mitchelli*	Makangaga	KX953935	0	0	0
CB 13.306	*H. mitchelli*	Makangaga	KX953936	0	0	0
CB 13.319	*H. mitchelli*	Makangaga	KX953937	0	0	0
CB 13.320	*H. mitchelli*	Makangaga	KX953938	0	0	0
CB 13.321	*H. mitchelli*	Makangaga	KX953939	0	1	0
CB 13.322	*H. mitchelli*	Makangaga	KX953940	0	0	0
CB 13.379	*H. mitchelli*	Kiwengoma	KX953941	0	0	0
CB 13.395	*H. mitchelli*	Kiwengoma	KX953942	0	0	0
CB 13.396	*H. mitchelli*	Kiwengoma	KX953943	1	1	1
BM 2005.127	*H. mitchelli*	Kasanga FR	KX953972	0	0	0
MTSN 7675	*H. mitchelli*	Kimboza	KX953973	0	0	1
MTSN 7676	*H. mitchelli*	Kimboza	KX953974	0	0	0
MTSN 7682	*H. mitchelli*	Kimboza	KX953975	0	0	0
MTSN 7683	*H. mitchelli*	Kimboza	KX953976	0	0	0
MTSN 7708	*H. mitchelli*	Kimboza	KX953977	0	0	0
MTSN 7709	*H. mitchelli*	Kimboza	KX953978	0	0	0
FMNH275039	*H. mitchelli*	Uluguru	0	0	HM772482.1	HM772346.1-HM772347.1
FMNH275030	*H. mitchelli*	Uluguru	KX953903	HM772426.1-HM772427.1	HM772487.1	HM772356.1-HM772357.1

FMNH275032	*H. mitchelli*	Uluguru	0	HM772428.1-HM772429.1	HM772488.1	HM772358.1-HM772359.1
FMNH275033	*H. mitchelli*	Uluguru	0	0	HM772489.1	0
FMNH275034	*H. mitchelli*	Uluguru	0	0	HM772490.1	HM772360.1-HM772361.1
FMNH275044	*H. mitchelli*	Uluguru north	0	0	HM772486.1	HM772354.1-HM772355.1
FMNH275043	*H. mitchelli*	Uluguru north	0	0	HM772485.1	HM772352.1-HM772353.1
FMNH275036	*H. mitchelli*	Uluguru	0	HM772430.1-HM772431.1	0	HM772362.1-HM772363.1
FMNH274990	*H. mitchelli*	Nkhata Bay	KX953902	HM77772434.1-HM772435.1	HM772496.1	HM772370.1-HM772371.1
FMNH274978	*H. mitchelli*	Nkhata Bay	0	0	0	HM772372.1-HM772373.1
FMNH274992	*H. mitchelli*	Nkhata Bay	0	HM772436.1-HM772437.1	HM772494.1	HM772374.1-HM772375.1
FMNH274989	*H. mitchelli*	Nkhata Bay	0	HM772438.1-HM772439.1	HM772495.1	HM772376.1-HM772377.1
FMNH274994	*H. mitchelli*	Nkhata Bay	0	0	0	HM772378.1-HM772379.1
FMNH275042	*H. mitchelli*	Uluguru north	0	HM772424.1-HM772425.1	HM772484.1	HM772350.1-HM772351.1
FMNH274979	*H. mitchelli*	Luwawa	0	1	1	1
FMNH274980	*H. mitchelli*	Luwawa	0	1	1	1
FMNH274982	*H. mitchelli*	Luwawa	0	1	1	0
FMNH274985	*H. mitchelli*	Luwawa	0	1	1	1
FMNH274995	*H. mitchelli*	Luwawa	0	0	1	1
NMK A5590/1	*H. rubrovermiculatus*	Shimba Hills	KX953904	1	1	0
NMK A5590/2	*H. rubrovermiculatus*	Shimba Hills	KX953905	1	1	1
NMK A5590/3	*H. rubrovermiculatus*	Shimba Hills	KX953906	1	1	0

MW 7913	*H. rubrovermiculatus*	Shimba Hills	1	0	0	0
NMK A5957/1	*H. rubrovermiculatus*	Shimba Hills	KX953986	0	0	0
NMK A5801/1	*H. rubrovermiculatus*	Shimba Hills	KX953987	1	1	1
NMK A5801/5	*H. rubrovermiculatus*	Shimba Hills	KX953988	1	1	1
NMK A5801/3	*H. rubrovermiculatus*	Shimba Hills	KX953989	1	1	1
NMK A5801/2	*H. rubrovermiculatus*	Shimba Hills	KX953990	1	1	1
NMK A5801/5	*H. rubrovermiculatus*	Shimba Hills	KX953991	1	1	1
NMK A5848	*H. rubrovermiculatus*	Shimba Hills	KX953992	0	1	1
NMK A5762/2	*H. rubrovermiculatus*	Shimba Hills	KX953993	1	1	1
NMK A5762/1	*H. rubrovermiculatus*	Shimba Hills	KX953994	1	1	1
NMK A5900/1	*H. rubrovermiculatus*	Shimba Hills	KX953995	1	1	1
NMK A5900/2	*H. rubrovermiculatus*	Shimba Hills	KX953996	1	1	1
NMK A5920	*H. rubrovermiculatus*	Shimba Hills	KX953997	0	1	0
NMK A5958/1	*H. rubrovermiculatus*	Shimba Hills	KX953998	1	1	1
NMK A5958/2	*H. rubrovermiculatus*	Shimba Hills	KX953999	1	1	1
NMK A5958/3	*H. rubrovermiculatus*	Shimba Hills	KX953999	0	1	1
NMK A5961/1	*H. rubrovermiculatus*	Shimba Hills	KX954001	1	1	1
NMK A5962/2	*H. rubrovermiculatus*	Shimba Hills	KX954002	1	1	1
NMK A5909	*H. rubrovermiculatus*	Shimba Hills	KX954003	0	0	0
MTSN 8643	*H. mitchelli*	North Pare Mountains	KX954008	0	0	0

CHAPTER III

Three new species of *Callulina* (Amphibia: Anura: Brevicipitidae) from East Africa with conservation and biogeographical considerations for the whole genus.

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Three new species of *Callulina* (Amphibia: Anura: Brevicipitidae) from East Africa with conservation and biogeographical considerations for the whole genus.

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Abstract

All known specimens of the genus *Callulina* were examined for this study. Three new species are described from (i) Ukaguru Mountains, (ii) Rubeho Mountains and (iii) a widely distributed submontane forest species from Central and Southern Eastern Arc Mountains in Tanzania. The species are diagnosed based on a combination of morphological, acoustic and molecular data. An updated key to all known *Callulina* species is provided. We also report on the assessment of *Callulina* population from Shimba Hills in Kenya and evaluate its taxonomic status. The diversity of the genus is twelve species with only 2 species having wide distributions across two or more mountain areas. The many narrowly distributed *Callulina* species are likely to be of high conservation concern given habitat change in the region.

Keywords: Brevicipitidae, Eastern Arc Mountains, lowland, Montane forests, Shimba Hills.

Introduction

Brevicipitids are a small radiation of frogs occurring in East and Southern Africa (Channing, 2001; Channing & Howell, 2006). The diversity of the family has expanded considerably in the last fifteen years with many new species described from Tanzania (Loader et al., 2006; Loader et al., 2014; Menegon et al., 2011). The family is comprised of five genera; *Balebreviceps, Breviceps, Callulina, Probreviceps* and *Spelaeophryne* (Parker, 1934; Largen & Drewes, 1989). The genus *Callulina* is mainly confined to the Eastern Arc Mountains of Tanzania and Kenya with a single population recorded from lowland forest (Shimba Hills) (Loader et al., 2010a). The genus has seen a rapid increase in numbers of species from one in 2003 (Poynton, 2003) to the nine species currently described (De Sa, et al., 2004; Loader et al., 2009a, 2010a, 2010b; Menegon et al., 2011).

Loader et al. (2014) published a phylogeny of brevicipitids that outlined a cryptic diversity of East African brevicipitids, which included known and undescribed species of *Callulina*. Two populations were noted as undescribed (see Loader et al., 2014 Appendix 1) and that for *Callulina* additional samples of “one Kenyan population from Shimba Hills” would be required to complete the understanding of this genus. In addition the taxonomic status of a *Callulina* collected at Mamiwa Kisara Ukaguru Mountains in 2005 remained unknown. In this paper we present new data on the morphology of these undescribed species and taxonomically assess them. We use genetic and acoustic data to provide further evidence towards their taxonomic distinction. A complete assessment of the genus provides an opportunity to assess the biogeographic and conservation patterns in *Callulina* and we briefly outline the implications of our findings.

Materials and methods

Specimens

We examined materials deposited in the following institutional collections: The British Museum of Natural History, London (BMNH); Museo Tridentino di Scienze Naturali, Trento (MTSN) and National Museums of Kenya (NMK). Specimens collected from recent fieldwork in the Shimba Hills Kenya (2015) were fixed in 10% formalin and subsequently stored in 70% ethanol. Samples of muscle tissue were taken from representative individuals and preserved in 95% ethanol. Comparative material comprised *Callulina* specimens as listed in Appendix 1 from previous publications (see Loader et al. 2010a).

Genetic diversity and species delimitation

DNA sequence data were generated for new samples using approaches outlined by Loader et al. (2006, 2009a, 2010a, 2014). Sequences were obtained for parts of the mitochondrial (mt) genes 12S, 16S and cytochrome b (*cytb*). GenBank (www.ncbi.nhi.gov/genbank/) accession numbers where available are given in Appendix 1. Alignments were constructed using MAFT (Katoh et al., 2002) in Geneious platform v.6.1.2 (http:www.geneious.com, Kearse et al., 2012), manually adjusted for obvious errors and ambiguously aligned sites removed and then insertions and deletions were removed using GBlocks (Castresena, 2001) for 12S and 16S while TranslatorX (Abascal et al., 2010) was used for Cytochrome b. Uncorrected pairwise comparisons were used to measure the genetic similarity of newly sampled *Callulina* populations against currently described taxa using Geneious software (v6.1.2) and the Species Delimitation plugin v1.04 for Geneious Pro (Masters et al., 2011).

Morphology

Measurements were taken to the nearest 0.1 mm using digital callipers. Following Loader et al. (2009a, 2010a, 2010b), the measurements taken were; horizontal eye diameter (ED); eye–tympanum distance (ETD); upper arm length (HL); head width at level of jaw articulation (HW); interorbital distance (IOD); length of finger 3, measured from the distal edge of the basal subarticular tubercle (LF3); length of toe 4, measured from the proximal edge of the basal subarticular tubercle (LT4); nostril diameter (ND); nostril–eye distance (NED); nostril–lip distance (NLD); snout–urostyle length (SUL); horizontal tympanum diameter (TD); tibiofibula length (TL); length of tarsus (TSL); width of disc of finger 3 (WDF3); width of finger 3 at level of distal subarticular tubercle (WDTF3). We used Principal component analyses (PCA) to establish the variation within the genus *Callulina* and the variables responsible for such variations if any. PCA was conducted using Statistica (STATSOFT v. 6) for species which had samples size of male and females above three. The effect of size was removed by first performing a regression analysis of all measurements against the SVL. The resulting residual scores were used as the new variables for calculating the PCA. Principal component analysis was conducted for males and females separately and also for both sexes together. In addition, the mean SVL for each sex was calculated and represented in the form of boxplots. Specimens examined and locality data are provided in each species account.

Bioaccoustics

Calls were recorded opportunistically in the field using a Marantz model PMD-430 stereo cassette tape recorder and a KE66 Sennheiser directional microphone. The following call properties, mean dominant frequency; mean signal duration and mean pause duration, were analyzed using seewave package in R (Sueur et al., 2008; R Core team, 2015).

Results

Taxonomy

The following taxonomic descriptions are preliminary and await formal publication.

Callulina new sp1.

Callulina sp 3 (Menegon et al., 2008).

Callulina sp "lowland" Loader et al., (2014).

Fig. 1: Dorsal and ventral views of the Holotype of *C. new sp1*.

Holotype.—MTSN 8597, an adult female from Nguru mountains in Tanzania. This specimen has been sequenced for 12S, 16S and Cytb genes. Specimen in good condition with midventral incision into coelom and incision around tympanic region on left and right.

Paratypes.—We restrict paratype material to localities within the Nguru on the basis that further detailed morphological/molecular analysis might uncover additional cryptic lineages (see Loader et al., 2014). MTSN 5153, MTSN 8237, MTSN 8242, MTSN 8597, MTSN 8598, MTSN 8599, MTSN 8600, MTSN 8601.

Referred material.—Ordered per locality: Nguru (MW7160, MW7162, MW 7164, MW7167, MW7168, MW7169, MW7170, MW7225, MW7227); Ukaguru (MW 03050, MW03052, MHNG 2624.5); Udzungwa (BM 1982.594, KMH 22478); Uluguru (CAM 808, KMH 21555, KMH 21557, KMH 21568, A 13611, A 13612, A 13613, A 13614, A 13617, A 13618, A 13620, A 13621, AMNH 37290, AMNH 37291).

Diagnosis.—The new species of *Callulina* is assigned to the *Callulina* genus based on the following characteristics: Truncated or expanded terminal phalanges (simple in *Spelaeophyrne, Probreviceps, Breviceps* and *Balebreviceps*); single posterior denticulated row in the palate of *Callulina* (two denticulated rows in *Probreviceps*, glandular mass in *Breviceps*). A large, stout and robust *Callulina*. Snout-urostyle distance reaching 44.3 mm. Snout to urostyle-tibia ratio 30–40%. Tympanum present though often slightly obscured by granular skin. Toe and finger tips truncate. *Callulina new sp1*. differ from *C. lamphami* and *C. shengena* in the presence of a tympanum. *Callulina new sp1*. has only slightly expanded toe tips (WDF3/WDTF3: >0.8) compared to *C. kreffti, C. kanga, Callulina new sp2*. and *Callulina new sp3*. nov. (WDF3/WDTF3: <0.8). Lack of colour in the ocular region in *C. new sp1*. compared to *C. laphami* and *C. dawida* (Loader et al., 2009). *Callulina new sp1*. lacks prominent glands on the arms and/or legs (*C. hanseni, C. meteora, C. lamphami* and *C. shengena*). Morphologically, *C. new sp1*. is most similar to *C. stanleyi, C. kisiwamsitu*. The distinctiveness of *C. new sp1*. from other *Callulina* is also supported by call (Figure 7; Table 2), distribution and DNA sequence data (2.7% distinct from *C. kanga*).

Description of holotype.—Body robust and stout. Tips of fingers truncate (slightly less than width of distal subarticular tubercle), rounded edges with lateral circummarginal grooves; first finger shortest, second and fourth finger equal, third finger longest. Inner metatarsal tubercle large, rounded and raised, separated by a middle palmar tubercle from an even larger, rounded outer metatarsal tubercle, which is raised and elongated along the margin of the hand. Smaller palmar tubercles present. Subarticular tubercles at the base of each finger, large subarticular tubercles on third and fourth finger at the phalangeal joints. Third finger with two small tubercles between basal articular tubercle and subarticular tubercle. Truncate and dorso-ventrally swollen toe tips without any lamellae on the ventral surface; tips of toes not expanded laterally, with circummarginal grooves; first toe same length as second. Third and fifth toes equal, fourth toe longest. Inner metatarsal tubercle large, rounded and raised, touching a smaller, rounded, raised, outer metatarsal tubercle. Palmar tubercles present on base of foot. Subarticular tubercles at the base of each toe, large subarticular tubercles on third and fourth toe at the phalangeal joints. All tubercles on hands and feet bluish/grey against a brown/grey background. Snout visible from ventral view.

Morphological and colour variation.—The paratype and non-paratype material is very similar to the holotype in the overall body proportions and key morphometric measures.

Colour in life.—Dorsum dark brown with darker glandular masses on side and back. Ventral surface pale brown. Tympanum pale brown, with irregular margins obscured by glandular warts. Loreal and canthal regions brown with lighter coloured warts. Nostrils, snout tip and jaw angle slightly darker brown.

Conservation status — *C. new sp1*. was collected across the Eastern Arc Mountains, from the Nguu to Mahenge. Given the large, but patchy distribution across the landscape the area of occurrence would be relatively large and therefore qualifying the species to be of least concern.

***Callulina new* sp2**.

Fig. 2: Ventral and dorsal views of the holotype of *Callulina new sp2*.

A female, ZMB83024 collected 8 December 2005 by Wilirk Ngalason and Alan Channing at Mamiwa Kisara North Forest Reserve, Ukaguru Mountains, Tanzania, 1854 m (6.425469 S; 36.967014 E). The specimen was found inside a decaying branch, 1 m above ground level with femoral incision and incision around tympanic region on left and right.

Paratypes.—MTSN 5553–5555 collected on January 25th 2004 by Michele Menegon at Mamiwa Kisara North Forest Reserve, Ukaguru Mountains, Tanzania, 1800 m.

Diagnosis.—The new species is assigned to the genus *Callulina* based on the following characteristics: Truncated or expanded terminal phalanges (simple in *Spelaeophyrne, Probreviceps, Breviceps* and *Balebreviceps*); single posterior denticulated row in the palate of *Callulina* (two denticulated rows in *Probreviceps*, glandular mass in *Breviceps*). A large, stout and robust *Callulina*. Snout-urostyle distance reaching 39.9 mm. Snout to urostyle-tibia ratio 33–39%. Tympanum present sometimes obscured by granular skin. Toe and finger tips truncate. *Callulina new sp2*. differ from *C. lamphami* and *C. shengena* in the presence of a tympanum. *Callulina new sp.2* has expanded toe tips (WDF3/WDTF3: <0.75) compared to *C. new sp1*., *C. lamphami, C. shengena, C. kisiwamsitu, C. stanleyi, C. dawida* and *C. hanseni* (WDF3/WDTF3: >0.8). Lack of colour in the ocular region in *C. new sp2*. compared to *C. laphami* and *C. dawida* (Loader et al., 2009). *Callulina new sp2.* lacks prominent glands on the arms and/or legs (*C. hanseni, C. meteora, C. lamphami* and *C. shengena*). Morphologically, *C. new sp2*. is most similar to *C. kreffti*, *C. new sp3*. and *C. kanga*. However, *C. new sp2*. has a smaller tympanum relative to the distance of tympanum to the eye, while in *C. kreffti* the tympanum diameter is more than the distance between tympanum and eye. *Callulina new sp2*. does not differ significantly from *C. kanga* and *C. new sp3*. The distinctiveness of *C. new sp2* from other *Callulina* is also supported by distribution and DNA sequence data. (3.2% sequence divergence from *C. new sp3*).

Description of holotype.—Body robust and stout (Figure 2). Measurements given in Appendix II. Tips of fingers truncate (wider than distal subarticular tubercle), rounded edges without lateral circummarginal grooves; first finger shortest, second and fourth finger equal, third finger longest. Inner metacarpal tubercle large, rounded and raised, outer metatarsal tubercle elongated, raised. Smaller flat, rounded palmar tubercles present. Subarticular tubercles at the base of each finger, large subarticular tubercles on third and fourth finger at the phalangeal joints. Third finger with two small tubercles between basal articular tubercle and subarticular tubercle. Truncate and dorsoventrally swollen toe tips; tips of toes slightly expanded laterally, without circummarginal grooves; relative toe lengths: 1 = 2<3<5<4. Inner metatarsal tubercle large, elongated and raised, touching a smaller, rounded, raised, outer metatarsal tubercle. Many small tubercles present on sole. Large subarticular tubercles at the base of each toe, with large subarticular tubercles on third, fourth and fifth toes at the phalangeal joints. In preservative, all tubercles on hands and feet bluish grey against a brown-grey background. Dorsal surfaces of wrists, arms, ankles and back covered with distinct low glandular warts. Snout visible from below.

Morphological and colour variation.—The paratype is very similar to the holotype in the overall body proportions and key morphometric measures. The paratype has a slightly smoother skin and has only a few dark symmetrical patches on the dorsum. Incision on left hand side of the tympanic region.

Colour in life.—Dorsum dark brown with tan glandular masses on side and back (Figure 2), with the sides purple with small white warts. Ventral surface pale brown. Tympanum pale brown, with irregular margins obscured by glandular warts. Loreal and canthal regions brown with grey warts. Nostrils, snout tip and jaw angle slightly darker grey. Snout visible from below.

Conservation status.—*Callulina new sp2*. was collected in Mamiwa Kisara North Forest Reserve at two localities at an elevation of 1800 m and 1851 m. Mamiwa Kisara was surveyed by two separate teams (totalling around two weeks of survey time), during which specimens were restricted to two small localities, despite searching many other locations and forest types and at different altitudes. If the species is localised to this particular band of montane forest then the species has an extremely narrow distributional range and would be of high conservation concern. The likely estimated area of occurrence would qualify the species to be critically endangered (CR B1b (iii)) under IUCN criteria.

Callulina new sp3.

Fig. 3: Dorsal and ventral views of the holotype of *Callulina new sp3*.

Callulina sp. "rubeho" Loader et al., (2014).

Holotype.—KMH 36024, an adult female collected from Mafwemiro Forest Reserve, Rubeho Mountains (06°56´19S, 36°35´04E, 1900 m a.s.l.) by Michele Menegon on 15th January 2006. This specimen has been sequenced for 12S, 16S and Cytb. Specimen in good condition, with femoral incision and incision around tympanic region on left and right.

Diagnosis.—The new species is assigned to the genus *Callulina* based on the following characteristics: Truncated or expanded terminal phalanges (simple in *Spelaeophyrne*, *Probreviceps*, *Breviceps* and *Balebreviceps*); single posterior denticulated row in the palate of *Callulina* (two denticulated rows in *Probreviceps*, glandular mass in *Breviceps*). A medium sized, stout and robust *Callulina*. Snout-urostyle distance reaching 28.2 mm. Snout to urostyle-tibia ratio 38%. Tympanum present though slightly obscured by granular skin. Toe and finger tips truncate. *C. new sp3*. differ from *C. lamphami* and *C. shengena* in the presence of a tympanum. *C. new sp3*. has expanded toe tips (WDF3/WDTF3: <0.75) compared to *C. new sp1*., *C. lamphami*, *C. shengena, C. kisiwamsitu, C. stanleyi*, *C. dawida* and *C. hanseni* (WDF3/WDTF3: >0.8). Lack of colour in the ocular region in *C. new sp3*. compared to *C. laphami* and *C. dawida* (Loader et al., 2009). *Callulina new sp3*. lacks prominent glands on the arms and/or legs (*C. hanseni, C. meteora, C. lamphami* and *C. shengena*). Morphologically, *C. new sp3*. is most similar to *C. kreffti*, *C. new sp2*. and *C. kanga*. However, *C. new sp3*. has a smaller tympanum relative to the distance of tympanum to the eye, while in *C. kreffti* the tympanum diameter is more than the distance between tympanum and eye. *Callulina new sp3*. does not differ significantly from *C. kanga* and *C. new sp2*. The distinctiveness of *C. new sp3*. from other

Callulina is also supported by distribution and DNA sequence data (5.1% sequence divergence from *C kreffti*).

Description of holotype.—Body robust and stout. Tips of fingers truncate (greater than width of distal subarticular tubercle), rounded edges with lateral circummarginal grooves; first finger shortest, second and fourth finger equal, third finger longest. Inner metatarsal tubercle large, rounded and raised, separated by a middle palmar tubercle from an even larger, rounded outer metatarsal tubercle, which is raised and elongated along the margin of the hand. Smaller palmar tubercles present. Subarticular tubercles at the base of each finger, large subarticular tubercles on third and fourth finger at the phalangeal joints. Third finger with two small tubercles between basal articular tubercle and subarticular tubercle. Truncate and dorso-ventrally swollen toe tips without any lamellae on the ventral surface; tips of toes not expanded laterally, with circummarginal grooves; first toe same length as second. Third and fifth toes equal, fourth toe longest. Inner metatarsal tubercle large, rounded and raised, touching a smaller, rounded, raised, outer metatarsal tubercle. Palmar tubercles present on base of foot. Subarticular tubercles at the base of each toe, large subarticular tubercles on third and fourth toe at the phalangeal joints. All tubercles on hands and feet bluish/grey against a brown/grey background. Snout visible from ventral view.

Morphological and colour variation.—The species is represented by a single specimen and hence no morphological or colour variation is known.

Colour in life.—Dorsum dark brown with darker irregular patches on the side and back. Ventral surface pale brown/cream. Tympanum pale brown, with irregular margins obscured by glandular warts. Loreal and canthal regions brown with dark grey warts. Nostrils, snout tip and jaw angle slightly darker grey. Snout visible from below.

Conservation status.— *Callulina new sp3*. was collected in Mafwemiro Forest Reserve at a single locality. Mafwemiro Forest Reserve was surveyed by single survey team (totalling around three weeks of survey time). If the species is localised to this particular band of montane forest then the species has an extremely narrow distributional range and would be of high conservation concern. The likely estimated area of occurrence would qualify the species to be critically endangered (CR B1b (iii)) under IUCN criteria.

Revised key to the species of Callulina

Externally, *Callulina* species are distinguished from other brevicipitids by their truncate to expanded toe and fingertips. The key below relies upon key morphological features and geographical distribution of species given the morphological similiarity of many species.

1a. Tympanum present, though may be slightly obscured by granular skin…2

1b. Tympanum absent…*9*

2a. Fingertips expanded (WDF3/WDTF3 <0.9), wider than the distal subarticular tubercle…3

2b. Fingertips slightly truncated (WDF3/WDTF3 >0.9).... *C. dawida*

3a. Fingertips truncated not expanded beyond the width of the first subarticular tubercle (WDF3/WDTF3 <0.75)...4

3b. Fingertips truncated wider than distal subarticular tubercle (WDF3/WDTF3 >0.75)….*C. new sp2.*

4a. Distance between tympanum and eye usually less than tympanum diameter. Distinctive call, known only from East Usambara Mountains and Shimba Hills…*C. kreffti*

4b. Distance between tympanum and eye usually greater than tympanum diameter. Distinctive call…*5*

5a. Known only from Rubeho Mountains…*C. new sp3.*

5b. Not found in Rubeho Mountains …*6, 7*

6a. Known only from Nguru Mountains…*C. kanga*

6b. Large, distinctive and continuous glands on arms and legs…*C. hanseni*

7a. Medium to large size with distinctive call, known from central and southern Eastern Arc Mountains………*C. new sp1.*

7b. Large, robust head known only from Northern Eastern Arc Mountains (South Pare Mountains, or West Usambara Mountains)…*8.*

8a. Large, robust head. Distinctive call, known only from South Pare Mountains……*C. stanleyi.*

8b. Less robust head. Distinctive call, known only from West Usambara Mountains…*C. kisiwamsitu.*

9a. Prominent glandular masses on arms and feet absent. Distinctive bright red (or green) interocular band connecting the opposite anterior and posterior margins of the eyelids. North Pare Mountains…*C. laphami.*

9b. Prominent, relatively pale glandular mass on arms and feet. Less distinct and less continuous interocular band. South Pare Mountain...*C. shengena.*

Species delimitation and pairwise divergence

Species divergence estimates based on the Geneious plugin using multi-locus alignment data ranged from 2.2–5.2% with highest divergence found between *C. new sp3.* and *C. kreffti*. The Shimba population of *Callulina* was not significantly divergent from *C. kreffti* in all the three partial genes examined and is therefore recognized as *C. kreffti* (Table 2).

Table 1: Uncorrected pairwise (p-) distances for 1170 base pairs of 12S, 16S rRNA and *cytb* mtDNA sequence data for *Callulina***.**

Species	Closest Species	Monophyletic?	Intra Dist	Inter Dist - Closest
C. hanseni	*C. meteora*	yes	0.006	0.025
C. stanleyi	*C. kisiwamsitu*	yes	0.004	0.022
C. sp. shimba	*C. kreffti*	yes	1.74E-04	7.38E-04
C. shengena	*C. laphami*	yes	0.002	0.05
C. kanga	*C. new sp1*	yes	0.002	0.027
C. new sp3	*C. new sp2*	yes	0.00E+00	0.032
*C. new sp3.*and *C. new sp2.*	*C. kreffti*	yes	0.032	0.051
C. new sp1.	*C. new sp1. (*1)	yes	0.009	0.013

Morphology

Based on the snout to vent length, *C. Kanga* Loader, Gower, Müller & Menegon, 2010 was smaller than the rest of the *Callulina* species, with a mean SVL (mm) of 25.5 while *C. meteora* Menegon, Gower & Loader, 2011 was the largest with mean at 35.9 (P = 0.008). Female *Callulina* were generally larger than the males. Only *C. kisiwamsitu* had mean SVL of males above 30 mm while for females only one *C. dawida* had the mean SVL of female samples around 30 mm (Figure 5).

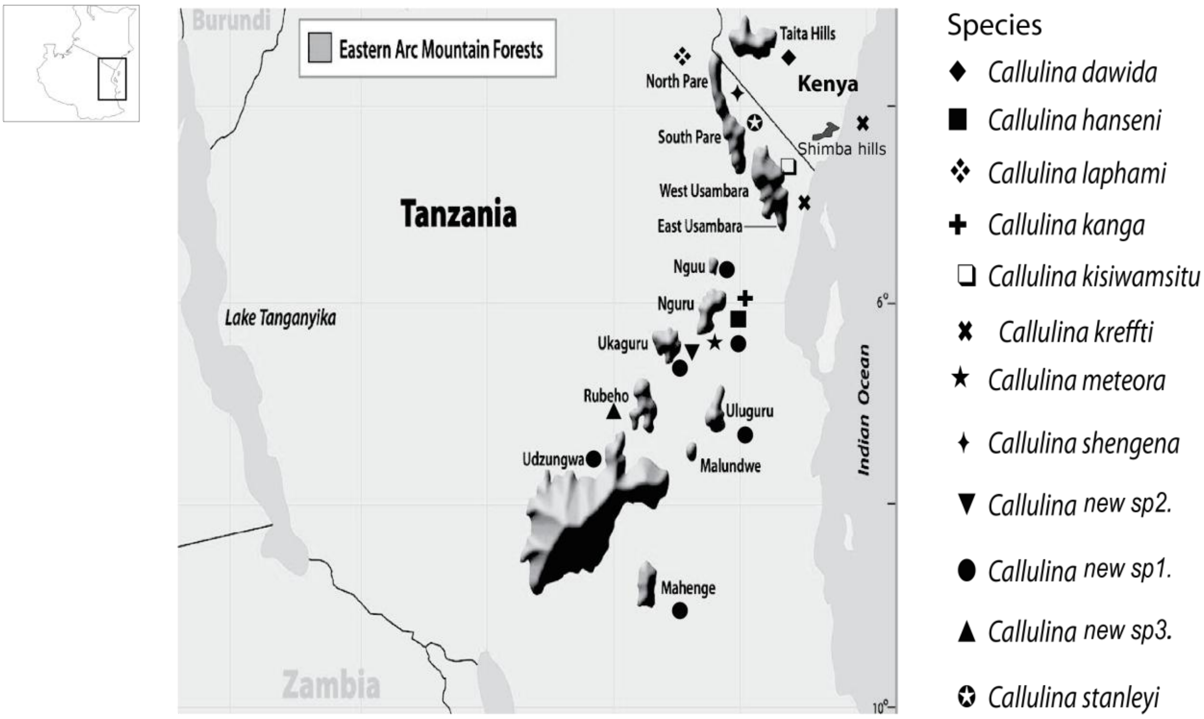

Fig. 4. Map of Kenya and Tanzania showing the distribution of the genus *Callulina*. Map modified from https://eamcef.wordpress.com/were-we-work/.

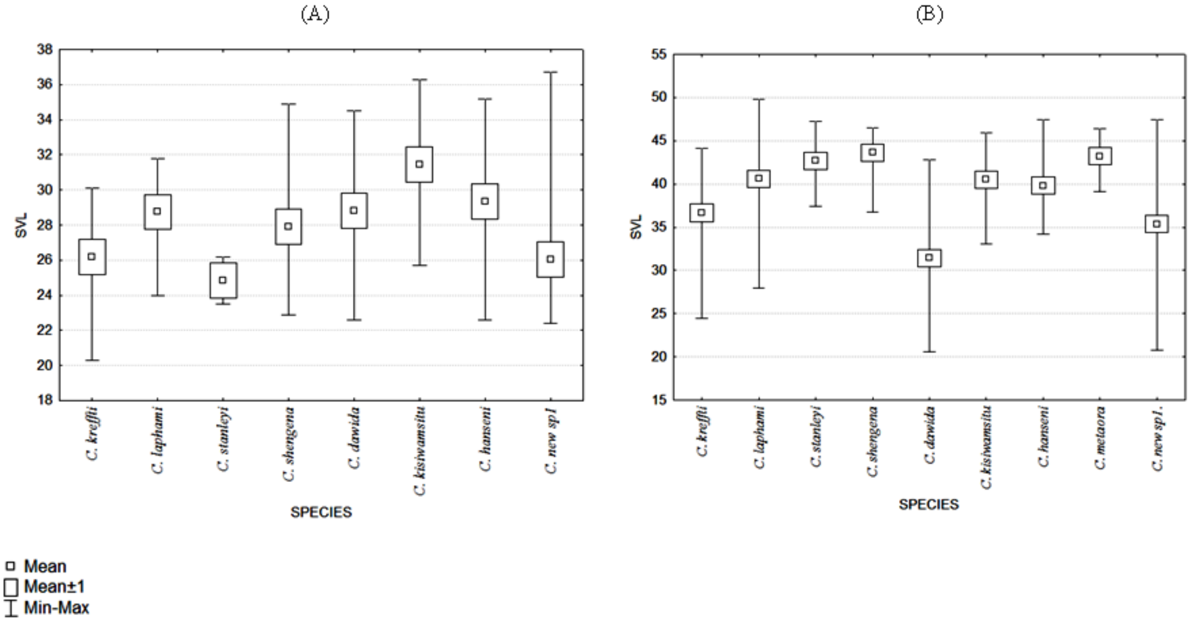

Fig. 5: Boxplot of snout to urostyle length (SUL) results of (A) eight male and (B) nine female *Callulina* species.

Principal component analysis (PCA) however could not differentiate the current twelve *Callulina* species. When separated into males and females, the females could still not be distinguished into the various species. However, it was possible to separate the males into four major groups (Figure 6). The following results are based only on the male PCA analysis. The variables responsible for the separation in the first principle component include Tibia, Nares, Jaw width, Humerous, Nare to lip distance while distal phalange width, width of first subarticular tubercle and infra-orbital distance accounted for the second component. *Callulina hanseni* Loader, Gower, Müller & Menegon, 2010 and *C. shengena* have larger jaw width and nares to lip distance while *C. kreffti* had smaller (Figure 6). *Callulina new sp1*. is separated into two groups one group has larger jaw width and nares to lip distance compared to the second group. *Callulina dawida*, specimens were mostly overlapping among three of the four major groups (Figure 6).

