THE HERPETOFAUNA OF THE EASTERN AFROMONTANE: EVOLUTIONARY HISTORY, BIOGEOGRAPHY AND CONSERVATION

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THE HERPETOFAUNA OF THE EASTERN AFROMONTANE: EVOLUTIONARY HISTORY, BIOGEOGRAPHY AND CONSERVATION

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Abstract

There is consensus that we are in the midst of the sixth great mass extinction (Barnosky et al., 2011), with a current rate of extinction around 1,000 times the background rate. Current extinction risk has, however, been formally evaluated for less than 5% of the world's described species, but information on the proportion of total species assessed and threatened offers a clear indication of the current trend of biodiversity loss (Baillie et al., 2004). The situation is particularly critical for the class Amphibia, with nearly one-third (32%) of the world’s amphibian species listed as threatened (IUCN, 2014). The Eastern Afromontane region is one of the most diverse areas on the Earth. A large proportion of its biodiversity and underlying evolutionary history is concentrated in small forest fragments on mountains, where both ancient lineages and more recently speciated taxa co-occur and a large proportion of the currently known taxa is formally undescribed. Compared to other tropical areas of the planet, East Africa has been characterized by pronounced climatic and geological turbulence, which, has led to a general faunal impoverishment. Despite this, the combination of the age of the forest fragments, their geographic and ecological isolation and the habitat persistence, makes the vast complex of forest fragments an ancient and stable archipelago of individually evolving sites, especially for the less vagile organisms. Research that assesses the actual patterns of diversity of the region and the definition of the most appropriate conservation strategies that can be effective in a world changing at unprecedented rate is crucial and timely. The aims of this PhD were to investigate historical and current biogeography of the herpetofauna of the Eastern Afromontane, to describe patterns of species richness and endemism across the region and to identify some of the main environmental drivers that have played a significant role in shaping the current scenario. Focused analyses on forest vipers (*Atheris*) and on dwarf forest bufonids (*Nectophrynoides*) were carried out in order to understand elements of evolutionary history of the herpetofauna across the region and to prioritize sites for conservation.

Chapter 2 focuses on the past and present of herpetological discoveries in the Eastern Afromontane region and identified priority areas where future research could lead to significant herpetofaunal discoveries. I discuss how survey effort, different taxonomic approaches and the use of biodiversity indexes, have important

implications for our understanding of the importance of individual sites since both species delimitation methods and the use of indices capture different aspects of biological diversity. The chapter includes 5 appendixes of which three are published papers – two on the description of species new to science and one is a herpetological inventory of a previously overlooked submontane forest in Tanzania. In the fourth appendix I identify priority sites for further inventories (e.g Gura Ferda in Ethiopia, Imatong Mts. in South Sudan, Itombwe and Misotshi-Kabogo ranges in DRC and Mt. Chiperone in Mozambique). The fifth appendix introduces a work-in-progress to publish an e-book on the region's amphibians and reptiles, which will aid inventory and ecological work across the region.

Chapter 3 investigates patterns of species richness and endemism of mountain-restricted amphibians across the Eastern Afromontane region by mapping the distributions of 3,488 geo-referenced records of 274 taxa. Results show a strong geographical congruence between species richness and endemism and how the Albertine Rift and the Eastern Arc mountains appear to have accumulated high amphibian diversity in different ways, with the latter having exceptional diversity despite their small sizes. Finally, results failed to support the idea that the Eastern Afromontane is a discrete zoogeographic region.

Chapter 4 focuses on the phylogenetic relationships and historical biogeography of the East African vipers in the genus *Atheris* and explores temporal and spatial relationships between the different species across Africa. Results showed congruent temporal patterns that link diversification to major tectonic and aridification events within East Africa over the last 15 million years. This points to a diversification pattern of the East African species consistent with a scenario of a delayed direct West-East colonization of the Eastern Arc Mountains, influenced by the formation of the western rift. Possible cryptic taxa in the *Atheris* genus are also identified. Chapter 5 examined radiations and evolution of forest-associated toad in the genus *Nectophyrnoides*, through phylogenetic reconstruction, finding that the Eastern Arc Mountains are characterized by the presence of both very ancient paleo-endemic species, along with more recent radiations. I then identify key conservation sites using different importance metrics. Congruence between richness of *Nectophrynoides* and amphibians as a whole was strongly positive, suggesting that this now better-known and conspicuous group could act as a proxy for assessing relative importance of sites for amphibians generally. The high spatial and elevational turnover of *Nectophrynoides* species indicates the importance of conserving forest at all altitudes and across the entire fragments in order to address the differences that exist between sites at different altitudes, within the same fragment, and at similar altitudes in different fragments on the same mountain block.

The results of this thesis highlight the biological importance of the Eastern Afromontane as a key area for the study of the evolution of life and biodiversity conservation, both at African and global levels. It provides original, updated knowledge on species occurrence and biogeographic pattern at regional level. Results also provide and interpretation of the signature left by geographic and climatic events in the pattern of species diversification, clarifying the importance of specific historical events in shaping what we see across the Eastern Afromontane today.

The recent surge in biodiversity studies, including the remarkable increase in species description, represents a significant advance in geographic sampling and this, coupled with the effectiveness of new methods for delimiting species, is helping in the assessment of the actual biological value of areas. Furthermore the increasing availability of genetic information on taxa should promote the use of phylogenetic indexes in order to move from a conservation approach solely based on species richness to a more inclusive one, that can inform conservation on the underlying functional diversity and evolutionary potential both at species and site level.

The Eastern Afromontane represents the most important area of mainland Africa for conservation of amphibians and reptiles and it offers an extraordinary conservation challenge. Because of the extreme species turnover across mountain ranges and individual forest fragments, a small number of protected areas, however well managed and resourced, will never fully capture the biodiversity of the region. Thus, a specific strategy aimed at identification and implementation of conservation initiatives at forest fragment scale must be considered. Moreover, as a reaction to a lucid assessment of global trends in population growth and associated habitat and species loss and increasing resource demands, there is an urgent need to try new conservation approaches. In the context of a more holistic and radical approach to biodiversity conservation, an active management of the surrounding matrix of the protected areas should be taken into consideration, with the aim of maintaining connectivity between areas of less disturbed habitat and to minimize damage to biodiversity, for unprotected lands, where resource extraction, agriculture, and other productive activities occur.

Chapter 1 : Introduction

Overview

The consensus from the scientific community is that we are in the midst of the sixth great mass extinction (Barnosky et al., 2011). This has been specifically attributed to human modifications of natural habitats, which have consequently impacted a range of organisms. The other five recognized great mass extinctions occurred during the history of life on this planet (Wake and Vredenburg, 2008; Barnosky et al., 2011) and were characterized by a profound loss of biodiversity during a relatively short period. Current extinction risk has, however, been evaluated for less than 5% of the world's described species, so figures provided by International Union for Conservation of Nature (IUCN, 2014) do not accurately represent the real number of the planet's species which are threatened. However, the information on the proportion of total species assessed and threatened offers a clear indication of the current trend of biodiversity loss (Baillie et al., 2004).

Biodiversity loss through local or global extinction is, of course not necessarily always human mediated, and is a natural process with more than 99 percent of all species that ever lived on Earth estimated to be extinct (Benton et al., 2003). In February 2015, there were 56,441 species on the International Union for Conservation of Nature Red List, of which 11,818 are threatened with extinction, with 11,008 data deficient, and 765 already extinct (IUCN, 2014). The situation is particularly critical for the class Amphibia. In 2004, the first major amphibian assessment found that 1,856 species or nearly one-third (32%) of the world's amphibian species are threatened. Furthermore, at least 2,469 (43%) species have populations that are declining (IUCN, 2014). The potential causes of these changes are often linked to human mediated processes (such as habitat decline) (Beebee and Griffiths, 2005) The decline in populations and number of threatened species indicate that the number of extinction events will probably continue to rise (Stuart et al. 2004). The detection of extinction rates above a 'natural' threshold and due to human mediated changes, is something extremely important to understand.

The pattern of extinction is not random, and is linked to a number of factors. Beyond human mediated changes (i.e. highest extinction rate is linked to highest habitat loss), the natural extinction rates are thought to have a latitudinal gradient pattern, with lower extinction rates in the tropics being reported in several groups of animals like beetles, birds, mammals, and bivalves (McKenna and Farrell, 2006; Weir and Schluter, 2007). Moreover, the greater resilience to extinction of the species in the tropics could be a factor that may have played an important role in the formation of the latitudinal biodiversity gradient (Mittelbach et al., 2007). The reasons for this latitudinal gradient are widely discussed in the literature (Brown and Lomolino, 1998). With the impact of human mediated changes the 'natural' extinction rates might be drastically impacted. For example, studies on species geographical range show that tropical species tend to have smaller ranges and lower population densities than temperate counterparts (Gaston and Rodrigues, 2003; Niklas et al., 2003) making tropical species more susceptible to disturbance caused by human activity and resulting in present day extinction occurring in areas that may have experienced low levels of natural extinction historically (Jablonski et al., 2006; Vamosi and Vamosi, 2008). The potential knock-on effects of anthropogenic habitat change, proportionally greater in tropical habitats, might therefore mean an even more drastic number of animal extinctions than previously estimated.

How extinction rates vary is further complicated by an uncertain understanding of how extinction precisely impacts a community of species, for example, does the loss of species increase or decrease the survivorship of another? In fact, we are just beginning to understand how compositional changes in species communities will affect phylogenetic community structure and phylogenetic diversity (Cavender-Bares et al., 2009; Laurance et al., 2012; Dirzo et al., 2014). Certain lineages clearly appear to be more susceptible to human impact than others. For example, Amphibians (43%) have a current proportion of threatened species higher than birds (17%), with mammals and reptiles experiencing intermediate threat levels (Schipper et al., 2008; Böhm et al., 2013). Although biodiversity loss occurs at global level, a recent study by Dirzo et al., (2014) shows that geographic distribution patterns of species loss are decidedly non-random. In fact in their evaluation of mammals and birds, the number of species per 10,000 km^2 in decline (IUCN population status 'decreasing') varied across regions, with highest numbers in tropical regions, with trends persisting even after factoring in the greater species diversity of the tropics (Dirzo et al., 2014).

The complex, non-random extinction processes poses hard but interesting questions on how best we should preserve the biodiversity that remains. Conservation biology and conservation planning, has increasingly began to appreciate the importance of these complex processes (Sechrest et al., 2002). Traditionally, conservation planning has been based on the evaluation of the number of species occurring in an area, mainly reflecting richness and endemism (Isaac, 2004) and to the subsequent designation of protected areas or the identification of biological valuable areas deserving specific conservation efforts (Le Saout et al., 2013). More recently effort have been focused on other aspects of diversity in order to protect not only species, but also the underlying functional and genetic diversity they represent (Tucker et al., 2012). This represents substantial progress in approaching biodiversity studies and conservation in a particularly critical period like the current. Also, as a reaction to a lucid assessment of global trends in population growth and associated habitat and species loss and increasing resource demands, there is an urgency to try new conservation approaches (Kareiva, 2014).

The Eastern Afromontane region is one of the most diverse areas on the Earth, with a large proportion of its biodiversity and underlying evolutionary history, concentrated in small forest fragments on mountains, representing a tiny part of the currently available productive land (Mittermeier et al., 2004). Between 2015 and 2100, the populations of Uganda, Malawi, Burundi, Zambia and United Republic of Tanzania are projected to increase at least five-fold, making some parts of the Eastern Afromontane one of the most densely populated regions of the world (United Nations, 2013). The projected increase in population will present an unprecedented challenge to habitat and species conservation across most of the Eastern Afromontane. Further research aimed to assess the actual patterns of diversity of the region and the definition of new conservation strategies that can be effective in a world changing at unprecedented rate, are necessary tasks to accomplish.

The whole thesis is focused on the area included in the Eastern Afromontane Hotpost of Biodiversity and, along with an account of herpetological discoveries, it reports an investigation on the general biogeographic patterns and evolutionary history of Amphibians, on the forest snakes genus *Atheris* in the rainforests of the Eastern Afromontane and a study on species diversity, pattern of speciation and evolution on the toad genus *Nectophrynoides*. A main goal of this thesis is to provide new information that could help the understanding and conservation of the herpetofauna across the Eastern Afromontane.