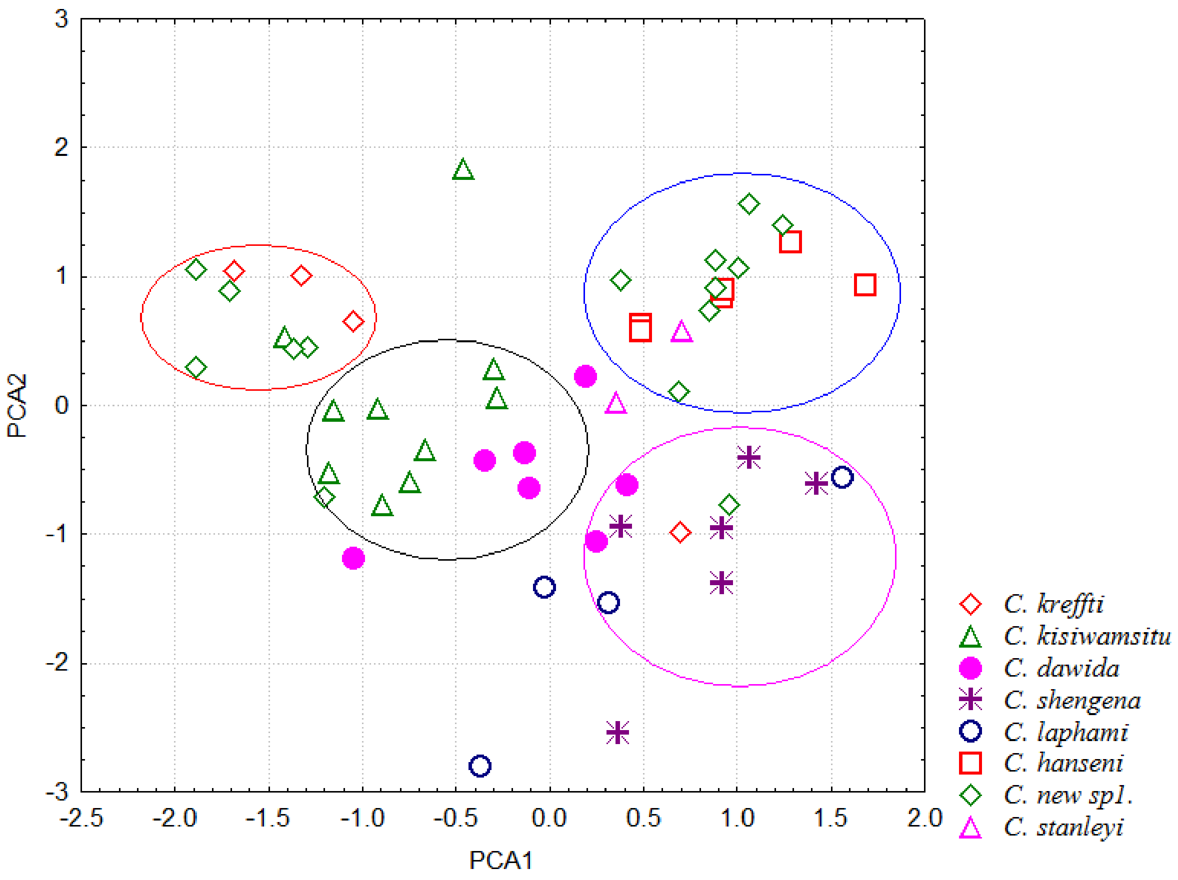

Fig. 6: A scatter plot of PCA analysis of *Callulina* species. The eight species analysed are represented by various shapes and colour codes.

Accoustics

Five calls from *C. new sp1*. from (Sali Forest Reserve, Nguu and Ukaguru Mountains) available while both *C. new sp2*. and *C.new sp3*. had no calls. The available calls were compared to previous calls for from *C. meteora* from Nguru Mountains Maskati, two calls from *C. laphami* (Kindoroko Forest Reserve), and four calls from *C. kanga* (Kanga). Mean dominant frequency was variable ranging from 0.81 kHz in *C. laphami* to 6.3 kHz in *C. kanga*. Spectrogram of *C. new sp1*. from Nguu Mountains seems to be different from that of Sali FR in Mahenge. Mean signal duration on the other hand was less variable ranging between 0.01 to 1 (see Figure 7 and Table 2 below).

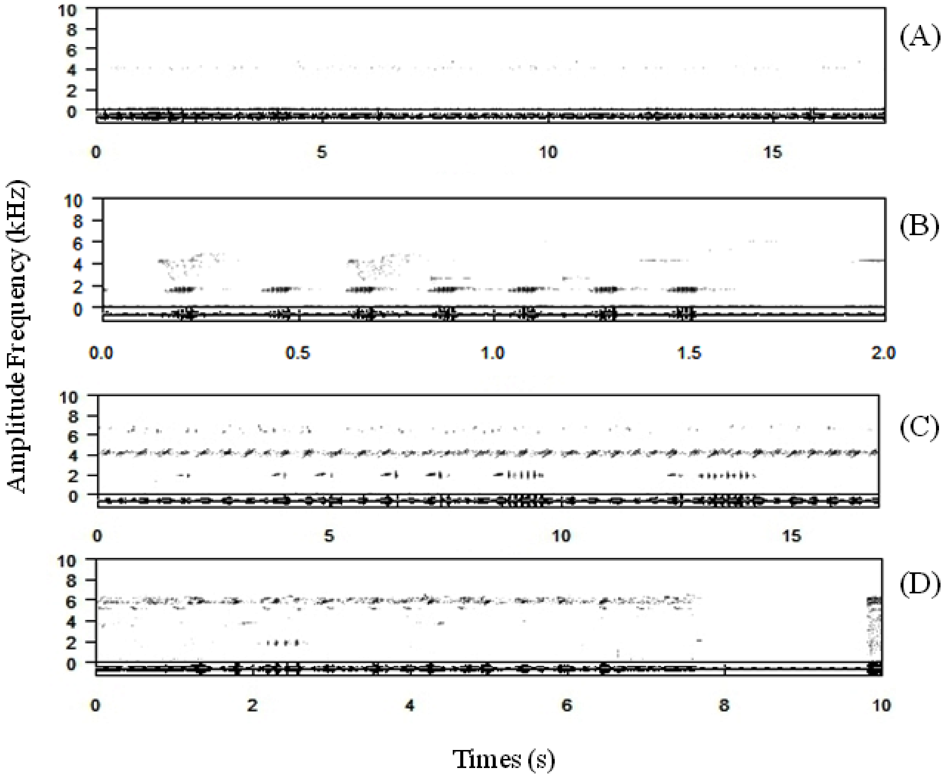

Fig. 7: Top to bottom: Oscilliogram and spectrogram of (A) *C. laphami*, (B) *C. meteora*, (C) *Callulina kanga* and (D) *Callulina new sp1*.

Table 2: Mean dominant frequency, signal duration and pause duration of some *Callulina* species 1 = *C. laphami*; 2 = *C. meteora*; 3 = *C. new sp1*., Ukaguru; 4 = *C. laphami*; 5 = *C. kanga*; 6 = *C. new sp1*., Nguu*;* 7 = *C. new sp1*., Mahenge; 8 = *C. new sp1*., Mahenge; 9 = *C. Kanga*; 10 = *C. Kanga1*; 11 = *C. kanga*; 12 = *C. new sp1*., Mahenge.

Locality	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Dominant frequency (mean)	0.81	1.01	2.29	3.85	3.88	5.77	2.41	2.45	4.31	6.33	3.27	3.55
Signal duration (mean)	0.12	0.06	3.42	0.01	0.46	0.19	0.02	0.08	0.34	0.08	0.13	0.06
Pause duration (mean)	0.01	0.03	NA	0.04	0.01	0.02	0.05	0.02	0.02	0.02	0.02	0.02

Discussion

Recent field work has seen the number of *Callulina* species increasing from one species (*C. kreffti* Nieden, 1910) over 100 years ago to nine (Loader et al., 2014). These species occur in Kenya (1) and

Tanzania (8). We outline three new species that are described in this study, based mainly from morphological and molecular data (see Taxonomy section above), thus increasing the currently known *Callulina* to 12. The various *Callulina* species are distinguishable from each other based on both morphological and molecular characters. Morphological characters such as presence or absence of tympanum, toe tips expanded or truncated among other characters can be used to broadly group *Callulina* species (Channing & Howell, 2006; Loader et al., 2010a and b, 2014). Principal Component analysis (PCA) of morphometric characters however only proved useful in distinguishing male *Callulina* species. Out of nine species in which sample size was more than one, four groups were identifiable (Figure 6). The genus is morphologicaly conservative and can be considered containing a number of cryptic species and for which molecular, acoustic and geographic data provide important additional information for their identification (Figure 4). The occurrence of cryptic species in amphibians is commonly shown in the literature and here we add another example (e.g. Loader et al., 2015; Barratt et al., 2017).

Acoustic data was useful for distinguishing some species. Even though the calls were not available for all the twelve species based on previous studies on *Callulina,* calls have proved to be distinctive and a good tool for distinguishing between the various *Callulina* species (De sa et al., 2004; Loader et al., 2010b). De sa et al., 2004 reported the differences between *C. kreffti* and *C. kisiwamsitu* based on their dominant frequency values. However in many cases only one call per species was recorded which make comparisons difficult and further work will be necessary to make more robust estimates of calls–and their variation.

Further taxonomic work will clearly be necessary. For example, *C. new sp1*. can be split into two groups based on morphometric analysis suggesting the possible presence of two species within this group. Based on the twelve morphometric characters examined, the Nguru and Nguu specimens were generally bigger than their sister taxa from southern EAM. This split was also evident in all the three genes and also in the multi-locus alignment where the population from Nguru and Nguu mountains were sister taxa to Mahenge, Uluguru, Ukaguru and Udzungwa populations of *C. new sp1.* with 1.3% sequence divergence. The geographical north south divide in *C. new sp1*. populations further supports the idea that these areas might represent distinct species. In addition two calls analysed from Sali Forest Reserve in Mahenge differ in call properties from calls recorded from Nguu Mountains. However no environmental parameters under which these calls were recorded are available making it difficult to tell whether the observed differences are not artefacts of prevailing environmental variables (See Giacoma & Castellano, 2001). Further analysis may be required to establish the taxonomic status of both populations of *C. new sp1*.

The Shimba Hills of Kenya population of *Callulina,* previously speculated to be a new species closer to either *C. kisiwamsitu* or *C. stanleyi* of West Usambara and South Pare Mountains respectively, were found to group with *C. kreffti* of East Usambara Mountains. The sequence divergence separating the two populations was only 0.05% confirming that the two populations likely belong to the same species. Further samples will need to be collected to understand the morphological variation in this species–and redefine this more widespread species.

Conservation

The genus *Callulina* comprise of range restricted species only known from the EAM of Kenya and Tanzania with one population known outside this area in the Shimba Hills in Kenya (De sa et al., 2004; Loader et al., 2009b; 2010 and b; 2014) (Figure 4). Apart from *C. kreffti* which is of Least Concern the rest of the previously described *Callulina* species are critically endangered (Loader et al., 2010b; IUCN, 2017). Out of the three proposed new species, *C. new sp2*. and *C. new sp3*. would likely qualify as critically endangered according to the IUCN Red List of threatened species while *C. new sp1*. would likely qualify as Least Concerned given its wider distribution. The continued survival of many species in this genus will require monitoring of populations and their habitats. Conservation of these amphibians is only likely to be successful if protection of their microhabitats is maintained.

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References

Abascal, F., Zardoya, R. & Telford, M.J. 2010. TranslatorX: multiple alignment of nucleotide sequences guided by amino acid translations. *Nucleic acids research* 291.

Barratt, C.D., Lawson, L.P., Bittencourt-Silva, G.B., Doggart, N., Morgan-Brown, T., Nagel, P., Loader, S.P. (2017): A new, narrowly distributed, and critically endangered species of spiny-throated reed frog (Anura: Hyperoliidae) from a highly threatened coastal forest reserve in Tanzania. *Herpetological Journal* **27**: 13-24.

Channing, A. 2001. *Amphibians of central and southern Africa*. Cornell University Press. Ithaca, New York.

Channing, A. &. Howell, K.M 2006. *Amphibians of East Africa*. Cornell University Press. Ithaca, New York.

Castresana, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**: 540–552.

De Sa, R.O., Loader, S.P. & Channing, A. 2004. A new species of *Callulina* (Anura : Microhylidae) from the West Usambara Mountains Tanzania. *Journal of Herpetology* **38**: 219-224. Doi http://dx.doi.org/10.1670/209-03A.

Giacoma, C. & Castellano, S. 2001. Advertisement call variation and speciation in the *Bufo viridis* Complex, in Ryan, M.J. (ed), *Anuran Communication.* Smithsonian Institution Press. Washington and London.

IUCN, 2017. IUCN Red List of Threatened Species. Version 2015. <www.iucnredlist.org>. [accessed 30th May 2017].

Kato, Misawa, Kuma & Miyata 2000. MAFT: A novel method for rapid sequence alignment based on fast Fourier transform. *Nucleic Acids Research*. **30**: 3059-3066.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C. & Thierer, T. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**: 1647-1649.

Largen, M.J. &. Drewes, R.C 1989. A new genus and species of brevicipitine frog (Amphibia, Anura, Microhylidae) from high altitude in the mountains of Ethiopia. *Tropical Zoology*. *Firenze* **2**:13–30.

Loader, S.P., Gower, D.J., Howell, K.M., Dorgat, N., Rodel, M.O., Clarke, B.T., De sa, R.O., Cohen, B.L. & Wilkinson, M. 2004. Phylogenetic relatioships of African microhylid frogs inffered from

DNA sequences of mitochondrial 12S and 16S rRNA genes. *Organisms and Diversity Evolution* **4:** 227-235.

Loader, S.P., Channing, A., Menegon, M., Davenport T.R.B. 2006. A new species of *Probreviceps* (Amphibia: Anura) from the Eastern Arc Mountains, Tanzania. *Zootaxa* **1237:** 45-60.

Loader, S.P., Measey, G.J., De Sa, R.O. & Malonza, P.K., 2009. A new brevicipitid species (Brevicipitidae*: Callulina*) from the fragmented forests of the Taita Hills, Kenya. *Zootaxa* **2123***:* 55.

Loader, S.P., Menegon, M., Müller, H., Gower, D.J., Wilkinson, M., Howell, K.M. & Orton, F. 2009. Remarkable amphibian diversity in the South Nguru Mountains, Tanzania. *Froglog* 8-10.

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

Loader, S.P., Gower, D.J., Ngalason, W. & Menegon, M. 2010. Three new species of *Callulina* (Amphibia: Anura: Brevicipitidae) highlight local endemism and conservation plight of Africa's Eastern Arc forests. *Zoological Journal of the Linnean Society* **160**: 496–514. http://doi.org/10.1111/j.1096-3642.2010.00652.x.

Loader, S.P., Ceccarelli, F.S., Wilkinson, M., Menegon, M., Mariaux, J., de Sá, R.O., Howell, K.M. & Gower, D.J. 2013. Species boundaries and biogeography of East African torrent frogs of the genus *Petropedetes* (Amphibia: Anura: Petropeditidae). *African Journal of Herpetology* **62**: 40-48.

Loader, S.P., Ceccarelli, F.S., Menegon, M. & Howell, K.M. 2014. Persistance and stability of the Eastern Afromontane forests: evidence from the brevicipitied frogs. *Journal of Biogeography* **41:** 1781-1792. http://wileyonlinelibrary.com/journal/jbi/doi:10.1111/jbi/12331.

Loader, S.P., Lawson, L.P., Portik, D.M., Menegon, M. 2015. Three new species of spiny throated reed frogs (Anura: Hyperoliidae) from evergreen forests of Tanzania. BMC Res. Notes, **8**: http://doi.org/10.1186/s13104-015-1050-y.

Masters, B.C., Fan, V., & Ross, H.A. 2011. Species delimitation: Geneious plugin for the exploration of species boundaries. *Molecular Ecology Resources* **11**: 154–157.

Menegon, M., Doggart, N. & Owen, N. 2008. The Nguru Mountains of Tanzania, an outstanding hotspot of herpetofaunal diversity. *Acta Herpetologica* **3**: 107-127.

Menegon, M., Bracebridge, C., Owen, N. & Loader, S.P. 2011. Herpetofauna of montane areas of

Tanzania. 4. Amphibians and reptiles of Mahenge Mountains, with comments on biogeography, diversity, and conservation. *Fieldiana Life and Earth Sciences*.103-111.

Parker, H.W. 1934. A monograph of the frogs of the family Microhylidae: British Museum London.

Poynton, J.C. 2003. Altitudinal species turnover in southern Tanzania shown by anurans: some zoogeographical considerations. *Systematics and Biodiversity* **1:**117-126.

R Development Core Team 2015. *R: A language and environment for statistical computing*. R foundation for statistical computing Vienna, Austria. Available from: <http://www.R-project.org>.

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

APPENDICES

Appendix 1: Specimen ID, molecular accession numbers, locality, GPS co-ordinates and Genbank accession numbers (where available) for all samples used in the phylogenetic analysis.

Species ID	Molecular Accession number	Locality	Lat	Long	12S,16S, Cytb
C. dawidae MW 03197	T446	Taita Hills	38.333	-3.367	FJ998385, FJ998386, FJ998387
C. dawidae JM 1234	T519	Taita Hills	38.333	-3.433	FJ998382, FJ998383, FJ998384
C. hanseni MTSN 8138	T500	Nguru	37.498	-6.066	
C. hanseni MTSN 8140	T503	Nguru	37.498	-6.066	FN81098, FN811033, FN811078
C. hanseni MW 06952	T708	Nguru	37.673	-3.575	FN81099, FN811042, FN811087
C. kreffti SL2775	T6265	Shimba Hills	39.396	-4.238	KX954012, KX954009
C. kreffti SL2783	T6266	Shimba Hills	39.396	-4.237	KX954013, KX954010
C. kreffti KMH 23534	T423	East Usambara	38.678	-4.916	AY531842, AY531865, FJ998381
C. laphami MW 03065	T429	North Pare	37.654	-3.729	FN563043, FN563044, FN563045
C. laphami MTSN 8617	T543	North Pare	37.644	-3.744	FN81099, FN811038, FN811038
C. new sp3. KMH 36024	T658	Rubeho	36.568	-6.831	FN563070, FN563071, FN811116
C. meteora MTSN 8130	T502	Nguru	37.498	-6.066	FN81098, FN811032, FN811077
C. meteora MTSN 8133	T504	Nguru	37.498	-6.066	FN81098, FN811034, FN811079
C. stanleyi MS 023	T452	South Pare	37.996	-4.328	FN563057, FN563058, FN563059
C. stanleyi MTNS 7540	T753	South Pare	37.996	-4.328	FN563070, FN563071, FN811116
C. kisiwamsitu MW 03215	T447	West Usambara	38.4	-5.066	AY531841, AY531864, FJ998379
C. kisiwamsitu MW 01968	T303	West Usambara	38.504	-4.813	AY531840, AY531863, FJ998380
C. shengena FM 251849	T683	South Pare	37.996	-4.328	FN56304, FN563050, FN563051
C. shengena MTSN 9285	T754	South Pare	37.996	-4.328	FN56306, FN563069, FN811117
C. shengena FM 255882	T685	South Pare	37.996	-4.328	FN56306, FN563067, FN811115
C. new sp1. MW03050	T426	Ukaguru	36.983	-6.342	FN81098, FN811027, FN811072
C. new sp1. MUSE 12303	T2973	Udzungwa	35.982	-8.395	
C. new sp1. MW07266	T719	Nguru	37.494	-5.479	FN81099, FN811044, FN811089

C. new sp1. KMH 26963	T524	Mahenge	36.663	-8.95	FN81099, FN811036, FN811081
C. new sp1. MW 03830	T467	Nguu	37.475	-5.480	FN81098, FN811029, FN811074
C. new sp1. MTSN 8242	T501	Nguru	37.526	-6.030	FN81098, FN811031, FN811076
C. new sp1. KMH 26969	T526	Mahenge	36.663	-8.95	
C. new sp1. MW 07161	T716	Nguru	37.525	-6.030	FN81099, FN811043, FN811088
C. new sp1. KMH 21555	T425	Uluguru	36.983	-6.342	FN81098, FN811026, FN811071
C. new sp1. KMH 22478	T448	Udzungwa	36.589	-7.817	FN81098, FN811028, FN811073
C. new sp2. ZMB83024	ZMB83024	Ukaguru	36.967	-6.425	KX954014, KX954011
C. kanga MTSN 8205	T505	Nguru	37.724	-6.004	FN81099, FN811035, FN811080
C. kanga KMH 36389	T697	Nguru	37.724	-6.004	FN81099, FN811041, FN811086

Appendix II: Morphometric data for the three proposed new species of *Callulina*, all measurements in mm. See Materials and Methods for explanation of abbreviations.

	Callulina new sp1. (N=15)				*Callulina new sp2*. (N=4)				*Callulina new sp3*. (N=1)
Measures	Min	Max	Mean	SD	Min	Max	Mean	SD	
SVL	19.8	47.4	28.79	7.09	28.8	41.3	34.03	5.4	29.9
TL	5	15.3	9.39	2.31	11.7	14.2	12.65	1.17	10.4
TD	0.5	1.9	1.19	0.36	1.2	1.7	1.5	0.24	1.4
ETD	0.9	2.7	1.46	0.42	0.9	1.6	1.23	0.33	1.7
ED	1.6	4.8	3.00	0.68	3.8	4.6	4.3	0.35	3.7
ND	1.2	3.1	1.90	0.43	1.9	2.7	2.2	0.35	2.2
NED	1.6	3.4	2.2	0.39	2.3	3.1	2.63	0.36	2.6
JW	4.1	13.8	7.98	2.29	11.6	16	13.53	2	10.9
LF3	2.2	6.7	3.76	1.09	4.8	6.6	5.65	0.89	6.1
LT4	2.9	8.7	4.87	1.34	7.7	8.5	8.08	0.39	6
TSL	4	11.9	6.85	1.79	8.1	10.6	9.1	1.22	7.6
HL	4.5	13.5	8.01	2.08	-	-	-	-	9.2
NLD	0.7	1.7	1.18	0.25	1.4	1.9	1.5	0.24	1.5
IOD	3.6	8.6	5.15	1.07	3.7	5.7	4.58	1.03	5.2
DPW	0.7	2	1.11	0.31	1.4	1.8	1.55	0.19	1.5
WST	0.5	1.3	0.86	0.22	0.9	1.2	1	0.14	0.9

CHAPTER IV

Phylogeography of amphibians of Shimba Hills, Kenya.

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Phylogeography of amphibians of Shimba Hills, Kenya.

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Abstract

Shimba Hills of Kenya (SHK) are located at the junction of two biodiversity hotspots; the coastal forests of eastern Africa (CFEA) and the Eastern Afromontane biodiversity region (specifically, the Eastern Arc Mountains-EAM). As a result the hills have been biogeographically linked to both hotspots based on their floral and faunal compositions. However no phylogeographic study has ever documented the biogeographic affiliation of the SHK with either the coastal forests and/or the Eastern Arc Mountains. We report on the biogeographic history of amphibians from the Shimba Hills based on a combination of phylogeographic analysis using the 16S rRNA mitochondrial gene (16S), population genetics and species distribution modeling. Based on a multispecies phylogeographic analysis, SHK were found to be more closely affiliated to the CFEA than to the EAM. Two previously undocumented phylogeographic breaks are recovered from the study area; one from the Kenya north coast and another in the Tanga region in Tanzania. Historical habitat stability and connectivity appear to play a significant role in species diversification in the area.

Key Words: Coastal forests of Eastern Africa, Eastern Afromontane biodiversity region, Eastern Arc Mountains, biogeography, species distribution modelling.

Introduction

The Shimba Hills (SHK) are found in the south eastern parts of Kenya and is located at the junction of two biodiversity hotspots (see Figure 1); the Coastal forests of Eastern Africa (CFEA) and the Eastern Afromontane Biodiversity Region (EABR), specifically the neighbouring Eastern Arc Mountains (EAM) (Myers et al., 2000; Sloan et al., 2014). SHK is an important conservation area along the Kenya coast comprising of both a national and a forest reserve. Biodiversity surveys conducted in the SHK over the years have revealed the presence of mixed assemblages of flora and fauna. There are some species endemic to SHK such as the Shimba butterfly *Charaxes acuminatus shimbanus* Van Someren, 1963, Shimba Hills reed frog *Hyperolius rubrovermiculatus*, Schiotz, 1975; others occur in both SHK and the CFEA but absent from the EAM such as the Persimmon tree *Diospyros shimbaensis* White, 1988, Changamwe caecilian *Boulengerula changamwensis* Loveridge, 1932. While some species only occur in SHK and the EAM but are absent from the CFEA such as Bergmans's collared fruit-bat *Myonycteris relicta* Bergmans, 1980*; Callulina spp.* (Harper et al., 2010) and the Usambara garter snake *Elapsoidea nigra* Günther, 1888. Still, other species occur in all the three areas such as the Black and Rufous sengi *Rhynchocyon petersi* Bocage, 1880 but with SHK as the northernmost limit. In addition SHK is known to have some species associated with West African forests (Burgess & Clarke, 2000 and references therein). Because of this diverse flora and fauna, the SHK has been classified differently as either part of the CFEA (*e.g.* Azeria et al., 2007) or the EAM (Lovett, 1998; Blackburn & Measey, 2009).

Despite the unique geographic location of SHK and its mixed assemblage of flora and fauna, no study had been conducted to understand the historical biogeographic patterns of this area. As previously documented, patterns of biodiversity distributions are complex and are known to be determined by a number of factors both current and historical. For example, environmental and geological history of an area (Crowe & Crowe, 1982; Fjeldså & Lovett, 1997; Ricklefs, 2003; Avise, 2004; Dornelas et al., 2006; Dimitrov et al., 2012), the individual species ecology and physiology (Duellman & Trueb, 1986; Hamilton, 1982; Hugget, 2004) all play a role in determining the biodiversity patterns, ranges and abundance of species in an area. Luke (2005) provided a detailed checklist of plants of SHK and noted the high diversity of plants present. He hypothesized that the close proximity of SHK to the Usambara Mountains through similar climatic history and altitude range could be responsible for high floral diversity in SHK. However this remains to be tested. Bwong et al. (*in press*) speculated the biogeographic history of SHK to be complex given the mixture of amphibian assemblages recorded there containing EABR, CFEA and widespread species. Several questions arise with regard to the evolutionary and biogeographical history of SHK and the biodiversity found therein; is the SHK a centre of species diversity and endemism? How are the overlapping species between SHK and CFEA and EABR phylogeographically structured? Which mechanisms can be invoked to describe the genetic patterns if any? Was SHK a biodiversity refugium? These questions have important implications for understanding the evolutionary history in this region and for the conservation of its biodiversity in general.

The biogeographic history of SHK can be understood by employing phylogeographic analyses. Phylogeography (Avise, 1987) is the branch of historical biogeography dealing with the analysis of the relationship between population genetic structure and geography (see also Avise, 2000, 2004) with the aim to characterize the roles played by environmental and historical factors in shaping the present species diversity patterns (Zink, 2002; Lomolino et al., 2004). However few phylogeographic studies have been conducted in Kenya especially those targeting such complex ecosystems. Phylogeographic studies however can form a good basis for biodiversity conservation. When integrated with other fields of studies such as Geographic Information Systems (GIS), phylogeographic analyses can help us understand the biogeographic history of an area. SDM (also known as climatic envelope models) estimate potential species distributions by deriving environmental envelopes from distributions and projecting into an interpolated potential climate of an area (Pearson, et al., 2007; Waltari & Guralnick, 2009). The models are produced by combining current environmental parameters and known occurrence data of a species fitted to a model to predict current distributions (Hugall et al., 2002; Elith & Leathwick, 2009). When projected to past climates, SDM can also be used to generate potential suitable habitats in past climatic conditions, i.e., the historical paleo-distributions of species (Hugall, et al., 2002; Carstens & Richards, 2007; Elith & Leathwick, 2009). Studies on paleo-distribution of species have proved useful as alternative ways of establishing potential historical factors determining the genetic structuring in species especially in taxa that lack good fossil representation such as amphibians. For example, species distribution modeling (SDM) can be employed to formulate *a priori* biogeographic hypotheses or validate phylogeographic results.

In order to understand the biogeographic history of SHK, we conducted a comparative phylogeographic analysis on its amphibian assemblage using the 16S mitochondrial rRNA (16S) gene. In addition, we incorporated the use of SDM (Hugall, et al., 2002; Carstens & Richards, 2007) and demographic analyses to better understand the resulting phylogeographic patterns. We also investigated the role of habitat stability, connectivity and isolation by distance (IBD) in structuring the observed phylogeographic patterns. Specifically we sought to answer the following;

1. Which are the closest relatives of SHK amphibian populations?

2. Do amphibian species currently occurring in SHK have similar phylogeographic patterns to each other?
3. Which historical processes, if any, account for the observed patterns of genetic diversity?

Material and methods

Study area

In order to answer the above questions, the study was designed to include areas of the CFEA from Tanga region north of the Pangani River, east and west Usambara Mountains (EAM) going north up to Mpeketoni in Lamu on the Kenyan north coast of Mombasa (Figure 1). This north to south transect includes the SHK. For species distribution modeling analysis this area was extended up to the Kenya and Somalia border as some species may extend to this region given suitable habitats. Four main study sites were included; Mpeketoni, Arabuko-Sokoke Forest and surrounding areas in the Kenya north coast, Coastal forests in Tanga while the EAM was represented by the Usambara Mountains (See Figure 1).

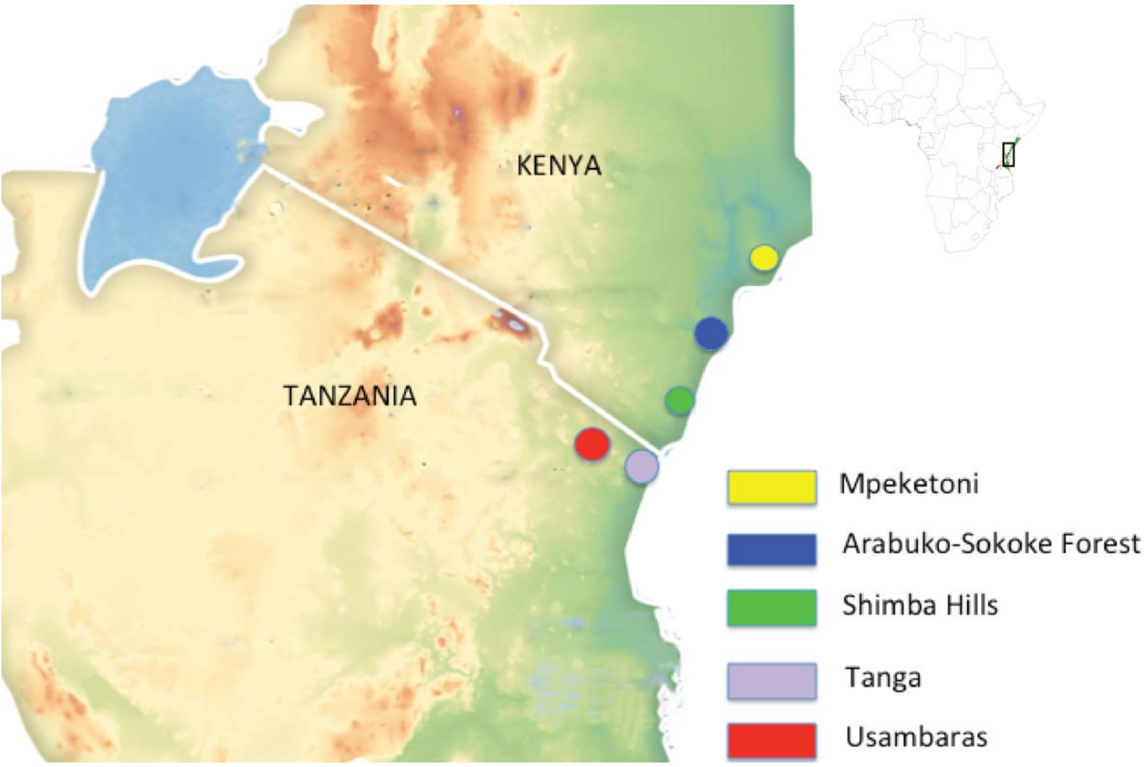

Fig 1: Map of Kenya and Tanzania showing the five major sampling sites colour coded as follows; Yellow = Mpeketoni, Blue = Arabuko-Sokoke Forest; Green = SHK; Purple = Tanga and Red = Usambara Mountains. Map modified from https://www.travcoa.com.

Data collection

Fieldwork in and around the SHK was conducted in December 2013, April and December 2014 and April–May 2015. Time-limited search, visual encounter survey methods were conducted as well as bucket pitfall traps with drift fences (Heyer et al., 1994). A representative of each species collected per locality was fixed in 10% formalin and stored in 70% ethanol and later deposited at the National Museums of Kenya herpetology reference collection. Tissues sample (toe clips, thigh and /or liver muscles) were preserved in 95% analytical ethanol. Specimen identification was made using standard field references (*e.g.* Schiøtz, 1999; Channing & Howell, 2006; Harper et al., 2010) and existing genetic data held in online repositories (www.ncbi.nhi.gov/genbank/). Furthermore, we conducted a 12-day survey in Arabuko-Sokoke Forest reserve in June and August 2015 and in Coastal forests in Tanzania in December, 2013, January–March 2014. Recent surveys were complemented by other field surveys conducted in the region over the last 15 years (Loader et al., unpublished data). See Appendix 1 for the species list and locality information for all the samples used in this study.

DNA extraction, amplification and sequencing

Total DNA was extracted from freshly collected muscle tissue and/or liver preserved in 95% ethanol using the DNeasy blood and tissue kit (Qiagen, Valencia, CA). Extraction, amplification and sequencing followed protocols described in Loader et al. (2010). Each individual was barcoded to verify its identity using the 16S gene. Sequences were multiply aligned in Geneious v6.1.2 (http:www.geneious.com, Kearse et al., 2012) using the MAFFT alignment method (Katoh et al., 2002) with default settings.

Phylogenetic analysis

We constructed alignments for understanding the phylogenetic relationships of amphibian species in the study area using 16S gene. The alignments per species included all available barcoded (partial ca. 600bp mtDNA fragment) samples of amphibian species so far recorded from SHK. For each species we used data from the current study and additional data available from previous fieldwork and the Sky Island database at the Biogeography group, University of Basel Switzerland. The evolutionary relationships of SHK amphibians based on the 16S alignment were reconstructed using both Bayesian (MrBayes 3.2; Ronquist & Huelsenbeck, 2003) and Maximum likelihood (RAxML v.8.0.0; Stamatakis, 2014) analyses with a single outgroup per species (*e.g.* a closely related congener). Substitution models for each species (Table S1) were determined using JModeltest v. 2.1.6 (Darriba et al., 2012) using the Bayesian Information Criterion. MrBayes analyses were implemented using parallel runs of four simultaneous Markov chains for 20 million generations, sampling every 1000 generations from the chain and discarding the first one million generations as burn-in. We also conducted Maximum Likelihood analysis on the same data using RAxML v. 8.0.0 (Stamatakis, 2014), applying the thorough bootstrap algorithm and the GTRMMA substitution model for 100 runs.

Haplotype reconstructions

To further examine population variation within the study area we employed haplotype network analysis using the program PopART (www://popart.otago.ac.nz). We used TCS networks (Templeton, 1992) to reconstruct the relationships among populations from Mpeketoni, Arabuko-Sokoke Forest, Coastal forests in Tanga, Usambara Mountains and the SHK.

Estimation of divergence time and sequence divergence

Relative divergence time between clades and subclades was estimated using rate-calibrated tree analysis in BEAST v. 2.1.3 (Bouckaert et al., 2014). We used strict molecular clock, a coalescent tree prior, log-normal mean of 0.01 and a lognormal standard deviation of 1.0. Because there are no appropriate amphibian fossils from the study taxa with which we could calibrate the tree, we used a substitution rate of 0.00277/lineage/mya for 16S based on Lemmon et al. (2007). Divergence time estimation was only conducted for species with geographically structured populations based on phylogeographic analysis described above. We used the program TRACER v. 1.6 (Rambaut & Drummond, 2015) to confirm if sampling has reached stationarity (Effective sample size has reached 200). BEAST runs ranged between 50–100 million generations, with sampling from the tree logged every 1000 generations. Sequence divergence was measured using the species delimitation plugin v1.04 for Geneious Pro (Masters et al., 2011).

Population genetics

We used Arlequin v. 3.5.2.2 (Excoffier & Lischer, 2010) to conduct analyses of haplotype, nucleotide and sequence diversity for each species as well as for each study site. Differentiation between sampling sites was calculated using the pair-wise F-statistic (F_{st}) analysis test (Wright, 1951). F_{st} values range from zero (identical populations) to one (populations fixed for different alleles). Tajima’s D (Tajima, 1989) and Fu’s F_s (Fu, 1997) tests were performed to check for signatures of recent population expansion. Negative significant values of F_s are interpreted as signatures of recent population expansion while negative values of Tajima’s D means selection (Fu, 1997). Mismatch distribution was used to compare the demographic history of the lineages where a recently expanded population shows a smooth wave-like mismatch distribution (Rogers & Happending, 1992). Deviations from sudden population expansions were tested using Harpendings raggedness index where significant *P* values indicate stability (Harpending, 1994).

Species distribution modeling

We modeled habitat suitability over time for species occurring in SHK to establish if the area has been a historically suitable habitat for all the species currently found there. Habitat suitability was modeled based on four climate scenarios; current (1950–2000), and three measures of historical habitat suitability; Holocene (6kya, years ago), Last Glacial Maximum (LGM), 25kya and Last Interglacial Maximum (LIG), 120kya. The study area consisted of a polygon extending from the coastline inland between 38.774, -6.055 and 37.607, -3.549 in the south and 41.337, -1.597 and 40.987, -0.832 in the north. We used data from Community Climate System Model research on climate (CCSM) for both Holocene and LGM climates and for LIG climate, data from Otto-Bleisner et al. (2008) was used. Climatic data consisted of the 19 bioclimatic variables (precipitation and temperature) available at the WorldClim database (Hijmans et al., 2005). We evaluated the Pearson's correlation among these variables using SDM Toolbox v. 1.1c (Brown, 2014) allowing only those that had a correlation coefficient of less than 0.8. After reducing Pearson's *r* to less than 0.8 the following nine variables were retained; annual mean temperature (Bio1), isothermality (Bio 3), temperature seasonality (Bio 4), annual precipitation (Bio 12), precipitation of the wettest month (Bio 13), precipitation of driest month (Bio 14), precipitation seasonality (Bio 15), precipitation of warmest quarter (Bio 18) and precipitation of coldest quarter (Bio 19). Presence data for species was obtained from all localities sampled for this study as well as verified co-ordinates based on National Museums of Kenya (NMK) herpetological collection database and from Global Biodiversity Facility (GBIF) online database (http://data.gbif.org). All geo-referenced localities were validated for co-ordinate errors. In total we used a total of 550 locality data points for 28 species (Appendix 1). We used Maxent, v. 3.3.3k (Philips et al., 2006) to model habitat suitability under the current climate and projected to the above three historical paleo-climatic conditions (Holocene, LGM and LIG). Maxent is a machine-learning algorithm, popular for predicting species and habitat distributions using presence only data. Models were trained using current climate and then projected to the paleo-climatic conditions for the study area. We used default parameters for Maxent with 10 replicate cross-validation runs. Model performance was evaluated using Area under Receiver Operating Characteristic curve (AUC) statistics with AUC >0.5 indicating better than random model prediction (Elith et al., 2006). The resulting suitability maps were compared with the phylogeographic results to establish congruence if any (Carstens & Richards, 2007). To produce habitat suitability stability maps we summed up the exponent of the averaged natural log of the predicted distributions corrected for LGM (Graham et al., 2010). Habitats that were consistently predicted as suitable in all the models were considered stable and were expected to have higher genetic diversity than unstable areas (Carnaval et al., 2009; Fitzpatrick et al., 2009). We further tested the stability theory by comparing the genetic diversity of all populations that were predicted as stable against unstable from the SDM analysis.

Habitat connectivity and Isolation by distance IBD

We evaluated whether habitat connectivity was responsible for patterns of genetic variation observed in the study area using Circuitscape theory (McRae & Shah, 2009). Habitat suitability maps for each species were generated from the SDMs and used as inputs for connectivity layers in this analysis, following Lawson, (2013). The suitability maps were first converted to connectivity maps where areas predicted as suitable conveyed less resistance to dispersal than unsuitable areas. We conducted pair-wise analysis selecting *Iterate across all pairs in the focal node* option in Circuitscape, which measures the connectivity between populations through environmental (habitat) space. Results were obtained in the form of cumulative and maximum current maps and voltages that were visualized in ArcGIS 10.2 (ESRI) and used to test the relationships with environmental variables.

We used Mantel (Mantel, 1967) and partial Mantel tests to evaluate the correlation between genetic structure (F_{st} values) and connectivity matrix results from Circuitscape analysis and geographical distance between populations. Geographical distance was measured in QGIS (v. 2.8.3-Wien). Mantel and partial Mantel tests were implemented using the Vegan package (Oksanen et al., 2011) in R (R core development team, 2015).

Results

Study species

We recorded a mixed amphibian assemblage from the SHK comprising two species of caecilians and 28 anurans species. SHK has one endemic amphibian species *H. rubrovermiculatus*; two EABR endemic species, *Callulina kreffti* Nieden, 1910 and *Scolecomorphus cf. vittatu*s, Boulenger, 1895 and 23 coastal forest and wide ranging species. Additionally we obtained sequences of overlapping species from Mpeketoni (9) and Arabuko-Sokoke Forest (16) both in Kenya north coast and 15 and 19 species from Coastal forests in Tanga and lowland forests in Usambara respectively.

Phylogenetic analysis was conducted based on 614 sequences of 16S. We recovered two main phylogenetic patterns from both MrBayes and RAxML analysis; (1) 15 species that lacked any phylogenetic resolution (Figures 2A and S1); (2) species that were divided into two well-supported clades. The second pattern can further be divided into two subgroups i.e. those with well resolved and geographically structured clades; *Arthroleptis stenodactylus* Pfeffer, 1893, (Figure 2B). *Arthroleptis xenodactyloides,* Hewitt, 1933, *Afrixalus delicatus* Pickersgill, 1984, *Afrixalus sylvaticus* Schiotz, 1974 (Figure S2), *Leptopelis concolor* Ahl, 1929, *Hyperolius pusillus* Cope 1862. *Hyperolius mariae* Barbour & Loveridge, 1928, *Hyperolius parkeri* Loveridge, 1933, *Hyperolius tuberilinguis* Smith, 1849, *Mertensophryne micranotis* Loveridge, 1925 (Figure S3), and *Sclerophrys pusilla* Mertens, 1937 and those with well resolved clades that lacked geographical structuring (*Chiromantis xerampelina* Peters, 1854 (Figure S4), *Phrynobatrachus acridoides* Cope, 1867 and *Kassina maculata* Duméril, 1853. Tree topologies from both Bayesian and Maximum Likelihood analyses were similar in all species except for *H. mariae* in which SHK samples were recovered as paraphyletic with respect to Mpeketoni samples in the MrBayes analysis. In addition major clades in MrBayes trees received good posterior probability (PP) support values where >95% PP is considered well supported and < 60% PP less supported while within clade relationships were less supported. Bootstrap values for RAxML analyses were equally high between the major splits ranging from 65 to 100%.

Phylogeography

We recovered different phylogeographic patterns from SHK amphibians as shown; in seven out of 16 species with overlapping samples in all the five study sites, the relationship among the study sites were unstructured (Figure 2A). In two species, *S. pusilla* and *A. stenodactylus* SHK samples were closer to Arabuko-Sokoke Forest than to Tanga and Usambara (Figure 2B). In four species, *L. concolor, A. delicatus* (Figure 2C), *H. mariae* and *H. tuberilinguis* samples from Mpeketoni, formed well supported monophyletic clades with respect to samples from Arabuko-Sokoke Forest, SHK, Tanga and Usambara whose relationships were unstructured.

In another four species, only samples from SHK to the south were available; for *C. kreffti* and *S. vittatus* SHK samples grouped with Usambara samples, *Leptopelis flavomaculatus* Günther, 1864 (Figure S5) the relationship between SHK, Tanga and Usambara was unstructured; however in *A. sylvaticus* (Figure S2) SHK samples and Usambara samples formed two well supported monophyletic clades. In addition, sequences were only available from the Kenya coast in five species; three lacked phylogeographic structure while *H. pusillus* (Figure S6) and *B. changamwensis*, SHK samples were divergent from Changamwe samples further north. Overall SHK amphibian populations formed well supported clades with CFEA to the exclusion of EAM in four species and none with EAM to the exclusion of CFEA. However, in another four species population from SHK, CFEA and EAM grouped together to the exclusion of population from the Kenya northcoast in Mpeketoni while in three other species SHK populations are monophyletic.

Two phylogeographic breaks were recovered in two different parts of the study area located in the north and south of SHK respectively. The northern break separated samples from Mpeketoni as monophyletic in *A. delicatus* (Figure 2C), *L. concolor* and *H. tuberilinguis*. In *H. parkeri,* samples from Mpeketoni and Arabuko-Sokoke Forest grouped together against samples to the south. Another phylogeographic break in the north was present between SHK and Arabuko-Sokoke Forest samples in *H. pusillus* (Figure S6). The southern phylogeographic break separates coastal Kenya from Tanga/Usambara samples (Figure 2B).

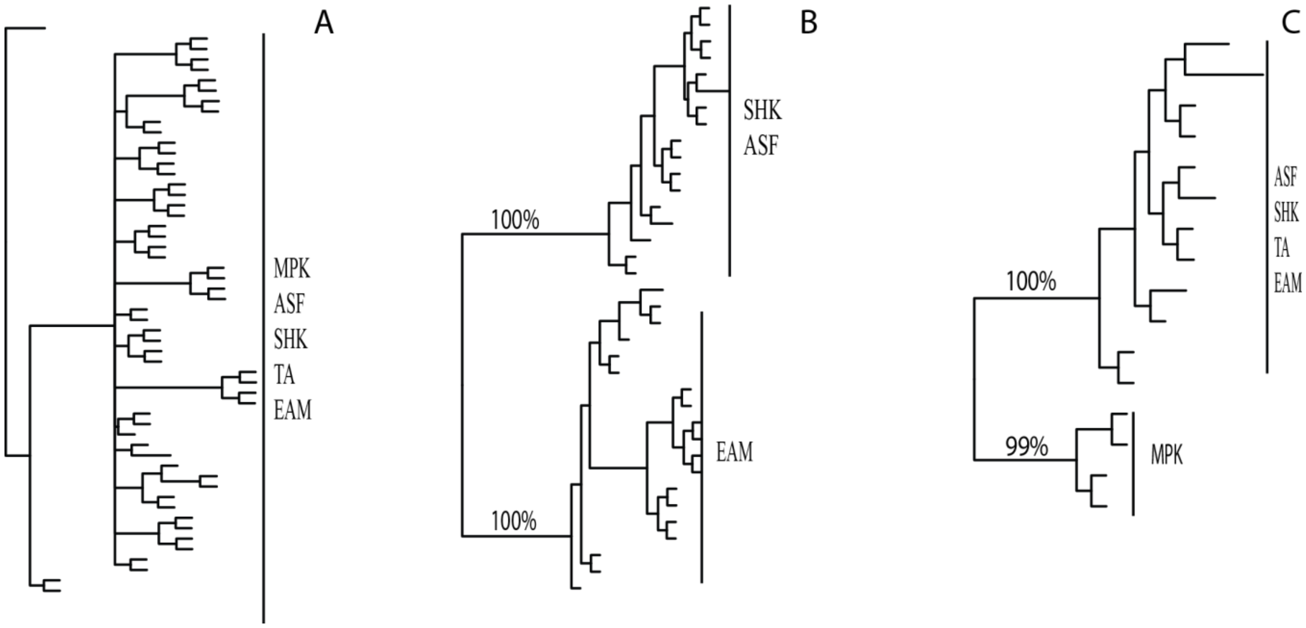

Fig. 2: MrBayes tree topologies showing the three phylogeographic patterns. A = *P. anchietae*-no clear geographic structuring. B and C = *A. stenodactylus* and *A. delicatus* topologies showing southern and northern region phylogeographic breaks respectively. Major sampling sites abbreviated as follows; MPK = Mpeketoni, ASF = Arabuko-Sokoke Forests, SHK = Shimba Hills of Kenya, TA = Tanga and EAM = Usambara.

Haplotype reconstructions

TCS haplotype numbers varied among species as well as among populations of the same species. Haplotypes recorded from species that lacked phylogeographic structures, ranged from one to four per species (Figure 3A) except in *Hyperolius argus* Peters, 1854 in which nine haplotypes were present–a central haplotype shared by samples from SHK, Arabuko-Sokoke Forest, Mpeketoni and Usambara plus eight other haplotypes connected to the central haplotype by one mutation step each (Figure S7). Geographically structured species recorded higher haplotype numbers ranging from three in *S. pusilla* to ten in *H. mariae.* As would be expected, there were higher numbers of mutation steps separating haplotypes in geographically structured species for example 17 steps separated Kenyan samples from Tanzanian samples in *A. stenodactylus* (Figure 3B). Haplotypes from Mpeketoni were separated by more mutation steps from the rest of the samples (Figure 3C). The networks for all species examined supported the results from the phylogenetic analysis and for *H. mariae* the haplotype network results were similar to results from RAxML analysis showing Mpeketoni samples as divergent from the rest and SHK samples as paraphyletic with respect to samples from Usambara.

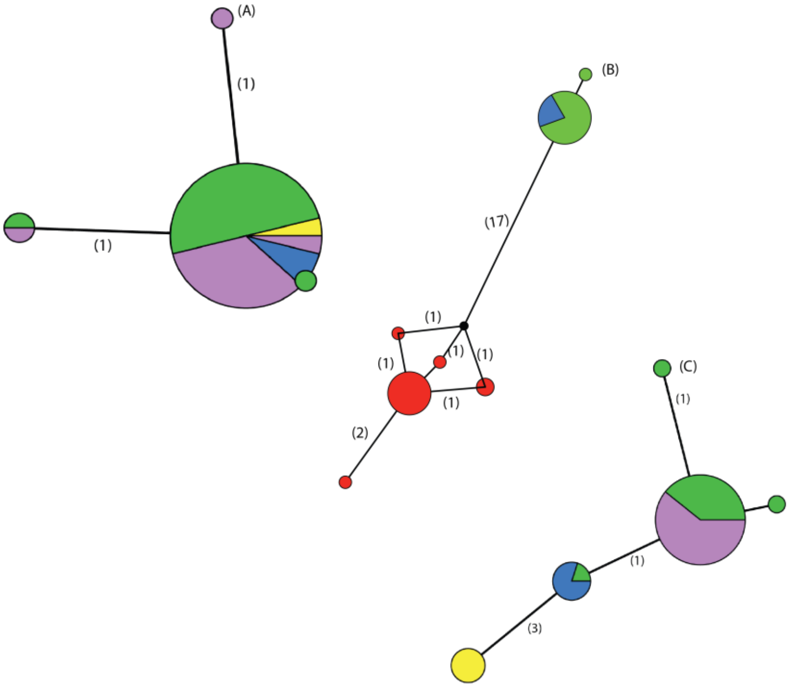

Fig. 3: TCS haplotype networks for (A). *P. anchietae;* (B). *A. stenodactylus;* (C) *A. delicatus* respectively. Colour coding stands for the various study sites as shown, Yellow= Mpeketoni, Blu e= Arabuko-Sokoke Forest, Green = Shimba Hills of Kenya, Purple = Tanga and Red = Eastern Arc Mountains.

Clade divergence times and sequence divergence

Molecular dating indicates all estimates between clades in the geographically structured species occurred from the late Miocene onwards. Both the oldest and the youngest lineage divergences occurred in the south separating Kenya and Tanzania samples around 7.3 million years ago (mya) (4.2–10.2, 95% Highest Posterior Density (HDP)) in *A. stenodactylus* (Figure 3A) and 1.4 mya (0.4–2.4, 95% HPD) in *S. pusilla*. Most of the divergences occurred in the Pliocene from 5 mya and only four species show clade divergences in the Pleistocene (see Table 1). Samples from Mpeketoni diverged from the rest much earlier as seen in *A. delicatus* 4.6 mya (2.4–6.9, 95% HPD), *H. mariae* 3.9 mya (2.0–6.1, 95% HPD), *H. tuberilinguis* 5.5 mya (3.2–8.2, 95% HPD), *H. parkeri* 5.4 mya (3.1–8.1, 95% HPD) and *L. concolor* 1.9 mya (0.8–3.2, 95% HPD) (Figure 3B). Even though the clade divergence varied among species, there seems to be some congruence, regarding subclade separation where most divergence are recent and occurred between 1.7 and 0.6 mya all predating the LGM (See Table 1). SHK shared its most recent common ancestor with West Usambara around 1.4 mya in *A. xenodactyloides*, while the most recent common ancestor with Usambara was 4.6 mya in *A. sylvaticus*. The most recent common ancestor between SHK and Arabuko- Sokoke Forest was 1.7 mya in *M. micranotis* and 2.3 mya in *H. pusillus* (Table 1). Sequence divergence varied from 4.3% in *A. stenodactylus* to 0.5% in *S. pussila* and samples from Mpeketoni recorded higher sequence divergence as shown in Table 1.

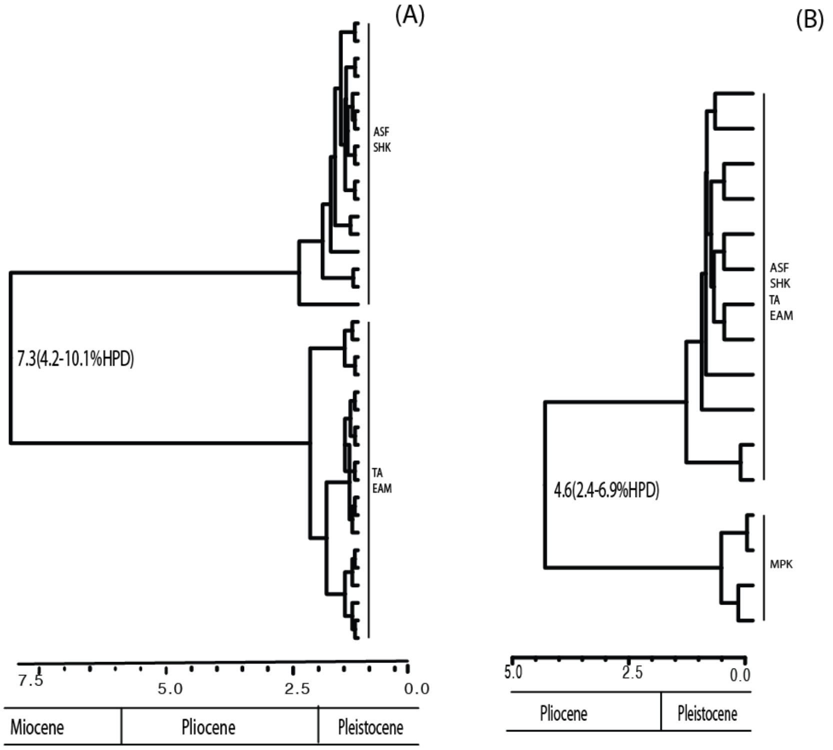

Fig. 4: BEAST tree topologies for (A) *A. stenodactylus* and (B) *L. concolor* showing the south and north clade divisions respectively. The abbreviations stand for the major study sites.

Table 1: Divergence time estimates and clade divergence for species that exhibited phylogeographic structuring. Major sampling sites are abbreviated as follows: ASF = Arabuko-Sokoke Forest, MPK = Mpeketoni, SHK = Shimba Hills, TA = Tanga and EAM = Usambara.

Species	Clade age (95%HPD)	Area	Subclade ages	Geologic time	Pairwise distance
S. pusilla	1.4 (0.5–2.4)	SHK, ASF vs. TA, EAM	Subclade1 0.4 Subclade2 0.7	Pleistocene	0.005
A. stenodactylus	7.3(4.2–10.1)	SHK, ASF vs. TA, EAM	Subclade1 1.3 Subclade2 1.1	Miocene	0.043
A. xenodactyloides	2.0(0.9–3.3)	SHK, EAM vs. EAM,TA	Subclade1 1.4 Subclade1 0.9	Plio-Pleistocene	0.005
L. concolor	1.9(0.8–3.3)	MPK vs. ASF,SHK,TA	Subclade1 0.8 Subclade2 0.3	Plio-Pleistocene	0.009
A. delicatus	4.6(2.4–6.9)	MPK vs. ASF,SHK, TA, EAM	Subclade1 1.5 Subclade2 0.9	Mio-Pleistocene	0.025
S. gutturalis	1.5(0.6–2.7)	ASF,SHK,TA vs. EAM	Subclade1 0.8 Subclade2 0.7	Pleistocene	0.007
H. pusillus	2.3(0.9–3.9)	ASF vs. SHK	Subclade1 0.8 Subclade2 0.6	Plio-Pleistocene	0.009
H. mariae	3.9(2.0–6.2)	MPK vs. SHK, EAM	Subclade1 1.3 Subclade2 1 Subclade3 0.2	Mio-Pleistocene	0.023
M. micranotis	2.6(1.3–4.0)	SHK, ASF, TA vs. EAM	Subclade1 1.1 Subclade2 1.7	Plio-Pleistocene	0.01
H. parkeri	5.5(3.1–8.1)	MPK vs. ASF, SHK, TA, EAM	Subclade1 1.2 Subclade2 1.3	Mio-Pleistocene	0.029
A. sylvaticus	4.3(2.3–6.4)	SHK vs EAM	Subclade1 0.8 Subclade2 1.4	Mio-Pleistocene	0.022
H. tuberilinguis	5.5(3.2–8.2)	MPK vs. ASF SHK, TA EAM	Subclade1 1.1 Subclade2 1.1	Mio-Pleistocene	0.031

Demographic analysis

Nucleotide diversity within species ranged between 0.0 (*e.g*. in *Afrixalus fornasini* Bianconi, 1849, *Ptychadena anchietae* Bocage, 1868) and 0.023 in *A. stenodactylus*. The highest nucleotide diversity was recorded from Arabuko-Sokoke Forest samples of *H. tuberilinguis* (Table 2). SHK recorded the highest nucleotide diversity in five species followed by Usambara and Arabuko-Sokoke Forest while Tanga and Mpeketoni had one each. Nucleotide diversity appeared to be higher in populations that had larger sample size (See Table 2). However all populations that had more than four samples recorded haplotype diversity of 1.0. (see Table 2). Tajima's D results were mostly negative and only significant in five populations (Table 2). On the other hand most of the Fu's F_s tests were negative. Tajima's D was only significant for one species from SHK while Fu's F_s test was significant for 10 out of 16 species. Demographic expansions were reported in seven populations in six species. However raggedness index which measures the smoothness of the mismatch distribution were all insignificant. Species that recorded stable populations from SHK based on the mismatch distribution include; *A. sylvaticus, C. xerampelin*a and *K. maculata* (See Figure 5). F_{st} values varied between populations where species without structuring recording mostly zero values between populations. SHK recorded significant F_{st} values in 11 species, three of which involved species with phylogeographic breaks in the south (*A. sylvaticus*, *A. stenodactylus*, *A. xenodactyloides*). Eight out of these 11 species showed significant F_{st} values between SHK and Arabuko-Sokoke Forest and/or Mpeketoni to the north (See Table S2).

Species distribution modelling

We modeled current and paleo distributions for 28 out of the 30 species currently found in SHK i.e. all except the two undetermined species. The AUC was high for all the species modeled and ranged from 0.837 to 0.993 with a mean of 0.947 implying better than random predictions. Our results points to the significance of precipitation in the distribution of amphibians in the area. For example Bio 19 (precipitation of coldest quarter) was most important for the prediction of 25 species while Bio14 (precipitation of driest month) was most important for *C. kreffti* and *S.* cf. *vittatus* and bio3 (isothermality) was most important for *A. xenodactyloides*. Bio 19 contributed a large percentage (>70%) for 11 species (See Table S3). Other variables that contributed to the prediction in either second or third position include, Bio12 (annual precipitation), Bio14 (precipitation of driest Month) and bio18 (precipitation of warmest quarter) the rest contributed much less.

Table 2: Demographic analysis for species that have overlapping ranges spanning more than two study sites, n = sample size, p = number of polymorphic sites, h = Haplotype diversity, Π = nucleotide diversity, TD Tajima's (D), Fs = Fu's Fs test and r = Harpending's Raggedness index. Populations predicted as stable and/ or unstable based on the SDM habitat stability modeling are indicated in letter S /US respectively in bracket after the locality. Significant values are highlighted in bold letters.

Species & population	n	p	h	Π	TD	FS	r
H. argus							
EAM (US)	1	0	1.000	0.000	NA	NA	NA
SHK (S)	14	3	1.000	0.002	-0.49	**-28.02**	0.09
ASF (S)	5	3	1.000	0.003	-1.05	-4.29	0.43
MPK (S)	1	0	1.000	0.000	NA	NA	NA
C. xerampelina							
TA (S)	4	1	1.000	0.001	-0.61	**-4.64**	0.25
EAM (S)	8	1	1.000	0.001	0.33	**-15.45**	0.20
SHK (S)	6	3	1.000	0.003	-0.16	**-5.76**	0.13
ASF (S)	4	0	1.000	0.000	0.00	34...	0.00
A. fornasini							
TA (S)	7	0	1.000	0.000	0.000	0.000	0.000
EAM (S)	3	0	1.0 00	0.000	0.000	0.000	0.000
SHK (S)	4	0	1.000	0.000	0.000	0.000	0.000
ASF (S)	5	0	1.000	0.000	0.000	0.000	0.000
H. marmoratus							
TA (S)	8	0	1.000	0.000	0.00	34..	0.00
US (S)	9	4	1.000	0.003	0.23	**-0.23**	0.75
SHK (S)	1	0	1.000	0.000	0.00	NA	NA
ASF (S)	3	1	1.000	0.001	0.00	**-2.30**	0.00
P. acridoides							
TA (S)	29	3	1.000	0.002	-0.38	**-34…**	0.20
EAM (S)	7	2	1.000	0.002	-0.28	**-9.76**	0.10
SHK (S)	21	3	1.000	0.001	-0.68	**-34…**	0.12
ASF (US)	4	0	1.000	0.000	0.000	34…	0.00
P. anchietae							
TA (S)	19	2	1.000	0.000	-1.51	**-34**…	0.39
EAM (S)	2	0	1.000	0.000	0.00	34...	0.00
SHK (S)	28	2	1.000	0.000	-1.51	**-34…**	0.54
ASF (US)	4	0	1.000	0.000	0.00	34…	0.00
MPK (US)	2	0	1.000	0.000	0.00	34…	0.00
S. pusilla							
TA (S)	4	0	1.000	0.000	0.00	34...	0.00
EAM (US)	3	0	1.000	0.000	0.00	34..	0.55
SHK (S)	3	1	1.000	0.001	0.000	-2.18	0.00
A. stenodactylus							
TA (US)	4	1	1.000	0.001	-0.61	**-4.64**	0.25
US (S)	13	5	1.000	0.002	**-1.86**	**-25.20**	0.07
SHK (S)	16	1	1.000	0.000	-1.16	**-34...**	0.58
ASF (US)	3	0	1.000	0.000	0.000	34…	0.00
L. concolor							
TA (S)	17	0	1.000	0.000	0.00	34	0.00
SHK (S)	14	3	1.000	0.001	**-1.67**	**-34...**	0.17
ASF (US)	4	0	1.000	0.000	0.00	34..	0.00
MPK (US)	4	0	1.000	0.000	0.00	34	0.00
A. delicatus							
TA (S)	1	0	1.000	0.000	0.00	0.00	NA
SHK (S)	7	6	1.000	0.001	-0.61	**-4.64**	0.21

ASF (S)	4	1	1.000	0.004	**-1.52**	**-6.32**	0.18
MPK (S)	4		1.000	0.001	1.63	**-3.99**	0.56
S. gutturalis							
TA (S)	4	3	1.000	0.004	-0.75	**-2.37**	0.08
EAM (US)	2	1	1.000	0.002	0.00	0.00	0.00
SHK (S)	5	1	1.000	0.001	-0.82	**-7.58**	0.02
ASF (US)	2	0	1.000	0.000	0.000	34...	0.00
H. mariae							
US (US)	4	0	1.000	0.000	0.00	34...	0.00
SHK (S)	18	8	1.000	0.005	-0.60	**-26.66**	0.03
MPK (US)	2	0	1.000	0.000	34…	34...	0.00
M. micranotis							
TA (S)	5	0	1.000	0.000	0.000	34…	0.00
SHK (S)	9	1	1.000	0.001	0.99	**-17.36**	0.25
EAM (S)	17	7	1.000	0.004	0.08	-23.89	0.13
ASF (US)	2	0	1.000	0.000	0.00	34…	0.00
H. parkeri							
TA (S)	3	1	1.000	0.002	0.000	**-2.20**	0.56
EAM (US)	1	0	1.000	0.000	0.000	NA	NA
SHK (S)	8	2	1.000	0.001	-1.31	-14.52	0.17
ASF (S)	3	0	1.000	0.000	0.00	34…	0.00
MPK(S)	2	3	1.000	0.007	0.00	1.20	0.00
H. tuberilinguis							
TA (S)	6	1	1.000	0.001	-0.93	**-10.9**	0.22
EAM (US)	2	1	1.000	0.002	0.00	0.00	0.00
SHK (S)	9	0	1.000	0.000	0.00	34…	0.00
ASF (S)	2	14	1.000	0.032	0.00	2.64	0.00
MPK (S)	7	3	1.000	0.002	-0.65	**-8.32**	0.31

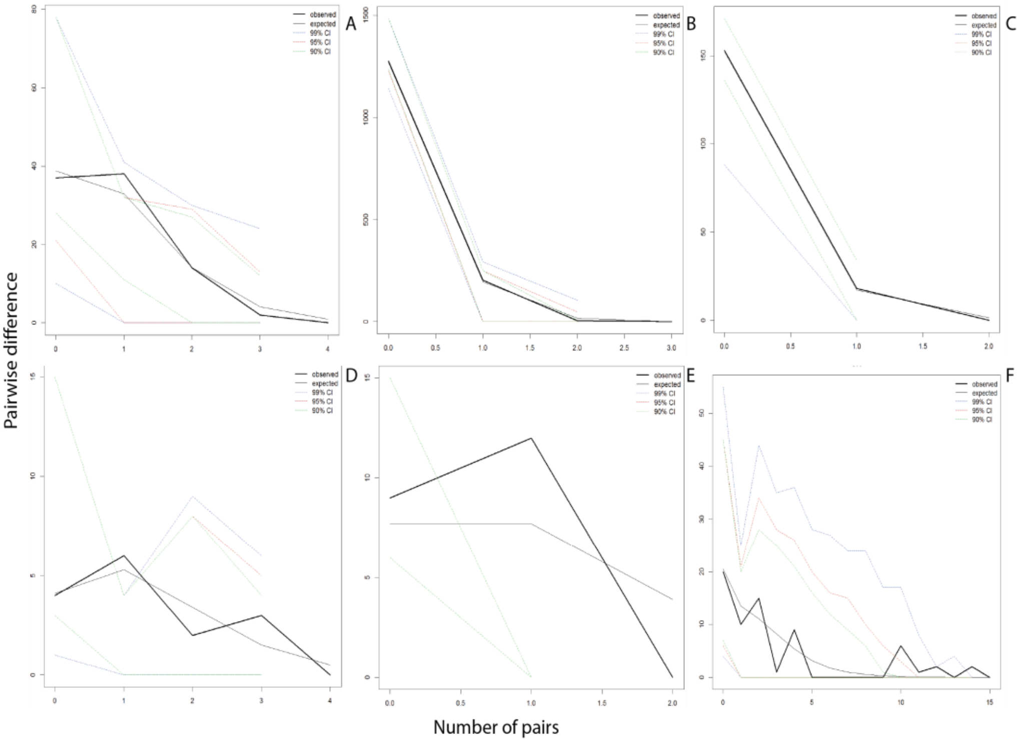

Fig. 5: Mismatch distribution for selected species from SHK. Top; sudden expansion; A, *H. argus;* B, *P. anchietae*; C, *A. stenodcatylus*. Bottom; stable populations; D, *C. xerampelina*; E, *K. maculata* and F, *A. sylvaticus.*

The current climate model predicted a suitable habitat from Usambara/Tanga to either Arabuko-Sokoke Forest or Mpeketoni among populations that did not have strong geographic structuring (Figure 6). For structured species a continuously suitable area was predicted in *S. pusilla*, *S. gutturalis* and *M. micranotis.* In *A. sylvaticus*, *A. stenodactylus* (Figure 6) and *A. xenodactyloides* (Figure S8), populations were disconnected around Tanga area while *H. mariae*, *A. delicatus* and *L. concolor* populations were disconnected in the north past Arabuko-Sokoke forest. The models predicted accurately for most species based on their current known occurrences however, it under predicted suitable habitats for *M. micranotis;* Arabuko-Sokoke Forest was predicted as very unsuitable yet the species is known to occur beyond Arabuko-Sokoke Forest to the north. The Holocene model prediction for phylogeographically unstructured species did not greatly differ from the current model except that Tanga region was predicted as less suitable for most species. In addition the presence and/or absence of unsuitable area in the north also varied among species. In the LGM all species had most of the study area predicted as suitable (Figure 6) except for *H. argus* (Figure S1) and *A. sylvaticus* Figure S2). For *L. concolor* and *M. micranotis* suitable area reduced from Arabuko-Sokoke Forest going north.

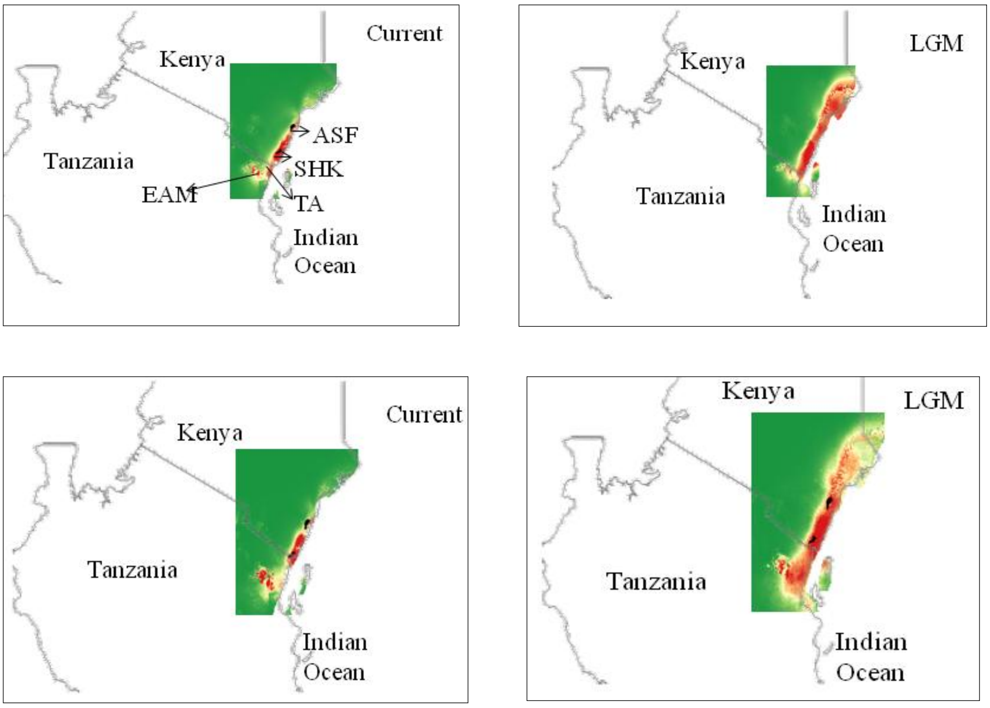

Fig. 6: Current and LGM Maxent habitat suitability predictions. Above; *P. anchietae* (no phylogeographic structure), below; *A. stenodactylus* (phylogeographically structured).