Diversity and ecology of the Herpetofauna

Classification

The kingdom Animalia is estimated to have a total of 1,525,548 extant, described species (Zhang, 2013). The phylum Craniata, including the vertebrates, represents 65,458 species among these 7,364 are Amphibians and 10,178 reptiles (Zhang, 2013; 2014, Frost, 2015, Uetz, 2015). The study of Amphibians and Reptiles has been traditionally grouped under the term Herpetology, despite the fact that the two classes of organisms involved are not closely related (Ahlberg and Milner, 1994). Moreover, over the past two decades, evidence has increasingly suggested that recognition of the Class Reptilia is not a monophyletic group (Modesto and Anderson, 2004; Iwabe, 2005; Velasco, 2013). The discovery that birds (Class Aves) are the closest modern relative to the crocodilians, or the sister clade to crocodilians and turtles, make the Class Reptilia an unnatural grouping (Modesto and Anderson, 2004). The present study therefore investigates patterns of distribution, species richness and evolutionary history of two distantly related, unnaturally grouped organisms that, in general share the fact of being poikilotherm, and more specifically, have associations with mountain environments, relatively low dispersal ability, and often a high degree of niche specialization (Zug et al, 2001).

Amphibians

Extant Amphibians (Class Amphibia) include 7416 species, divided into three major orders: Anura (frogs and toads with 54 families) with more than 6536 known species; Urodela (salamanders and newts with nine families) with about 676 species, and Gymnophiona (the caecilians, with ten families) with 204 species known species (AmphibiaWeb Species Numbers. Available from: http://amphibiaweb.org/amphibian/speciesnums.html). There is consensus on considering all extant Amphibians as descendants of a common ancestor and forming a monophyletic clade, the Lissamphibia, with an estimated origin between 350 and 367 mya, predating the breakup of the supercontinent Pangaea, and some 100 my before the oldest known amphibian fossil (Wells, 2007) Of these three orders, anurans and caecilians are represented in East Africa, while newts and salamanders mainly occur north of the equator and are lacking in Africa south of the Sahara Desert (Duellman and Trueb, 1986, Wells, 2007).

Frogs (Order *Anura*)

Anurans represent the most speciose group and occur on most of the major landmasses of the planet, being absent only on the most remote oceanic islands and in the very cold regions like Greenland and Antarctica (Duellman and Trueb, 1986). Frogs occupy a variety of habitats, from the first layer of soil to the tree canopy. They are currently classified as 28 families, and 16 of these occur across the Eastern Afromontane Region. The higher-level systematics of African anuran lineages has fluctuated considerably in the past, especially after the important contributions to higher-level phylogeny produced by recent molecular studies (Frost et al., 2006; Pyron and Wiens, 2011; San Mauro et al., 2014).

Anurans are essentially animals built for jumping, with an external morphology characterized by several derived characteristics compared to early terrestrial Amphibians (Zug, 2001), which probably had locomotion similar to modern salamanders. Other locomotion modes known among frogs (hopping, walking or burrowing) are all modifications of jumping. Some frog species have become almost completely aquatic, like those belonging to the genus *Xenopus*, which are quite poorly equipped for locomotion on land (Duellman and Trueb, 1986).

A remarkable proportion of Anuran species occurring in the Eastern Afromontane are burrowers, being able to dig with hind feet and descend backward into soil (the genus *Breviceps* and *Probreviceps*) or by using the so-called ‘headfish’ burrowing. This is generally much less common and known for the genus *Hemisus*, which bend the head downward and keep the backbone straight while moving forward into soil (Emerson, 1976, Duellman and Trueb, 1986). Arboreal frogs have a body morphology that contrasts that seen in burrowing species, characterized by large heads, large eyes, long legs ending with expanded digital pads. In rainforest, a large proportion of frog species in different genera and families often are arboreal (Duellman and Trueb, 1986, Wells, 2007). Interestingly, arboreal species tend to have larger brains compare to their aquatic or terrestrial counterparts; despite very few studies carried out so far on the subject, this could related to the fact that they live in a complex, three dimensional environment, which requires neural and sensory capacities not found in species occurring in simpler environments (Wells, 2007).

Figure 1-1. Eastern Afromontane endemic anurans (clockwise): *Hyperolius pictus*, *Ericabatrachus baleensis*, *Leptopelis grandiceps* and *Nectophrynoides viviparus*

Caecilians (Order *Gymnophiona*)

Caecilians are the least understood of the three orders of Amphibians (San Mauro et al., 2014) for a number of reasons. The main factors are probably their cryptic habits, tropical distribution, and a relative scarcity of dedicated study (Nussbaum and Wilkinson, 1989, San Mauro et al., 2014). Caecilians are distributed throughout much of the wet tropics, occurring in South America, Africa, the Seychelles, the Indian subcontinent and parts of SE Asia (Wells, 2007, San Mauro et al., 2014) They are absent (or at least unknown) from Madagascar and Australasia. Although one of the recognised families (the South American *Typhlonectidae*) includes aquatic species, most caecilians are found in the soil, spending their time in their subterranean burrows, and probably only coming to the surface during heavy rains and/or possibly at night. As a result, caecilians are rarely seen in the wild (Duellman and Trueb, 1986).

Caecilians are often regarded as a small, conservative, and even ‘primitive’ vertebrate group (Duellman and Trueb, 1986, Wells). However, despite numbering only about 203 currently recognised species worldwide, caecilians are extremely diverse, and many novel morphological features and natural histories are evidence of this (Wilkinson et al., 2003; 2011). They have developed a remarkable array of reproductive strategies, which spans from typical oviparity with free-living aquatic larvae, to oviparity with direct development, and true viviparity, the latter characterized by the recently discovered maternal dermatophagy, in which hatchlings eat their mother's specially modified skin. Furthermore several forms of parental care are known, including egg attendance (Gower and Wilkinson, 2009; Kouete et al., 2012; Gomes et al., 2012).

Figure 1-2. Eastern African endemic caecilians (clockwise): *Boulengerula fisheri*, *Scolecomorphus uluguruensis*, *Schistometopum gregorii* and *Scolecomorphus kirkii* (detail of the mouth)

Reptiles

The Class Reptiles is a paraphyletic group (Modesto and Anderson, 2004) containing over 10,178 known species (Uetz, 2015). Most of the information available on the global distribution and conservation status of Amphibians is not yet available for Reptiles. In fact, they are the only terrestrial vertebrates without a complete known species-level global assessment (Böhm et al., 2013). The gap should be filled soon, since the Global Reptile Assessment was launched in 2004, and assessments are published for almost 40% of the world's reptile species (IUCN, 2014).

Living reptiles belong to phylogenetic groups characterized by remarkable differences in number of species, with the non-squamate reptiles combined (turtles, crocodilians and tuataras) not reaching 350 species in total, and squamate reptiles exceeding 9000 species, being the more successful lineage within the group (Pincheira-Donoso et al., 2013).

Like Amphibians, reptiles generally have narrower distributional ranges and narrow niche requirements than other vertebrates (Anderson and Marcus, 1992; Gibbons et al., 2000) making them more sensitive to threat processes of anthropogenic origin. They are therefore a group of conservation concern (Böhm et al., 2013). Regional assessments in Europe (Cox and Temple, 2009) and southern Africa (Bates et al., 2014) indicate that 1/5 and 1/10 of reptile species respectively are threatened with extinction. It has also been proposed that declines in reptiles are similar in taxonomic breadth, geographic coverage and intensity to the ones currently observed in Amphibians (Gibbons et al., 2000), although there are still little quantitative data supporting this claim.

Living reptiles are currently classified in around 87 families, of which 18 occur across the Eastern Afromontane Region (Uetz et al., 2011).

Figure 1-3. Eastern African endemic reptiles (clockwise): *Trioceros laterispinis, Leptosiaphos kilimensis, Atheris rungweensis* and *Prosymna ornatissima*.

Ecology and breeding behaviour of Amphibians and Reptiles

Amphibians pioneered the vertebrate colonization of the terrestrial environment and this necessitated major adaptation of their morphology, physiology and behaviour (Pough et al., 1998). Most extant Amphibians have an aquatic larval stage, and are therefore dependent on water for their reproduction. Amniotes, like reptiles, on the other hand, are generally considered to have completed the transition to land through the 'invention' of the 'drought resistant' terrestrial amniotic egg (Zug, 2011). Among vertebrates, Reptiles, and Amphibians in particular, exhibit by far the greatest diversity of reproductive strategies with 39 documented reproductive modes for anurans alone (Haddad and Prado, 2005; Müller et al., 2013). Reptiles display a great diversity in modes of reproduction and in the life-history traits diversity (Shine, 2005). In particular, reptiles of both sexes can display flexible maternal control influenced by reproductive mode and frequency, seasonality and multiple mating, allowing females to manipulate both genotypes and phenotypes of their offspring (Shine, 2003).

Although the ancestral biphasic life history in Amphibians has tremendous potential for adaptive diversification (Hanken et al., 1992), living in aquatic habitats also carries significant risks because of possible desiccation and predation (Duellman and Trueb, 1986) In fact, in tropical and/or montane habitats, many Amphibians, and especially frogs, have developed reproductive modes that are increasingly terrestrialized. This is attained by controlling the timing of reproduction to avoid desiccation, and also through prolonging embryonic development leading to larger offspring and/or decreasing the time spent in the aquatic environment (Duellman and Trueb 1986, Haddad and Prado, 2005).

The independent terrestrialization of reproduction in amniotes and in several lineages of Amphibians (Müller et al., 2013) constitutes a possible key innovation. Among the most striking possible adaptations to terrestrial development are direct development and viviparity (Tinkle and Gibbons, 1977; Pyron and Burbrink, 2013). Direct development in Amphibians, as the name suggests, is characterized by the absence of a free-living, usually aquatic larva. Most adult features that do not form until metamorphosis (or later) develop during the embryonic period (Wake and Hanken, 1996). By the time of hatching, a tiny but fully formed amphibian rather than a tadpole larva emerges from the egg. Direct development is an evolutionarily successful strategy, as suggested by its multiple re-occurrence in all three orders of modern Amphibians with a particularly high rate of occurrence across the genera and species in the Eastern Arc Mountains of Tanzania, where different modes of terrestrialized reproduction have been documented (Müller et al., 2013).

Figure 1-4. Example of breeding strategy of amphibian species occurring in the Eastern Afromontane (clockwise): a female *Hyperolius* on its egg clutch, *Hyperolius kihangensis* tadpoles in their suspended water bowl, the semi-fossorial *Probreviceps* sp. with its egg clutch in a subterranean chamber, *Leptopelis grandiceps* tadpole in shallow water.

Global patterns of herpetofaunal diversity

Patterns of species richness among terrestrial vertebrates are broadly concordant, with a high proportion of species occurring in the tropics (Lamoreux et al., 2005). It has been documented for a wide spectrum of taxonomic groups that moving from high to low latitudes the average species richness within an area of a given size increases (Gaston, 2000). This latitudinal pattern is thought to be the product of various ecological factors and historical processes (Wiens, 2007; Fritz and Rahbek, 2012) and a major research agenda has been to explain the important factors influencing species diversity. Are there common patterns, do ecological or historical processes best explain such patterns, and are these shared across taxonomic groups?

Despite latitudinal variation being the best-known variable affecting species diversity at species level, the latitudinal gradient is probably a gross abstraction, and this has led to much debate and dispute about what are the important factors influencing species diversity. From an ecological point of view, latitudinal patterns are often disrupted by variation in species richness at a regional or local scale, driven by factors such as elevation, terrain topography and rainfall (Gaston, 2000). Latitudinal patterns are further influenced by available environmental energy (Hawkins et al., 2003; Dowle et al., 2013). In fact, at more local scales, the relationship between species richness and available energy is positive, with species richness increasing from low to higher levels of energy and then declining again beyond a threshold of high energy levels (Rosenzweig, 1992; Gaston, 2000). Environmental energy is usually estimated indirectly from other variables, and used interchangeably with 'net primary productivity' (Gaston, 2000; Hawkins et al., 2003). From an ecological perspective, latitudinal patterns are therefore hard to consistently recover across taxonomic groups and geographic landscapes.

Differences in species diversity between areas, which are not explained by contemporary environmental variables, have then often been attributed to effects of macroevoutionary historical processes, such as diversification and dispersal (Rahbek and Graves, 2001; Rahbek et al., 2007). One explanation for high tropical species richness in general, and more specifically in Amphibians is dubbed the Tropical Conservatism Hypothesis (Wiens and Donoghue, 2004). This assumes that the diversification rate (rate of speciation minus rate of extinction) is generally similar between tropical and temperate regions, and hypothesizes that many species-rich clades originated in tropical regions and spread to temperate regions infrequently and more recently, or not at all, leaving little time for species richness to accumulate (Wiens and Donoghue, 2004b; Wiens, 2007). In contrast to the predictions of the Tropical Conservatism Hypothesis, more recent studies on macroevolutionary patterns suggest that the latitudinal diversity gradient may be caused more by differences in diversification rates between temperate and tropical regions than by differences in the timing of biogeographic dispersal, as suggested by the remarkably higher rates of diversification found in predominately tropical clades compared to temperate ones (Wiens, 2007). Such historical macro-evolutionary processes, coupled with contemporary ecological processes, might explain variations in predicted latitudinal patterns observed, and this remains hotly debated in the literature (Rosenzweig, 1992; Roy et al., 1998; Gaston, 2000).