The study area was suitable for only a few species during the LIG; *A. delicatus*, *H. mariae*, *H. argus*, *H. tuberilinguis* and *Sclerophrys steindachneri* Pfeffer, 1893. There was no suitable habitat connecting Tanga/Usambara and SHK in *S. pusilla*, *A. stenodactylus*, *A.*

xenodactyloides and *M. micranotis*, while no suitable habitat existed from SHK to the north for *L. concolor*. A small strip along the coastline from the Kenya north to Tanga-Usambara area was predicted as stable for species with no structuring (*C. xerampelina, Hemisus marmoratus* Peters, 1854, *K. maculatus, S. steindachneri*) (Figure 7 A and B). SHK was stable for all species that exhibited phylogeographic structuring (Figure 7 C and D), however only *A. delicatus* had a suitable habitat predicted in the whole study area. Not all populations which were predicted as stable based on SDM estimations recorded higher than usual nucleotide diversity compared to unstable areas. Only four species where SHK was predicted as stable had higher nucleotide diversity than unstable areas (see Table 2).

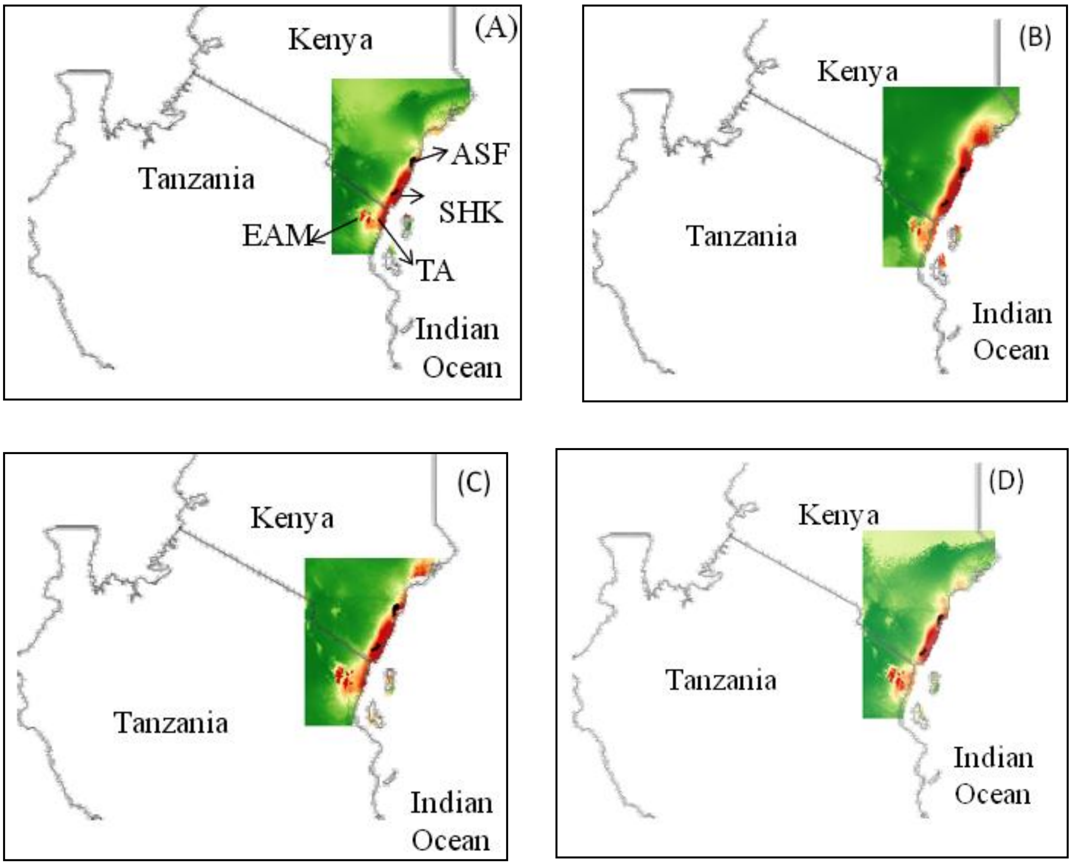

Fig. 7: Predicted habitat suitability stability areas for A, *P. anchietae* no phylogeographic structuring but displayed recent population expansion in SHK; B, *C. xerampelina* no phylogeographic structuring and no recent population expansion in SHK. C, *H. mariae* phylogeographic break in the north; D, *A. stenodactylus* display phylogeographic break in the south.

Isolation by distance and habitat connectivity

Mantel tests to detect correlations between genetic differentiation and geographical distance, current and LGM habitat connectivity produced mixed results. Ten out of 23 species had highly correlated but non-significant values between geographical distance and genetic differentiation however *C. xerampelina, H. argus*, *H. marmoratus* and *S. pusilla* had a negative correlation with geographical distance. Both *L. concolor* and *M. micranotis* had significant correlations with current and LGM habitat connectivity. When distance was controlled for in partial Mantel test, still habitat connectivity was highly and significantly correlated with genetic differentiation in *M. micranotis* however *L. concolor* was only significantly correlated with current habitat connectivity. In *H. parkeri* there was a significant correlation between genetic differentiation and LGM habitat connectivity in both Mantel and partial Mantel tests (Table S4).

Discussion

Phylogeographic patterns within SHK and adjacent areas

We present the first intra-specific phylogeographic data for amphibians depicting relationships between SHK and the biodiversity hotspots of the CFEA and EABR (specifically the EAM). Despite our analysis being based on a single molecular marker, which may have some limitations for such analysis (Ballard & Whitlock, 2004; Gutierrez-Garcia & Vazquez-Domingues, 2011), using multiple co-distributed species plus the integration of SDM provides an important first step in understanding the phylogeographic patterns of SHK.

Overlapping amphibian assemblages of the study area exhibited mixed phylogeographic patterns, which is to be expected for an assemblage-wide study of ecologically different species. However subsets of the data using particular species show congruent patterns of phylogeographic breaks across the study area. SHK population was recovered as well supported clades in *A. sylvaticus, A. xenodactyloides* and *H. pusillus* (Figures S2 and S6). The monophyly of *H. pusillus* is tentative as samples were only available from the Kenyan coast. Increased sampling from Tanga and Usambara areas where it is known to occur (Channing & Howell, 2006; Harper et al., 2010) may be required to prove the status of the SHK samples. When only species with phylogeographically structured samples from all the five study sites were considered (9), SHK amphibian population grouped with CFEA against EAM in four species while no species from SHK grouped with EAM to the exclusion of CFEA. Few studies exist in this area to compare our results, however comparison can be made with a study by Dimitrov (2012) on African violets (*Saintpaulia* spp), in which coastal Kenya population including SHK grouped together against those of EAM. Another study of *A. xenodactyloides* by Blackburn & Measey, (2009), also showed SHK population divergent from those of the Usambaras.

The two phylogeographic boundaries recovered in this study shed light on the historical biogeography of the area. The first barrier in Kenya's north coast coincided with the Tana River Delta seemingly isolating Mpeketoni populations as monophyletic across species. This barrier was also predicted from the SDM analysis in which the region was consistently predicted as unsuitable both in the Current and Holocene climate models. The break however was absent in the LGM predictions of the affected species (Figure 2C). Estimated divergence time across this phylogeographic break is in the Mio-Pleistocene period (Table 1). Sequence divergences across the barrier are relatively high ranging between 0.9 to 3.1% perhaps reflecting the individual species specific responses to the effect of this barrier. Demographic analysis showed differences in population parameters across this barrier further supporting its existence and old age. For example there were high and significant F_{st} structure between Mpeketoni population and those to the north of the barrier (Table S2) (Avise, 1987). We speculate that the Tana River Delta may have acted as the barrier to gene flow in this area. Rivers, especially ancient drainage basins, are known to act as barriers to gene flow to some amphibians (Lampert et al., 2003; Dias-Terciero et al., 2015; Moraes et al., 2016). Contrasting this, other studies also show that rivers are not necessarily a hindrance to gene flow in amphibians e.g. Lougheed (1998), Gascon (2000). The ability to cross or not to cross a river barrier therefore may depend on both ecological and physiological requirements of each species (Schneider et al., 1998). As can be seen from the current study, the Tana River Delta barrier did not affect species like *C. xerampelina, P. anchietae* and *Kassina maculata* which maintained gene flow across it. Alternatively, the modern-day Tana River Delta may not be an absolute barrier to gene flow especially to recent immigrants in the area (Newman & Rissler, 2011). In addition the position and size of the delta may have changed over the years causing some species to make secondary contact after a long period of separation causing the observed inconsistencies. Apart from the Tana Delta, there is a dry or arid coastal zone between the Sabaki and Tana Rivers going up to the shoreline, consisting mainly of dry bushland, which may be unfavourable with amphibians. However the fact that some amphibians were able to disperse through this dry area make it doubtful that it is solely responsible for the phylogeograhic breaks in this area. No barrier had previously been identified in the northern Kenya part of the coastal forest making our study the first to identify this barrier.

The second barrier mostly separates Usambara and Tanga populations from those along the Kenyan Coast. The exact position is not clear based on the existing data as it seems to vary among species. In *A. sylvaticus, H. mariae, M. micranotis* and *S. gutturalis*, it separates Usambara samples as monophyletic while in other species, samples from both Tanga and Usambara group together against those of SHK going north (*A. stenodactylus* and

S. pusilla). The lack of clear position of this boundary could be associated with the paucity of genetic data in our sampling but the historical unsuitability of this area over long periods of time was also evident from some SDM predictions. The area between SHK and Tanga was predicted as unsuitable for some of these species; for *A. stenodactylus, A. sylvaticus, A. xenodactyloides* and *S. pusilla* during the Holocene and *M. micranotis* in the LIG (See Figure S8). This barrier, unlike the previous one is not obvious as there are no significant geographic features in the area with which it can be associated. It appears slightly above the Pangani River which has been associated with the separation of *A. xenodactyloides* populations between the northern and Southern EAM (Blackburn & Measey, 2009). The break could be associated with long term climatic events that have operated in the area (Driscoll, 1998) causing areas to be isolated. The estimated divergence period of this range from 7.3 to 1.5 mya (Table 1). The only barrier previously reported in the entire CFEA was the Rufiji River for species boundaries (See Burgess & Clarke 2000 and references therein) and Pangani Rivers for intra species genetic diversification (Blackburn & Measey, 2009). Similar studies involving different taxa should be conducted to shed more light on these putative phylogeographic barriers. In addition the effects of rivers on intraspecific phylogeography of different taxa along the CFEA needs to be investigated.

There were no phylogeographic patterns in nine of the studied species, which can be interpreted in two ways; (1) broad scale dispersal and (2) continuous gene flow between SHK and adjacent areas over time (Avise et al., 1987; Carpenter et al., 2010). Distinguishing the two scenarios within these species may be hampered by lack of sufficient data from some localities; however mismatch distribution for sudden population expansion was exhibited in three species (*H. argus* from SHK, *L. flavomaculatus* from Usambara and *P. anchietae* from SHK and Usambara). Furthermore these populations reported low nucleotide diversity consistent with populations undergoing range expansions (Hewitt, 2000). Our SDMs predicted that SHK was unsuitable for *P. anchietae* in LIG but suitability has progressively increased since the LGM while Usambara as suitable habitat for *L. flavomaculatus* reduced significantly during the Holocene compared to the LGM and current models. However the same is not evident in *H. argus*. The remaining species lacking phylogeographic structure did not exhibit population expansions and some may have undergone high connectivity and gene flow for a long period of time. For example our SDM estimations for *C. xerampelina, P. acridoides* and *H. marmoratus* indicate the presence of a continous suitable habitat in all the five study sites from the LGM to the current, supporting the continuous gene flow within the study area. Most of these species are wide spread with distributions in the entire coastal forests of Eastern Africa and beyond (Poynton, 1991). The connectivity of their populations may have therefore been maintained by their ability to disperse through the region over the years. As observed by Poynton (2000), for the wide spread species in CFEA, 'the mosaic nature of the area encourage their ability to adapt to a variety of habitats and this may explain current patterns, having enabled the maintenance of continuous gene flow during the oscillating wet and dry climate of the Pleistocene (Hamilton, 1982). Future studies examining genomic scale data sets might be able to test these preliminary findings.

Clade divergence

Estimated divergences using molecular clocks shows that about 60% (8 out of 13) of the species diverged before the Pleistocene, indicating that divergence within the area was not solely due to Pleistocene climatic fluctuations. Six species diverged in the Mio-Pliocene and five diverged from Pleistocene onwards (Table 1). While three of the Pleistocene divergences occurred in the south except for *L. concolor* (Tana River delta) and *H. pusillus* (break between SHK and Arabuko-Sokoke Forest). Studies within the CFEA are minimal but our results are comparable to some in the neighbouring EAM, Blackburn & Measey (2009) found the divergence between the southern EAM and southern Malawi to have occurred between 0.2–2 mya. This is comparable with our data on the split between SHK-West Usambara and East Usambara that diverged between 0.9–3 mya. Lawson (2013) reported the divergence between East and West Usambara populations of *Hyperolius substriatus* Ahl, 1931 to have occurred less than 1 mya which is consistent with subclade divergences in some species in the current study (Table 1).

Pleistocene climatic oscillations that led to the expansion of savanna ecosystems have been associated with many diversification events in East Africa, (Moreau, 1933; Hamilton, 1982; de Menocal, 1995). Our study supports this, and in addition shows that some lineages diverged long before the Pleistocene. Savanna species showed increased divergence at the onset of Pleistocene when their habitat expanded as the forest sizes reduced (2–3 mya with peak periods between 1.2–1.7mya) and this can explain the major splits observed within the clades for some species. The majority of SHK amphibian species are mostly savanna and/or farmbush (Schiotz, 1975; Channing & Howell, 2006; Harper et al., 2010; Bwong et al., *in press*) and so their expansion may have increased during the Pleistocene.

The two species that form monophyletic clades in SHK e.g. *A. sylvaticus* and *A. xenodactyloides* were already present in SHK during the Mio-Pleistocene and Plio-Pleistocene respectively and the deep divergence from other clades indicate their long term persistence in the SHK. Additionally, SHK and Arabuko–Sokoke Forest samples of *A. stenodactylus* show that this species has also been present in these areas for a very long time (Table 1).

Habitat stability, habitat connectivity and isolation by distance

Ten species had all their populations predicted as stable, recording higher nucleotide diversity than non-stable areas similar to studies elsewhere (Carnaval et al., 2009; Qu et al., 2014). For *A. delicatus, L. concolor* and *A. sylvaticus* none of the populations predicted as stable had higher nucleotide diversity values. However some species showed mixed results where populations predicted as stable had lower diversity and vice versa (Table 2). This lack of concordance between habitat stability and genetic diversity is not unique to this study. Discordance may be a result of low sample size in our genetic data and/or over prediction of suitable habitats by the SDM. However a study by Tonini et al. (2013) that compared concordance between habitat stability and genetic diversity found that wide-spread species (good dispersers) resulted in concordance but this was not true for range-restricted amphibians. Similarly, in our study, all species that showed concordance between stability and genetic diversity were wide spread species such as *P. acridoides* while range-restricted species such as *A. sylvaticus*, only known from Usambara, Tanga and SHK did not exhibit high nucleotide diversity in areas predicted as stable. Additional studies with increased sample sizes may prove if this is true for other species. Habitat connectivity both currently and during LGM was found to be positively correlated with genetic structure in three species however, isolation by distance did not explain the genetic patterns observed in this study.

Conclusion

It is evident that SHK and indeed the entire study area has a complex biogeographic history and no single pattern can explain the current amphibian assemblage in the area previously speculated upon. The fact that we recovered both structured and unstructured phylogeographic patterns shows that the species occupying this area have responded differently to past environmental conditions and/or geographical barriers present in the area. For the two EAM species (*C. kreffti* and *S.* cf. *vittatus*), there was no structuring between SHK and East Usambara populations in the former but *S.* cf. *vittatus* from SHK are shown to have separated from EAM around 0.8 mya. The relationships among the study sites were unresolved in nine species. SHK amphibian populations were closer to the CFEA, grouping with 4 species to the exclusion of EAM while no SHK amphibian population grouped with EAM to the exclusion of the CFEA out of nine species that were geographically structured and occurred across all of the five study sites. Demographic analysis further shows that some SHK amphibian populations have been stable while others have undergone recent population expansions pointing to the possibility that the currently co-distributed species in SHK are results of recent gene flow.

Habitat stability, Current and LGM habit connectivity appear to play a role in the diversification processes in the area however clear signals are obscured by low sampling effort in some areas, which may be confirmed or discredited in future studies. Results presented here are preliminary and form a baseline for understanding the historical biogeography of the wider CFEA and neighbouring EABR. Similar studies incorporating more samples and additional molecular markers at wider geographic scale may show a clearer picture of the biogeographic history of this fascinating region, which would be useful for improved conservation efforts.

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Supplementary material

Fig. S1–S3: MrBayes phylogenetic tree topologies for *H. argus, A. sylvaticus* and *M. micranotis.* Study sites have been abbreviated as shown; ASF = Arabuko-Sokoke Forest; TA = Coastal forests in Tanga north eastern Tanzania; SHK = Shimba Hills MPK = Mpeketoni and EAM- East and West Usambara.

Fig.S4–S6: MrBayes phylogenetic tree topologies for *C. xerampelina*, *L. flavomculatus* and *H. pusillus*

Fig.S7: 16S TCS haplotype network for *H. argus*. The colour coding for the study sites are as follows; Yellow = Mpeketoni; Blue = Arabuko-Sokoke Forest; Green = Shimba Hills; Purple = Tanga; Red = Usambaras.

Fig. S8: Predicted species distributions in Maxent showing the position of the southern barrier. A–C Predicted distribution for *A. sylvaticus*; *A. xenodactyloides* and *S. pusilla* during the Holocene*;* D prediction for *M. micranotis* during the LIG.

Table S1: Substitution models based on JModeltest analysis for each species used in the Bayesian analysis.

Table S2: Pair wise distances (Fst), 16S MtDNA between populations of species that occurred in more than two study sites. Significant values are shown in bold.

TableS3: Climatic variables contribution to habitat suitability predictions in Maxent.

Table S4: Mantel and Partial Mantel tests results for isolation by distance and Isolation by resistance. Significant values are indicated in bold.

References

Avise J.C., Arnold, J., Ball, R.M. et al. 1987. Intraspecific phylo-geography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* **18**: 489–522.

Avise, J.C. 2000. *Phylogeography: The history and formation of species*. Harvard University Press, Cambridge, MA.

Avise, J.C. 2004. *Molecular markers, natural selection, and evolution* 2nd edition. SinauerAssociates Inc. 23 Plumptree road. Sunderland ma 01375 USA.

Azeria, E.T., Sanmartín, I. A.S., Carlson, A. & Burgess, N. 2000. Biogeographic patterns of the East African coastal forest vertebrate fauna. In *Vertebrate Conservation and Biodiversity* (pp. 57–86). Springer Netherlands.

Ballard, J.W.O. & Whitlock, M.C. 2004. The incomplete natural history of mitochondria. *Molecular Ecology* **13**: 729–744.

Blackburn, D.C. & Measey, G.J. 2009. Dispersal to or from an African biodiversity hotspot? *Molecular Ecology* **18**: 1904–1915. http://doi.org/10.1111/j.1365-294X.2009.04156.x.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A. & Drummond, A.J. 2014. BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* **10**: e1003537. http://dx.doi.org/10.1371/journal. pcbi.1003537Brown, 2014.

Burgess, N.D. & Clarke, G.P. (eds) 2000. *Coastal forests of Eastern Africa*, Xiii

+443pp. IUCN, Gland, Switzerland and Cambridge, UK.

Bwong, B.A., Malonza, P.K., Wasonga, D.V., Nyamache, J.O., Nagel, P. & Loader, S.P. 2014. At a biogeographical crossroads: Amphibian paradise in Shimba Hills of Kenya. *FrogLog* **22:** 111:72–73.

Channing, A. & Howell, K.M. 2006. *Amphibians of East Africa*. Cornell University Press Ithaca, New York.

Carnaval, A.C., Hickerson, M.J., Haddad, C.F., Rodriges, M.T. & Moritz, C. 2009.

Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. *Science* **323**: 785–789.

Carpenter, K.E., Barber, P.H., Crandall, E.D., Ablan-Lagman, M.C.A., Mahardika, G.N., Manjaji-Matsumoto, B.M., Juinio-Meñez, M.A., Santos, M.D., Starger, C.J. & Toha, A.H.A. 2010. Comparative phylogeography of the Coral Triangle and implications for marine management. *Journal of Marine Biology* **2011.**

Carstens, B.C. & Richards, C.L. 2007. Integrating coalescent and ecological niche modeling in comparative phylogeography. *Evolution* **61**: 1439–1454. http://doi.org/10.1111/j.1558-5646.2007.00117.x.

Crowe, T.M. & Crowe, A.A. 1982. Patterns of distribution, diversity and endemism in Afrotropical birds. *Journal of Zoology London* **198**:417–442.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. 2012. JModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* **9**: 772.

Dias-Terceiro, R.G., Kaefer, I.L., Fraga, R., Araújo, M.C., Simões, P.I. & Lima, A.

P. 2015. A matter of scale: Historical and environmental factors structure anuran assemblages from the Upper Madeira River, Amazonia. *Biotropica* **47**: 259–266.

Dimitrov, D., Nogués-Bravo, D. & Scharff, N. 2012. Why do tropical Mountains support exceptionally high biodiversity? The Eastern Arc Mountains and the drivers of *Saintpaulia* diversity. *PLoS ONE* **7**(11). http://doi.org/10.1371/journal.pone.0048908.

Dornelas, M., Connolly, S.R., & Hughes, T.P. 2006. Coral reef diversity refutes the neutral theory of biodiversity. *Nature* **440**: 80–82.

Driscoll, D.A. 1998. Genetic structure of the frogs *Geocrinia lutea* and *Geocrinia rosea* reflects extreme population divergence and range changes, not dispersal barriers. *Evolution* 1147–1157.

Duellman, W.E. & Trueb, L. 1986. Biology of Amphibians. McGraw-Hill, New York.

Excoffier, L. & Lischer, H.E.L. 2010. Arlequin suite version 3.5.2.2: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**: 564–567.

Elith, J., Graham, C., the NCEAS species distribution modelling group 2006. Novel methods improve prediction of species' distributions from occurrence data. *Ecography* **29**: 129–151.

Elith, J. & Leathwick, J.R. 2009. Species distribution models: Ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution, and Systematics* **40**: 677–697. http://doi.org/10.1146/annurev.ecolsys.110308.120159.

Fitzpatrick, S.W., Brasileiro, C.A., Haddad, C.F. & Zamudio, K.R. 2009. Geographical variation in genetic structure of an Atlantic Coastal Forest frog reveals regional differences in habitat stability. *Molecular Ecology* **18**: 2877–2896.

Fjeldsaå, J. & Lovett, J.C. 1997. Geographical patterns of old and young species in African forest biota: The significance of specific montane areas as evolutionary centres. *Biodiversity and Conservation*, **6**: 325–346. http://doi.org/10.1023/a:1018356506390.

Fu, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. *Genetics* **147**: 915–925.

Gascon, C., Malcolm, J.R., Patton, J.L., da Silva, M.N., Bogart, J.P., Lougheed, S. C., Peres, C.A., Neckel, S. & Boag, P.T. 2000. Riverine barriers and the geographic distribution of Amazonian species. *Proceedings of the National Academy of Sciences*, **97**: 13672–13677.

Graham, C.H., VanDerWal, J., Phillips, S.J., Moritz, C. & Williams, S.E. 2010. Dynamic refugia and species persistence: tracking spatial shifts in habitat through time. *Ecography* **33:** 1062–1069.

Gutiérrez-García, T.A. & Vázquez-Domínguez, E. 2011. Comparative phylogeography: Designing studies while surviving the process. *BioScience* **61**: 857–868.

Hamilton, A.C.1982. Environmental history of Eastern Africa; a study of the Quaternary. Academic Press Inc. (London) Ltd., London NW1.

Harpending, H. 1994. Signature of ancient population growth in a low- resolution mitochondrial DNA mismatch distribution. *Human Biology: An international Record Research* **66:** 4.

Harper, E.B., Measey, G.J. Patrick, D.A., Menegon, M. & Vonesh, J.R. 2010. *Field guide to amphibians of the Eastern Arc Mountains and Coastal Forests of Tanzania and Kenya.* Camerapix Publishers International, Nairobi, Kenya.

Hewitt, G.M. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907–913.

Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., Hayek, L.-A. & Foster, M.S. 1994. Measuring and Monitoring biological diversity. Standard Methods for amphibians. Washington and London.

Hijmans, R.J., Cameron, S.E., Para, J.L., Jones, P. & Jarvis, A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**: 1965–1978.

Hugall, A., Moritz, C., Moussalli, A. & Stanisic, J. 2002. Reconciling paleodistribution models and comparative phylogeography in the Wet Tropics rainforest land snail Gnarosophia bellendenkerensis (Brazier 1875). *Proceedings of the National Academy of Sciences of the United States of America* **99**: 6112–6117. http://doi.org/10.1073/pnas.092538699

Hugget, R.J. 2004. Fundamentals of biogeography. Routledge Fundamentals of Physical Geography, New York.

Kato, Misawa, Kuma & Miyata 2002. MAFT: Anovel method for rapid sequence alighnment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059–3066.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C. & Thierer, T. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**: 1647–1649.

Lampert, K.P., Rand, A.S., Mueller, U.G. & Ryan, M.J. 2003. Fine-scale genetic

pattern and evidence for sex-biased dispersal in the túngara frog, *Physalaemus pustulosus*. *Molecular Ecology* **12**: 3325–3334.

Lawson, L.P. 2013. Diversification in a biodiversity hot spot: Landscape correlates

of phylogeographic patterns in the African spotted reed frog. *Molecular Ecology* **22**: 1947–1960.

Lemmon, E.M., Lemmon, A.R. & Cannatella, D.C. 2007: Geological and climatic forces driving speciation in the continentally distributed trilling chorus frogs (*Pseudoacris*). *Evolution* **61**: 2086–2103.

Loader, S.P., Gower, D.J., Ngalason, W. & Menegon, M. 2010. Three new species of *Callulina* (Amphibia: Anura: Brevicipitidae) highlight local endemism and conservation plight of Africa’s Eastern Arc forests. *Zoological Journal of the Linnean Society* **160**: 496–514. http://doi.org/10.1111/j.1096-3642.2010.00652.x.

Lomolino, M.V., Sax, D.F. & Brown, J.H. 2004. Foundations of biogeography classic papers with commentaries. University of Chicago Press, Chicago, Illinois.

Luke, Q. 2005. Annotated checklist of the plants of the Shimba Hills, Kwale District, Kenya. *Journal of East African Natural History* **94**: 5–120. http://doi.org/10.2982/0012-8317(2005)94[5:ACOTPO]2.0.CO;2.

Mantel, N. 1967. The detection of disease clustering and generalized regression approach. *Cancer Research* **27**: 209–220.

McRae, B.H. & Shah, V.B. 2009. Circuitscape user’s guide. *The University of*

California, Santa Barbara.

de Menocal, P.B. 1995. Plio-Pleistocene African climate. *Science* **270**: 53–59.

Moraes, L.J., Pavan, D., Barros, M.C. & Ribas, C.C. 2016. The combined influence of riverine barriers and flooding gradients on biogeographical patterns for amphibians and squamates in south-eastern Amazonia. *Journal of Biogeography* **43**: 2113–2124.

Moreau, R.E. 1933. Pleistocene climatic changes and the distribution of life in East Africa. *Journal of Ecology* **21**: 415–435.

Myers, N., Mittermeier, R.A., da Fonseca, G.A.B. & Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**: 853–58. doi:10.1038/35002501.

Newman, C.E. & Rissler, L.J. 2011. Phylogeographic analyses of the southern leopard frog: The impact of geography and climate on the distribution of genetic lineages vs. subspecies. *Molecular Ecology* **20**: 5295–5312.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R.B. & Oksanen, M.J. 2013. Package 'vegan'. *Community ecology package version*, **2**(9).

Pearson, R.G., Raxworthy, C.J., Nakamura, M. & Townsend, P.A. 2007. Predicting species distributions from small numbers of occurrence records: A test case using cryptic geckos in Madagascar. *Journal of Biogeography* **34**: 102–117. http://doi.org/10.1111/j.1365-2699.2006.01594.x.

Phillips, S.J., Anderson, R.P. & Schapire, R.E. 2006. Maximum entropy modeling of species distribution. *Ecological Models* **190**: 231–259.

Poynton, J.C. 1999. Distribution of amphibians in sub-Saharan Africa, Madagascar, and Seychelles. *Patterns of distribution of amphibians, a global perspective. Johns Hopkins University Press, Baltimore* 483–539.

Poynton, J.C. 2000. Amphibians. *The coastal forests of eastern Africa. IUCN, Gland,* 201–210.

Qu, Y., Ericson, P.G., Quan, Q., Song, G., Zhang, R., Gao, B. & Lei, F., 2014. Long-term isolation and stability explain high genetic diversity in the Eastern Himalaya. *Molecular Ecology* **23**: 705–720.

R Development Core Team 2015. R*:* A language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria. v3.2.1. Available from: <http://www.R-project.org>.

Rambaut, A., Suchard, M.A., Xie, D. & Drummond, A.J. 2014. Tracer v1.6, Available from: <http://beast.bio.ed.ac.uk/Tracer>.

Ricklefs, R.E. 2004. A comprehensive framework for global patterns in biodiversity. *Ecology letters* **7**: 1–15.

Ronquist, F., Teslenko, M., Van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. 2012. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.

Rogers, A.R. & Harpending, H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology Evolution* **9**:552–569.

Schiøtz, A. 1975. The Treefrogs of Eastern Africa. Steenstrupia, Copenhagen.

Schiøtz, A. 1999. *The Treefrogs of Africa*. Ed. Chimaira. Frankfurt am Main.

Stamatakis, A. 2014. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.

Schneider, C.J., Cunningham, M. & Moritz, C. 1998. Comparative phylogeography and the history of endemic vertebrates in the wet tropics rainforests of Australia. *Molecular Ecology* **7**: 487–498.

Sloan, S., Jenkins, C.N., Joppa, L.N., Gaveau, D.L.A. & Laurance, W.F. 2014. Remaining natural vegetation in the global biodiversity hotspots. *Biological Conservation* **177**: 12–24.

Templeton, A.R., Crandall, K.A. & Sing, C.F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132**: 619–633.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**: 585–595.

Tonini, J.F.R., Costa, L.P. & Carnaval, A.P. 2013. Phylogeographic structure is always strong in the Atlantic Forest; predictive power of correlative paleodistribution models, not always. *Journal of Zoological systematics and Evolutionary Research* **51**: 114–121.

Waltari, E. & Guralnick, R.P. 2009. Ecological niche modelling of montane mammals in the Great Basin, North America: Examining past and present connectivity of species across basins and ranges. *Journal of Biogeography* **36**: 148–161. http://doi.org/10.1111/j.1365–2699.2008.01959.x.

Wright, S. 1951. The genetical structure of populations. *Annals of Eugenics*, **15:** 323–354.

Zink, R.M. 2002. Methods in comparative phylogeography and their application to

studying evolution in the North American aridlands. *Integrative and Comparative Biology* **42**: 953–959.

Appendix 1: A list of all the samples used in phylogeographic analysis, their tissues identification, voucher number, sampling area, counry of origin plus geographic co-ordinates.