Pattern of species diversity in Amphibians and Reptiles are generally consistent with those of other terrestrial vertebrates, with higher numbers of species in the tropics (Wiens, 2007) and proxy measures of contemporary processes, like available environmental energy, generally correlating with global patterns of species richness (Hawkins et al., 2003; Fritz and Rahbek, 2012). However, large areas such as tropical mountains with exceptionally high richness and regions containing many small-ranged species, represent statistical outliers in such models (Rahbek and Graves, 2001; Jetz and Rahbek, 2001). The pattern of global phylogenetic diversity in Amphibians and reptiles both highlight the importance of biogeographic barriers to dispersal/colonization, and of diversification processes (Vieites et al., 2009; Fritz and Rahbek, 2012) and shows as many islands and archipelagos contain low phylogenetic diversity for the number of species present.

The presence of large, isolated radiations in islands implies the successful colonization by relatively few amphibian lineages, which have radiated to unusually high species richness. At the other end of the spectrum, areas like Central America and southern China, contain unusually high phylogenetic diversity for a relatively low number of species present, suggesting that they are centres of diversification, where old lineages are still present and many new lineages have arisen and/or high immigration rates of multiple lineages (Fritz and Rahbek, 2012).

Overall, Amphibians and Reptiles offer an interesting perspective of the relative importance of ecological and historical processes. It should, however, be noted that their longevity in the fossil record (Duellmann and Trueb, 1994; Pough et al., 2003), their often relatively poor dispersal abilities (Duellmann and Trueb, 1994; Pough et al., 2003), and highly conserved niches (Wiens et al., 2010) might make them exceptions (see also below) rather than rules, for understanding patterns of species diversity. This is particularly the case for Amphibians that show particularly specific characteristics – that differ strikingly with other vertebrates such as mammals and birds.

Dispersal

Current patterns of diversity across the globe are strongly influenced by spatial and temporal aspects of animal movements (Smith and Green, 2005; Dobrovolski et al., 2011). Furthermore, dispersal ability influences metapopulation dynamics, population regulation and long-term persistence of species on the planet (Semlitsch, 2008). Amphibians and reptiles are known to have relatively poor dispersal ability, as a consequence of their physiology and behaviour (Duellman and Trueb, 1999).

Amphibians especially show a high degree of site loyalty (Blaustein et al., 1994) resulting in a limited capability to cope with rapid climatic and environmental changes, along with a slower speed of recolonization of areas subjected to pronounced changes (Dobrovolski et al., 2011).

Dispersal movements of species influences gene flow, that ultimately significantly affects population genetic differentiation of animal species in nature (Lee-Yaw et al., 2009) and plays an important role in, both as a constraint and a promoter, by preventing or promoting local adaptation and radiations (Slatkin 1987, Wang et al., 2009). Amphibians are the ideal group for investigating the effects of landscape and geography on genetic structure as they generally have low individual mobility (leading to highly structured populations) and respond strongly to a variety of climatic and elevation gradients reviewed by Zeisset and Beebee (2008). Several studies have shown that some form of 'least-cost path' distance fits patterns of genetic structure more closely than geographic distance (Michels et al., 2001; Coulon et al., 2004; Gherghel and Papeş, 2015), demonstrating the value of the cost- weighted approach (Wang et al., 2009) in the understanding of the dispersal patterns of Amphibians. In fact, landscape heterogeneity plays a fundamental role in dispersal and in maintaining or limiting gene flow between fragmented population and can both create opportunities for local adaptation (Lawson, 2013) and reveal much about the effect of environment on the distribution of genetic variation in natural populations (Stevens et al., 2006; Storfer et al., 2007).

Oceanic dispersal had, until recently, been thought to be impossible for Amphibians because they do not tolerate the osmotic stress of salt water (Vences et al., 2003; de Queiroz, 2005). For Reptiles, oceanic dispersal is possible given their ability to tolerate salt water, and this is evidence in numerous examples of recent dispersal (Vences et al., 2003) as well as older vicariant patterns (de Queiroz, 2005). The distribution patterns of Amphibians have, therefore, generally been explained by vicariance biogeography (Biju and Bossuyt, 2003; Vences et al., 2003; San Mauro, 2010). However, Recent studies on the origin of insular species assemblages of Amphibians have shown that overseas dispersal exists and is not a rare exception (de Queiroz, 2005). Tectonic vicariance certainly remains the most important explanation for continental level amphibian biogeography (Vences et al., 2003; Upchurch, 2008; Pyron, 2014).

Why are Amphibians so threatened?

In 1989, at the first World Congress of Herpetology, in Canterbury, U.K., the herpetological community became aware of the global amphibian decline. During the congress, data were presented on the abrupt and simultaneous disappearance of montane species including in apparently pristine habitats of Costa Rica, Ecuador and Venezuela (Stuart et al., 2004). Initial skepticism about the attribution of these declines and disappearances to an unidirectional decline process rather than to natural population fluctuation, disappeared when test of probabilistic null models showed that the decline was much more widespread and severe than expected (Pounds et al., 1997) and convinced the herpetological community that something was threatening Amphibians on a global scale.

Along with habitat destruction, alteration and fragmentation, which is known to cause amphibian species declines and extinctions (Stuart et al., 2004; Beebee and Griffiths, 2005; Blaustein et al., 2011; Dodd and Smith, 2003), several enigmatic declines were also reported. For example, the loss of species in protected areas such as Yosemite National Park in US, the Monteverde Cloud forest preserve in Costa Rica or the Eungella National Park in Australia (IUCN, 2014). In cases of species threatened by habitat destruction the causes could be easily attributed, and mitigating strategies put in place at least at local scale. However, for enigmatic species declines the strategies to conserve species were much more challenging (Stuart et al., 2004).

Due to the devastating decline in many amphibian species, a global assessment of the class Amphibia was instigated in 2000. The assessment found that over one-third of the world's species were highly threatened, with nearly 168 species believed to have become extinct, and at least 2,469 (43%) more having populations that are declining (Stuart et al., 2004; IUCN, 2014). Factors linked to population crashes and extinctions include environmental changes such as increased UV radiation (Blaustein et al., 2003) hydrological and climatic changes (McCallum, 2007; Seimon et al., 2007; McMenamin et al., 2008) and emerging infectious diseases (Daszak et al., 1999). Furthermore and more worryingly, more complex causes involving combination of different factors (Blaustein and Kiesecker, 2002; Beebee and Griffiths, 2005; Blaustein et al., 2011b). This indicates that the number of extinct and threatened species will probably continue to rise (Stuart et al., 2004, IUCN, 2014) and humans are both, at least partly, the cause and can be, hopefully, the solution.

Habitat loss and land use change

Temporal patterns of amphibian decreases reflect the major impact of habitat destruction in Europe during the mid-twentieth century; in contrast with more recent declines elsewhere, especially in tropical regions (Houlahan et al., 2000; Beebee and Griffiths, 2005). In the last 100 years, land conversion to agriculture brought about the loss of half of the world's wetlands (Gallant et al., 2007). Since the beginning of the 20th century, population growth in tropical regions has been exponential and caused a broad scale change in land cover and land use, mainly associated with agriculture (Gallant et al., 2007). The full consequences of habitat destruction over time and space have been difficult to assess. For example, it has been shown that road construction might not instantly impact amphibian populations, typically devastating declines in populations are only manifested over decades (Findlay and Bourdages, 2000). Long-term consequences of land cover destruction and land are extremely difficult to neutralize and the chances of reversing the current trend of amphibian decline at global scale due to habitat alteration seem very poor (Beebee and Griffith, 2005).

A vast majority of world's amphibian species occur across the tropical rainforests that are currently being lost at an alarming rate (Burivalova, 2014). Conversion to agricultural land, degradation and fragmentation are threatening the forest ecosystems worldwide and with it their inhabitants. Being ectothermic and relying on external environment for the regulation of body temperature and moisture loss (Duellman and Trueb, 1986) among vertebrates, amphibians are particularly sensitive to environmental changes (Pough et al., 2004). Amphibian species richness has been found to decrease with decreasing canopy cover and leaf litter thickness, with systematic decline along land use modification gradients (Wanger et al., 2009).

Amphibians are steeply declining also in selective logged areas, as a possible consequence of their sensitivity to the hotter and drier microclimates generated by the logging activity. A study by Gallant and colleagues (2007) generated a composite map including combined information on land cover, land use and human population growth, showing that many of the regions of the earth supporting the richest assemblages of amphibians are undergoing the highest rate of landscape and habitat alterations (Gallant et al., 2007), including major areas of tropical rainforests.

Figure 1-5 Clearance on forest in the biologically poorly explored Mount Ribauè in 2015, Northern Mozambique.

Emerging diseases

Emerging fungal and viral pathogens, such as the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Bd), ranavirus and, more recently, *Batrachochytrium salamandrivorans*, have been causally associated with rapid die-offs and disappearances of numerous amphibian species in the Neotropics and other regions globally (Stuart et al., 2004; Lips et al., 2006; Gray et al., 2009; Vredenburg et al., 2010; Martel et al., 2013). They clearly represent a significant and growing threat to biodiversity in a globalizing world (Scheele et al., 2014). The chytridiomycosis has been identified as an important driver of global amphibian declines over 15 years ago, and is considered the most devastating wildlife disease recorded (Berger et al., 1998; Skerratt et al., 2007) causing a massive negative impact on global amphibian communities. Chytridiomycosis infection has been detected in 516 of 1240 (42%) amphibian species sampled (Olson et al., 2013), and a conservative estimate suggests that it has caused the severe decline or extinction of over 200 species (Skerratt et al., 2007; Scheele et al., 2014). In Africa, recent research investigations have identified chytridiomycosis in Nigeria, Democratic Republic of Congo, Ethiopia, Uganda, Kenya, Cameroon, South Africa, Gabon, Tanzania and Rwanda (Weldon et al., 2004;

Greenbaum et. al., 2008, Soto-Azat et al., 2010, Bell et al., 2011, Seimon et al., 2011, Gower et al., 2013).

Climate change

A recent review on the effect of climate change on Amphibians by Li et al., (2013) shows that there is little clear evidence that climate change is directly contributing to amphibian declines. This study stressed the need for a shift from primarily inductive, correlational approaches, to studies that evaluate alternative climate change related hypotheses for declines (Li et al., 2013). Despite this, many tropical organisms, including Amphibians, are suspected to be thermally specialized, being adapted to respond to limited temperature variability (Janzen, 1967; Huey, 1976) and species that experience low temperature variation in nature are thought to have reduced thermal tolerances (van Berkum, 1988; Addo-Bediako et al., 2000; Ghalambor et al., 2006; Jiguet et al., 2006; Deutsch et al., 2008).

A key consequence of thermal specialization is represented by the many organisms occurring in narrow elevational ranges (Janzen, 1967; Ghalambor et al., 2006; Colwell et al., 2008; McCain, 2009; Laurance et al., 2011), resulting in striking species endemicity in several montane areas like in the Eastern Afromontane (Fjeldså and Lovett, 1997; Rahbek, 1997; Ricketts et al., 2005, Menegon et al., 2008, 2011, Rovero et al., 2014).

Tropical high-elevation specialists are thought to be among the most threatened species on Earth due to global warming (Williams et al., 2003; Hilbert et al., 2004; Ricketts et al., 2005; Thomas et al., 2004; Williams et al., 2007; Raxworthy et al., 2008; Sekercioglu et al., 2008). Upslope displacement of species, also called the Escalator effect, could lead to the extinction of species by pushing them towards and off the tops of mountains (Grabherr et al., 1994; Parmesan, 1996; Pauli et al., 1996; Kullman, 2001; Erasmus et al., 2002; Epps et al., 2003; Konvicka et al., 2003; Pauli et al., 2007). Few studies have addressed the vulnerability of the herpetofauna of tropical mountains, despite results seeming to confirm that Amphibians and reptiles are highly susceptible to global warming, due to their physiological and ecological constraints (Wells, 2007, Raxworthy et al., 2008). The herpetofauna of tropical montane regions are particularly vulnerable. These usually show high levels of endemicity, and the areas inhabited by species adapted to live in narrow elevational zones close to summits (Ricketts et al., 2005) have been poorly investigated (Rull and Vegas-Vilarrúbia, 2006).

Figure 1-6 (left to right) *Nectophrynoides poyntoni* and *N. wendyae*, both species are restricted to a single forest fragment in the southern Udzungwa Mountains, while the latter is relatively abundant within its known range constituted by few valleys, the former, despite dedicated investigation, hasn't been seen in the single locality where it was known to occur since 2003, the year of its discovery.

The Eastern Afromontane region

Biogeography of Africa

Over much of the Cenozoic period (present - 65 million years ago), the continent of Africa was subjected to intense climatic and geological changes. These climatic fluctuations are thought to have had a strong influence on habitat composition, and subsequently species richness (Tolley et al., 2008; Murienne et al., 2013; Voelker et al., 2010). These changes have been so distinct that Africa has been dubbed 'the odd man out' in comparison to the Neotropics and Asian equatorial region (Richards, 1973). With its unique history and because of its diverse range of habitats (including rainforest hotspots) with different dates of origin, Africa is an excellent study area for testing hypotheses on species accumulation providing multiple opportunities to test the effects of extrinsic (e.g. climatic history) and intrinsic (e.g. ecology) controls on species accumulation. Another compelling reason for studying the biogeography of the African continent is the fact that it is poorly known and seemingly contains a rich biodiversity based on estimates and predictions (Burgess et al., 2007; Menegon et al., 2008; rovero et al., 2014; Loader et al., 2014), though not as diverse as other tropical regions (Richards, 1973).

Establishing a coherent biogeographical classification for Africa has challenged scientists over the past decades (Moreau, 1969; White, 1978; 1981; Linder et al., 2012). Sub-Saharan Africa can be divided into a number of distinct biomes (e.g. desert, savanna, and rainforest), however, beyond this ecological regionalization of Africa, each region shows distinct fauna and flora, indicating a separate biogeographical history and has led biogeographers to further sub-divide areas (Linder et al. 2012).

For tropical rainforests of Africa there has been a long tradition of understanding the different rainforest assemblages from a phytochronological perspective (White, 1983) and also from a historical biogeographic point of view (Lovett and Wasser, 1993). These two views are not mutually exclusive and have provided important complementary findings in our understanding of the biogeographic relations among areas. From these older works, African rainforests have been divided into four main areas on the basis of climate, and assemblage similarities (e.g. White 1983): Guinea-Congolian; Afromontane Archipelago; Lake Victoria Mosaic; and Zanzibar-Inhambane regional mosaic. Recent studies have provided less compelling evidence for their precise recognition (Linder et al., 2012; Holt et al., 2013) and modifications have been made (Burgess et al. 2004). Importantly for work outlined in this study, East African rainforests can be divided into three relatively distinct regions, and this includes Coastal Forests of East Africa, Guinea-Congolian Rainforests and the Eastern Afromontane Region (EAR), the latter a major strand of this thesis (see Chapter 3).

The exceptionally biodiverse EA is highly fragmented (Figure 1-7) and is considered to be among the most important areas for conservation globally (e.g. Mittermeier et al. 2004) Biodiversity patterns within EA are still generally poorly understood, but a substantial variation in local species richness across the region is known (Mittermeier et al. 2004), with some areas holding disproportionate amounts of diversity (Rovero et al., 2014). Various historical factors have been suggested as causes of local differences in diversity across EAR (Fjeldså and Lovett, 1997; Fjeldså et al., 1999; Fjeldså et al., 2011; Loader et al., 2014). Generally, however, the most pervading hypothesis is that higher diversity is explained by long-term persistence of habitat, as indicated by the presence of multiple 'old' lineages (Lovett et al., 2005; Fjeldså et al., 2011; Loader et al., 2014). Palaeoenvironmental data have provided important information on the stability and persistence of forest habitats in East Africa, including the EA, during the last 150,000 years. Dramatic shifts between lowland and highland forest and savannah habitats have been documented across numerous sites in Malawi, Western Tanzania, Southern and Western Uganda, throughout Kenya, and Ethiopia (e. g. Hamilton 1982, Scholz et al. 2007, Umer et al. 2007, Lamb et al. 2004, Tiercelin et al., 2008; Hamilton and Perrott, 1981). Interestingly, in contrast to most East African sites, rainforest habitats persisted in the Eastern Arc Mountains (EAM) even during the last glacial maximum, although it should be noted that marked compositional assemblage changes have been detected in montane forest habitats (Mumbi et al., 2008). Though only a few EAM areas have been sampled, such insights provide evidence to support the longer-term persistence of rainforest in at least some parts of the (Loader et al., 2014).

Phylogenetic data have provided complementary information to the palaeoenvironmental records for East Africa (e.g. Ruiz Guajardo et al., 2010). Such data have confirmed the presence of palaeoendemics, but also revealed species- rich radiations in forests (Lovett et al., 2005; Dimitrov et al., 2012). Of particular significance are areas for which palaeoenvironmental data are absent or relatively incomplete, which is the case for large parts of the EA. Recent work by Loader et al. (2014) have identified large variation in phylogenetic diversity of forest Brevicipitids across the EA and show that areas with the longest evolutionary history and highest species richness correlate to locations previously identified as areas where forest habitats are likely to have persisted over a long period of time. In fact, the Eastern Arc Mountains of Tanzania, where the highest Brevicipitid diversity occurs, has famously been dubbed the “oldest rainforest in Africa” (Lovett et al., 2005).

Figure 1-7 Map of East Africa with shaded relief of the Eastern Afromontane ranges.

The geography of the region and its biodiversity

The African section of the Eastern Afromontane region (EAR) covers more than one million km^2 and includes three mountain ranges: (1) the Eastern Arc Mountains and Southern Highlands, that stretch from south-eastern Kenya to southern Tanzania and northern Malawi, with smaller outliers in eastern Zimbabwe and northern and central Mozambique; (2) the Albertine Rift that includes parts of Rwanda, Burundi, Uganda, Tanzania and the Democratic Republic of Congo and the (3) Ethiopian Highlands that covers much of Ethiopia, with smaller parts in South Sudan, Eritrea and Djibouti and is bisected by the Great Rift Valley (Plumptre et al., 2007). In addition to these three main massifs, a number of other outlying mountains are part of the hotspot, like the volcanic northern highlands of Tanzania and the neogene volcanic highlands of Kenya (Brooks et al., 2004).

The Eastern Afromontane is a vast archipelago of fragmented mountains arranged in different ways. The three main mountain ranges are, to different degrees, composed of isolated peaks on vast highlands as in the case of the Albertine Rift, or several widely scattered isolated mountains, as in the case of the Eastern Arc Mountains or, as for the Ethiopia highlands, by a huge plateau dissected by the Great Rift valley with small outliers. The age of these mountains, the extremely varied morphology and the array of habitats has produced extraordinary biodiversity (Brooks et al., 2004).

The Albertine Rift has been identified as a region of exceptionally high species diversity, with more endemic and threatened vertebrates than elsewhere in Africa (Plumptre et al., 2007). It also contains at least 16% of Africa's plant species with over 6,400 species identified from the region. As a result, it is recognized as part of the Eastern Afromontane Biodiversity hotspot (Brooks et al, 2004), an endemic bird area (Stattersfield et al., 1998) and an ecoregion (Olson & Dinerstein, 1998). While it is recognized that, within the Albertine Rift, there are sites that may have been Pleistocene refugia, which have contributed to this diversity in the past, it is not clear exactly where these refugia occurred, and what other mechanisms of speciation may have occurred.

The Eastern Arc Mountains stretch from the Taita Hills in south-coastal Kenya for some 900 km to the Makambako Gap, southwest of the Udzungwa Mountains in southern Tanzania (Lovett and Wasser 1993; CEPF 2003). They comprise a chain of thirteen main mountain blocks, from south to north, are: Mahenge; Udzungwa;

Rubeho; Uluguru; Malundwe; Ukaguru; Nguru South; Nguu; East Usambara; West Usambara; North Pare; South Pare and Taita Hills (Fig 1-7.) The highest point is Kimhandu Peak in the Ulugurus, which is more than 2,600 m a.s.l., with most of the other peaks ranging between 2,200-2,500 m (CEPF 2003) The mountains are mainly formed by Pre-Cambrian rocks uplifted, about 100 million years ago (Griffiths 1993) with the last uplifting event dating about 7 myr ago (Tolley et al., 2011). The Indian Ocean ensures high rainfall, up to 3,000 mm per year on the eastern slopes of the Ulugurus, falling to 600 mm per year in the western rain shadow; GEF 2002) Climatic conditions are believed to have been relatively stable for at least the past 30 million years (Axelrod and Raven 1978). The high orogenetic rainfall coupled with long-term climatic stability, together with the fragmentation of both the mountain blocks and the forest cover, have resulted in forests habitats that are ancient and biologically diverse (Loader et al., 2014, Rovero et al., 2014).

In Ethiopia, there are two main forested areas, respectively on the Western and Eastern side of the Great Rift Valley. On The eastern side, the Bale Mountains are a high (up to nearly 4,400 m) and extensive massif that harbours an impressive biodiversity with many unique, rare, spectacular and threatened taxa. Hillman (1986) considered the Bale Mountains “a centre of faunal endemicity, probably with the highest rate of animal endemicity for a terrestrial habitat anywhere in the world”. The region incorporates the largest single area (> 4,000 km^2) in Africa over 3,000 m: consequently it is a crucially important reservoir of Afromontane and Afroalpine habitats, both of which are globally threatened (Largen and Spawls, 2010). The summit of the Bale Mountains is the vast, undulated Sanetti Plateau, characterized by expanses of Afroalpine grasslands, with their own highly endemic biota. To the South, the Sanetti Plateau abruptly terminates with the Harenna escarpment that drops from 3,200 to 2,000 m in just 8 km. The vegetation zonation across the escarpment is dramatic, spanning from the plateau grasslands through narrow belts characterized by markedly different vegetation types, including giant heather (*Erica* spp.) forest, vast stands of *Hagenia* trees, bamboo in the genus *Arundinaria* and *Podocarpus* forest, high altitude woodland and into medium altitude rainforest (Friis, 1986; Miehe and Miehe, 1993, 1994) On the southwestern side of the rift, there are vast and still biologically poorly known expanses of sub-montane and montane forests (Friis, 1986).

The Mozambican portion of the Eastern Afromontane Hotspot is characterized by the presence of small, isolated granite inselbergs, covered in small to medium size montane forest fragments (Portik et al., 2013a; Bayliss et al., 2014). Four of these montane isolates (Namuli, Mabu, Chiperone, Gorongosa) are recognised as Key Biodiversity Areas (Langhammer, 2007). Recently, preliminary investigations of the biological diversity for two of these mountain blocks (Mabu and Namuli), have stimulated considerable interest regarding their biological importance, and also hinted at the potential for exceptional diversity in nearby, but unknown forests. Indeed, several of the lesser-known, scattered montane forest have received little or no attention (Mount Njesi, Mt. Inago, Mt. Cucutea, Mt. Mercula) despite their potential as biologically rich montane forests.

Figure 1-8 Typical montane habitat in the Eastern Afromontane Region, (clockwise): Nyungwe forest at about 1,800 m in Rwanda, Harenna forest at about 3,000 m in Ethiopia, Montane forest on Mt. Bigugu at about 2,600 m in Rwanda, Lukwangule Plateau, on the Uluguru Mts. at about 2400 m in Tanzania.

General patterns of diversity in the Eastern Afromontane

In mainland Africa, the rainforests of the Congo River basin are generally species-poor in vertebrates, and forest Amphibians in particular (Linder et al., 2012). In contrast, the East Afromontane forests across the tropical belt are species-rich (Lamoreux et al., 2006; Burgess et al., 2007; Plumptre et al., 2007). In fact, East

Africa contains one of the highest known concentrations of endemic plants and vertebrates on Earth (Myers et al., 2000; Plumptre et al., 2007; Burgess et al., 2007b), due in part to the highly diverse montane floras and faunas of the Eastern Arc Mountains of Tanzania and Kenya, the Albertine Rift (Plumptre et al., 2007; Burgess et al., 2007) and the Ethiopian Highlands (Bussmann, 2006). Hypotheses put forward to explain the extraordinary biodiversity of East African mountains, generally point to post-Oligocene climatic shifts as triggers for lineage diversification in the presence of refugia, with major lineage diversification in the Pleistocene (Bowie et al., 2006; Couvreur et al., 2008).