Species	Tissue ID	Voucher	Site	Country	Long	Lat
Afrixalus delicatus	T5188	SL1256	Mpeketoni	KE	40.686	-2.409
Afrixalus delicatus	T5189	SL 1260	Mpeketoni	KE	40.686	-2.409
Afrixalus delicatus	T5190	SL 1261	Mpeketoni	KE	40.686	-2.409
Afrixalus delicatus	T5191	SL 1262	Mpeketoni	KE	40.686	-2.409
Afrixalus delicatus	T5379	MUC 0151	Shimba Hills	KE	39.144	-4.552
Afrixalus delicatus	T5380	MUC 0153	Shimba Hills	KE	39.144	-4.552
Afrixalus delicatus	T6343	SL 1448	Shimba Hills	KE	39.419	-4.181
Afrixalus delicatus	T6283	SL 1480	Arabuko-Sokoke Forest	KE	39.976	-3.263
Afrixalus delicatus	T6284	SL 1481	Arabuko-Sokoke Forest	KE	39.976	-3.263
Afrixalus delicatus	T6285	SL 1482	Arabuko-Sokoke Forest	KE	39.976	-3.263
Afrixalus delicatus	T6286	SL 1483	Arabuko-Sokoke Forest	KE	39.976	-3.263
Afrixalus delicatus	T6287	SL 1484	Arabuko-Sokoke Forest	KE	39.976	-3.263
Afrixalus delicatus	T6367	SL 2777	Shimba Hills	KE	39.463	-4.43
Afrixalus delicatus	T4236	CB:886	Tanga	TZ	39.102	-4.632
Afrixalus delicatus	T5394	MVZ:226254	Arabuko-Sokoke Forest	KE	39.867	-3.333
Afrixalus delicatus	T5395	MVZ:226255	Arabuko-Sokoke Forest	KE	39.867	-3.333
Afrixalus fornasini	T4165	CB:13:815	Tanga	TZ	38.924	-5.034

Afrixalus fornasini	T4166	CB:13:816	Tanga	TZ	38.924	-5.034
Afrixalus fornasini	T4167	CB:13:817	Tanga	TZ	38.924	-5.034
Afrixalus fornasini	T4168	CB:13:818	Tanga	TZ	38.924	-5.034
Afrixalus fornasini	T4169	CB:13:819	Tanga	TZ	38.924	-5.034
Afrixalus fornasini	T4170	CB:13:820	Tanga	TZ	38.924	-5.034
Afrixalus fornasini	T4178	CB:13:828	Tanga	TZ	38.924	-5.034
Afrixalus fornasini	T4426	BM:2000.825	East Usambara Mountains	TZ	38.733	-4.923
Afrixalus fornasini	T4427	BM:2002.551	East Usambara Mountains	TZ	38.663	-4.904
Afrixalus fornasini	T4428	BM:2002.552	East Usambara Mountains	KE	38.663	-4.904
Afrixalus fornasini	T5304	SL 1143	Shimba Hills	KE	39.431	-4.276
Afrixalus fornasini	T6277	SL 1474	Shimba Hills	KE	39.976	-3.263
Afrixalus fornasini	T6279	SL 1476	Arabuko-Sokoke Forest	KE	39.976	-3.263
Afrixalus fornasini	T6280	SL 1477	Arabuko-Sokoke Forest	KE	39.976	-3.263
Afrixalus fornasini	T6281	SL 1478	Arabuko-Sokoke Forest	KE	39.976	-3.263
Afrixalus fornasini	T6352	SL 2700	Shimba Hills	KE	39.419	-4.181
Afrixalus fornasini	T6353	SL 2701	Shimba Hills	KE	39.419	-4.181
Afrixalus fornasini	T2461	MW 7784	Shimba Hills	KE	39.422	-4.443
Afrixalus fornasini	T6344	SL 1450	Shimba Hills	KE	39.419	-4.181
Afrixalus sylvaticus	T4924	MTSN 9517	East Usambara Mountains	TZ	38.758	-4.981
Afrixalus sylvaticus	T4925	MTSN 9518	East Usambara Mountains	TZ	38.758	-4.981

Afrixalus sylvaticus	T4926	MTSN 9519	East Usambara Mountains	TZ	38.758	-4.981
Afrixalus sylvaticus	T4942	MTSN 9528	East Usambara Mountains	TZ	38.758	-4.981
Afrixalus sylvaticus	T4938	MTSN 9524	East Usambara Mountains	TZ	38.758	-4.981
Afrixalus sylvaticus	T4955	MTSN 9547	East Usambara Mountains	TZ	38.762	-4.976
Afrixalus sylvaticus	T4978	MTSN 9574	East Usambara Mountains	TZ	38.762	-4.976
Afrixalus sylvaticus	T5025	CB 14.1012	East Usambara Mountains	TZ	38.663	-4.904
Afrixalus sylvaticus	T5169	SL 1197	Shimba Hills	KE	39.425	-4.375
Afrixalus sylvaticus	T5168	SL 1196	Shimba Hills	KE	39.425	-4.375
Afrixalus sylvaticus	T5158	SL 1160	Shimba Hills	KE	39.417	-4.158
Afrixalus sylvaticus	T5184	SL 1241	Shimba Hills	KE	39.445	-4.176
Afrixalus sylvaticus	T5195	SL 1302	Shimba Hills	KE	39.485	-4.225
Afrixalus sylvaticus	T5196	SL 1303	Shimba Hills	KE	39.485	-4.225
Afrixalus sylvaticus	T5197	SL 1304	Shimba Hills	KE	39.485	-4.225
Afrixalus sylvaticus	T5199	SL 1324	Shimba Hills	KE	39.431	-4.276
Afrixalus sylvaticus	T5200	SL 1325	Shimba Hills	KE	39.431	-4.276
Afrixalus sylvaticus	T5392	MVZ:Herp:234560	Shimba Hills	KE	39.341	-4.266
Afrixalus sylvaticus	T5393	MVZ:Herp:234561	Shimba Hills	KE	39.341	-4.266
Arthroleptis stenodactylus	T2319	BM 2002.594	East Usambara Mountains	TZ	38.693	-4.928
Arthroleptis stenodactylus	T2492	MTSN 9510	East Usambara Mountains	TZ	38.758	-4.981
Arthroleptis stenodactylus	T2732	MCZ 148848	Tanga	TZ	38.141	-4.923

Arthroleptis stenodactylus	T2733	MCZ 148849	Tanga	TZ	38.141	-4.923
Arthroleptis stenodactylus	T2734	MCZ 148850	Tanga	TZ	39.13	-4.765
Arthroleptis stenodactylus	T4450	CB 14.1038	East Usambara Mountains	TZ	38.693	-4.928
Arthroleptis stenodactylus	T4930	MTSN 9512	East Usambara Mountains	TZ	38.758	-4.981
Arthroleptis stenodactylus	T4933	MTSN 9515	East Usambara Mountains	TZ	38.758	-4.981
Arthroleptis stenodactylus	T4934	MTSN 9516	East Usambara Mountains	TZ	38.758	-4.981
Arthroleptis stenodactylus	T4939	MTSN 9525	East Usambara Mountains	TZ	38.758	-4.981
Arthroleptis stenodactylus	T4940	MTSN 9526	East Usambara Mountains	TZ	38.758	-4.981
Arthroleptis stenodactylus	T4943	MTSN 9529	East Usambara Mountains	TZ	38.758	-4.981
Arthroleptis stenodactylus	T4945	MTSN 9535	East Usambara Mountains	TZ	38.758	-4.981
Arthroleptis stenodactylus	T4946	MTSN 9536	East Usambara Mountains	TZ	38.762	-4.976
Arthroleptis stenodactylus	T4950	MTSN 9540	East Usambara Mountains	TZ	38.762	-4.976
Arthroleptis stenodactylus	T4964	MTSN 9556	East Usambara Mountains	TZ	38.762	-4.976
Arthroleptis stenodactylus	T2712	MCZ 138370	West Usambara	TZ	NULL	NULL
Arthroleptis stenodactylus	T5322	SL 1206	Shimba Hills	KE	39.429	-4.392
Arthroleptis stenodactylus	T5319	SL 1117	Shimba Hills	KE	39.473	-4.416
Arthroleptis stenodactylus	T5154	SL 1122	Shimba Hills	KE	39.396	-4.238
Arthroleptis stenodactylus	T5155	SL 1123	Shimba Hills	KE	39.396	-4.238
Arthroleptis stenodactylus	T5156	SL 1124	Shimba Hills	KE	39.396	-4.238
Arthroleptis stenodactylus	T5157	SL 1125	Shimba Hills	KE	39.396	-4.238

Arthroleptis stenodactylus	T5162	SL 1165	Shimba Hills	KE	39.425	-4.231
Arthroleptis stenodactylus	T5320	SL 1161	Shimba Hills	KE	39.396	-4.238
Arthroleptis stenodactylus	T5161	SL 1164	Shimba Hills	KE	39.396	-4.238
Arthroleptis stenodactylus	T5321	SL 1172	Shimba Hills	KE	39.431	-4.276
Arthroleptis stenodactylus	T5323	SL 1243	Shimba Hills	KE	39.425	-4.231
Arthroleptis stenodactylus	T5324	SL 1294	Shimba Hills	KE	39.396	-4.238
Arthroleptis stenodactylus	T6337	SL 1441	Shimba Hills	KE	39.384	-4.249
Arthroleptis stenodactylus	T6356	SL 2726	Shimba Hills	KE	39.419	-4.181
Arthroleptis stenodactylus	T6363	SL 2771	Shimba Hills	KE	39.363	-4.287
Arthroleptis stenodactylus	T6307	SL 2827	Arabuko-Sokoke Forest	KE	39.891	-3.326
Arthroleptis stenodactylus	T6308	SL 2828	Arabuko-Sokoke Forest	KE	39.891	-3.326
Arthroleptis stenodactylus	T6309	SL 2829	Arabuko-Sokoke Forest	KE	39.891	-3.326
Arthroleptis stenodactylus	T6325	SL 2864	Arabuko-Sokoke Forest	KE	39.972	-3.281
Arthroleptis xenodactyloides	T2441	MTSN 7515	East Usambara Mountains	TZ	38.761	-4.977
Arthroleptis xenodactyloides	T2716	MTSN 7516	East Usambara Mountains	TZ	38.761	-4.976
Arthroleptis xenodactyloides	T2717	MCZ 138385	West Usambara	TZ	38.42	-5.068
Arthroleptis xenodactyloides	T2729	MCZ 138386	West Usambara	TZ	38.378	-5.054
Arthroleptis xenodactyloides	T2730	MCZ 148840	Tanga	TZ	38.141	-4.923
Arthroleptis xenodactyloides	T2731	MCZ 148841	Tanga	TZ	38.141	-4.923
Arthroleptis xenodactyloides	T4121	CB 13.770	Tanga	TZ	39.048	-5.073

Arthroleptis xenodactyloides	T4122	CB 13.771	Tanga	TZ	39.048	-5.073
Arthroleptis xenodactyloides	T4123	CB 13.772	Tanga	TZ	39.048	-5.073
Arthroleptis xenodactyloides	T4131	CB 13.773	Tanga	TZ	39.048	-5.073
Arthroleptis xenodactyloides	T4132	CB 13.781	Tanga	TZ	39.048	-5.073
Arthroleptis xenodactyloides	T4133	CB 13.782	Tanga	TZ	39.048	-5.073
Arthroleptis xenodactyloides	T4135	CB 13.784	Tanga	TZ	39.048	-5.073
Arthroleptis xenodactyloides	T4186	CB 13.785	Tanga	TZ	39.048	-5.073
Arthroleptis xenodactyloides	T4187	CB 13.836	Tanga	TZ	38.925	-5.034
Arthroleptis xenodactyloides	T4201	CB 13.837	Tanga	TZ	38.925	-5.034
Arthroleptis xenodactyloides	T4202	CB 13.851	Tanga	TZ	38.925	-5.034
Arthroleptis xenodactyloides	T4203	CB 13.852	Tanga	TZ	38.925	-5.034
Arthroleptis xenodactyloides	T4461	CB 13.853	Tanga	TZ	38.925	-5.034
Arthroleptis xenodactyloides	T4953	MTSN 9527	East Usambara Mountains	TZ	38.758	-4.981
Arthroleptis xenodactyloides	T4967	MTSN 9543	East Usambara Mountains	TZ	38.762	-4.976
Arthroleptis xenodactyloides	T4975	MTSN 9560	East Usambara Mountains	TZ	38.762	-4.976
Arthroleptis xenodactyloides	T5040	MTSN 9569	East Usambara Mountains	TZ	38.737	-4.973
Arthroleptis xenodactyloides	T5041	BM 2002.325	East Usambara Mountains	TZ	38.752	-5.058
Arthroleptis xenodactyloides	T5042	BM 2002.326	East Usambara Mountains	TZ	38.748	-5.059
Arthroleptis xenodactyloides	T5049	BM 2002.329	East Usambara Mountains	TZ	38.748	-5.059
Arthroleptis xenodactyloides	T5050	BM 2000.840	East Usambara Mountains	TZ	38.751	-4.921

Arthroleptis xenodactyloides	T5051	BM 2002.883	East Usambara Mountains	TZ	38.813	-4.792
Arthroleptis xenodactyloides	T5052	BM 2002.884	East Usambara Mountains	TZ	38.813	-4.792
Arthroleptis xenodactyloides	T5053	BM 2002.885	East Usambara Mountains	TZ	38.813	-4.792
Arthroleptis xenodactyloides	T5054	BM 2002.886	East Usambara Mountains	TZ	38.813	-4.792
Arthroleptis xenodactyloides	T5056	BM 2002.597	East Usambara Mountains	TZ	38.693	-4.928
Arthroleptis xenodactyloides	T5057	BM 2002.598	East Usambara Mountains	TZ	38.665	-4.911
Arthroleptis xenodactyloides	T5059	BM 2002.600	East Usambara Mountains	TZ	38.659	-4.944
Arthroleptis xenodactyloides	T5480	BM 2002.601	East Usambara Mountains	TZ	38.659	-4.944
Arthroleptis xenodactyloides	T5599	MUSE 11090	East Usambara Mountains	TZ	38.61	-5.113
Arthroleptis xenodactyloides	T5170	MUSE 11099	East Usambara Mountains	TZ	38.6	-5.092
Arthroleptis xenodactyloides	T5151	SL 1194	Shimba hills	KE	39.429	-4.375
Arthroleptis xenodactyloides	T5152	SL 1119	Shimba hills	KE	39.425	-4.231
Arthroleptis xenodactyloides	T5153	SL 1120	Shimba hills	KE	39.425	-4.231
Arthroleptis xenodactyloides	T5160	SL 1121	Shimba hills	KE	39.425	-4.231
Arthroleptis xenodactyloides	T5182	SL 1162	Shimba hills	KE	39.425	-4.231
Arthroleptis xenodactyloides	T6338	SL 1430	Shimba hills	KE	39.414	-4.233
Arthroleptis xenodactyloides	T5183	SL 1230	Shimba Hills	KE	39.425	-4.231
Arthroleptis xenodactyloides	T6336	SL 1430	Shimba Hills	KE	39.414	-4.233
Arthroleptis xenodactyloides	T2425	MTSN 7515	East Usambara Mountains	TZ	38.762	-4.976
Arthroleptis xenodactyloides	T5194	SL 1298	Shimba Hills	KE	39.485	-4.225

Boulengerula changamwensis	T491	A4129	Shimba Hills	KE	39.485	-4.225
Boulengerula changamwensis	T2511	VW 648	Shimba Hills	KE	NULL	NULL
Boulengerula changamwensis	T5325	SL 1126	Shimba hills	KE	39.396	-4.238
Boulengerula changamwensis	T5326	SL 1127	Shimba hills	KE	39.396	-4.238
Boulengerula changamwensis	T5327	SL 1167	Shimba hills	KE	39.425	-4.231
Boulengerula changamwensis	T5328	SL 1168	Shimba hills	KE	39.425	-4.231
Boulengerula changamwensis	T5329	SL 1242	Shimba hills	KE	39.425	-4.231
Boulengerula changamwensis	T5330	SL 1300	Shimba hills	KE	39.485	-4.225
Boulengerula changamwensis	T5331	VW 00649	Shimba hills	KE	39.485	-4.225
Boulengerula changamwensis	T6379	SL 1364	Shimba hills	KE	39.396	-4.231
Boulengerula changamwensis	T6390	SL 1408	Shimba hills	KE	39.396	-4.238
Boulengerula changamwensis	T6391	SL 1409	Shimba hills	KE	39.396	-4.238
Boulengerula changamwensis	T6395	SL 1425	Shimba hills	KE	39.396	-4.238
Boulengerula changamwensis	T6339	SL 1443	Shimba hills	KE	39.396	-4.237
Boulengerula changamwensis	T6340	SL 2734	Shimba hills	KE	39.396	-4.237
Boulengerula changamwensis	T6357	SL 2738	Shimba hills	KE	39.485	-4.225
Boulengerula changamwensis	T6358	SL 2739	Shimba hills	KE	39.485	-4.225
Boulengerula changamwensis	T6364	SL 2772	Shimba hills	KE	39.396	-4.231
Boulengerula changamwensis	T6365	SL 2773	Shimba hills	KE	39.396	-4.231
Boulengerula changamwensis	T6369	SL 2781	Shimba hills	KE	39.414	-4.232

Boulengerula changamwensis	T6370	SL 2782	Shimba hills	KE	39.414	-4.232
Boulengerula changamwensis	T6377	SL 2791	Shimba hills	KE	39.405	-4.462
Boulengerula changamwensis		FN652722	Arabuko-Sokoke Forest	KE	39.557	-4.232
Chiromantis xerampelina	T4262	CB 13.915	Tanga	TZ	38.646	-5.583
Chiromantis xerampelina	T4263	CB 13.916	Tanga	TZ	39.117	-4.749
Chiromantis xerampelina	T4264	CB 13.917	Tanga	TZ	39.117	-4.749
Chiromantis xerampelina	T4270	CB 13.924	Tanga	TZ	39.117	-4.749
Chiromantis xerampelina	T4484	BM 2002.862	Tanga	TZ	39.117	-4.749
Chiromantis xerampelina	T4485	BM 2002.620	East Usambara Mountains	TZ	38.813	-4.791
Chiromantis xerampelina	T4486	BM 2002.621	East Usambara Mountains	TZ	38.663	-4.908
Chiromantis xerampelina	T4487	BM 2002.765	East Usambara Mountains	TZ	38.663	-4.908
Chiromantis xerampelina	T4948	MTSN 9538	East Usambara Mountains	TZ	38.663	-4.908
Chiromantis xerampelina	T4949	MTSN 9539	East Usambara Mountains	TZ	38.762	-4.976
Chiromantis xerampelina	T4963	MTSN 9555	East Usambara Mountains	TZ	38.762	-4.976
Chiromantis xerampelina	T4977	MTSN 9573	East Usambara Mountains	TZ	38.762	-4.976
Chiromantis xerampelina	T6041	MW 07916	East Usambara Mountains	TZ	38.762	-4.976
Chiromantis xerampelina	T5332	SL 1180	Shimba Hills	KE	39.423	-4.443
Chiromantis xerampelina	T5333	SL 1225	Shimba Hills	KE	39.437	-4.382
Chiromantis xerampelina	T5334	SL 1247	Shimba Hills	KE	39.44	-4.128
Chiromantis xerampelina	T5335	SL 1329	Mpeketoni	KE	40.664	-2.409

Chiromantis xerampelina	T6380	SL 1365	Shimba Hills	KE	39.431	-4.276
Chiromantis xerampelina	T6302	SL 2815	Shimba Hills	KE	39.396	-4.238
Chiromantis xerampelina	T6303	SL 2816	Arabuko-Sokoke Forest	KE	39.950	3.316
Chiromantis xerampelina	T6311	SL 2841	Arabuko-Sokoke Forest	KE	39.950	3.316
Chiromantis xerampelina	T6319	SL 2853	Arabuko-Sokoke Forest	KE	39.952	-3.333
Hemisus marmoratus	T4226	CB:13.876	Tanga	TZ	39.102	-4.632
Hemisus marmoratus	T4227	CB:13.877	Tanga	TZ	39.102	-4.632
Hemisus marmoratus	T4228	CB:13.878	Tanga	TZ	39.102	-4.632
Hemisus marmoratus	T4233	CB:13.883	Tanga	TZ	39.102	-4.632
Hemisus marmoratus	T4250	CB:13.903	Tanga	TZ	39.117	-4.749
Hemisus marmoratus	T4251	CB:13.904	Tanga	TZ	39.117	-4.749
Hemisus marmoratus	T4252	CB:13.905	Tanga	TZ	39.117	-4.749
Hemisus marmoratus	T4271	CB:13.925	Tanga	TZ	39.117	-4.749
Hemisus marmoratus	T4490	BM 2002.881	East Usambara Mountains	TZ	38.813	-4.792
Hemisus marmoratus	T4491	BM 2002.882	East Usambara Mountains	TZ	38.681	-4.809
Hemisus marmoratus	T4499	BM 2002.579	East Usambara Mountains	TZ	38.652	-4.929
Hemisus marmoratus	T4928	MTSN 9509	East Usambara Mountains	TZ	38.758	-4.981
Hemisus marmoratus	T4947	MTSN 9537	East Usambara Mountains	TZ	38.762	-4.976
Hemisus marmoratus	T4968	MTSN 9561	East Usambara Mountains	TZ	38.762	-4.976
Hemisus marmoratus	T4969	MTSN 9562	East Usambara Mountains	TZ	38.762	-4.976

Hemisus marmoratus	T4973	MTSN 9566	East Usambara Mountains	TZ	38.762	-4.976
Hemisus marmoratus	T4986	MCZ A32138	Tanga	TZ	38.141	-4.923
Hemisus marmoratus	T6042	A-148853	Arabuko-Sokoke Forest	KE	38.141	-4.923
Hemisus marmoratus	T5336	SL 1109	Shimba Hills	KE	39.557	-3.981
Hemisus marmoratus	T6662	SL2733	Arabuko-Sokoke Forest	KE	39.976	-3.263
Hemisus marmoratus	T2467		Arabuko-Sokoke Forest	KE	39.557	-4.232
Hyperolius argus	T4501	BM 2000.857	East Usambara Mountains	TZ	38.736	-4.923
Hyperolius argus	T6039	MW 07912	Shimba Hills	KE	39.422	-4.443
Hyperolius argus	T5341	SL 1112	Shimba Hills	KE	39.462	-4.429
Hyperolius argus	T5344	SL 1135	Shimba Hills	KE	39.396	-4.238
Hyperolius argus	T5345	SL 1136	Shimba Hills	KE	39.396	-4.238
Hyperolius argus	T5346	SL 1137	Shimba Hills	KE	39.396	-4.238
Hyperolius argus	T5206	SL 1144	Shimba Hills	KE	39.396	-4.238
Hyperolius argus	T5347	SL 1146	Shimba Hills	KE	39.396	-4.238
Hyperolius argus	T5348	SL 1264	Mpeketoni	KE	40.684	-2.402
Hyperolius argus	T5338	SL 1286	Shimba Hills	KE	39.396	-4.238
Hyperolius argus	T5356	SL 1287	Shimba Hills	KE	39.396	-4.238
Hyperolius argus	T5368	SL 1288	Shimba Hills	KE	39.396	-4.238
Hyperolius argus	T5369	SL 1289	Shimba Hills	KE	39.396	-4.238
Hyperolius argus	T5370	SL 1290	Shimba Hills	KE	39.396	-4.238

Hyperolius argus	T6274	SL 1454	Shimba Hills	KE	39.419	-4.181
Hyperolius argus	T6275	SL 1471	Arabuko-Sokoke Forest	KE	39.976	-3.263
Hyperolius argus	T6276	SL 1472	Arabuko-Sokoke Forest	KE	39.976	-3.263
Hyperolius argus	T6312	SL 1473	Arabuko-Sokoke Forest	KE	39.976	-3.263
Hyperolius argus	T6313	SL 2842	Arabuko-Sokoke Forest	KE	39.952	-3.333
Hyperolius argus	T5342	SL1133	Shimba Hills	KE	39.396	-4.238
Hyperolius argus	T5343	SL1134	Shimba Hills	KE	39.396	-4.238
Hyperolius mariae	T4508	BM 2000.858	East Usambara Mountains	TZ	38.733	-4.923
Hyperolius mariae	T4512	BM 2000.859	East Usambara Mountains	TZ	38.733	-4.923
Hyperolius mariae	T5359	BM 2005.1302	East Usambara Mountains	TZ	38.708	-4.820
Hyperolius mariae	T5358	SL 1190	Shimba Hills	KE	39.4292	-4.392
Hyperolius mariae	T5373	SL 1189	Shimba Hills	KE	39.4292	-4.392
Hyperolius mariae	T5374	SL 1248	Mpeketoni	KE	40.664	-2.409
Hyperolius mariae	T5360	SL 1249	Mpeketoni	KE	40.664	-2.409
Hyperolius mariae	T5375	SL 1291	Shimba Hills	KE	39.396	-4.238
Hyperolius mariae	T5339	SL 1295	Shimba Hills	KE	39.396	-4.238
Hyperolius mariae	T5376	SL 1309	Shimba Hills	KE	39.431	-2.393
Hyperolius mariae	T6385	SL 1334	Shimba Hills	KE	39.431	-4.276
Hyperolius mariae	T6386	SL 1395	Shimba Hills	KE	39.396	-4.238
Hyperolius mariae	T6351	SL 1396	Shimba Hills	KE	39.396	-4.238

Hyperolius mariae	T6403	SL 1461	Shimba Hills	KE	39.419	-4.181
Hyperolius mariae	T6360	SL 2732	Shimba Hills	KE	39.485	-4.225
Hyperolius mariae	T6361	SL 2759	Shimba Hills	KE	39.419	-4.181
Hyperolius mariae	T6368	SL2779	Shimba Hills	KE	39.485	-4.225
Hyperolius mariae	T6262	SL2733	Shimba Hills	KE	39.485	-4.225
Hyperolius mariae	T4525		East Usambara Mountains	TZ	38.733	-4.923
Hyperolius mariae	T4502	BM. 2000.858	East Usambara Mountains	TZ	38.733	-4.923
Hyperolius mariae	T6362	SL2765	Shimba Hills	KE	39.419	-4.181
Hyperolius mariae	T6259	SL2704	Shimba Hills	KE	39.419	-4.181
Hyperolius mariae	T6260	SL2706	Shimba Hills	KE	39.419	-4.181
Hyperolius mariae	T6383	SL1391	Shimba Hills	KE	39.396	-4.238
Hyperolius pusillus	T6294	SL 1490	Arabuko-Sokoke Forest	KE	39.976	-3.263
Hyperolius pusillus	T6373	SL 1491	Arabuko-Sokoke Forest	KE	39.976	-3.263
Hyperolius pusillus	T6691	SL 2787	Arabuko-Sokoke Forest	KE	39.440	-4.442
Hyperolius pusillus	T6692	SL 2919	Shimba Hills	KE	39.462	-4.429
Hyperolius pusillus	T6681	SL 2920	Shimba Hills	KE	39.462	-4.429
Hyperolius pusillus	T6693	SL 2921	Shimba Hills	KE	39.462	-4.429
Hyperolius pusillus	T6310	SL 2833	Arabuko-Sokoke Forest	KE	39.976	-3.263
Hyperolius pusillus	T6299	SL 2830	Arabuko-Sokoke Forest	KE	39.976	-3.263
Hyperolius parkeri	T2474	BM 2002.634	East Usambara Mountains	TZ	38.652	-4.929

Hyperolius parkeri	T4179	CB 13.829	Tanga	TZ	38.924	-5.034
Hyperolius parkeri	T4180	CB 13.830	Tanga	TZ	38.924	-5.034
Hyperolius parkeri	T4237	CB 13.887	Tanga	TZ	39.102	-4.632
Hyperolius parkeri	T5362	SL 1192	Shimba Hills	KE	39.429	-4.392
Hyperolius parkeri	T5361	SL 1191	Shimba Hills	KE	39.429	-4.392
Hyperolius parkeri	T5357	SL 1258	Mpeketoni	KE	40.686	-2.409
Hyperolius parkeri	T5363	SL 1259	Mpeketoni	KE	40.686	-2.409
Hyperolius parkeri	T5364	SL 1314	Shimba Hills	KE	39.451	-2.398
Hyperolius parkeri	T5365	SL 1315	Shimba Hills	KE	39.451	-2.398
Hyperolius parkeri	T5367	SL 1317	Shimba Hills	KE	39.451	-2.398
Hyperolius parkeri	T6295	SL 1492	Shimba Hills	KE	39.976	-3.263
Hyperolius parkeri	T6371	SL 2785	Shimba Hills	KE	39.440	-4.442
Hyperolius parkeri	T6372	SL 2786	Shimba Hills	KE	39.440	-4.442
Hyperolius parkeri	T6300	SL 2813	Arabuko-Sokoke Forest	KE	39.976	-3.263
Hyperolius parkeri	T5366	SL 1316	Shimba Hills	KE	39.451	-2.398
Hyperolius tuberilinguis	T4164	CB 13.814	Tanga	TZ	38.924	-5.034
Hyperolius tuberilinguis	T4171	CB 13.821	Tanga	TZ	38.924	-5.034
Hyperolius tuberilinguis	T4183	CB 13.833	Tanga	TZ	38.924	-5.034
Hyperolius tuberilinguis	T4184	CB 13.834	Tanga	TZ	38.924	-5.034
Hyperolius tuberilinguis	T4185	CB 13.835	Tanga	TZ	38.924	-5.034

Hyperolius tuberilinguis	T4192	CB 13.842	Tanga	TZ	38.924	-5.034
Hyperolius tuberilinguis	T4519	BM 2002.668	East Usambara Mountains	TZ	38.652	-4.929
Hyperolius tuberilinguis	T4521	BM 2002.670	East Usambara Mountains	TZ	38.643	-4.955
Hyperolius tuberilinguis	T5218	SL 1199	Shimba Hills	KE	39.425	-4.375
Hyperolius tuberilinguis	T5349	SL 1265	Mpeketoni	KE	40.684	-2.402
Hyperolius tuberilinguis	T5350	SL 1266	Mpeketoni	KE	40.684	-2.402
Hyperolius tuberilinguis	T5351	SL 1267	Mpeketoni	KE	40.684	-2.402
Hyperolius tuberilinguis	T5352	SL 1268	Mpeketoni	KE	40.684	-2.402
Hyperolius tuberilinguis	T5353	SL 1269	Mpeketoni	KE	40.684	-2.402
Hyperolius tuberilinguis	T5354	SL 1270	Mpeketoni	KE	40.684	-2.402
Hyperolius tuberilinguis	T5355	SL 1271	Mpeketoni	KE	40.684	-2.402
Hyperolius tuberilinguis	T5219	SL 1311	Shimba Hills	KE	39.431	-2.393
Hyperolius tuberilinguis	T5220	SL 1392	Shimba Hills	KE	39.396	-4.238
Hyperolius tuberilinguis	T6296	SL 1493	Arabuko-Sokoke Forest	KE	39.976	-3.263
Hyperolius tuberilinguis	T6297	SL 1497	Arabuko-Sokoke Forest	KE	39.976	-3.263
Hyperolius tuberilinguis	T6354	SL 2710	Shimba Hills	KE	39.419	-4.181
Hyperolius tuberilinguis	T6355	SL 2712	Shimba Hills	KE	39.419	-4.181
Hyperolius tuberilinguis	T6304	SL 2818	Arabuko-Sokoke Forest	KE	39.950	3.316
Hyperolius tuberilinguis	T6350	SL1459	Shimba Hills	KE	39.419	-4.181
Hyperolius tuberilinguis	T6347	SL 1454	Shimba Hills	KE	39.419	-4.181

Hyperolius tuberilinguis	T6261	SL2707	Shimba Hills	KE	39.419	-4.181
Kassina maculatus	T5225	SL 1110	Shimba Hills	KE	39.462	-4.429
Kassina maculatus	T5226	SL 1235	Shimba Hills	KE	39.445	-4.176
Kassina maculatus	T5227	SL 1236	Shimba Hills	KE	39.445	-4.176
Kassina maculatus	T5228	SL 1237	Shimba Hills	KE	39.445	-4.176
Kassina maculatus	T5229	SL 1238	Shimba Hills	KE	39.445	-4.176
Kassina maculatus	T5230	SL 1274	Mpeketoni	KE	40.684	-2.402
Kassina maculatus	T5231	SL 1275	Mpeketoni	KE	40.684	-2.402
Kassina maculatus	T5232	SL 1276	Mpeketoni	KE	40.684	-2.402
Kassina maculatus	T5233	SL 1328	Shimba Hills	KE	39.431	-4.276
Kassina maculatus	T6341	SL 1445	Shimba Hills	KE	39.419	-4.181
Kassina maculatus	T6324	SL 2858	Arabuko- Sokoke Forest	KE	39.891	-3.324
Leptopelis argenteus	T4136	CB 13.786	Coastal Region	TZ	39.048	-5.073
Leptopelis argenteus	T4137	CB 13.787	Tanga	TZ	39.048	-5.073
Leptopelis argenteus	T4138	CB 13.788	Tanga	TZ	39.048	-5.073
Leptopelis argenteus	T4139	CB 13.789	Tanga	TZ	39.048	-5.073
Leptopelis argenteus	T4148	CB 13.798	Tanga	TZ	39.048	-5.073
Leptopelis argenteus	T4189	CB 13.839	Tanga	TZ	38.924	-5.034
Leptopelis argenteus	T4193	CB 13.843	Tanga	TZ	38.924	-5.034
Leptopelis argenteus	T4194	CB 13.844	Tanga	TZ	38.924	-5.034

Leptopelis argenteus	T4195	CB 13.845	Tanga	TZ	38.924	-5.034
Leptopelis argenteus	T4234	CB 13.884	Tanga	TZ	39.102	-4.632
Leptopelis argenteus	T4256	CB 13.909	Tanga	TZ	39.117	-4.749
Leptopelis argenteus	T4257	CB 13.910	Tanga	TZ	39.117	-4.749
Leptopelis argenteus	T4258	CB 13.911	Tanga	TZ	39.117	-4.749
Leptopelis argenteus	T4259	CB 13.912	Tanga	TZ	39.117	-4.749
Leptopelis argenteus	T4260	CB 13.913	Tanga	TZ	39.117	-4.749
Leptopelis argenteus	T4261	CB 13.914	Tanga	TZ	39.117	-4.749
Leptopelis argenteus	T4273	CB 13.930	Tanga	TZ	39.117	-4.749
Leptopelis argenteus	T5163	SL 1175	Shimba Hills	KE	39.425	-4.425
Leptopelis concolor	T5164	SL 1176	Shimba Hills	KE	39.425	-4.425
Leptopelis concolor	T5165	SL 1177	Shimba Hills	KE	39.425	-4.425
Leptopelis concolor	T5166	SL 1188	Shimba Hills	KE	39.425	-4.425
Leptopelis concolor	T5171	SL 1209	Shimba Hills	KE	39.417	-4.158
Leptopelis concolor	T5172	SL 1210	Shimba Hills	KE	39.417	-4.158
Leptopelis concolor	T5173	SL 1211	Shimba Hills	KE	39.417	-4.158
Leptopelis concolor	T5175	SL 1213	Shimba Hills	KE	39.417	-4.158
Leptopelis concolor	T5176	SL 1214	Shimba Hills	KE	39.417	-4.158
Leptopelis concolor	T5177	SL 1217	Shimba Hills	KE	39.417	-4.158
Leptopelis concolor	T5178	SL 1218	Shimba Hills	KE	39.44	-4.128

Leptopelis concolor	T5179	SL 1219	Shimba Hills	KE	39.44	-4.128
Leptopelis concolor	T5181	SL 1221	Shimba Hills	KE	39.44	-4.128
Leptopelis concolor	T5185	SL 1252	Shimba Hills	KE	39.44	-4.128
Leptopelis concolor	T5186	SL 1253	Mpeketoni	KE	40.697	-2.385
Leptopelis concolor	T5187	SL 1254	Mpeketoni	KE	40.697	-2.385
Leptopelis concolor	T5192	SL 1272	Mpeketoni	KE	40.697	-2.385
Leptopelis concolor	T6348	SL 1455	Mpeketoni	KE	40.684	-2.402
Leptopelis concolor	T5396	MVZ:234054	Arabuko-Sokoke Forest	KE	39.863	-2.17
Leptopelis concolor	T5397	MVZ:234055	Arabuko-Sokoke Forest	KE	39.863	-2.17
Leptopelis concolor	T5398	MVZ:234056	Arabuko-Sokoke Forest	KE	39.863	-2.17
Leptopelis concolor	T5401	MVZ:234591	Arabuko-Sokoke Forest	KE	39.863	-2.17
Leptopelis concolor	T6402	SL 2730	Shimba Hills	KE	39.485	-4.225
Leptopelis concolor	T6359	SL 2756	Shimba Hills	KE	39.419	-4.181
Leptopelis flavomaculatus	T2624	MTSN 9522	East Usambara Mountains	TZ	38.75	-4.983
Leptopelis flavomaculatus	T4096	CB 13.746	Tanga	TZ	38.645	-5.583
Leptopelis flavomaculatus	T4097	CB 13.747	Tanga	TZ	38.645	-5.583
Leptopelis flavomaculatus	T4098	CB 13.748	Tanga	TZ	38.645	-5.583
Leptopelis flavomaculatus	T4099	CB 13.749	Tanga	TZ	38.645	-5.583
Leptopelis flavomaculatus	T4100	CB 13.750	Tanga	TZ	38.645	-5.583
Leptopelis flavomaculatus	T4101	CB 13.751	Tanga	TZ	38.645	-5.583

Leptopelis flavomaculatus	T4196	CB 13.846	Tanga	TZ	38.924	-5.034
Leptopelis flavomaculatus	T4197	CB 13.847	Tanga	TZ	38.924	-5.034
Leptopelis flavomaculatus	T4198	CB 13.848	Tanga	TZ	38.924	-5.034
Leptopelis flavomaculatus	T4199	CB 13.849	Tanga	TZ	38.924	-5.034
Leptopelis flavomaculatus	T4200	CB 13.850	Tanga	TZ	38.924	-5.034
Leptopelis flavomaculatus	T4532	CB 14.1160	East Usambara Mountains	TZ	38.748	-5.059
Leptopelis flavomaculatus	T4533	CB 14.1161	East Usambara Mountains	TZ	38.649	-4.930
Leptopelis flavomaculatus	T4534	CB 14.1163	East Usambara Mountains	TZ	38.663	-4.904
Leptopelis flavomaculatus	T4536	CB 14.1164	East Usambara Mountains	TZ	38.662	-4.906
Leptopelis flavomaculatus	T4935	MTSN 9520	East Usambara Mountains	TZ	38.645	-4.971
Leptopelis flavomaculatus	T4936	MTSN 9521	East Usambara Mountains	TZ	38.758	-4.981
Leptopelis flavomaculatus	T4944	MTSN 9530	East Usambara Mountains	TZ	38.758	-4.981
Leptopelis flavomaculatus	T4960	MTSN 9552	East Usambara Mountains	TZ	38.762	-4.976
Leptopelis flavomaculatus	T4970	MTSN 9563	East Usambara Mountains	TZ	38.61	-5.113
Leptopelis flavomaculatus	T5235	SL 1181	Shimba Hills	KE	39.396	-4.236
Leptopelis flavomaculatus	T5236	SL 1182	Shimba Hills	KE	39.396	-4.236
Leptopelis flavomaculatus	T5237	SL 1183	Shimba Hills	KE	39.396	-4.236
Leptopelis flavomaculatus	T5238	SL 1184	Shimba Hills	KE	39.396	-4.236
Leptopelis flavomaculatus	T5239	SL 1185	Shimba Hills	KE	39.396	-4.236
Leptopelis flavomaculatus	T6396	SL 1431	Shimba Hills	KE	39.396	-4.236