The forests of EAM are relicts of a once widespread pan-African forest, which became fragmented due to aridification starting in the early Oligocene (Lovett et al., 2005; Fjeldså et al., 2011). They have persisted through Pleistocene glaciation cycles, due to Indian Ocean circulation bringing reliable orographic rainfall (Murienne et al., 2004; Tolley et al., 2011). In general, the forests are confined to the summits and upper slopes of mountain blocks and volcanic cones, with the exception of few sites in the Eastern Arc, where forest still covers the full altitudinal gradient from valley bottoms to the highest peaks (Lovett, 1996; Lovett et al., 2006).

The Afromontane archipelago of Mozambique is still very poorly known, but potentially biologically very rich) The system of isolated mountain blocks form a very poorly known linkage between the better studied Eastern Arc Mountains in the north and the southernmost Cape components of the entire Afromontane archipelago. Recent surveys suggest the ‘sky island’ of Mozambique contain high levels of biodiversity particularly among reptiles and in terms of single site endemics (Branch and Bayliss, 2009; Portik et al., 2013b; Bayliss et al., 2014). Therefore, it is expected that rarely-sampled montane forests are underestimated in terms of their biological diversity. The result is that narrow endemics are at risk of extinction, even before they are discovered, making it critical that these forests receive attention in terms of species documentation and discovery (Branch and Bayliss, 2009; Portik et al., 2013; Bayliss et al., 2014).

Brief summary of herpetological discoveries in the EAM

The earliest amphibian species to be described from East Africa were collected and described in the mid-19th century, when missionaries and explorers sent specimens to European museums, where they were described and named by museum zoologists

(Harper et al., 2010). Subsequently, in the early 1900s, the German administration established a botanical garden in the East Usambara Mountains of Tanzania, which became the first site within the Eastern Afromontane, intensively investigated for Amphibians and Reptiles. From the early- to mid-1900s, the history of herpetological exploration in the Eastern Afromontane reflects also the history of few dedicated herpetologists/explorers, especially Arthur Loveridge and Raymond Laurent. Chapter 2 discusses the full history of discovery of amphibians and reptiles in the region.

In general, issues related to colonialism, were the main drivers of the early accumulation of knowledge on amphibians and reptile diversity across the Eastern Afromontane. In fact, most of the early gazetteers' (Schiøtz, 1975, Broadley and Howell, 1991) main collecting localities were along the sea or great lakes coast, close to big cities or major geographic features. Large areas were remote and remained largely unexplored until very recently. During the 1990s, while intensive taxonomic and ecological studies on herpetological fauna were underway in many regions of West Africa, tropical America and tropical Asia (as documented by the publication of comprehensive books and studies covering entire regions: Inger, 1996, 1997, 1999; Duellman, 1997, 1980, Channing and van Dijk, 1976; Amiet, 1983), forests in East Africa were still largely undocumented and unstudied (Howell, 1993).

Ecological studies of the herpetofauna of East Africa are still very scarce, but during the last 20 years, a new wave of field investigations and taxonomic description greatly raised the number of species discovered and described, boosting the biological importance of the Eastern Afromontane at global level (Pumptre et al., 2007, Rovero et al., 2014). For the Eastern Arc mountains of Tanzania, many of these studies are summarised in Burgess et al., (2007) and Rovero et al., (2014), while information on biological exploration and species diversity on Ethiopian highlands are summarized in Largen and Spawls (2010) and Gower et al. (2013).

Conservation within the Eastern Afromontane

The mountains of the Eastern Afromontane region are suffering rapid degradation through anthropogenic deforestation and subsequent erosion, and forest loss will likely be exacerbated by climate change (Colwell et al., 2008). The reduction in montane habitats consequently influences the already restricted distributions of many endemic montane taxa, substantially increasing their susceptibility to extinction (Raxworthy, 2008). As well as being restricted to narrow altitudinal bands on individual mountaintops, several endemic amphibian and reptile species are known from just a few valleys covering <5 km^2 (Menegon et al., 2008) so it will take only tiny losses of forest or microhabitat changes to extirpate species (Sekercioglu et al., 2008). There is a clear need for predictions to be made as to future responses of key amphibian and reptile species to environmental change, and the mapping of multi-taxon evolutionary information into protected area design processes (Possingham et al., 2000).

Despite being remote and poorly investigated, some montane forests in Mozambique are known to be impacted due to agriculture (Timberlake et al., 2009, Bayliss et al., 2010), and it is likely that these same threats exist for other montane isolates in the region (e.g. Timberlake et al., 2007). At present, it is not possible to fully prioritise these mountains in terms of conservation, due to the large gap in information for indicator taxa. To date, herpetological collections have been opportunistic and no dedicated surveys for these target groups have been carried out. In northern Mozambique, there is still a massive information gap on species occurrence (e.g. Schneider et al., 2005).

How to measure herpetological importance in Eastern Afromontane?

Traditional and modern taxonomic approaches to species delimitation

Understanding whether a population should be recognized as being a distinct species is, of course, of high interest both for purely taxonomic purposes and for all the disciplines that uses species as a reference unit. Species can be identified by using multiple lines of evidence, morphological molecular and, when possible, ecological; this approach is often refered to 'integrative taxonomy' (Padial et al., 2010, Dupuis et al., 2012), where congruence between the various lines of evidence is considered a necessary requisite for an effective species delimitation (Padial et al., 2010). Exceptions could exist in so-called cryptic species that originated allopatrically, where a long-standing geographic isolation between populations leads to reproductive isolation and lineage divergence. Cryptic species do not necessarily have detectable morphological differences, especially when the habitat of the two populations is characterized by high stability through time (Losos, 2009). In the latter case, populations representing clear divergent lineages could remain morphological very stable, preventing their recognition based on morphological estimates alone (Stuart et al., 2006, Vieites et al., 2009). This is interesting in itself, but also revealing the differences and similarities is interesting for understanding the causal mechanism behind the processes of evolutionary change (Simpson, 1953; Givnish and Systma, 1997; Schluter 2000; Kozak and Wiens, 2006).

Classical morphological and morphometric analysis, often complemented by the use of multivariate statistical methods to analyse differences, involves the detection of morphological differences through direct measurement of various characters that, together, are considered an indirect evidence of reproductive isolation or divergent lineage evolution (Mayr and Ashlock, 1991). Such techniques represented the most widely use method to species delimitation until methods based on the phylogenetic reconstruction become available (Hanken, 1999). Morphological and acoustic data have been used to describe an ever-increasing number of species, and, among vertebrates, have one of the highest rates of species discovery (Vieites et al., 2009). Totaling > 7,400 species, the number of species is likely to increase substantially and these are thought to be not the result of taxonomic inflation but rather correspond to real divergent species (Hanken, 1999, Vieites et al., 2009).

The high number of new species descriptions has been significantly impacted by the relatively recent development of molecular systematic tools (e.g. Stech et al. 2013; Bickford et al., 2007; Vieites et al., 2009). Molecular systematic approaches have provided reliable, rapid and objective means to evaluate species (e.g. Palumbi and Cipriano, 1998; Vieites et al., 2009), and molecular data have alerted biologists to the potentially misleading or cryptic morphological variation exhibited by populations (e.g. Bickford et al., 2007).

Figure 1-9 Cryptic species in the genus *Hyperolius*, recently assessed through DNA based species delimitation, they were both formerly considered *Hyperolius spinigularis*.

Objectively defining species using molecular data has been undertaken using particular parts of the genome, and has also uncovered problems in defining species (Will et al., 2004) Certain molecular data have provided important information in delimiting species, in particular partial mitochondrial data (mtDNA). However, gene tree/species tree conflicts continue to be identified in analyses of mtDNA data and this has, in some cases, yielded inaccurate estimations (e.g. Zardoya and Meyer, 1996), highlighting the danger of relying on single lines of evidence. In response to this, nuclear DNA has been used to resolve species relationships alongside mtDNA (e.g. Rokas et al., 2003). However, given the low resolution found in many nuclear-gene trees, species delimitation often still relies on mtDNA results alone (Spinks and Shaffer, 2005). Also, analytical methods have been developed whereby putative species can be identified using threshold models (e.g. general mixed Yule-coalescent model (GMYC; Esselstyn et al., 2012), which can subsequently used to describe a species (Gehring et al., 2012) or delimit species units for evolutionary studies (Hey, 2006).

Approaches have recently been developed to delimit species that integrate molecular, morphological and acoustic data. Such integrative approaches have not developed a novel method, rather they have provided a framework within which putative species can be evaluated (Dayrat, 2005; Vieites et al., 2009). Such approaches will be important (Carstens et al., 2013), particularly for providing well- supported taxonomies for areas rich in biodiversity, such as tropical rainforests. A better understanding of species delimitation and mechanisms for improving the quality (e.g. molecular) and efficiency of species (e.g. web based) description are crucial to address the so-called 'Linnean and Wallacean shortfalls' we currently face (Bini et al., 2006).

Should species be the currency of conservation science?

The taxonomists have always looked to the category "species" as having unique properties, in fact many scientists believed that the species is the only real unity in nature; the only 'real', thus fundamental, division of biological diversity (de Queiroz, 2007). The hierarchical structure of the higher taxonomic categories reflects the evolutionary or 'genealogical' nature of the diversity of the life on Earth, but the categories themselves are deliberately artifacts: human decisions about how to split, group or classify life in an effective taxonomic system (Mayr, 1996). The species has, therefore, become a privileged object of analysis of evolutionary biology - the study of the patterns of speciation (birth of a species) and extinction (death of a species) over time; is the currency in studies of biogeography - in the investigation of processes of diversification and dispersal of species in the history of life. Species has also been, and currently is, the unit of measurement in conservation biology; it represents infact the current building block in the estimates of biodiversity, in the identification of extinction risk and in setting priorities for conservation (Hey, 2006).

Despite this, there is a never solved, so-called 'problem of the species', that mainly refers to a philosophical debate on the definition and nature of the species (Dobzhansky, 1935; de Queiroz, 2005). The debate focuses on a crucial topic, fundamental to the theoretical justification of biology. Despite the fact that each of us would be able to recognize and describe several animal and plant species, the definition of the unit to which we refer is very controversial. Charles Darwin himself, in a famous letter sent on Christmas Eve 1856 to his friend Joseph Dalton Hooker, wrote that, a naturalist, attempting to define species is equivalent to an "attempt to define the indefinable."

This long and problematic debate on the term 'species' and on associated concepts, seems not to have led to the recognition of the species as the natural and fundamental entity of biology, rather, it has raised new problems (Hey, 2006). In fact, with the advancement of diversity studies, it has become clear how the species, however it is defined, represents only a portion of the actual biodiversity, a portion that is influenced by intrinsic biological characteristics of the species itself in a variable manner, which makes impossible to identify a constant relationship between species and the rest of biological diversity. Because of this, the species cannot be used as a reliable proxy of biodiversity (Bernardi and Menegon, 2014, Bernardi et al., in print). This generates a series of practical implications. For example, the criteria that are the basis of the identification of the 34 global biodiversity hotspots are based on the assumption that the species (Myers et al., 2000), particularly if endemic, should represent the cornerstone of conservation efforts. In fact, over the past decades, most conservation actions aimed at the protection of species or territory inhabited by species, considered the latter as the main currency (Fleishmann et al., 2006). To one species was implicitly assigned a value of one, therefore in the evaluation of the biological value of an area, four species have always had a value double that of two species. On the basis of species richness and, in case, of the relative ratio of endemic species, areas have been ranked in function of their biological importance, their position in the rankings often influenced the scale of investment by governments or conservation agencies (Possingham and Wilson, 2005). Underlying the reasons that led to the prioritization of species, are the limited resources available for conservation, not sufficient to prevent or counteract the loss of biodiversity in place or predicted (Wilson et al., 2006). The relationship between the limited funds available and the excess of demand, the latter represented by the number of species that require investments for their conservation, have forced the investors in conservation to rank the species according to their biological or evolutionary value. This is in order to maximize the investments, highlighting some features intrinsic to the species that until a few years ago had remained secondary in the debate, both philosophical and biological, on the identification of the unit called species.

As a result, today, one species not always count one, and this leads to what has been defined as the agony of choice (Vane-Wright et al., 1991) that requires a prioritization of species on the basis of their intrinsic value, identifying those that are more worth than others of conservation investments. Today objectives of conservation measures are both species, and the genetic and functional diversity that each of them represents (Tucker, 2012). In fact, in recent times, the phylogenetic diversity has become a key component in the measurements of the values associated with the term biodiversity, as it reflects its evolutionary heritage, its functional diversity and represents the potential ability of organisms to adapt to future conditions (Vane-Wright et al., 1991, Cadotte and Davies, 2010, Winter et al., 2012).