Leptopelis flavomaculatus	T2466	MW 7915	Shimba Hills	KE	39.422	-4.443
Mertensophryne micranotis	T1882	MTSN 9558	East Usambara Mountains	TZ	38.762	-4.976
Mertensophryne micranotis	T2243	PK 118	Arabuko-Sokoke Forest	KE	39.669	-3.844
Mertensophryne micranotis	T2245	PK 064	Shimba Hills	KE	39.264	-4.487
Mertensophryne micranotis	T2246	VW 00465	Shimba Hills	KE	39.422	-4.235
Mertensophryne micranotis	T2247	VW 00462	Shimba Hills	KE	39.451	-4.215
Mertensophryne micranotis	T2291	BM 2002.343	East Usambara Mountains	TZ	38.744	-5.070
Mertensophryne micranotis	T2518	VW 679	Shimba Hills	KE	39.374	-4.665
Mertensophryne micranotis	T2519	VW 680	Shimba Hills	KE	39.374	-4.665
Mertensophryne micranotis	T3242	CB 13.889	Tanga	TZ	39.125	-4.773
Mertensophryne micranotis	T3243	CB 13.890	Tanga	TZ	39.125	-4.773
Mertensophryne micranotis	T3244	CB 13.891	Tanga	TZ	39.125	-4.773
Mertensophryne micranotis	T3252	CB 13.920	Tanga	TZ	39.125	-4.773
Mertensophryne micranotis	T4548	BM 2002.328	East Usambara Mountains	TZ	38.748	-5.059
Mertensophryne micranotis	T4549	BM 2002.891	East Usambara Mountains	TZ	38.807	-4.757
Mertensophryne micranotis	T4927	MTSN 9557	East Usambara Mountains	TZ	38.762	-4.976
Mertensophryne micranotis	T4965	MTSN 9558_double	East Usambara Mountains	TZ	38.762	-4.976
Mertensophryne micranotis	T4966	MTSN 9559	East Usambara Mountains	TZ	38.762	-4.976
Mertensophryne micranotis	T4974	MTSN 9568	East Usambara Mountains	TZ	38.737	-4.972
Mertensophryne micranotis	T5243	SL 1226	Shimba Hills	KE	39.425	-4.231

Mertensophryne micranotis	T5244	SL 1227	Shimba Hills	KE	39.425	-4.231
Mertensophryne micranotis	T5245	SL 1228	Shimba Hills	KE	39.425	-4.231
Mertensophryne micranotis	T5246	SL 1297	Shimba Hills	KE	39.485	-4.225
Mertensophryne micranotis	T5377	VW 00677	Shimba Hills	KE	39.374	-4.665
Mertensophryne micranotis	T5378	VW 00681	Shimba Hills	KE	39.374	-4.665
Mertensophryne micranotis	T6388	SL 1404	Shimba Hills	KE	39.396	-4.237
Mertensophryne micranotis	T6389	SL 1405	Shimba Hills	KE	39.396	-4.237
Mertensophryne micranotis	T6394	SL 1423	Shimba Hills	KE	39.396	-4.237
Mertensophryne micranotis	T6378	SL 2792	Shimba Hills	KE	39.405	-4.462
Mertensophryne micranotis	T6305	SL 2820	Arabuko-Sokoke Forest	KE	39.891	-3.324
Mertensophryne micranotis	T6306	SL 2822	Arabuko-Sokoke Forest	KE	39.891	-3.324
Mertensophryne micranotis	T4547	BM:2002.342	East Usambara Mountains	TZ	38.754	-5.059
Mertensophryne micranotis	T6405	SL 2735	Shimba Hills	KE	39.485	-4.225
Mertensophryne micranotis	T3253	CB 13.892	Tanga	TZ	39.125	-4.773
Phrynobatrachus acridoides	T4128	CB 13.778	Tanga	TZ	39.048	-5.073
Phrynobatrachus acridoides	T4129	CB 13.779	Tanga	TZ	39.048	-5.073
Phrynobatrachus acridoides	T4143	CB 13.793	Tanga	TZ	39.048	-5.073
Phrynobatrachus acridoides	T4144	CB 13.794	Tanga	TZ	39.048	-5.073
Phrynobatrachus acridoides	T4145	CB 13.795	Tanga	TZ	39.048	-5.073
Phrynobatrachus acridoides	T4146	CB 13.796	Tanga	TZ	39.048	-5.073

Phrynobatrachus acridoides	T4147	CB 13.797	Tanga	TZ	39.048	-5.073
Phrynobatrachus acridoides	T4154	CB 13.804	Tanga	TZ	38.924	-5.034
Phrynobatrachus acridoides	T4155	CB 13.805	Tanga	TZ	38.924	-5.034
Phrynobatrachus acridoides	T4174	CB 13.824	Tanga	TZ	38.924	-5.034
Phrynobatrachus acridoides	T4177	CB 13.827	Tanga	TZ	38.924	-5.034
Phrynobatrachus acridoides	T4191	CB 13.841	Tanga	TZ	38.924	-5.034
Phrynobatrachus acridoides	T4216	CB 13.866	Tanga	TZ	39.102	-4.632
Phrynobatrachus acridoides	T4218	CB 13.868	Tanga	TZ	39.102	-4.632
Phrynobatrachus acridoides	T4235	CB 13.885	Tanga	TZ	39.102	-4.632
Phrynobatrachus acridoides	T4239	CB 13.892	Tanga	TZ	39.117	-4.749
Phrynobatrachus acridoides	T4240	CB 13.893	Tanga	TZ	39.117	-4.749
Phrynobatrachus acridoides	T4241	CB 13.894	Tanga	TZ	39.117	-4.749
Phrynobatrachus acridoides	T4242	CB 13.895	Tanga	TZ	39.117	-4.749
Phrynobatrachus acridoides	T4243	CB 13.896	Tanga	TZ	39.117	-4.749
Phrynobatrachus acridoides	T4244	CB 13.897	Tanga	TZ	39.117	-4.749
Phrynobatrachus acridoides	T4245	CB 13.898	Tanga	TZ	39.117	-4.749
Phrynobatrachus acridoides	T4246	CB 13.899	Tanga	TZ	39.117	-4.749
Phrynobatrachus acridoides	T4247	CB 13.900	Tanga	TZ	39.117	-4.749
Phrynobatrachus acridoides	T4248	CB 13.901	Tanga	TZ	39.117	-4.749
Phrynobatrachus acridoides	T4249	CB 13.902	Tanga	TZ	39.117	-4.749

Phrynobatrachus acridoides	T4267	CB 13.921	Tanga	TZ	39.117	-4.749
Phrynobatrachus acridoides	T4268	CB 13.922	Tanga	TZ	39.117	-4.749
Phrynobatrachus acridoides	T4557	BM 2002.347	East Usambara Mountains	TZ	38.748	-5.059
Phrynobatrachus acridoides	T4558	BM 2002.230	East Usambara Mountains	TZ	38.733	-4.923
Phrynobatrachus acridoides	T4559	BM 2002.231	East Usambara Mountains	TZ	38.733	-4.923
Phrynobatrachus acridoides	T4560	BM 2002.739	East Usambara Mountains	TZ	38.643	-4.955
Phrynobatrachus acridoides	T4954	MTSN 9546	East Usambara Mountains	TZ	38.762	-4.976
Phrynobatrachus acridoides	T4958	MTSN 9550	East Usambara Mountains	TZ	38.762	-4.976
Phrynobatrachus acridoides	T6033	MW 07777	Shimba Hills	KE	39.422	-4.443
Phrynobatrachus acridoides	T6034	MW 07778	Shimba Hills	KE	39.422	-4.443
Phrynobatrachus acridoides	T6040	MW 07779	Shimba Hills	KE	39.422	-4.443
Phrynobatrachus acridoides	T5257	SL 1200	Shimba Hills	KE	39.425	-4.375
Phrynobatrachus acridoides	T5258	SL 1201	Shimba Hills	KE	39.425	-4.375
Phrynobatrachus acridoides	T5247	SL 1147	Shimba Hills	KE	39.396	-4.238
Phrynobatrachus acridoides	T5248	SL 1153	Shimba Hills	KE	39.417	-4.158
Phrynobatrachus acridoides	T5249	SL 1155	Shimba Hills	KE	39.417	-4.158
Phrynobatrachus acridoides	T5251	SL 1156	Shimba Hills	KE	39.417	-4.158
Phrynobatrachus acridoides	T5252	SL 1157	Shimba Hills	KE	39.417	-4.158
Phrynobatrachus acridoides	T5253	SL 1158	Shimba Hills	KE	39.417	-4.158
Phrynobatrachus acridoides	T5254	SL 1159	Shimba Hills	KE	39.417	-4.158

Phrynobatrachus acridoides	T5255	SL 1173	Shimba Hills	KE	39.431	-4.276
Phrynobatrachus acridoides	T5256	SL 1174	Shimba Hills	KE	39.431	-4.276
Phrynobatrachus acridoides	T5259	SL 1215	Shimba Hills	KE	39.417	-4.158
Phrynobatrachus acridoides	T5260	SL 1239	Shimba Hills	KE	39.445	-4.176
Phrynobatrachus acridoides	T5261	SL 1306	Shimba Hills	KE	39.485	-4.225
Phrynobatrachus acridoides	T5262	SL 1337	Shimba Hills	KE	39.425	-4.375
Phrynobatrachus acridoides	T6345	SL 1452	Shimba Hills	KE	39.419	-4.181
Phrynobatrachus acridoides	T6346	SL 2723	Shimba Hills	KE	39.396	-4.237
Phrynobatrachus acridoides	T6317	SL 2850	Arabuko-Sokoke Forest	KE	39.891	-3.324
Phrynobatrachus acridoides	T6318	SL 2895	Arabuko-Sokoke Forest	KE	39.891	-3.324
Phrynobatrachus acridoides	T6672	SL 2896	Arabuko-Sokoke Forest	KE	39.891	-3.324
Phrynobatrachus acridoides	T4998	MCZ A-32137	Tanga	TZ	38.141	-4.923
Phrynobatrachus acridoides	T6258	SL 1342	Arabuko-Sokoke Forest	KE	39.891	-3.324
Phrynobatrachus acridoides	T4124	CB:13:774	Tanga	TZ	39.048	-5.073
Phrynobatrachus acridoides	T6398	SL 2732	Shimba Hills	KE	39.396	-4.237
Ptychadena anchietae	T2976	CB 13.927	Tanga	TZ	39.117	-4.749
Ptychadena anchietae	T4114	CB 13.764	Tanga	TZ	38.646	-5.583
Ptychadena anchietae	T4149	CB 13.799	Tanga	TZ	39.048	-5.073
Ptychadena anchietae	T4173	CB 13.823	Tanga	TZ	38.924	-5.034
Ptychadena anchietae	T4176	CB 13.826	Tanga	TZ	38.924	-5.034

Ptychadena anchietae	T4205	CB 13.855	Tanga	TZ	39.102	-4.632
Ptychadena anchietae	T4206	CB 13.856	Tanga	TZ	39.102	-4.632
Ptychadena anchietae	T4207	CB 13.857	Tanga	TZ	39.102	-4.632
Ptychadena anchietae	T4208	CB 13.858	Tanga	TZ	39.102	-4.632
Ptychadena anchietae	T4209	CB 13.859	Tanga	TZ	39.102	-4.632
Ptychadena anchietae	T4210	CB 13.860	Tanga	TZ	39.102	-4.632
Ptychadena anchietae	T4230	CB 13.880	Tanga	TZ	39.102	-4.632
Ptychadena anchietae	T4231	CB 13.881	Tanga	TZ	39.102	-4.632
Ptychadena anchietae	T4253	CB 13.906	Tanga	TZ	39.117	-4.749
Ptychadena anchietae	T4254	CB 13.907	Tanga	TZ	39.117	-4.749
Ptychadena anchietae	T4255	CB 13.908	Tanga	TZ	39.117	-4.749
Ptychadena anchietae	T4961	MTSN 9553	East Usambara Mountains	TZ	38.762	-4.976
Ptychadena anchietae	T4962	MTSN 9554	East Usambara Mountains	TZ	38.762	-4.976
Ptychadena anchietae	T5002	MCZ A-32101	Tanga	TZ	39.059	-5.074
Ptychadena anchietae	T5004	MCZ A-32132	Tanga	TZ	38.115	-4.991
Ptychadena anchietae	T6037	MW 07786	Shimba Hills	KE	39.422	-4.443
Ptychadena anchietae	T6038	MW 07789	Shimba Hills	KE	39.422	-4.443
Ptychadena anchietae	T5282	SL 1193	Shimba Hills	KE	39.422	-4.443
Ptychadena anchietae	T5271	SL 1129	Shimba Hills	KE	39.396	-4.238
Ptychadena anchietae	T5272	SL 1130	Shimba Hills	KE	39.396	-4.238

Ptychadena anchietae	T5273	SL 1131	Shimba Hills	KE	39.396	-4.238
Ptychadena anchietae	T5274	SL 1148	Shimba Hills	KE	39.417	-4.158
Ptychadena anchietae	T5275	SL 1149	Shimba Hills	KE	39.417	-4.158
Ptychadena anchietae	T5276	SL 1150	Shimba Hills	KE	39.417	-4.158
Ptychadena anchietae	T5277	SL 1151	Shimba Hills	KE	39.417	-4.158
Ptychadena anchietae	T5278	SL 1152	Shimba Hills	KE	39.417	-4.158
Ptychadena anchietae	T5279	SL 1170	Shimba Hills	KE	39.431	-4.276
Ptychadena anchietae	T5280	SL 1171	Shimba Hills	KE	39.431	-4.276
Ptychadena anchietae	T5281	SL 1187	Shimba Hills	KE	39.396	-4.238
Ptychadena anchietae	T5283	SL 1223	Shimba Hills	KE	39.440	-4.128
Ptychadena anchietae	T5287	SL 1246	Mpeketoni	KE	40.697	-2.385
Ptychadena anchietae	T5288	SL 1255	Mpeketoni	KE	40.686	-2.409
Ptychadena anchietae	T5264	SL 1281	Shimba Hills	KE	39.396	-4.238
Ptychadena anchietae	T5265	SL 1282	Shimba Hills	KE	39.396	-4.238
Ptychadena anchietae	T5284	SL 1283	Shimba Hills	KE	39.396	-4.238
Ptychadena anchietae	T5266	SL 1284	Shimba Hills	KE	39.396	-4.238
Ptychadena anchietae	T5267	SL 1285	Shimba Hills	KE	39.396	-4.238
Ptychadena anchietae	T5268	SL 1326	Shimba Hills	KE	39.431	-4.276
Ptychadena anchietae	T5269	SL 1327	Shimba Hills	KE	39.431	-4.276
Ptychadena anchietae	T6392	SL 1410	Shimba Hills	KE	39.485	-4.225

Ptychadena anchietae	T6273	SL 1470	Arabuko-Sokoke Forest	KE	39.976	-3.263
Ptychadena anchietae	T6401	SL 2729	Shimba Hills	KE	39.485	-4.225
Ptychadena anchietae	T6320	SL 2854	Arabuko-Sokoke Forest	KE	39.891	-3.324
Ptychadena anchietae	T6328	SL 2869	Arabuko-Sokoke Forest	KE	39.904	-3.200
Ptychadena anchietae	T6329	SL 2870	Arabuko-Sokoke Forest	KE	39.904	-3.200
Ptychadena anchietae	T6661	SL 2888	Arabuko-Sokoke Forest	KE	39.976	-3.263
Ptychadena anchietae	T2975	CB 13.926	Tanga	TZ	39.117	-4.749
Ptychadena anchietae	T6381	SL 1370	Shimba Hills	KE	39.396	-4.238
Ptychadena anchietae	T6035	MW 07787	Shimba Hills	KE	39.422	-4.443
Sclerophrys gutturalis	T4116	MW 7922	Tanga	KE	39.557	-3.981
Sclerophrys gutturalis	T4117	CB 13.766	Tanga	TZ	38.646	-5.583
Sclerophrys gutturalis	T4118	CB 13.767	Tanga	TZ	38.646	-5.583
Sclerophrys gutturalis	T4272	CB 13.768	Tanga	TZ	38.646	-5.583
Sclerophrys gutturalis	T4468	CB 13.929	East Usambara Mountains	TZ	39.117	-4.749
Sclerophrys gutturalis	T4469	BM 2005.1298	East Usambara Mountains	TZ	38.703	-4.81
Sclerophrys gutturalis	T5309	SL 1114	Shimba Hills	KE	39.462	-4.429
Sclerophrys gutturalis	T5310	SL 1231	Shimba Hills	KE	39.445	-4.176
Sclerophrys gutturalis	T5311	SL 1232	Shimba Hills	KE	39.445	-4.176
Sclerophrys gutturalis	T5312	SL 1233	Shimba Hills	KE	39.445	-4.176
Sclerophrys gutturalis	T5313	SL 1234	Shimba Hills	KE	39.445	-4.176

Sclerophrys gutturalis	T6314	SL 1250	Mpeketoni	KE	40.686	-2.409
Sclerophrys gutturalis	T6330	SL 2873	Arabuko-Sokoke Forest	KE	39.904	-3.2
Sclerophrys pusilla	T2457	MW 7780	Shimba Hills	KE	39.422	-4.443
Sclerophrys pusilla	T4119	CB 13.769	Tanga	TZ	39.048	-5.073
Sclerophrys pusilla	T4125	CB 13.775	Tanga	TZ	39.048	-5.073
Sclerophrys pusilla	T4127	CB 13.777	Tanga	TZ	39.048	-5.073
Sclerophrys pusilla	T4188	CB 13.838	Tanga	TZ	38.924	-5.034
Sclerophrys pusilla	T4476	CB 14.1104	East Usambara Mountains	TZ	38.751	-5.054
Sclerophrys pusilla	T4477	CB 14.1105	Tanga	TZ	38.727	-4.950
Sclerophrys pusilla	T4478	CB 14.1106	East Usambara Mountains	TZ	38.814	-4.791
Sclerophrys pusilla	T5315	SL 1208	Kwale	KE	39.429	-4.392
Sclerophrys pusilla	T5314	SL 1207	Shimba Hills	KE	39.429	-4.392
Sclerophrys pusilla	T6331	SL 2874	Arabuko-Sokoke Forest	KE	39.904	-3.200
Sclerophrys pusilla	T6332	SL 2875	Arabuko-Sokoke Forest	KE	39.904	-3.200
Sclerophrys pusilla	T6333	SL 2876	Arabuko-Sokoke Forest	KE	39.904	-3.200
Sclerophrys pusilla	T6334	SL 2877	Arabuko-Sokoke Forest	KE	39.904	-3.200
Sclerophrys pusilla	T6335	SL 2878	Arabuko-Sokoke Forest	KE	39.904	-3.200
Sclerophrys pusilla	T2219	PK 126	Arabuko-Sokoke Forest	KE	39.669	-3.844
Sclerophrys steindachneri	T2516	VW 597	Shimba Hills	KE	39.433	-4.275
Sclerophrys steindachneri	T2517	VW 614	Shimba Hills	KE	39.440	-4.128

Sclerophrys steindachneri	T5318	SL 1199	Shimba Hills	KE	39.417	-4.158
Sclerophrys steindachneri	T5316	SL 1245	Mpeketoni	KE	40.686	-2.409
Sclerophrys steindachneri	T5317	SL 1257	Mpeketoni	KE	40.686	-2.409
Sclerophrys steindachneri	T6674	SL 2898	Arabuko-Sokoke Forest	KE	39.976	-3.263
Sclerophrys steindachneri	T5312	SL 1234	Shimba Hills	KE	39.396	-4.238
Sclerophrys steindachneri	T6334	SL 2877	Arabuko-Sokoke Forest	KE	39.904	-3.200
Sclerophrys steindachneri	T6331	SL 2874	Arabuko-Sokoke Forest	KE	39.904	-3.200
Sclerophrys steindachneri	T6332	SL 2875	Arabuko-Sokoke Forest	KE	39.904	-3.200
Sclerophrys steindachneri	T6333	SL 2876	Arabuko-Sokoke Forest	KE	39.904	-3.200
Sclerophrys steindachneri	T6335	SL 2878	Arabuko-Sokoke Forest	KE	39.904	-3.200
Scolecomorphus vittatus	T226	KMH 21262	East Usambara Mountains	TZ	NULL	NULL
Scolecomorphus vittatus	T228	KMH 21263	East Usambara Mountains	TZ	NULL	NULL
Scolecomorphus vittatus	T441	MW 03040	East Usambara Mountains	TZ	NULL	NULL
Scolecomorphus vittatus	T4790	WTS 1572	East Usambara Mountains	TZ	38.717	-4.95
Scolecomorphus vittatus	T4791	WTS 1548	East Usambara Mountains	TZ	38.717	-4.95
Scolecomorphus vittatus	T5299	SL 1244	Shimba hills National reserve	KE	39.396	-4.236
Xenopus muelleri	T2977	CB 13.928	Tanga	TZ	39.117	-4.749
Xenopus muelleri	T4126	CB 13.776	Tanga	TZ	39.048	-5.073
Xenopus muelleri	T4140	CB 13.790	Tanga	TZ	39.048	-5.073
Xenopus muelleri	T4141	CB 13.791	Tanga	TZ	39.048	-5.073

Xenopus muelleri	T4142	CB 13.792	Tanga	TZ	39.048	-5.073
Xenopus muelleri	T4150	CB 13.800	Tanga	TZ	39.048	-5.073
Xenopus muelleri	T4190	CB 13.840	Tanga	TZ	38.924	-5.034
Xenopus muelleri	T4211	CB 13.861	Tanga	TZ	39.102	-4.632
Xenopus muelleri	T4212	CB 13.862	Tanga	TZ	39.102	-4.632
Xenopus muelleri	T4213	CB 13.863	Tanga	TZ	39.102	-4.632
Xenopus muelleri	T4214	CB 13.864	Tanga	TZ	39.102	-4.632
Xenopus muelleri	T4215	CB 13.865	Tanga	TZ	39.102	-4.632
Xenopus muelleri	T4232	CB 13.882	Tanga	TZ	39.102	-4.632
Xenopus muelleri	T4265	CB 13.918	Tanga	TZ	39.117	-4.749
Xenopus muelleri	T4266	CB 13.919	Tanga	TZ	39.117	-4.749
Xenopus muelleri	T4931	MTSN 9513	East Usambara Mountains	TZ	38.758	-4.981
Xenopus muelleri	T4932	MTSN 9514	East Usambara Mountains	TZ	38.758	-4.981
Xenopus muelleri	T5300	SL 1205	Shimba Hills	KE	39.425	-4.375
Xenopus muelleri	T5301	SL 1224	Shimba Hills	KE	39.440	-4.128
Xenopus muelleri	T5302	SL 1240	Shimba Hills	KE	39.445	-4.176
Xenopus muelleri	T6399	SL 2727	Shimba Hills	KE	39.396	-4.237
Xenopus muelleri	T4598	CB 14.1224	East Usambara Mountains	TZ	38.807	-4.757

Synthesis

Introduction

The Shimba Hills of Kenya (SHK) is located at the crossroads of two biodiversity hotspots; the Coastal Forest of Eastern Africa (CFEA) and the Eastern Afromontane Biodiversity Region (EABR). In addition mixed assemblages of flora and fauna have been recorded in SHK including; endemic species, species only shared between SHK and EAM, species shared between SHK and CFEA plus overlapping species found in the three areas and even species shared with west and Central African countries (Burgess & Clarke, 2000). However no study has ever investigated the biogeographical affiliation of SHK to these hotspots. In this thesis I sought to understand the biogegraphic history of SHK using a combination of molecular and spatial analysis of its amphibian assemblage.

Before I could perform analysis of phylogeographic patterns of amphibians of SHK, it was necessary to first sample the area extensively and compile a species list of its amphibians and this is the subject of the first chapter. Despite the fact that SHK is an important conservation area along the coastal Kenya tourist circuit, information about its amphibian fauna was very scarce prior to this thesis. I dedicated the first chapter to compiling recent fieldwork and all known amphibian records from SHK, consolidating them into the first ever annotated checklist of amphibians of Shimba Hills National Reserve. The reserve plus the entire SHK area contains the highest number of amphibian diversity for any known locality in Kenya (compare, Malonza &Veith, 2012; Wasonga et al., 2007). Therefore its continued conservation will ensure about 30% of Kenya's amphibian species are preserved. Apart from the checklist, Chapter 1 also reports on two interesting records; a new country record for the caecilian, *Scolecomorphus* cf. *vittatus* an EAM endemic species (Howell, 1993; Poynton, 2000; Harper et al., 2010). In addition I report on the rediscovery of a *Callulina sp.* lastly collected in 1961 (Loader et al., 2010). Amphibian surveys began in the SHK around 1960's (Chapter 1) however, new records and/or species rediscoveries are still being made implying that, through systematic sampling, more species may still be recovered in this area and thus the checklist is not an end in itself to the search for more amphibian species and/or new records in this area.

Major findings

The taxonomic status of *H. rubrovermiculatus* has been uncertain as the species has been synonimized with *H. mitchelli* (Channing & Howell, 2006) a wide ranging species from Tanzania to Zimbabwe (Poynton & Broadley, 1967). In Chapter 2, I showed that *H. rubrovermiculatus* is genetically and morphologically distinct from *H. mitchelli.* Furthermore, *H. mitchelli* was recovered as paraphyletic with a population occurring in north eastern Tanzania genetically closer to *H. rubrovermiculatus* than to *H. mitchelli* from Central and Southern Tanzania to Malawi. I propose description of the population from north eastern Tanzania as a new species- (*H. new sp.*). Using dorsal colour patterns and skin texture I showed that *H. rubrovermiculatus* is distinct from its sister species *H. new sp*. These findings raise interesting questions regarding what drives dorsal colour pattern evolution within Hyperoliid frogs which may be worthy of future investigations. In this chapter, I have demonstrated the benefits of applying integrated taxonomic analysis approaches in unravelling cryptic diversity in Hyperollids in the region. The same approach may be used in other species in the area in which high genetic distances was recorded between sister clades (see chapter 4). The confirmation of the species status of *H. rubrovermiculatus* is important since apart from being endemic to the SHK, the species is also listed as endangered (IUCN, accessed on 8th March 2017) and therefore its conservation requires targeted approaches which can only be done if its taxonomic status is known. While the proposed description of *H. new sp.* from the once wide spread *H. mitchelli* brings to our attention the need to re-visit the taxonomy of similar wide spread species in the area using integrated taxonomic tools.

Loader et al. (2010) reported on the presence of a *Callulina* sp. from SHK based on a single specimen recovered from the American Museum of Natural History and speculated it to be either *C. kisiwamsitu* or *C. stanleyi* if not a new species. In chapter 3, I report on the rediscovery of this *Callulina.* Using Bayesian and maximum Likelihood analysis of three different genes (12S, 16S, Cytochrome b) and a concatenated analysis of these three genes, I show that the *Callulina* from the Shimba Hills is genetically similar to *C. kreffti.* Three *Callulina* records from SHK have been made within the SHNR, all of them recorded from Makadara forest fragment indicating their restricted distribution in the area. In addition, the fact that just a few specimens have been recovered may indicate low population size of this species within the SHNR. The chapter also includes proposed description of three new *Callulina* found in the EAM and points to the importance of combining morphology and DNA analysis to identify the currently recognized 12 *Callulina* species.

Combining molecular and spatial analysis, I have showed in Chapter 4 that SHK amphibians have different biogeographic histories. Lack of concordant phylogeographic breaks showed that these species lack a common biogeographic history. While some species appear to have occupied the SHK for long periods (*H. rubrovermiculatus*, *A. sylvaticus*, *A. stenodactylus*) other species seem to be recent dispersals. Based in multispecies comparisons, I found SHK amphibians to be more closely related to the CFEA than the EABR. This is demonstrated by the number of species that formed groupings with CFEA (4) and EABR (0) based on the species with overlapping samples across the five study sites. The divergence patterns among species however varied between species and areas with recent (1.9 myr) and old (5.5 myr) divergences within northern phylogeographic break and recent (1.5myr) and old (7.3 myr) divergences within the southern phylogeographic break. The seeming closer affiliation between SHK and CFEA is an important finding, especially since along the coastal Kenya, there is rapid and increasing habitat destruction which may pertub amphibian population. Given that SHK has been connected to the neighbouring CFEA relatively recently, migration corridors between these forests should be maintained if species are likely to maintain viable populations.

Conservation and management implications

Understanding genetic variations within species is important for conservation purposes as areas harbouring evolutionarily unique populations may be considered more valuable for conservation purposes. In addition understanding historical factors that have shaped the genetic patterns of an area is important as such information can be used to formulate management strategies to meet conservation challenges such as climate change and habitat destruction. Recent studies have demonstrated how species level conservation greatly underestimates intraspecific genetic diversity which is equally important for conservation (Rissler et al., 2006; Barrat et al., 2017). Such studies call for the need to identify the unique evolutionary units within wide ranging species and the underlying factors that generated them in order to inform both current and future conservation measures. Current and projected future climate change plus habitat destruction within the Coastal forests of eastern Africa and Eastern Afromontane biodiversity region (Burgess & Clarke, 2000) require that measures should be taken to conserve these important repositories for biodiversity. One such measure is to identify areas with unique evolutionary units for targeted conservation action. At a species level, only *H. rubrovermiculatus* is endemic to SHK, however results from this thesis (Chapter 4) points to more than one unique lineages that are independently evolving within SHK; populations of *A. sylvaticus, A. xenodactyloides* and *H. pusillus* have been recovered as monophyletic. These populations separated from neighbouring populations a long time ago (Pliocene) and the levels of sequence divergence reported within some species are significant (Chapter 4). These populations are speculated to be evolutionary significant units (ESU) *sensu* Moritz (1994). Rigorous analysis involving more sample size, additional molecular markers plus morphological and bioaccoustic analysis may be required to confirm their ESU or otherwise status. If these categories are confirmed then conservation activities geared towards their protection area necessary.

Limitations

Even though efforts were made to acquire as much data as possible for this study, one of the major constraints is incomplete sampling across the region. This therefore means that some of the phylogeographic patterns reported in this thesis remain tentative pending further more spatially comprehensive sampling. For example, the two EAM species that have been recorded from SHK, only one sequence was available for *S. cf. vittatus* and two for *C. kreffti*. More data are needed to better understand the phylogeography of these two species to shed more light on the relationship between SHK and EAM. For some species only DNA data from the Kenyan side was available, *Hyperolius pusillus*, *Kassina maculata*, *Sclerophrys steindachneri.* Given the complex phylogeographic patterns exhibited by various amphibian species from the study area, more data are needed to get the better picture of their phylogeographic patterns. GPS co-ordinates for some species were not representative and therefore the habitat suitability maps predicted for such species should be interpreted with much caution.

The dating of divergence time given in this thesis may be considered tentative since it is based on single genes and no amphibian fossil exists in the area with which we could provide primary calibration dates for the phylogenetic trees. However our results are comparable with other studies on amphibians in the area (Lawson et al., 2010, 2013; Blackburn & Measey, 2009).

Future directions

Almost nothing is known about the ecology of the only endemic amphibian from SHK. Since *H. rubrovermiculatus* is also listed as endangered by the IUCN Red List of threatened species, it is important that the information necessary for its effective conservation is documented and this should be done as a matter of urgency.

The two EAM species so far recorded from SHK are both known from Makadara forest within the SHNR. In addition, only three records of *C. kreffti* and one of *S. cf. vittatus* are known. More intensive studies need to be conducted in all remaining forest fragments in SHK to establish their population status and also to shed more light on their phyogeographic affiliations.

A. sylvaticus is listed as vulnerable based on the IUCN Redlist and its range is given as from SHK to Central Usambara, based on this study the SHK population is divergent (2.2%) from the Tanzania samples. Estimated divergence time places the SHK population at about 2.8 million years old and has not undergone any recent population expansions. Evidence shows that species formerly thought to be widespread in the region might actually represent cryptic species (Loader et al., 2015; Barrat et al., 2017; Chapter 2). Studies incorporating bioacoustics, morphology and multilocus DNA analysis is required to confirm the taxonomic status of the SHK population as it may be endemic to just the SHK and hence deserves taxonomic recognition and the likely changes to its IUCN RedList status.

Conclusion

Based on its amphibian assemblages, SHK is an important area for biodiversity conservation.

Apart from the fact that it holds the highest amphibian diversity in Kenya, it also holds a diverse assemblage of amphibians, such as potential ESU, one endemic species, EAM, coastal and wide ranging species. No other area in Kenya is known to hold such a mixed diversity of amphibians. Concerted efforts are therefore required to protect this unique diversity at SHK.

In this thesis I have established based on multi-species phylogeographic and spatial analysis, that SHK is more closely related to the CFEA through habitat connectivity both current and in the past. The hills have also been relatively stable to allow for the evolution of an endemic species plus several potential ESUs. The conservation of SHK is therefore a matter of high importance. The results presented here are the first to establish the biogeographic affinity of the SHK with the adjacent hotspots. It is important that similar studies to be carried out using other taxa for more insight on the biogeographic history of SHK.

References

Barratt, C.D., Bwong, B.A., Ostein, R.E., Rosauer, D.F., Doggart N., Nagel, P., Kissling, W.D & Loader S.P. 2017. Environmental correlates of phylogenetic endemism in amphibians and conservation of refugia in the Coastal Forests of Eastern Africa. *Diversity and distributions* **23**:875–888.

Barrat, C.D., Lawson L.P., Bittencourt-Silva, G.B., Doggart, N., Morgan-Brown, T. Nagel, P & Loader, S.P. 2017. Anew narrowly distributed and critically endangered species of spiny reed-frog (Anura, Hyperoliidae) from a highly threatened coastal forest reserve in Tanzania. *Herpetological Journal* **27**: 13–24.

Bennun, L. & Njoroge, P. 1999. *Important Bird Areas*. East Africa Natural History, Nairobi. Pp. 116–120.

Blackburn, D.C. & Measey, G.J. 2009. Dispersal to or from an African biodiversity hotspot? *Molecular Ecology* **18**: 1904–1915. http://doi.org/10.1111/j.1365-294X.2009.04156.x.

Burgess, N.D. & Clarke, G.P. (eds) 2000. *Coastal forests of Eastern Africa*, Xiii +443pp. IUCN, Gland, Switzerland and Cambridge, UK.

Channing, A. & Howell, K.M. 2006. *Amphibians of East Africa*. Cornell University Press Ithaca, New York.

Harper, E.B., Measey, G.J., Patrick, D.A., Menegon, M. & Vonesh, J.R. 2010. *Field guide to amphibians of the Eastern Arc Mountains and Coastal Forests of Tanzania and Kenya.* Camera Prix Publishers International, Nairobi, Kenya.

Howell, K.M. 1993. Herpetofauna of the East African Forests. In J.C. Lovett & S.K. Wasser (eds.), *Biogeography of the Rain Forests of Eastern Africa*. Cambridge University Press, Cambridge. Pp. 173–201.

Lawson, L.P. 2010. The discordance of diversification: Evolution in the tropical-montane frogs of the Eastern Arc Mountains of Tanzania. *Molecular Ecology* **19**: 4046–4060. http://doi.org/10.1111/j.1365-294X.2010.04788.x2013.

Lawson, L.P. 2013. Diversification in a biodiversity hot spot: landscape correlates

of phylogeographic patterns in the African spotted reed frog. *Molecular Ecology* **22**: 1947–1960.

Luke, Q. 2005. Annotated checklist of the plants of the Shimba Hills, Kwale District, Kenya. *Journal of East African Natural History* **94:** 5–120. http://doi.org/10.2982/0012–8317(2005)94[5:ACOTPO]2.0.CO;2.

Loader, S.P., Gower, D.J., Ngalason, W. & Menegon, M. 2010. Three new species of *Callulina* (Amphibia: Anura: Brevicipitidae) highlight local endemism and conservation plight of Africa's Eastern Arc forests. *Zoological Journal of the Linnean Society* **160**: 496–514. http://doi.org/10.1111/j.1096-3642.2010.00652.x.

Loader S.P., Lawson, L.P., Portick, D.M. & Menegon, M. 2015. Three new species of Spiny throated reed frogs (Anura: Hyperoliidae) from ever green forests of Tanzania. *BMC Research notes.* **8**: 167 doi 10.1186/s13104-015-1050-y.