The Zoological Society of London introduced the concept of EDGE (Evolutionary Distinct and Globally Endangered) species (Isaac et al, 2007). The EDGE species are globally threatened and that have few or no close relative in the tree of life, so they usually have distinct characteristics of uniqueness, often even in appearance and behaviour, with genomes containing a considerable amount of non-shared evolutionary history. The disappearance of one species EDGE therefore, would result in a disproportionate loss of evolutionary history. In terms of conservation, investing in EDGE species could appear particularly profitable; as equal value invested yields a disproportionately high amount of biodiversity conserved (Isaac et al, 2007, 2012).

The mismatch between the taxon species and the evolutionary units existing in reality, led conservation biologists, driven by the need to measure the effectiveness of their actions, to circumnavigate the taxon species. The units on which, increasingly, conservation actions act are becoming ‘packages of diversity’ identified by indices (Faith, 1992, Tucker et al., 2011, Winter et al., 2012) sometimes without clear bounds with the Linnaean nomenclature, and characterized by higher affinity to evolutionary units than to taxa.

Alternative biodiversity metrics

Available phylogenetic metrics can be grouped in those relevant for addressing the distinctiveness of a single species, like the taxonomic distinctness (Winter et al., 2012) and evolutionary distinctiveness (Isaac, 2007), reflecting a branching order on a phylogenetic tree within a monophyletic group, weighted according to its distinctiveness (Isaac, 2007, 2012, Winter et al., 2012) and community based indexes. These are calculated as the sum of the lengths of all those branches that are members of the corresponding minimum spanning path in which 'branch' is a segment of a phylogenetic tree and the minimum spanning path is the minimum distance between the two nodes (Faith, 1992). For conservation purposes, phylogenetic diversity or its endemism-weighted version of phylogenetic diversity, are particularly suited for a complementarity approach (Faith, 1992; Rosauer, 2009; Winter, 2012) The first introduced phylogenetically based index of diversity was Phylogenetic Diversity (PD), a measure that represents the sum of branch lengths within a tree connecting all species of interest at a site, commonly measured with Faith's index (Faith, 1992) Since then, several other indexes have been identified and introduced. Among them, the most widely used are the Phylogenetic endemism (PE) which is a site-based index of the amount of evolutionary history uniquely represented within a given area and Evolutionary Distinctiveness (ED), a species-based measure that identifies species with less redundancy for the unique genetic information they contain (Faith, 1992; Faith and Baker, 2006; Cadotte et al., 2010).

Aims and Objectives of the PhD

Over the last 20 years, the author and colleagues have made a large number of herpetological discoveries across the Eastern Afromontane region. This offers the opportunity to examine, in a depth never possible before, aspects of the evolutionary history and biogeography of the EA herpetofauna, and to use these findings to inform conservation planning across the region.

This PhD aims to examine important aspects of evolutionary history and biogeography of the Eastern Afromontane herpetofauna by achieving the following objectives:

- To summarize the past and present of herpetological investigations in the area and to identify priority areas where future research could lead to significant herpetofaunal discoveries (Chapter 2).
- To investigate patterns of diversity, to examine zoogeographical partitions and explore relationships between diversity measures and environmental predictors, across the different mountain ranges (Chapter 3).
- To investigate the phylogenetic relationships and historical biogeography of the viper genus *Atheris*, and assess the impact of palaeoclimatic fluctuations and tectonic movements on the cladogenesis of the genus (Chapter 4).
- To investigate the radiation and evolution of forest-associated dwarf toad of East Africa through phylogenetic reconstruction, and to identify key conservation sites (Chapter 5).
- To contestualize this work into a wider discussion on evolutionary, biogeographical and conservation implications of the findings, and recommend future research and conservation actions in the region (Chapter 6).

Chapter 2 documents the past and present of herpetological discovery in the Eastern Afromontane region and identifies priority areas where future research could lead to significant herpetofaunal discoveries. The chapter describes and summarizes the history of herpetological contributions in the last 150 years and more recently since 1998 and the present. It then discusses how different taxonomic approaches and the use of biodiversity indexes have had important implications for our understanding of biological values of areas and lead to differences in site prioritization. Finally it introduces a work-in-progress to publish an e-guide on the region's amphibians and reptiles, which will aid inventory and ecological work across the region. The chapter also contains three recently published papers as appendixes: The recent description of two species new to science, a bush viper in the genus *Atheris* and a frog in the genus *Callulina*, respectively from the Southern Highlands of Tanzania and the Nguru Mountains. The third appendix is a paper reporting a herpetological inventory in the Mahenge Mountains, Tanzania, with comments on the occurrence of several species new to science and biogeography of the area.

Michele Menegon collected all the original data and wrote the published papers (co-authors are indicated in the respective papers), MM with Stuart Marsden conceived the study. MM, with the supervision of Stuart Marsden, wrote the chapter.

Chapter 3 investigates patterns of species richness and endemism of mountain-restricted amphibians across the Eastern Afromontane region, and explores relationships between species richness/endemism in the different mountain ranges and environmental predictors. It also examines zoogeographical partitions through the use of dissimilarity cluster analyses. Finally, it ranks sites according to importance in terms of taxon richness and endemism and highlight their protection status.

Michele Menegon collected all the original data of species occurrence and selected, downloaded and cleaned data obtained from global databases (e.g. GBIF); Climatic data were selected and downloaded by Guy Pichton Phillipps. MM with Stuart Marsden conceived the study and analyised the data. MM, with the supervision of Stuart Marsden, wrote the chapter.

Chapter 4 focuses on the phylogenetic relationships and historical biogeography of the East African vipers in the genus *Atheris,* and explores temporal and spatial relationships between the different species species across Africa. It then investigates the impact of palaeoclimatic fluctuations and tectonic movements on the cladogenesis of the genus.

Michele Menegon conceived the study, analysed part of the data and run spatial and biogeographic analysis. Sylvain Ursenbacher and MM analysed molecular data. MM wrote the chapter (with the supervision of Stuart Marsden).

Chapter 5 investigates the radiations and evolution of forest-associated dwarf toad of East Africa, with a main focus on the genera *Nectophynoides* and *Churamiti,* through phylogenetic reconstruction. The chapter identifies the key conservation sites using different importance metrics and assesses the congruence between richness of *Nectophrynoides* and amphibians as a whole.

Michele Menegon and Simon Loader conceived the study, MM, SL, Cristiano Vernesi and Christopher Liedtke analyised molecular data. MM run spatial and macroecological analysis and wrote the chapter (with the supervision of Stuart Marsden).

Chapter 6 is a summary of the main findings of the thesis, and a wider discussion on evolutionary, biogeographical and conservation implications of the findings, with recommendation for future research and conservation action in the region. MM wrote the chapter.

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Chapter 2 : Biological exploration

Herpetological discovery in the Eastern Afromontane: taxonomic and conservation implications

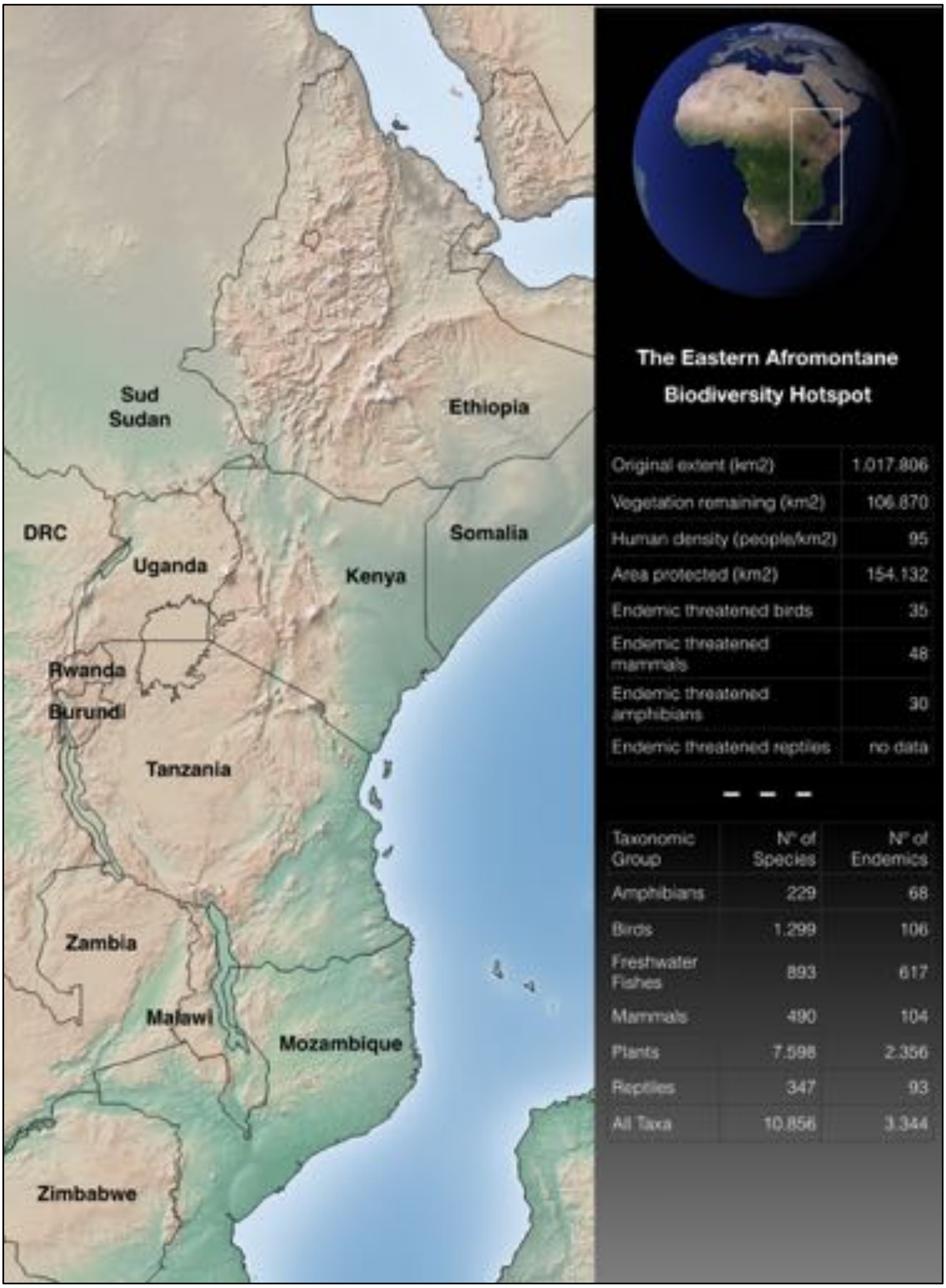
Abstract

The Eastern Afromontane (EA) is now known to be globally important for amphibian and reptile diversity. This chapter documents the past, present and future of herpetological discovery in the region. First, I examine the history of discovery across the region, in the last 150 years and more recently since 1998, when MM became active within the region. I describe field methods used to collect herpetological specimens, and morphological and DNA analyses used to identify taxa. I chronicle discoveries of 101 new species at 39 East African sites between 1998 and the present. These additions to the herpetofauna of EA boost its gamma diversity by around 30%. Survey effort, different taxonomic approaches and the use of biodiversity indexes, have important implications for our understanding of the importance of individual sites since both, species delimitation methods and the use of indexes aimed to capture different aspects of biological diversity, could lead to differences in site prioritization. I identify poorly studied sites, which are priorities for further inventories. These include Gura Ferda plateau (Ethiopia), Imatong Mountains (South Sudan), Itombwe Massif and Misotshi-Kabogo range (DRC), and Mt. Chiperone (Mozambique) Finally, I introduce a work-in-progress to publish an e-book on the region's amphibians and reptiles, which will aid inventory and ecological work across the region.

Introduction

The Eastern Afromontane (EA) includes a system of widely scattered mountain ranges, from the Asir Mountains of Saudi Arabia and the highlands of Yemen in the north to the Eastern Highlands of Zimbabwe and Mozambique in the south (see chapter 3 for a more detailed description of the area). In terms of biodiversity, EA is among the richest places on earth (Mittermeier et al., 2004). It is home of nearly 7600 species of plants, of which more than 2300 are endemic, and 1200 bird species, of which over 100 are endemic. The Eastern Arc Mountains of Kenya and Tanzania alone hosts over 1,100 endemic species of plants in about 40 endemic genera (Lovett and Wasser, 1993), the Ethiopian Highlands harbour an estimated 5200 plant species, of which 555 are endemic (Friis, 1992, Lovett and Friis, 1997, Nievergelt et al., 1998, Gereau et al., 2010). The Albertine Rift is home to 14% (around 5800 species) of mainland Africa's plant species, with more than 550 endemic species and 3 endemic genera (Burgess et al., 2007; Plumptre et al., 2007; Rovero et al., 2014).