Malonza, P.K. & Veith, M. 2012. Amphibian community along elevational and habitat

disturbance gradients in the Taita Hills, Kenya. *Herpetotropicos* **7**: 7–16.

Masters, B.C., Fan, V., & Ross, H.A. 2011. Species delimitation: Geneious plugin for the exploration of species boundaries. *Molecular Ecology Resources* **11**: 154–157.

Moritz, C. 1994. Defining 'evolutionarily significant units' for conservation. *Trends in Ecology & Evolution*, **91**: 373–375.

Poynton, J.C. & Broadley, D.G. 1987. Amphibia Zambesiaca 3. Rhacophoridae and Hyperoliidae. *Annals of the Natal Museum* **28**: 161–229. Retrieved from http://content.ajarchive.org/cgibin/showfile.exe?CISOROOT=/03040798&CISOPTR=421%5Cn http://hdl.handle.net/10499/AJ401.

Poynton, J.C. 2000. Amphibians. *The Coastal forests of Eastern Africa. IUCN, Gland*, 201–210.

Rissler, L.J., Hijmans, R.J., Graham, C.H., Moritz, C. & Wake, D.B. 2006. Phylogeographic lineages and species comparisons in conservation analysis: A case study of California herpetofauna. *American Naturalist* **167**: 655–666.

Schiøtz, A. 1975. *The Treefrogs of Eastern Africa*. Steenstrupia, Copenhagen.

Schiøtz, A. 1999. *The Treefrogs of Africa*. Ed. Chimaira. Frankfurt am Main.

Spawls, S., Howell, K. & Drewes, R.C. 2006. *Reptiles and amphibians of East Africa* Princeton University Press.

Wasonga, V.D., Bekele, A., Lötters, S. & Balakrishnan, M. 2007. Amphibian abundance and diversity in Meru national park, Kenya. *African Journal of Ecology* **451**: 55–61.

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Supplementary Materials

Supplementary chapter 1

Appendix 1. A list of all known amphibian records from Shimba Hills National Reserve indicating museum number, collector name, date and locality. Records with stars were obtained from the HerpNet.

Museum ID	Species	Collection date	Collector	Locality
*LACM 50633	*Hyperolius rubrovermiculatus*	2 Apr 1968	A. Williams	Shimba Hills Rainforest
NMK A737/1–2	*Xenopus muelleri*	May 1968	A. D. Mackay	Shimba Hills
NMK A739/1–9	*Kassina maculata*	Jun 1968	A. D. Mackay	Shimba Hills
NMK A3003/1–5	*Kassina maculata*	20 May 1968	A. Schiøtz & A.D. Mackay	Shimba Hills
NMK A787	*Leptopelis flavomaculatus*	Nov 1968	D. Sheldrick	Near Giriama point
NMK A788	*Hyperolius rubrovermiculatus*	Dec 1968	D. Sheldrick	Near Giriama point
NMK A3041/1–2	*Hyperolius argus*	20 May 1968	A. Schiøtz & A.D. Mackay	Shimba Hills
NMK A3096/1–39	*Hyperolius mariae*	20 Jun 1968	A. Schiøtz & A.D. Mackay	Shimba Hills
ZMUC-R 73916–937	*Hyperolius acuticeps*	20 May 1968	A. Schiøtz	Shimba Hills
ZMUC-R73854	*Hyperolius rubrovermiculatus*	20 May 1968	A Schiøtz	Shimba Hills
ZMUC-R73855	*Afrixalus delicatus*	20 May 1968	A. Schiotz	Shimba Hills
ZMUC-R73948/49	*Afrixalus delicatus*	20 May 1968	A. Schiøtz	Shimba Hills
ZMUC-R77457/458	*Afrixalus delicatus*	20 May 1968	A. Schiøtz	Shimba Hills
NMK A3169	*Hyperolius rubrovermiculatus*	no date	A. D. Mackay,	Sheldrick Falls
NMK A1150/1–9	*Mertensophryne micranotis*	Apr-Jun 1977	A. D. Mackay	Makadara Forest
BMNH 1980.195	*Mertensophryne micranotis*	1977	?	Shimba Hills

BMNH 1980.197	*Mertensophryne micranotis*	1977	L. P. Lounibos	Shimba Hills
BMNH 1982.395–396	*Mertensophryne micranotis*	Apr 1977	L. P. Lounibos	Makadara Forest
*CAS 153633–40	*Leptopelis flavomaculatus*	12 Apr 1981	S. Reilly	Makadara Forest, picnic site
*CAS 153694	*Xenopus muelleri*	13 Apr 1981	S. Reilly	Shimba Hills
*CAS 153695	*Kassina senegalensis*	14 Apr 1981	S. Reilly	200 m. S of Risley's Ridge turnaround
*CAS 153697	*Ptychadena anchietae*	15 Apr 1981	S. Reilly	200 m. S of Risley's Ridge turnaround
*CAS 153698	*Mertensophryne micranotis*	16 Apr 1981	S. Reilly	Shimba Hills, campsite 1
*CAS 153709–11	*Hyperolius tuberilinguis*	17 Apr 1981	S. Reilly	Shimba Hills
BMNH 1982.842	*Sclerophrys gutturalis*	6 May 1981	A. Grandison	Shimba Hills
BMNH1982.857–859	*Afrixalus sylvaticus*	6 May 1981	A. Grandison	Sheldrick Falls
BMNH 1982.860–887	*Hyperolius rubrovermiculatus*	6 May 1981	A. Grandison	Sheldrick Falls
*CAS 155604	*Arthroleptis xenodactyloides*	6 Jul 1981	M. Tandy	Makadara Forest
*CAS 155606	*Sclerophrys pusilla*	6 Jul 1981	M. Tandy	Marere head works
*CAS 155613–20	*Sclerophrys pusilla*	6 Jul 1981	M. Tandy	Marere head works
*CAS 155621–23	*Phrynobatrachus acridoides*	6 Jul 1981	M. Tandy	Marere head works
*CAS 155624	*Ptychadena anchietae*	6 Jul 1981	M. Tandy	Marere head works
*CAS 155626–29	*Xenopus muelleri*	6 Jul 1981	M. Tandy	Shimba Hills National Reserve
*CAS 155630–31	*Leptopelis flavomaculatus*	6 Jul 1981	M. Tandy	Marere head works
*CAS 155632–34	*Phrynobatrachus acridoides*	6 Jul1981	M. Tandy	Marere head works
*CAS 155635–46	*Hyperolius rubrovermiculatus*	12 Jul 1981	M. Tandy	Marere head works

*CAS 155647–50	*Sclerophrys pusilla*	12 Jul 1981	M. Tandy	Marere head works
*CAS 155652–54	*Afrixalus sylvaticus*	13 Jul 1981	M. Tandy	Marere head works
*CAS 155655–67	*Sclerophrys pusilla*	17 Jul 1981	M. Tandy	Marere head works
*CAS 155668–69	*Xenopus muelleri*	17 Jul1981	M. Tandy	Marere head works
*CAS 155671–77	*Arthroleptis stenodactylus*	18 Jul 1981	M. Tandy	Makadara forest
*CAS 155883	*Sclerophrys pusilla*	17 Jul 1981	M. Tandy	Marere head works
*CAS 155932–46	*Hyperolius rubrovermiculatus*	11 Jul 1981	M. Tandy	Below Marere head works
*CAS 155947	*Afrixalus sylvaticus*	11 Jul 1981	M. Tandy	5 km N main gate - Kwale entrance into SHNR
*CAS 157491	*Ptychadena anchietae*	Feb 1984	M. Ryan	6 km N main gate - Kwale entrance into SHNR
*CAS 157492	*Afrixalus fornasini*	Feb 1984	M. Ryan	7 km N main gate - Kwale entrance into SHNR
*CAS 157493	*Sclerophrys pusilla*	Feb 1984	M. Ryan	8 km N main gate - Kwale entrance into SHNR
*CAS 157494–95	*Phrynobatrachus acridoides*	Feb 1984	M. Ryan	9 km N main gate - Kwale entrance into SHNR
*CAS 157496–98	*Hyperolius mariae*	Feb 1984	M. Ryan	10 km N main gate - Kwale entrance into SHNR
MVZ 233935	*Hyperolius rubrovermiculatus*	5 Jun 1998	Dan R. Buchholz et al	Shimba Hills
MVZ 233824	*Afrixalus sylvaticus*	5 Jun 1998	Dan R. Buchholz et al	Shimba Hills
MVZ 233910	*Hyperolius parkeri*	5 Jun 1998	Dan R. Buchholz et al	Shimba Hills
MVZ 233825	*Afrixalus sylvaticus*	5 Jun 1998	Dan R. Buchholz et al	Shimba Hills
MVZ 233909	*Hyperolius parkeri*	5 Jun 1998	Dan R. Buchholz et al	Shimba Hills
NMK A3550/1–7	*Ptychadena anchietae*	3 Jul 1998	A. Wise, Weatherby, C. & Ross, K.	Shimba Hills
NMK A3553/1–6	*Xenopus muelleri*	3 Jul 1998	A. Wise, Weatherby, C. & Ross, K.	Shimba Hills

NMK A3582/1–2	*Sclerophrys pusilla*	3 Jul 1998	A. Wise, Weatherby, C. & Ross, K.	Shimba Hills
NMK A4448/1–6	*Arthroleptis xenodactyloides*	29–30 Nov 2005	P. K. Malonza & J.G. Measey	Longomwagandi Forest
NMK A4395/1–11	*Boulengerula changamwensis*	29–30 Nov 2005	P. K. Malonza & J.G. Measey	Longomwagandi Forest
NMK A4401/1–6	*Arthroleptis stenodactylus*	29–30 Nov 2005	P. K. Malonza & J.G. Measey	Longomwagandi Forest
NMK A4440	*Afrixalus sylvaticus*	28 Nov 2005	P. K. Malonza & J.G. Measey	Sheldrick Falls
NMK A4442	*Xenopus muelleri*	29 Nov 2005	P. K. Malonza & J.G. Measey	Kivumoni Swamp
NMK A4443/1–5	*Ptychadena anchietae*	28 Nov 2005	P. K. Malonza & J.G. Measey	Bufallo River
NMK A4448/1–6	*Arthroleptis xenodactyloides*	29–30 Nov 2005	P. K. Malonza & J.G. Measey	Longomwagandi Forest
NMK A4449	*Hyperolius pusillus*	29 Nov 2005	P. K. Malonza & J.G. Measey	Kivumoni Gate Swamp
NMK A4452	*Sclerophrys steindachneri*	28 Nov2005	P. K. Malonza & J.G. Measey	Sheldrick Falls
NMK A4455/1–2	*Kassina maculata*	29 Nov 2005	P. K. Malonza & J.G. Measey	Kivumoni Gate Swamp
NMK A4458/1–4	*Afrixalus fornasini*	29 Nov 2005	P. K. Malonza & J.G. Measey	Kivumoni Gate Swamp
NMK A4459/1–8	*Arthroleptis xenodactyloides*	28–29 Nov 2005	P. K. Malonza & J.G. Measey	Makadara Forest
NMK A4461	*Sclerophrys steindachneri*	28–29 Nov 2005	P. K. Malonza & J.G. Measey	Kivumoni Gate Swamp
NMK A4450/1–5	*Hyperolius tuberilinguis*	29 Nov 2005	P. K. Malonza & J.G. Measey	Kivumoni Gate Swamp
NMK A4653/1–2	*Arthroleptis xenodactyloides*	29 Nov 2005	P. K. Malonza & J.G. Measey	Makadara Forest
NMK A4653/1–2	*Arthroleptis stenodactylus*	30 Nov 2005	P. K. Malonza & J.G. Measey	Longomwagandi Forest
NMK A4460/1–3	*Arthroleptis stenodactylus*	30 Nov 2005	P. K. Malonza & J.G. Measey	Longomwagandi Forest
NMK A4445	*Hyperolius rubrovermiculatus*	28 Nov 2005	P. K. Malonza & J.G. Measey	Sheldrick Falls
NMK A4447/1–3	*Hyperolius rubrovermiculatus*	29 Nov 2005	P. K. Malonza & J.G. Measey	Kivumoni Gate Swamp

NMK A4450/1–5	*Hyperolius tuberilinguis*	29 Nov 2005	P. K. Malonza & J.G. Measey	Kivumoni Gate Swamp
NMK A4615/1–6	*Afrixalus fornasini*	22 Apr 2006	B. Bwong, J.G. Measey & Venu	Kivumoni Gate Swamp
NMK A4613	*Arthroleptis stenodactylus*	22 Apr 2006	B. Bwong, J.G. Measey & Venu	Longomwagandi Forest
NMK A4619/1–7	*Hyperolius argus*	23 Apr 2006	B. Bwong, J.G. Measey & Venu	Kivumoni Gate Swamp
NMK A4686/1–3	*Ptychadena anchietae*	13–16 Sep 2006	Jos Kielgast	Shimba Hills
NMK A4689/1	*Sclerophrys steindachneri*	13–16 Sep 2006	Jos Kielgast	Shimba Hills
NMK A4690/1–7	*Afrixalus fornasini*	13–16 Sep 2006	Jos Kielgast	Shimba Hills
NMK A4693/1–4	*Xenopus muelleri*	13–16 Sep 2006	Jos Kielgast	Shimba Hills
NMK A4694	*Xenopus muelleri*	13–16 Sep 2006	Jos Kielgast	Shimba Hills
NMK A4696	*Kassina senegalensis*	13–16 Sep 2006	Jos Kielgast	Shimba Hills
NMK A4697/1–4	*Kassina maculatus*	13–16 Sep 2006	Jos Kielgast	Shimba Hills
NMK A4698/1–35	*Xenopus muelleri*	13–16 Sep 2006	Jos Kielgast	Shimba Hills
NMK A4699/1–7	*Leptopelis concolor*	13–16 Sep 2006	Jos Kielgast	Shimba Hills
NMK A4745/1–6	*Hyperolius argus*	13–16 Sep 2006	Jos Kielgast	Shimba Hills
NMK A4703/1–7	*Afrixalus sylvaticus*	13–16 Sep 2006	Jos Kielgast	Shimba Hills
NMK A4705/1–5	*Chiromantis xerampelina*	13 Sep 2006	Jos Kielgast	Shimba Hills
NMK A4700/1–6	*Hyperolius argus*	13 Sep 2006	Jos Kielgast	Shimba Hills
NMK A4623/1–2	*Hyperolius rubrovermiculatus*	22 Apr 2006	B. Bwong & J.G. Measey	Kivumoni Gate Swamp
NMK A4704	*Hyperolius rubrovermiculatus*	13–16 Sep 2006	Jos Kielgast	Shimba Hills
NMK A5241	*Ptychadena anchietae*	17–18 Dec 2010	Miloslav Jirku	Shimba Lodge Swamp

NMK A5252	*Afrixalus fornasini*	17–18 Dec 2010	Miloslav Jirku	Shimba Lodge Swamp
NMK A5256	*Arthroleptis stenodactylus*	17–19 Dec 2010	Miloslav Jirku	Shimba Lodge Swamp
NMK A5243	*Ptychadena anchietae*	18 Dec 2010	Miloslav Jirku	Shimba Hills National Reserve
NMK A5269	*Hyperolius tuberilinguis*	17–18 Dec 2010	Miloslav Jirku	Shimba Lodge Swamp
NMK A5268	*Hyperolius rubrovermiculatus*	17–18 Dec 2010	Miloslav Jirku	Shimba Lodge Swamp
NMK A5451	*Chiromantis xerampelina*	7 Apr 2012	V. Wasonga & J. Nyamache	Mkongani west Forest
NMK A5452	*Ptychadena anchietae*	10 Apr 2012	V. Wasonga & J. Nyamache	Marere circuit
NMK A5453/1–2	*Hemisus marmoratus*	8 Apr 2012	V. Wasonga & J. Nyamache	Mkongani west Forest
NMK A5459/1–2	*Arthroleptis stenodactylus*	5 Apr 2012	V. Wasonga & J. Nyamache	Sheldrick Falls
NMK A5460	*Mertensophryne micranotis*	4 Apr 2012	V. Wasonga & J. Nyamache	Sable Bandas
NMK A5461	*Ptychadena anchietae*	9 Apr 2012	V. Wasonga & J. Nyamache	Mkongani west Forest
NMK A5462	*Chiromantis xerampelina*	2 Apr 2012	V. Wasonga & J. Nyamache	Sable Bandas
NMK A5463	*Ptychadena anchietae*	5 Apr 2012	V. Wasonga & J. Nyamache	Sheldrick Falls
NMK A5464	*Mertensophryne micranotis*	4 Apr 2012	V. Wasonga & J. Nyamache	Longomwagandi Forest
NMK A5501	*Arthroleptis stenodactylus*	21 Jun 2012	V. Wasonga & J. Nyamache	Mwele Forest
NMK A5502	*Arthroleptis stenodactylus*	23 Jun 2012	V. Wasonga & J. Nyamache	Sheldrick Falls
NMK A5465	*Boulengerula changamwensis*	3 Apr 2012	V. Wasonga & J. Nyamache	Sheldrick Falls
NMK A5505	*Arthroleptis stenodactylus*	3 Apr 2012	V. Wasonga & J. Nyamache	Mwele Forest
NMK A5507/1–2	*Sclerophrys pusilla*	23 Jun 2012	V. Wasonga & J. Nyamache	Sheldrick Falls
NMK A5504	*Boulengerula changamwensis*	24 Jun 2012	V. Wasonga & J. Nyamache	Sheldrick Falls

NMK A5511	*Hemisus marmoratus*	23 Jun 2012	V. Wasonga & J. Nyamache	Sheldrick Falls
NMK A5513	*Hyperolius argus*	19 Apr 2012	V. Wasonga & J. Nyamache	Shimba Lodge Swamp
NMK A5515	*Arthroleptis xenodactyloides*	5 Jun 2012	V. Wasonga & J. Nyamache	Sheldrick Falls
NMK A5514	*Hyperolius tuberilinguis*	19 Jun 2012	V. Wasonga & J. Nyamache	Shimba Lodge Swamp
NMK A5633	*Mertensophryne micranotis*	12–16 Nov 2012	J. Mueti & C. Ofori	Kaya Forest
NMK A5458	*Mertensophryne micranotis*	5 Nov 2012	V. Wasonga & J. Nyamache	Sheldrick Falls
NMK A5631/1–2	*Arthroleptis xenodactyloides*	12–16 Nov 2012	J. Mueti & C. Ofori	Kaya Forest
NMK A5510	*Boulengerula changamwensis*	19 Jun 2012	V. Wasonga & J. Nyamache	Mwele Forest
NMK A5506	*Hyperolius rubrovermiculatus*	19 Jun 2012	V. Wasonga & J. Nyamache	Shimba Lodge Swamp
NMK A5809/1–3	*Arthroleptis xenodactyloides*	18 Dec 2013	J. Nyamache & P. Mwasi	Longomwagandi Forest
NMK A5805/1–4	*Arthroleptis xenodactyloides*	17 Dec 2013	J. Nyamache & P. Mwasi	Makadara Forest
NMK A5803/1–2	*Boulengerula changamwensis*	17 Dec 2013	J. Nyamache & P. Mwasi	Longomwagandi Forest
NMK A5818/1–4	*Ptychadena anchietae*	20 Dec 2013	J. Nyamache & P. Mwasi	Shimba Lodge Swamp
NMK A5800	*Ptychadena sp.*	18 Dec 2013	J. Nyamache & P. Mwasi	Shimba Lodge Swamp
NMK A5812/1–6	*Hyperolius argus*	18 Dec 2013	J. Nyamache & P. Mwasi	Shimba Lodge Swamp
NMK A5801/1–5	*Hyperolius rubrovermiculatus*	18 Dec 2013	J. Nyamache & P. Mwasi	Shimba Lodge Swamp
NMK A5810/1–2	*Afrixalus fornasini*	18 Dec 2013	J. Nyamache & P. Mwasi	Shimba Lodge Swamp
NMK A5808	*Phrynobatrachus acridoides*	18 Dec 2013	J. Nyamache & P. Mwasi	Shimba Lodge Swamp
NMK A5819	*Mertensophryne micranotis*	20 Dec 2013	J. Nyamache & P. Mwasi	Longomwagandi Forest
NMK A5820/1–3	*Arthroleptis xenodactyloides*	20 Dec 2013	J. Nyamache & P. Mwasi	Longomwagandi Forest

NMK A5817/1–2	*Boulengerula changamwensis*	20 Dec 2013	J. Nyamache & P. Mwasi	Longomwagandi Forest
NMK A5816	*Arthroleptis xenodactyloides*	20 Dec 2013	J. Nyamache & P. Mwasi	Makadara Forest
NMK A5811	*Mertensophryne micranotis*	23 Dec 2013	J. Nyamache & P. Mwasi	Sheldrick Falls
NMK A5802/1–2	*Ptychadena anchietae*	23 Dec 2013	J. Nyamache & P. Mwasi	Sheldrick Falls
NMK A5806	*Arthroleptis stenodactylus*	23 Dec 2013	J. Nyamache & P. Mwasi	Sheldrick Falls
NMK A5804/1–2	*Phrynobatrachus acridoides*	23 Dec 2013	J. Nyamache & P. Mwasi	Sheldrick Falls
NMK A5917/1–4	*Sclerophrys pusilla*	19 Jun 2014	V. Wasonga & J. Ochong	Shimba Hills National Reserve
NMK A5911	*Mertensophryne micranotis*	19 Jun 2014	V. Wasonga & J. Ochong	Makadara Forest
NMK A5915	*Arthroleptis xenodactyloides*	16 Jun 2014	V. Wasonga & J. Ochong	Mkanda River, Lokore Forest
NMK A5913	*Arthroleptis stenodactylus*	14 Jun 2014	V. Wasonga & J. Ochong	Mwele Grassland
NMK A5912	*Arthroleptis xenodactyloides*	18 Jun 2014	V. Wasonga & J. Ochong	Sable Bandas
NMK A5953/1–2	*Ptychadena anchietae*	2 Sep 2014	J. Nyamache	Sheldrick Falls
NMK A5844/1–5	*Leptopelis flavomaculatus*	30 Apr 2014	J. Nyamache & P. Mwasi	Shimba Lodge Swamp
NMK A5848	*Hyperolius rubrovermiculatus*	30 Apr 2014	J. Nyamache & P. Mwasi	Shimba Lodge Swamp
NMK A5835	*Ptychadena anchietae*	30 Apr 2014	J. Nyamache & P. Mwasi	Shimba Lodge Swamp
NMK A5838/1–3	*Mertensophryne micranotis*	3 May 2014	J. Nyamache & P. Mwasi	Longomwagandi Forest
NMK A5851/1–2	*Arthroleptis xenodactyloides*	3 May 2014	J. Nyamache & P. Mwasi	Longomwagandi Forest
NMK A5855/1–4	*Sclerophrys gutturalis*	3 May 2014	J. Nyamache & P. Mwasi	Shimba Hills National Reserve HQ
NMK A5846	*Phrynobatrachus acridoides*	3 May 2014	J. Nyamache & P. Mwasi	Shimba Hills National Reserve HQ
NMK A5840	*Xenopus muelleri*	3 May 2014	J. Nyamache & P. Mwasi	Shimba Hills National Reserve HQ

NMK A5837	*Afrixalus sylvaticus*	3 May 2014	J. Nyamache & P. Mwasi	Shimba Hills National Reserve HQ
NMK A5850	*Boulengerula changamwensis*	4 May 2014	J. Nyamache & P. Mwasi	Longomwagandi Forest
NMK A5849	*Arthroleptis stenodactylus*	4 May 2014	J. Nyamache & P. Mwasi	Longomwagandi Forest
NMK A5854	*Scolecomorphus vittatus*	4 May 2014	J. Nyamache & P. Mwasi	Makadara Forest
NMK A5896/1–5	*Ptychadena anchietae*	12 Jun 2014	J. Nyamache & J. Ochong	Shimba Lodge Swamp
NMK A5904/1–2	*Hyperolius argus*	12 Jun 2014	J. Nyamache & J. Ochong	Shimba Lodge Swamp
NMK A5907/1–3	*Hyperolius puncticulatus*	12 Jun 2014	J. Nyamache & J. Ochong	Shimba Lodge Swamp
NMK A5899	*Hyperolius mariae*	12 Jun 2014	J. Nyamache & J. Ochong	Shimba Lodge Swamp
NMK A5900/1–2	*Hyperolius rubrovermiculatus*	12 Jun 2014	J. Nyamache & J. Ochong	Shimba Lodge Swamp
NMK A5897	*Arthroleptis stenodactylus*	12 Jun 2014	J. Nyamache & J. Ochong	Shimba Lodge Swamp
NMK A5905	*Hyperolius rubrovermiculatus*	12 Jun 2014	J. Nyamache & J. Ochong	Shimba Lodge Swamp
NMK A5901/1–2	*Arthroleptis xenodactyloides*	13 Jun 2014	J. Nyamache & J. Ochong	Kivumoni Forest
NMK A5898	*Mertensophryne micranotis*	13 Jun 2014	J. Nyamache & J. Ochong	Kivumoni Forest
NMK A5908/1–2	*Boulengerula changamwensis*	13 Jun 2014	J. Nyamache & J. Ochong	Kivumoni Forest
NMK A5903	*Afrixalus fornasini*	13 Jun 2014	J. Nyamache & J. Ochong	Kivumoni Gate Swamp
NMK A5902/1–3	*Afrixalus sylvaticus*	14 Jun 2014	J. Nyamache & J. Ochong	Kivumoni Gate Swamp
NMK A5909	*Hyperolius rubrovermiculatus*	14 Jun 2014	J. Nyamache & J. Ochong	Kivumoni Gate Swamp
NMK A5906/1–2	*Phrynobatrachus acridoides*	14 Jun 2014	J. Nyamache & J. Ochong	Kivumoni Gate Swamp
NMK A5961/1–4	*Hyperolius tuberilinguis*	2 Sep 2014	J. Nyamache & J. Ochong	Sheldrick Falls
NMK A5958/1–3	*Hyperolius rubrovermiculatus*	2 Sep 2014	J. Nyamache & J. Ochong	Sheldrick Falls

NMK A5957/1–3	*Afrixalus sylvaticus*	2 Sep 2014	J. Nyamache	Sheldrick Falls
NMK A5953/1–2	*Ptychadena anchietae*	2 Sep 2014	J. Nyamache	Sheldrick Falls
NMK A5960	*Kassina maculata*	2 Sep 2014	J. Nyamache	Sheldrick Falls
NMK A5956	*Chiromantis xerampelina*	2 Sep 2014	J. Nyamache	Sheldrick Falls
NMK A5954	*Afrixalus fornasini*	2 Sep 2014	J. Nyamache	Sheldrick Falls
NMK A5918/1–3	*Boulengerula changamwensis*	19–20 Jun 2014	V. Wasonga & J. Ochong	Makadara Forest and picnic site
NMK A6019/1–3	*Arthroleptis xenodactyloides*	27 Apr 2015	B. Bwong & J. Nyamache	Longomwagandi Forest
NMK A6020	*Boulengerula changamwensis*	27 Apr 2015	B. Bwong & J. Nyamache	Longomwagandi Forest
NMK A6021	*Chiromantis xerampelina*	27 Apr 2015	B. Bwong & J. Nyamache	Shimba Lodge Swamp
NMK A6022/1–4	*Leptopelis flavomaculatus*	27 Apr 2015	B. Bwong & J. Nyamache	Shimba Lodge Swamp
NMK A6026/1–4	*Ptychadena anchietae*	27 Apr 2015	B. Bwong & J. Nyamache	Shimba Lodge Swamp
NMK A6025	*Ptychadena anchietae*	27 Apr 2015	B. Bwong & J. Nyamache	Shimba Lodge Swamp
NMK A6023/1–7	*Hyperolius argus*	27 Apr 2015	B. Bwong & J. Nyamache	Shimba Lodge Swamp
NMK A6024/1–9	*Hyperolius rubrovermiculatus*	27 Apr 2015	B. Bwong & J. Nyamache	Shimba Lodge Swamp
NMK A6030/1–4	*Hyperolius tuberilinguis*	27 Apr 2015	B. Bwong & J. Nyamache	Shimba Lodge Swamp
NMK A6027/1–2	*Hyperolius mariae*	27 Apr 2015	B. Bwong & J. Nyamache	Shimba Lodge Swamp
NMK A6028	*Afrixalus sylvaticus*	27 Apr 2015	B. Bwong & J. Nyamache	Shimba Lodge Swamp
NMK A6029/1–5	*Phrynobatrachus acridoides*	27 Apr 2015	B. Bwong & J. Nyamache	Shimba Lodge Swamp
NMK A6031	*Arthroleptis xenodactyloides*	27 Apr 2015	B. Bwong & J. Nyamache	Shimba Lodge Swamp
NMK A6038/1–2	*Mertensophryne micranotis*	28 Apr 2015	B. Bwong & J. Nyamache	Makadara Forest

NMK A6037/1–2	*Arthroleptis xenodactyloides*	28 Apr 2015	B. Bwong & J. Nyamache	Makadara Forest
NMK A6039/1–6	*Boulengerula changamwensis*	28 Apr 2015	B. Bwong & J. Nyamache	Makadara Forest
NMK A6032	*Ptychadena anchietae*	28 Apr 2015	B. Bwong & J. Nyamache	Kivumoni Gate Swamp
NMK A6035/1–4	*Phrynobatrachus acridoides*	28 Apr 2015	B. Bwong & J. Nyamache	Kivumoni Gate Swamp
NMK A6033/1–5	*Afrixalus sylvaticus*	28 Apr 2015	B. Bwong & J. Nyamache	Kivumoni Gate Swamp
NMK A6034	*Hyperolius rubrovermiculatus*	28 Apr 2015	B. Bwong & J. Nyamache	Kivumoni Gate Swamp
NMK A6040	*Arthroleptis stenodactylus*	29 Apr 2015	B. Bwong & J. Nyamache	Makadara Forest
NMK A6041/1–3	*Arthroleptis xenodactyloides*	29 Apr 2015	B. Bwong & J. Nyamache	Makadara Forest
NMK A6042	*Arthroleptis xenodactyloides*	30 Apr 2015	B. Bwong & J. Nyamache	Marere Hill
NMK A6044	*Leptopelis flavomaculatus*	30 Apr 2015	B. Bwong & J. Nyamache	Sheldrick Falls
NMK A6043/1–4	*Afrixalus sylvaticus*	30 Apr 2015	B. Bwong & J. Nyamache	Sheldrick Falls
NMK A6046/1–5	*Phrynobatrachus acridoides*	30 Apr 2015	B. Bwong & J. Nyamache	Sheldrick Falls
NMK A6045	*Arthroleptis stenodactylus*	30 Apr 2015	B. Bwong & J. Nyamache	Sheldrick Falls
NMK A6048	*Arthroleptis stenodactylus*	1 May 2015	B. Bwong & J. Nyamache	Pengo Forest
NMK A6049	*Arthroleptis xenodactyloides*	1 May 2015	B. Bwong & J. Nyamache	Risley Forest
NMK A6047/1–2	*Boulengerula changamwensis*	1 May 2015	B. Bwong & J. Nyamache	Pengo Forest
NMK A6057	*Kassina maculata*	1 May 2015	B. Bwong & J. Nyamache	Mwadabara Swamp
NMK A 6055/1–4	*Afrixalus delicatus*	1 May 2015	B. Bwong & J. Nyamache	Mwadabara Swamp
NMK A6054	*Afrixalus delicatus*	1 May 2015	B. Bwong & J. Nyamache	Mwadabara Swamp
NMK A6052/1–3	*Phrynobatrachus acridoides*	1 May 2015	B. Bwong & J. Nyamache	Mwadabara Swamp

NMK A6053	*Hyperolius argus*	1 May 2015	B. Bwong & J. Nyamache	Mwadabara Swamp
NMK A6051	*Leptopelis concolor*	1May 2015	B. Bwong & J. Nyamache	Mwadabara Swamp
NMK A6050/1–5	*Hyperolius rubrovermiculatus*	1 May 2015	B. Bwong & J. Nyamache	Mwandabara Swamp
NMK A6056	*Hyperolius mariae*	1 May 2015	B. Bwong & J. Nyamache	Mwadabara Swamp
NMK A6058/1–2	*Hyperolius tuberilinguis*	1 May 2015	B. Bwong & J. Nyamache	Mwadabara Swamp
NMK A6059/1–2	*Arthroleptis xenodactyloides*	2 May 2015	B. Bwong & J. Nyamache	Makadara Forest
NMK A6062/1–2	*Afrixalus fornasini*	12 May 2015	J. Nyamache	Mwadabara Swamp
NMK A6065	*Hyperolius argus*	12 May 2015	J. Nyamache	Mwadambara Swamp
NMK A6064/1–2	*Hyperolius rubrovermiculatus*	12 May 2015	J. Nyamache	Mwadabara Swamp
NMK A6066/1–3	*Hyperolius rubrovermiculatus*	12 May 2015	J. Nyamache	Mwadabara Swamp
NMK A6063/1–9	*Hyperolius tuberilinguis*	12 May 2015	J. Nyamache	Mwadabara Swamp
NMK A6067/1–2	*Hyperolius mariae*	12 May 2015	J. Nyamache	Mwadabara Swamp
NMK A6068/1–4	*Afrixalus delicatus*	12 May 2015	J. Nyamache	Mwadabara Swamp
NMK A6069/1–2	*Phrynobatrachus acridoides*	12 May 2015	J. Nyamache	Mwadabara Swamp
NMK A6071	*Phrynobatrachus acridoides*	12 May 2015	J. Nyamache	Makadara Forest
NMK A6070/1–3	*Arthroleptis xenodactyloides*	12 May 2015	J. Nyamache	Mwadabara Swamp
NMK A6072	*Xenopus muelleri*	12 May 2015	J. Nyamache	Makadara Forest
NMK A6073	*Ptychadena oxyrhynchus*	13 May 2015	J. Nyamache	Kivumoni Gate Swamp
NMK A6074	*Ptychadena anchietae*	13 May 2015	J. Nyamache	Kivumoni Gate Swamp
NMK A6075	*Leptopelis concolor*	13 May 2015	J. Nyamache	Kivumoni Gate Swamp

NMK A6076/1–2	*Hyperolius mariae*	13 May 2015	J. Nyamache	Kivumoni Gate Swamp
NMK A6077	*Hyperolius rubrovermiculatus*	13 May 2015	J. Nyamache	Kivumoni Gate Swamp
NMK A6078	*Boulengerula changamwensis*	13 May 2015	J. Nyamache	Makadara Forest
NMK A6079/1–3	*Arthroleptis xenodactyloides*	13 May 2015	J. Nyamache	Kivumoni Gate Swamp
NMK A6080/1–2	*Boulengerula changamwensis*	13 May 015	J. Nyamache	Kivumoni Tower
NMK A6081/1–4	*Hyperolius rubrovermiculatus*	14 May 2015	J. Nyamache	Mwadabara Swamp
NMK A6083/1–8	*Hyperolius tuberilinguis*	14 May 2015	J. Nyamache	Mwadabara Swamp
NMK A6082/1–2	*Hyperolius rubrovermiculatus*	14 May 2015	J. Nyamache	Mwadabara Swamp
NMK A6084/1–2	*Leptopelis concolor*	14 May 2015	J. Nyamache	Mwadabara Swamp
NMK A6085	*Afrixalus fornasini*	14 May 2015	J. Nyamache	Mwadabara Swamp
NMKA 6086/1–2	*Hyperolius mariae*	14 May 2015	J. Nyamache	Mwadabara Swamp
NMK A6109	*Hyperolius rubrovermiculatus*	23 May 2015	J. Nyamache & P. K. Malonza	Mwadabara Swamp
NMK A6111	*Arthroleptis stenodactylus*	24May 2015	J. Nyamache & P. K. Malonza	Mwele Forest
NMK A6112/1–2	*Boulengerula changamwensis*	24 May 2015	J. Nyamache & P. K. Malonza	Mwele Forest
NMK A6108	*Ptychadena oxyrhynchus*	23 May 2015	J. Nyamache & P. K. Malonza	Mwadabara Swamp
NMK A6113	*Callulina sp.*	25 May 2015	J. Nyamache & P. K. Malonza	Makadara Forest
NMK A6061/1–2	*Boulengerula changamwensis*	30 Apr 2015	B. Bwong & J. Nyamache	Marere Hill
NMK A6060	*Callulina sp.*	30 Apr 2015	B. Bwong & J. Nyamache	Makadara Forest

Supplementary Material chapter II

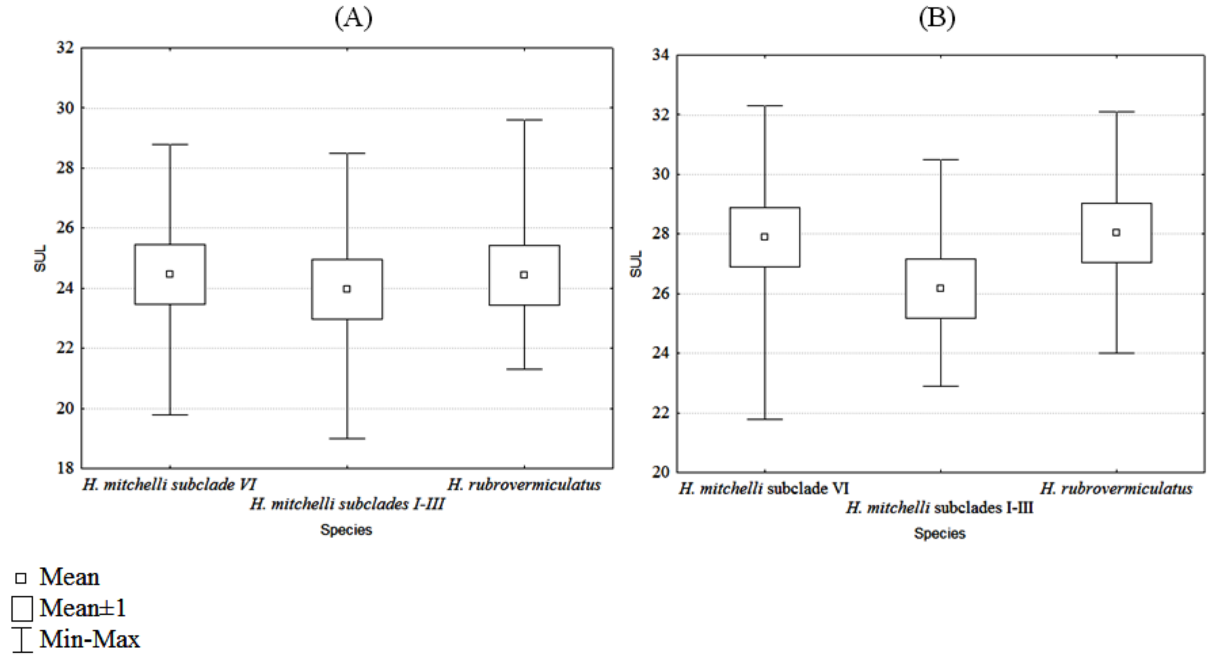

Fig. S1: Top, boxplot of snout to urostyle length (SUL) of males; bottom female samples of *H. mitchelli* subclades I-III, *H. mitchelli*, subclade VI and *H. rubrovermiculatus*.