Precise patterns of biodiversity within the EA are generally poorly understood, but local species richness is known to vary substantially across the region (Mittermeier et al., 2004), with some areas holding disproportionate amounts of diversity like the Eastern Arc Mountains of Kenya and Tanzania (Burgess et al., 2007; Rovero et al., 2014). Various historical factors have been suggested as causes of local differences in diversity across the EA (Lovett and Wasser, 2008; Rovero et al., 2014). Generally, however, the most prevalent hypothesis is that higher diversity is explained by long-term persistence of habitats, as indicated by the presence of multiple ‘old’ lineages (Fjeldså and Lovett, 1997; Loader et al., 2014).



The Eastern Afromontane Biodiversity Hotspot

Original extent (km2)	1.017.806
Vegetation remaining (km2)	106.870
Human density (people/km2)	95
Area protected (km2)	154.132
Endemic threatened birds	35
Endemic threatened mammals	48
Endemic threatened amphibians	30
Endemic threatened reptiles	no data

Taxonomic Group	N° of Species	N° of Endemics
Amphibians	229	68
Birds	1.299	106
Freshwater Fishes	893	617
Mammals	490	104
Plants	7.598	2.356
Reptiles	347	93
All Taxa	10.856	3.344

Figure 2-1 Map of the countries interested by the Eastern Afromontane Hotspot and main facts about regional biodiversity (data from: http://www.cepf.net)

Despite the recognized importance of EA's biodiversity, and the area's designation as a Biodiversity Hotspot, the region remains poorly studied for various taxa, and new species are frequently documented (Rovero et al., 2014). These include the remarkable discovery of a new genus of primate in 2005 (Jones et al., 2005; Davenport et al., 2006). A sharp increase in species description reflects the general underestimation of biodiversity in this region, advanced recently by more thorough geographic sampling and new methods for delimiting species (Dimitrov et al., 2012; Ceccarelli et al., 2014). DNA-based approaches have revealed the presence of many 'cryptic' species overlooked by morphological estimates (Loader et al., 2011; Ceccarelli et al., 2014; Loader et al., 2014). However, the appropriate use of such species-delimiting methods is currently debated (Vieites et al., 2009; Monaghan et al., 2009) and determining species diversity in biodiverse areas such as the Eastern Afromontane, is still a 'work in progress' for most groups of organisms. Overall, despite a substantial increase in our understanding of the region, significant gaps in our knowledge exist and continued innovative approaches to describe the diversity are required.

The aim of this chapter is to examine and discuss the state of play regarding our knowledge of the herpetofauna of the Eastern Afromontane. To do this, I have the following objectives.

1. To review the history of knowledge accumulation on the region's herpetofauna since the mid 19th Century, and including the period since 1998, when MM became an active researcher in the region.

2. To catalogue the herpetofaunal discoveries made by MM, and to use case studies to illustrate important issues exemplifying methods of description, accumulation of new species at important sites, and implications for novel and charismatic new species.

3. To discuss the implications of these discoveries for conservation of key sites across EA and to identify priority sites for further herpetofaunal work.

Methods

Historical review of herpetofaunal research from the late 1800s to 1998

A comprehensive review of discovery across different parts of the EA was achieved by trawling available literature concerning the history of herpetological accumulation of knowledge across the Eastern Afromontane. In some cases, information was obtained directly from the authors, or from original annotation in museum labels. Museum collections were also examined, mainly through electronic museum catalogues.

Post-1998 surveys by MM and others

I reviewed and summarized recent contributions by MM and colleagues to the current knowledge of the herpetofauna of the EAM, including those on biological inventories, and discovery and formal description of species new to science and taxonomic updates.

Site selection

Since 1998, survey sites have been selected using a few general criteria: being among the least known or least sampled across the EAR based on available literature, being geographically isolated; encompassing large altitudinal gradient and being relevant from a biogeographic point of view on the basis of the previously accumulated knowledge. Survey times varied considerably – with some sites visited for single nights whereas others for up to three weeks. Earlier dated surveys tended to be longer due to extended time periods spent in Tanzania. Surveys remained consistent in the basic methodology carried out per locality. Usually the surveying areas and night transects were located within an hour's walk of the camp. Satellite camps have been also used with the aim of investigating important habitats/spots found during day reconnaissance walks and too far away from the main camp for a standard night walk. Figure 2-2 shows the sites covered by MM and colleagues between 1998 and 2014.

In early years preliminary site selection was usually done by using Government maps (for examples the 1:50,000 scale Tanzania Government maps), and consultation with other researchers and literature to identify potential study sites. More recently, satellite images available online have almost completely replaced topographic maps in the preliminary selection of sites and, especially when associated with vegetation maps from remote sensing, facilitating the identification of small, remote and poorly known forest fragments. Through 3D projection of the landscape, it is possible to identify forest characteristics, and to see in which direction the forest fragment is oriented (e.g. facing the ocean or a large lake, a possible indication of long-term climate stability) Once all the available information was gathered, sites were selected in order to sample all or most of the habitats within the area.

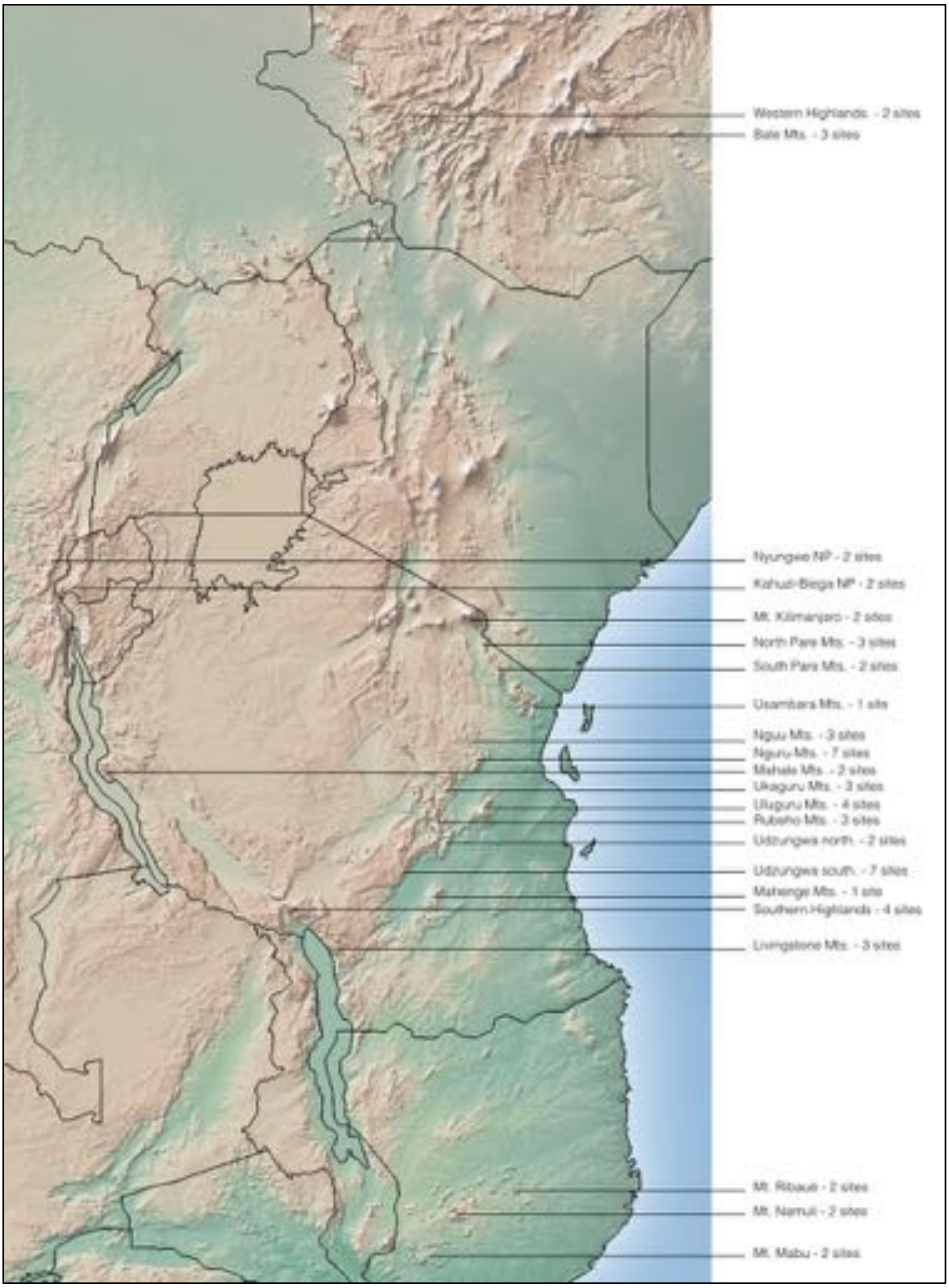


Figure 2-2 Location of post-1998 herpetefaunal surveys conducted by MM and colleagues in the Eastern Afromontane.

Field sampling techniques

Amphibians and reptiles are generally relatively easy to sample compare to other classes of vertebrates like birds and mammals (Menegon, 2006) Frogs usually occur at high densities in forest habitats or congregate around ponds and along streams (Crump and Scott, 1994). However, some fossorial amphibians species, lizards and most snake species are more difficult to locate, and to produce accurate inventories of the amphibian and reptile fauna, it is necessary to undertake a variety of survey methods, which will sample different niches. For example, digging is essential to sample subterranean amphibian and reptiles, including burrowing frogs, caecilians, amphisbaenians, snakes and lizards (Mcdiarmid et al., 2012) During the field investigation carried out since 1998, in order to optimize the number of species contacted and, in some cases, collect abundance data, MM employed four main methods: (1) visual encounter surveys – with digging; (2) pitfall trapping; (3) acoustic sampling and (4) opportunistic captures.

1. Visual Encounter Survey

Visual Encounter Surveys (VES) can be used to estimate species richness of an area and the species composition of a local assemblage, and to estimate relative abundances of species within an assemblage (Crump and Scott, 1994). The method was formalized by Campbell and Christman (1982) and Corn and Bury (1990), and are based on a number of assumptions: 1) equal observability among species and among individuals; 2) no between-sampling visit effects i.e. there is an equal likelihood of each species being observed on each sampling visit; 3) individuals are recorded only once per survey; and 4) there are no observer-related effects. There are three standard sampling designs for visual encounter surveys: opportunistic or randomized walk, transects, or a quadrat design (see Crump and Scott 1994 for details). Visual encounter surveys can determine species richness, be applied in long term monitoring projects, provide information for compilation of a species list, and provide data used to estimate proportion of area surveyed that is occupied by target species. VES data are expressed as numbers of individuals of a certain species found in an area per unit time. For practical reasons, 'man-hours' can be used, which can be adjusted to the complexity of the habitats being sampled (see also Rovero et al., 2014). According to Corn and Bury (1990), VES can only provide information on the presence or absence of a species in an area and are inadequate for determining abundances, the methods were therefore only used as a qualitative or semi-quantitative tool. VES transect, usually in form of opportunistic walks, were usually carried out every day, after dusk for about three hours, along transect usually defined during the previous day and aimed to sample all available habitats at the site.

2. Pitfall trapping

Although widely used and recommended, especially in long-term field studies within temperate regions, pit fall traps and drift fences have proved to be the least effective method in forest habitats (Rödel and Ernst, 2004). However, this method can be useful to determine species richness of epigeic organisms or active surface forms (Rödel and Ernst, 2004). Capture success may vary greatly between species, with, for example, frogs that are strong jumpers difficult to trap in buckets (Enge, 2001; Rödel and Ernst, 2004). In the Eastern Afromontane forests only a specific subset of the amphibian and reptile assemblage is likely to be captured in such a way, typically the skinks in the genera *Melanoseps* and *Leptosiaphos*, small leaf litter snakes such as *Buhoma*, *Aparallactus* and *Crotaphopeltis*, Caecilians, especially in the genus *Scolecomorphus*, and leaf litter and the burrowing frogs *Arthroleptis* and *Probreviceps* (Menegon, 2006). In fact, we have used this method in the case of surveys lasting longer than 5-7 days per site, because it was found that virtually all the species that are usually captured in the buckets were found also by active searching. The pitfall trap lines were made up of a series of 11 buckets, positioned five meters apart. The traps work by channeling animals that are crossing the forest floor along the drift fencing until they fall into the bucket. Trapping sites are located with a preference for areas of forest with low levels of disturbance and close to water sources. Where multiple sites were being surveyed within a forest reserve, these were distributed in such a way as to sample forest from the maximum possible altitudinal range and in order to cover different type of habitat.