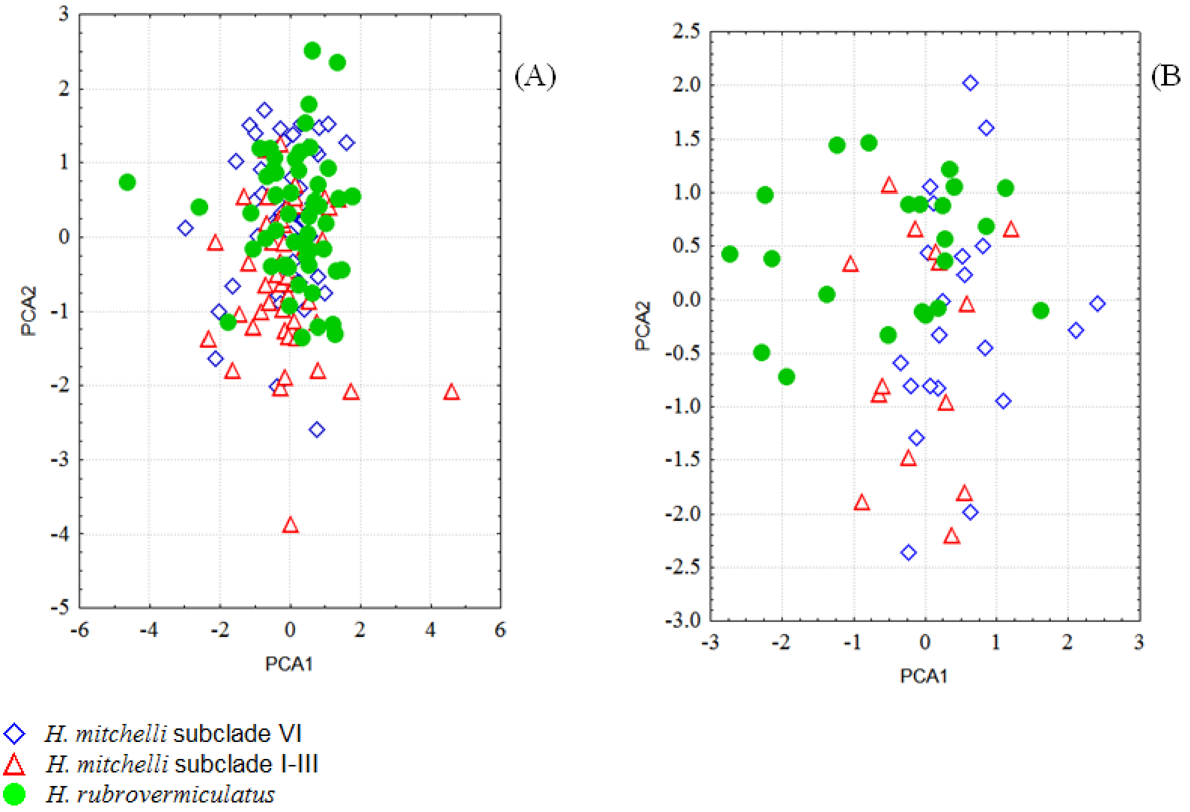

Fig. S2: A, PCA of males and B, females of *H. mitchelli* subclades VI (blue), *H. mitchelli* subclade I-III (red) and *H. rubrovermiculatus* (green) showing lack of differentiation among the samples.

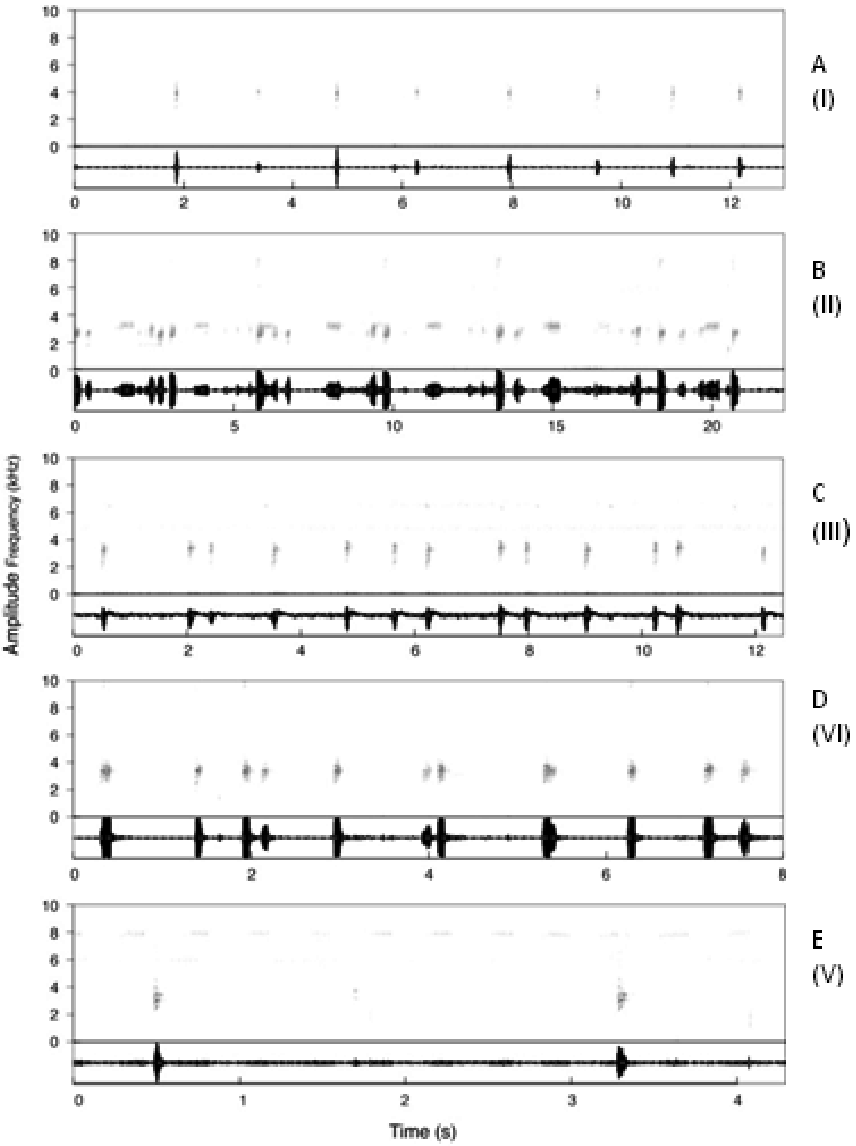

Fig. S3: Oscillograms and spectrograms showing call properties of *H. mitchelli* subclades I, II, III, VI and *H. rubrovermiculatus* (subclade V).

Table S1: Substitution models from jModelTest v2.1.6 used in the multi-locus analysis 1 and 2 respectively.

	Partition	Model
Analysis 1	16S and ND2_1	HKY + G
	ND2_2	HKY + I
	ND2_3	HKY
	C-myc exon1_1, 2 and 3	JC
	POMC_1, Cmyc_exon2_1 and non- Cmyc non coding region	K80 + I +G
	POMC_2 and 3, Cmyc exon 2_2 and 3	HKY + I + G
	ND2_1	HKY + I
Analysis 2		
	ND2_2	HKY + I
	ND2_3	HKY
	Cmyc exon1_1 and 2, POMC_1 and 3	F81 + G
	Cmyc exon1_3 and POMC _2	HKY + G
	Cmyc non coding region	JC + I

Table S2: Topology test results of alternative phylogenetic relationships based (A) 16S and (B) multi-locus alignment. 16S: Optimal – optimal tree, Constraint 1 – *H. mitchelli* subclades I-III + subclades IV and VI. Constraint 2 – subclades VI + subclades I-III. Multi-gene dataset (ND2, C-myc, POMC): Optimal – optimal tree, Constraint1 – subclade VI + subclades I-III. obs – the observed log-likelihood difference, bp – bootstrap probability, np – bootstrap probability calculated from multiscale bootstrap, pp = Bayesian posterior probability. AU – Approximately Unbiased test, KH, Kishino-Hasegawa test, SH – Shimodaira-Hasegawa test, WKH – Weighted Kishino-Hasegawa test, WSH – Weighted Shimodaira-Hasegawa test.

(A)

Tree	obs	au	np	bp	pp	kh	sh	wkh	wsh
Optimal	-1.2	.687	.666	.657	.630	.689	.690	.689	.690
Constraint 1	1.2	.321	.339	.155	.190	.311	.311	.311	.484
Constraint 2	1.2	.319	.338	.188	.190	.311	.311	.311	.487

(B)

Tree	obs	au	np	bp	pp	kh	sh	wkh	wsh
Optimal	-27.0	0.996	0.995	0.995	1.000	0.993	0.993	0.993	0.993
Constraint 1	27.0	0.004	0.005	0.005	2e-12	0.007	0.007	0.007	0.007

Table S3: Summary of call properties for *H. mitchelli* from subclade I =Makangala forest, subclade II = Udzungwa Mountains, subclade III = Uluguru Mountains, subclade VI from Nguru Mountains and *H. rubrovermiculatus* from Shimba Hills.

	H. mitchelli (I)	*H. mitchelli* (II)	*H. mitchelli* (III)	*H. rubrovermiculatus* (V)	*H. mitchelli* (VI)
Dominant Frequency (mean)	4.486	5.141	4.498	3.161	3.512
Signal duration (mean)	0.032	0.040	0.038	0.029	0.090
Pause duration (mean)	1.644	1.443	0.524	0.152	0.614

Table S4. Factor loadings and standard deviation of the first four principal components (PC) of the 19 bioclim variables used in SDM.

Bioclim	PC1	PC2	PC3	PC4
Bio1	-0.359	0.032	-0.018	0.140
Bio2	0.209	-0.051	-0.216	0.447
Bio3	0.015	-0.250	-0.307	-0.067
Bio4	0.192	0.155	0.147	0.472
Bio5	-0.322	-0.007	0.012	0.356
Bio6	-0.362	-0.056	0.044	-0.023
Bio7	0.228	0.097	-0.067	0.557
Bio8	-0.346	0.064	-0.035	0.188
Bio9	-0.328	-0.106	0.111	0.031
Bio10	-0.353	0.034	0.005	0.194
Bio11	-0.363	-0.007	-0.031	0.080
Bio12	0.046	0.145	0.452	0.006
Bio13	-0.002	0.269	0.390	-0.055
Bio14	0.074	-0.334	0.300	0.137
Bio15	-0.055	0.447	-0.027	-0.087
Bio16	-0.004	0.309	0.385	-0.016
Bio17	0.071	-0.356	0.324	0.069
Bio18	0.066	0.349	-0.082	-0.013
Bio19	0.017	-0.364	0.329	0.019
Std. dev.	2.7E+00	2.0E+00	1.9E+00	1.3E+00

Supplementary Chapter IV

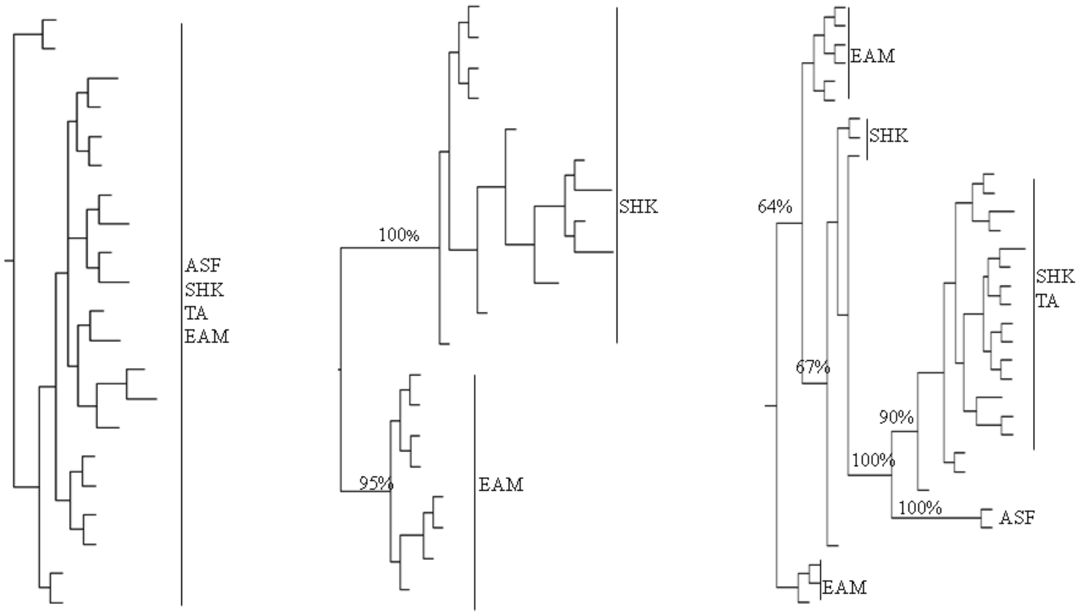

Fig. S1 **Fig**. S2 **Fig**. S3

Fig. S1–**S3**: MrBayes phylogenetic tree topology for *H. argus*, *A. sylvaticus* and *M. micranotis.* Study sites have been abbreviated as shown; ASF = Arabuko-Sokoke Forest; TA = Coastal forests in Tanga north eastern Tanzania; SHK = Shimba Hills MPK = Mpeketoni and EAM-East and West Usambara.

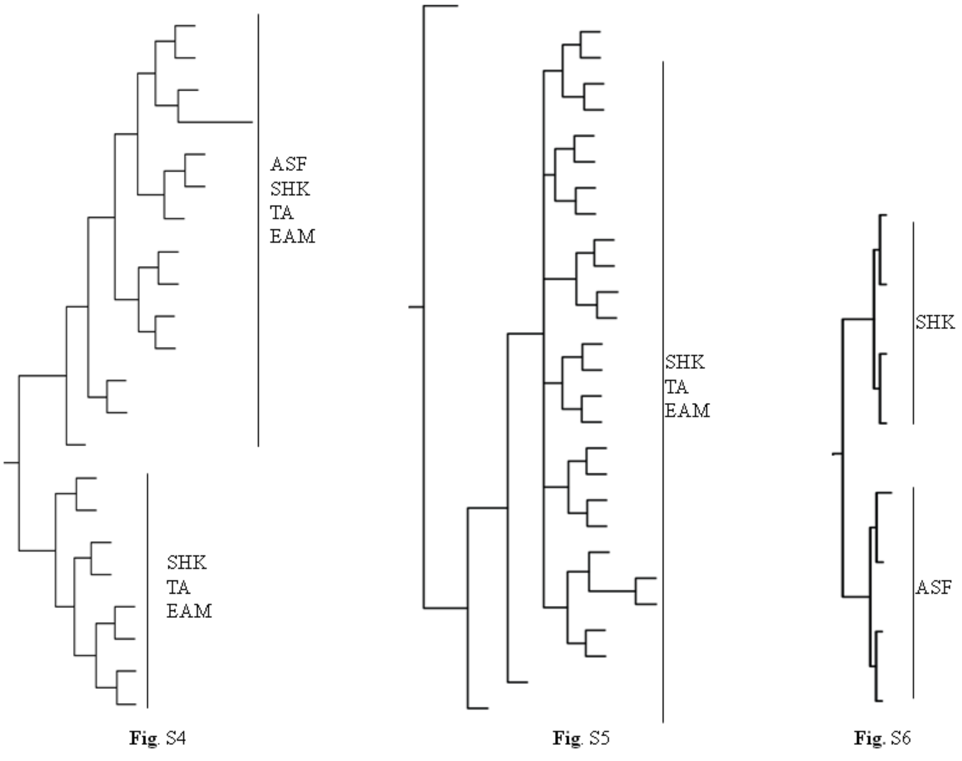

Fig. S4 **Fig**. S5 **Fig**. S6

Fig.S4-S6: MrBayes phylogenetic tree topology for *C. xerampelina*, *L. flavomculatus* and *H. pusillus*

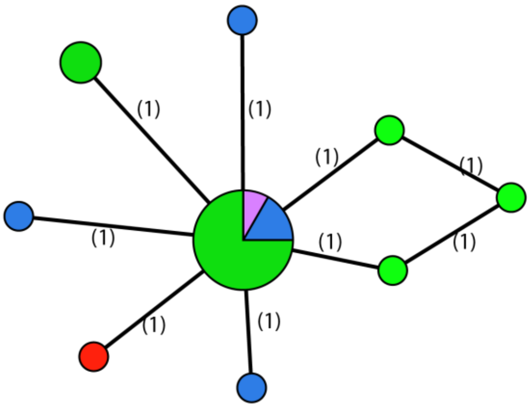

Fig.S7: 16S TCS haplotype network for *H. argus*. The colour coding for the study sites are as follows; Yellow = Mpeketoni; Blue = Arabuko-Sokoke Forest; Green = Shimba Hills; Purple = Tanga; Red = Usambaras.

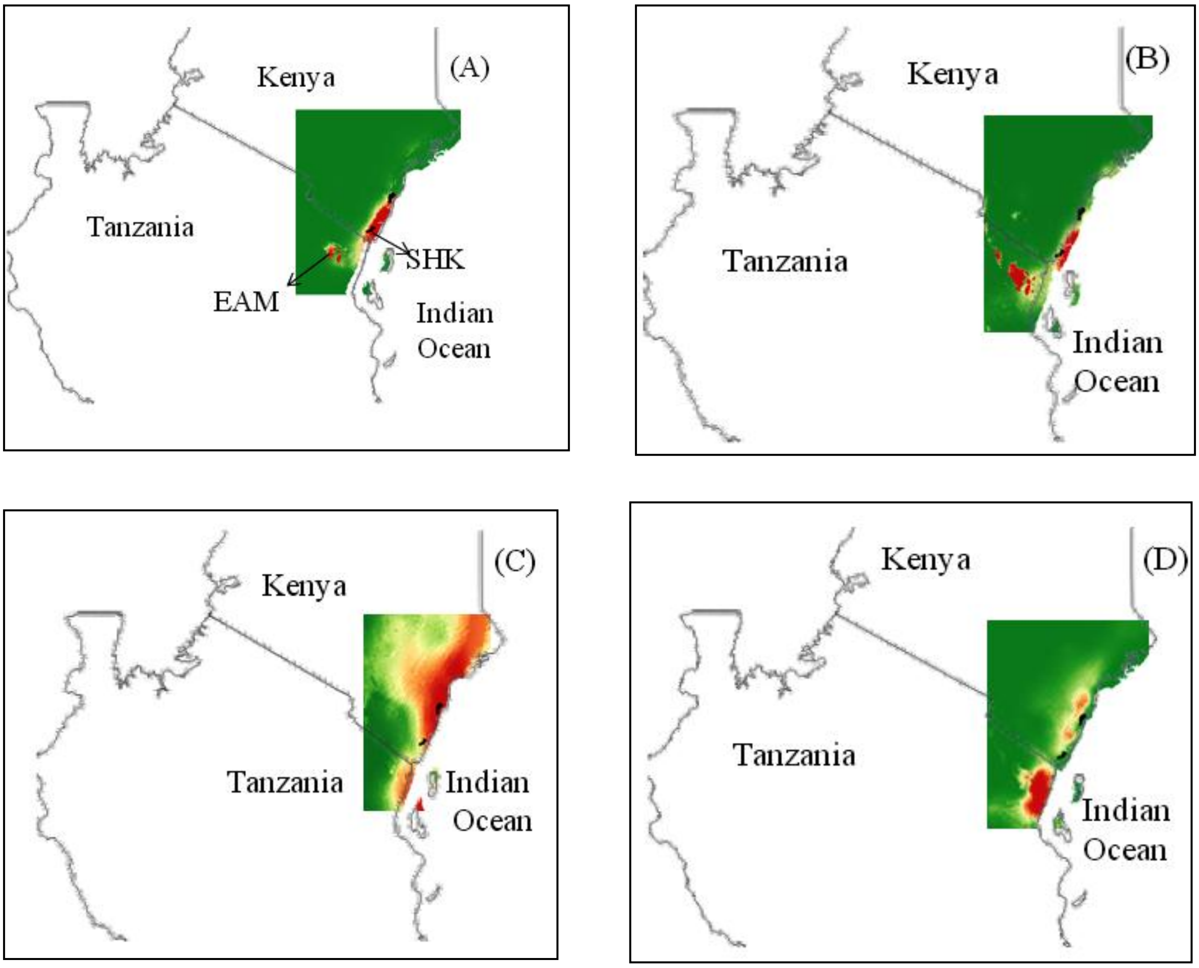

Fig. S8: Predicted species distributions in Maxent showing the position of the southern barrier. A–C Predicted distribution for *A. sylvaticus*; *A. xenodactyloides* and *S. pusilla* during the Holocene. D prediction for *M. micranotis* during the LIG.

Table S1: Substitution models based on JModeltest analysis for each species used in the Bayesian analysis.

No	Species	Substitution model
1	*Afrixalus delicatus*	TPM2UF +I+G
2	*Afrixalus fornasini*	GTR+G
3	*Afrixalus sylvaticus*	TIM2+G
4	*Arthroleptis stenodactylus*	TIM2+G
5	*Arthroleptis xenodactyloides*	TRN+G
6	*Boulengerula changamwensis*	TrN
7	*Chiromantis xerampelina*	TIM2+I
8	*Hyperolius argus*	GTR+G
9	*Hyperolius mariae*	TIM2+I
10	*Hyperolius marmoratus*	TIM2ef+I
11	*Hyperolius parkeri*	TIM2+G
12	*Hyperolius pussilus*	TIM2+G
13	*Hyperolius tuberilinguis*	TVM+I
14	*Kassina maculata*	GTR+I
15	*Leptopelis concolor*	TIM2+I
16	*Leptopelis flavomaculatus*	TIM2+I
17	*Mertensophryne micranotis*	TIM2+I
18	*Phrynobatrachus acridoides*	HKY+G
19	*Ptychadena anchietae*	TIM2+G
20	*Sclerophrys gutturalis*	GTR+G
21	*Sclerophrys pusilla*	TIM2+I+G
22	*Sclerophrys steindachneri*	TIM2+G
23	*Xenopus muelleri*	TVM+I

Table S2: Pair wise distances (Fst), 16S MtDNA between populations of species that occurred in more than two study sites. Significant values are highlighted in bold. The study sites have been abbreviated as follows; ASF = Arabuko-Sokoke Forest, EAM = Eastern Arc Mountains; MPK = Mpeketoni, SHK = Shimba Hills, TA = Tanga.

S. pusilla	**SHK**	**TA**	**EAM**	
SHK	0	0.16667	0.52	
TA	0.16667	0	0.07692	
EAM	0.52	0.07692	0	
H. argus	**US**	**SHK**	**MPK**	**ASF**
US	0	0.44615	1	0.25
SHK	0.44615	0	-0.84615	0.06411
MPK	1	-0.84615	0	-0.1
ASF	0.25	0.06411	-0.1	0
A. stenodactylus	**SHK**	**ASF**	**TA**	**EAM**
SHK	0	-0.06667	**0.97695**	**0.96958**
ASF	-0.06667	0	**0.98402**	**0.96518**
TA	**0.97695**	**0.98402**	0	-0.0971
EAM	**0.96958**	**0.96518**	-0.0971	0
A. xenodactyloides	**SHK**	**TA**	**EAM**	
SHK	0	**0.41982**	**0.3179**	
TA	**0.41982**	0	-0.02019	
EAM	**0.3179**	-0.02019	0	
C. xerampelina	**TA**	**EAM**	**ASF**	**SHK**
TA	0	0.26904	0.66667	-0.9621
EAM	0.26904	0	0.02041	0.00592
ASF	0.66667	0.02041	0	0.015084
SHK	-0.9621	0.00592	0.015084	0
L. concolor	TA	SHK	MPK	ASF
TA	0	0.1434	1	1
SHK	0.1434	0	**0.91639**	**0.70103**
MPK	**1**	**0.91639**	0	**1**
ASF	**1**	**0.70103**	**1**	0
A. delicatus	**SHK**	**ASF**	**MPK**	
SHK	0	-0.12327	**0.943093**	
ASF	-0.12327	0	**0.87636**	
MPK	**0.943093**	**0.87636**	0	
L. flavomaculatus	**TA**	**EAM**	**SHK**	
TA	0	0	0.28795	
EAM	0	0	0.23497	
SHK	0.28795	0.23497	0	
A. fornasini	**SHK**	**TA**	**ASF**	**EAM**
SHK	0	0	0	0

TA	0	0	0	0	
ASF	0	0	0	0	
EAM	0	0	0	0	
S. gutturalis	**SHK**	**TA**	**ASF**	**EAM**	
SHK	0	0.23851	-0.15702	0.21162	
TA	0.23851	0	0.42029	0.1111	
ASF	-0.15702	0.42029	0	0.5	
EAM	0.21162	0.1111	0.5	0	
H. marmoratus	**SHK**	**ASF**	**TA**	**EAM**	
SHK	0	-1	0	-1.16667	
ASF	-1	0	0.34247	0.21298	
TA	0	0.34247	0	**0.39605**	
EAM	-1.16667	0.21298	**0.39605**	0	
H. pusillus	**SHK**	**ARBK**			
SHK	0	0.94118			
ARBK	**0.94118**	0			
K. maculatus	**ASF**	**SHK**	**MPK**		
ASF	0	-0.33333	1		
SHK	-0.33333	0	0.34375		
MPK	1	0.34375	0		
H. mariae	**SHK**	**EAM**	**MPK**		
SHK	0	0.13485	**0.7778**		
EAM	0.13485	0	1		
MPK	**0.7778**	1	0		
M. micranotis	**TA**	**SHK**	**EAM**	**ASF**	
TA	0	0.0442	**0.93819**	1	
SHK	0.0442	0	**0.67076**	**0.74031**	
EAM	**0.93819**	**0.67076**	0	**0.94721**	
ASF	**1**	**0.74031**	0.94721	0	
P. acridoides	**ASF**	**EAM**	**TA**	**SHK**	
ASF	0	0.45894	**0.31317**	**0.44672**	
EAM	0.45894	0	0.0464	-0.08442	
TA	**0.31317**	-0.0464	0	0.01207	
SHK	**0.44672**	-0.08442	-0.01207	0	
P. anchietae	**SHK**	**TA**	**EAM**	**ASF**	**MPK**
SHK	0	-0.01904	-0.33216	-0.14027	-0.33216
TA	-0.01904	0	-0.33074	-0.13716	-0.33074
EAM	-0.33216	-0.33074	0	0	0
ASF	-0.14027	-0.13716	0	0	0
MPK	-0.33216	-0.33074	0	0	0
H. parkeri	**MPK**	**ASF**	**TA**	**EAM**	**SHK**
MPK	0	0.25	0.8863	0.76	**0.93416**
ASF	0.25	0	0.97297	1	**0.96846**
TA	0.8863	0.97297	0	-1	0.03467

EAM	0.76	1	-1	0	-1	
SHK	**0.93416**	**0.96846**	0.03467	-1	0	
S. steindachneri	**ASF**	**SHK**	**MPK**			
ASF	0	-1.015385	0.50318			
SHK	-1.015385	0	0.76923			
MPK	0.50318	0.76923	0			
A. sylvaticus	**US**	**SHK**				
US	0	**0.68253**				
SHK	**0.68253**	0				
H. tuberilinguis	**SHK**	**TA**	**EAM**	**ASF**	**MPK**	
SHK	0	0.07216	0.66038	0.65621	0.96581	
TA	0.07216	0	0.22581	0.50439	0.94635	
EAM	0.66038	0.22581	0	0	0.92454	
ASF	0.65621	0.50439	0	0	0.46788	
MPK	**0.96581**	**0.94635**	0.92454	0.46788		0
X. muelleri	**SHK**	**TA**	**EAM**			
SHK	0	-0.09589	0			
TA	-0.09589	0	-0.15385			
EAM	0	-0.15385	0			

TableS3: Climatic variables contribution to habitat suitability predictions in Maxent.

Species	Highest variable contributing	% Contribution
Sclerophrys pusilla	Bio19	79.8
Hyperolius argus	Bio19	85.7
Arthroleptis stenodactylus	Bio19	41.5
Arthroleptis xenodactyloides	Bio3	47.8
Callulina kreffti	Bio14	56.8
Boulengerula changamwensis	Bio19	50.9
Chiromantis xerampelina	Bio19	85.2
Leptopelis concolor	Bio19	62.9
Afrixalus delicatus	Bio19	82.7
Leptopelis flavomaculatus	Bio19	71.1
Afrixalus fornasini	Bio19	67.7
Sclerophrys gutturalis	Bio19	48
Hemisus marmoratus	Bio19	76.2
Hyperolius pusillus	Bio19	85.8
Hyperolius rubrovermiculatus	Bio19	59.3
Kassina maculatus	Bio19	82.9
Kassina senegalensis	Bio19	86.7
Hyperolius mariae	Bio19	61
Mertensophryne micranotis	Bio19	69.4
Phrynobatrachus acridoides	Bio19	68.4
Ptychadena anchietae	Bio19	67.3
Hyperolius parkeri	Bio19	62.6
Scolecomorphus vittatus	Bio19	54.1
Sclerophrys steindachneri	Bio19	84.9
Afrixalus sylvaticus	Bio19	52.9
Hyperolius tuberilinguis	Bio19	90.6
Xenopus muelleri	Bio19	48.7

Table S4: Mantel and Partial Mantel tests results for isolation by distance and habitat connectivity. Significant values are indicated in bold.

	Mantel Test			Partial Mantel test	
Species	**Distance**	**Current climate**	**LGM**	**Current climate**	**LGM**
S. pussila	r=-0.170,P=0.25	r=-0.141,P=0.458	r=-0.099,P=0.458	r=-0.615,P=0.917	r=-0.504,P=0.875
H. argus	r=-0.840,P=0.333	r=-0.356,P=0.667	r=-0.998,P=1	r=-1,P=1	r=-1,P=2
A. stenodactylus	r=0.560,P=0.33	r=0.792,P=0.333	r=0752,P=0.333	r=-0.916,P=0.208	r=0.97,P=0.083
A. xenodactyloides	r=0.853,P=0.333	r=0.961,P=0.333	r=0.895,P=033	r=1,P=0.667	r=1,P=0.667
C. xerampelina	r=-0.167,P=0.417	r=-0.099,P=0.75	r=0.033,P=0.417	r=0.027,P=0.417	r=0.678 P=0.208
L. concolor	r=0.525,P=0.167	**r=0.908,P=0.042**	**r=0.428,P=0.042**	**r=0.884,P=0.042**	r=-0.118,P=0.708
A. delicatus	r=0.863,P=0.333	r=0.967,P=0.333	r=0.820,P=0.333	r=1,P= 0.667	r=-1,P=0.667
L. flavomaculatus	r=0.853,P=0.333	r=0.847,P=0.333	r=0.738,P=0.333	r=-1, P=1	r=-1,P=1
A. fornasini	0	0	0	0	0
S. gutturalis	r=0.921,P=0.083	r=0.192,P=0.333	r=-0.069,P=0.5	r=-0.617,P=1	r=-0.617,P=1
H. marmoratus	r=-0.927,P=1	r-0.655,P=1	r=-0518,P=1	r=0.174,P=0.333	r=0.227,P=0.292
K. maculatus	r=0.034,P=0.5	r=0.272,P=0.5	r=-0.145,P=0.5	r=1,P=0.667	r=-1,P=1
H. mariae	r=0.999,P=0.167	r=0.994,P=0.167	r=0.977,P=0.167	r=-1,P=1	r=1,P=1
M. micranotis	r=0.342P=0.208	**r=0.611,P=0.041**	**r=0.642,P=0.042**	**r=0.669,P=0.042**	**r=0.759,P=0.042**
P. acridoides	r=0.212,P=0.417	r=-0.299,P=0.875	r=-0.329,P=0.875	r=-0.224,P=0.833	r=-0.261,P=0.833
P. anchietae	0	0	0	0	0
H. parkeri	r=0.223,P=0.317	r=0.124, P=0.25	**r=0.750, P=0.033**	r=-0.111, P=0.483	**r=0.737, P=0.05**
S. steindachneri	r=0.683,P=0.33	r=0.728,P=0.333	r=-0.460,P=0.667	r=1,P=0.667	r=1,P=0.667
H. tuberilinguis	r=0.998,P=0.167	r=-0.723,P=1	r=0.867,P=0.167	r=1,P=0.5	r=-1,P=1