3. Acoustic sampling

In the majority of frog species, males use distinctive species-specific calls to advertise their position to potential mates and rivals during breeding periods (Ryan and Rand, 1993; Gerhardt, 1994). These species-specific vocalizations can be used to determine the presence of species at night, sometimes from distances up to several hundred meters. Frog calls were recorded using different devices, following the improvements of recording devices availability, as traditional analogic recorders with audiocassette, DAT or minidisc recorder, equipped with a directional microphone, yet in recent times all these devices have been replaced by digital recorders, in our case equipped with good quality, inbuilt microphone, that was proven to be sensitive enough given that calling males are usually easy to approach at short distance.

Sound analyses were conducted using specific software such as Raven, developed by the Bioacoustics Research Program of the Cornell University (Bioacoustics Research Program, 2014), or Avisoft, developed by Avisoft Bioacoustics (Specht, 1996), that generate spectrograms. These indicate frequency versus time, and amplitude, Fast Fourier Transform (FFT) spectra - amplitude/pressure in arbitrary units versus frequency - and oscillograms - amplitude versus time (Bee et al., 2001). Through sound analysis, it was possible to obtain precise measurements of call features that help in species ID. Measurements included call duration, number of units, unit duration, fundamental frequency, highest frequency and the frequency of visible harmonics (Gerhardt, 1994). The advertisement calls, including their numerical data on temporal and spectral features, are often included in species description as one of the diagnostic characters (Channing, 2004; Loader et al., 2010; Menegon et al., 2011).

Most amphibians call mostly at night, exceptions including Puddle frogs (*Phrynobatrachus*) and some Squeakers (*Arthroleptis*) and a few other species that could, occasionally call during the day, especially before, during or immediately after heavy rains (pers. obs) Males of many species call while sitting on exposed sites, such as branches or leaves, sometimes high on trees. Other species, like some *Arthroleptis*, call from concealed sites in the leaf litter. The recently described *Probreviceps durirostris* call from under rotten logs or from holes in the ground and is extremely hard to locate, even while is calling (Loader et al., 2006). *Callulina* sp. sometimes call from tree trunks, where they resemble bark knobs (de Sa et al., 2004). Recording a call is usually quite easy, since frogs keep calling even if approached closely, the recorder with built in microphone or the external microphone is then oriented toward the call is order to maximize call quality and reduce the background noise.

4. Opportunistic captures

Snakes species are difficult to detect (McDiarmid, 2011) as they are often secretive or nocturnal, some species can sit, well camouflaged and motionless, for hours or days. Others can easily elude capture once detected (McDiarmid, 2011) Therefore, a complete list of snake species can be difficult to obtain. Snakes are often encountered during walks or can be observed during the night while perched on tree branches. In any case, the number of species collected or encountered during a survey will be far from a complete list and help from local inhabitants can provide important supplementary information. Since local inhabitants are often working at the forest edge and they are willing to kill snakes found in their crop fields, an agreement with the farmers in order to obtain those specimens can greatly increase the number of snake species recorded in a particular area (Menegon, 2006). Species obtained in this way were not included in analyses of abundance or species richness estimates but can provide useful information when compiling the species lists by areas.

Species identification

Species were identified by using multiple lines of evidence, morphological molecular and, when possible, ecological; this approach is often refereed as "integrative taxonomy" (Padial et al., 2010; Dupuis et al., 2012), where congruence between the various lines of evidence is considered a necessary requisite for an effective species delimitation (Padial et al., 2010). Exceptions could exist in the so-called 'cryptic species' that originated allopatrically, where a long-standing geographic isolation between populations leads to reproductive isolation and lineage divergence but do not necessarily produce detectable morphological differences, especially when the habitat of the two separated population is characterized by high stability through time (Losos, 2009). In the latter case, populations representing clear divergent lineages could remains morphological very stable, preventing their recognition based on morphological estimates alone (Stuart et al., 2006; Vieites et al., 2009).

Classical morphological and morphometric analysis involve the detection of morphological differences through direct measurement of various characters that, together, are considered an indirect evidence of reproductive isolation or divergent lineage evolution (Mayr, 1969), and these are the methods we have mostly used to define species boundaries of the collected samples, coupled in more recent years, with DNA species delimitation approaches. In case of species, which are morphologically very similar, we have analyzed differences by using multivariate methods (Mutanen and Pretorius, 2007) and the technique was successful in separating species with a certain degree of individual character overlap (Meegaskumbura and Manamendra-Arachchi, 2005; Kaliontzopoulou and Carretero, 2007). Quantitative morphometric analyses could be as accurate as molecular analyses in the delimitation of morphologically challenging species (Meegaskumbura and Manamendra-Arachchi, 2005; Kaliontzopoulou and Carretero, 2007) especially when coupled with geometric morphometric analysis, useful to detect overall changes in shape by using landmarks of homologous structures (Schwarzfeld and Sperling, 2014).

Molecular-based systematic approaches

Along with various morphological approaches, as previously described, DNA barcode and DNA-based species delimitation were carried out using several methods, most of which require a phylogeny of the taxonomic group in question, in order to assess the congruence between morphological and molecular results in species delimitation.

The General Mixed Yule-Coalescent (GMYC) method identifies the point of transition between a coalescent and a speciation branching pattern on an ultrametric phylogeny (Pons et al., 2006). Although this method has proven useful in rapid biodiversity assessments of mega-diverse groups (Monaghan et al., 2009), it has been found in certain cases to be highly dependent upon the tree-building method and parameters used (Ceccarelli et al., 2014). Because the GMYC method also relies only on a single consensus tree, it is prone to phylogenetic error, and it has been found to return dissimilar estimates of species numbers compared to other methods in some cases (Miralles and Vences, 2013). To counter this problem, a Bayesian implementation of the GMYC was developed (bGMYC; Reid and Carstens, 2012) that samples over the posterior of the output trees. Another alternative to GMYC based species delimitation approaches is the Bayes Factor species Delimitation method (BFD, Grummer et al., 2014). BFD analysis relies on the coalescent species tree algorithm (for a review see (Degnan and Rosenberg, 2009) to test different hypotheses of ‘species groups’ defined by the user (i.e. different *BEAST runs, each with individuals placed in alternative groupings). The appropriate use or not of different species delimitation methods (GYMC, bGYMC, and BFD) is still debated, and thus researchers are currently being encouraged to use several different methods and compare consistencies between various approaches (Miralles and Vences, 2013; Carstens et al., 2013). Further details on the molecular analysis methods used in phylogenetic reconstruction and species delimitation are included in chapters 4 and 5.

Results

Annotated chronology of herpetofaunal discoveries in the EAR

The earliest amphibian species to be described from East Africa were collected and described in the mid-19th century, when missionaries and explorers sent specimens to European museums, where they were described and named by museum zoologists (Harper et al., 2010). Subsequently, in the early 1900s, the German administration established a botanical garden in the East Usambara Mountains of Tanzania, which became the first site within the Eastern Afromontane, intensively investigated for Amphibians and Reptiles. From the early- to mid-1900s, the history herpetological exploration in the Eastern Afromontane reflects also the history of the dedicated life of few herpetologists/explorers, especially Arthur Loveridge and Raymond Laurent.

In general, issues related to colonialism, were the main drivers of the early accumulation of knowledge on amphibians and reptile diversity across the Eastern Afromontane. In fact, most of the early gazetteers' (Schiøtz, 1975, Broadley and Howell, 1991) main collecting localities were along the sea or great lakes coast, close to big cities or major geographic features. Large areas were remote and remained largely unexplored until very recently. During the 1990s, while intensive taxonomic ecological studies on herpetological fauna were underway in many regions of West Africa, tropical America and tropical Asia (as documented by the publication of comprehensive books and studies covering entire regions: Inger, 1996, Duellman, 1997, 1980, Channing and van Dijk, 1976, Amiet, 1983), forests in East Africa were still largely undocumented and unstudied (Howell, 1993).

Summary: Chronology of early discovery and taxonomy of EA amphibian and reptiles

1. In mid 1800s to early 1900s collection of the first amphibian and reptile specimens in East Africa by missionaries and explorers, especially from Coastal Africa. The specimens were shipped to Europe where museum based zoologist described and named them.

2. 1909 -1911. George Albert Boulenger based at British Museum in London and Fritz Neiden based at Berlin Museum of Natural Science described the first forest associated species from the Eastern Afromontane.

3. Between 1926-27, the mammalogist W.H. Osgood, collected some amphibians in the Bale

Mountains, Ethiopia.

4. 1928. Barbour and Loveridge published a paper with the description of 11 new species of amphibians and 7 new species of reptiles collected in the Usambara and Uluguru Mountains.

5. Between 1924 and 1931 Ernst Ahl, based at the Berlin Museum of Natural Science, described several species from Eastern Afromontane mountain ranges in Ethiopia, Uganda, Rwanda and Tanzania from specimens hosted at the museum.

6. Between 1927 and 1936 W. Uthmöller surveyed for reptiles the northern volcanic mountains of Tanzania

7. In 1941 Raymond Laurent described the first species from the Albertine Rift area in early 40s, and has continued with herpetological exploration and species description till early the 70s. Laurent was the greatest contributor to the herpetological knowledge of the Albertine Rift with dozens of new species discovered and described currently valid.

8. Arthur Loveridge continued to explore East African Mountain and describe over a hundred new amphibian and reptile species until 1957 when he published a comprehensive Checklist of the Reptiles and Amphibians of East Africa.

9. Between 1971 and 1975 Malcolm J. Largen, D.W. Yalden and P.A. Morris surveyed the northern side of the Bale Mountains.

10. During the early 70s Jean-Luc Perret, based at the Natural History Museum in Geneva, described several reptiles and amphibians from Tanzania, including two *Nectophrynoides* species.

11. In 1975 Arne Schiøtz, based at Danish Aquarium, published the book Treefrogs of East Africa, where he summarized all the available information collected so far and described several new species from the Eastern Afromontane area.

12. In 1986 a recently built road allowed to access the Harenna forest in the southern side of the Bale Mts., M.J. Largen, D.W. Yalden collected a remarkable number of new species of amphibians.

13. In 1988 Hinkel and Fischer compiled the first list of amphibian and reptile species for the Virunga volcanoes and Nyungwe forest in Rwanda based on field surveys.

14. Between 1992 and 1998 Bob Drewes and Jens A. Vindum from the California Academy of Sciences, conducted several surveys in the Impenetrable forest in Uganda and published the description of some new species

15. In 1998 John Vonesh surveyed the Kibale forest in Uganda updating the species checklist for amphibians and adding new distribution records.

16. In 1999 Arne Schiøtz published the book Treefrogs of Africa, representing a state of the art for the knowledge about African treefrogs and description of more new species from the Eastern Afromontane.

The first specimens from the Albertine Rift region of East Africa were secured from broader natural history studies and exploration in the region from German and British naturalists in the late 1800s, while the first account of the amphibian fauna of several sites in the region, including the Virunga Mountains, was mainly the result of the herpetological explorations carried out by Raymond Laurent, between 1952 and 1972, the main contributor to the current knowledge on herpetofauna of the region. Investigation between late 1980s and mid 1990s complemented early studies by Laurent, especially in Rwanda and Uganda (Hinkel and Fisher, 1988, Drewes and Vindum, 1991) but without adding new species associated with the Eastern Afromontane. Vast mountain ranges like Itombwe and Kabobo in eastern DRC and Mahale in western Tanzania were still virtually unexplored (Plumptre et al., 2007).

As with the Albertine Rift, German and British exploration of East Africa at the turn of the 20th Century provided the first understanding of species from the Eastern Arc Mountains of Tanzania. In particular, specimens secured from Amani, in the East Usambara, were described by people working in British and German natural history museums (e.g. Boulenger, Ahl, Nieden, Tornier). Arthur Loveridge is often considered the greatest contributor to early East African herpetology. Loveridge collected specimens during World War I and onwards during the 1920s and 1930s, and continued describing new species until the 1957 when he published a comprehensive Checklist of the Reptiles and Amphibians of East Africa (see Table 3).

The publication of the book ‘Biogeography and Ecology of the rain forest of East Africa’, provided the first comprehensive work on East African forests and summarized most of the available knowledge (Lovett and Wasser, 1993). Prof. Kim M. Howell, author of the chapter on the herpetofauna, listed 47 amphibian and 59 reptile species associated to the East African forests, including coastal forests of Kenya and Tanzania, and montane forests of Tanzania, Kenya, Mozambique and

Zimbabwe, of which respectively 39 and 35 are associated to the Eastern Afromontane (Howell, 1993).

In 1989, the non-government organization Frontier (http://www.frontier.ac.uk) started biodiversity surveys in Tanzania. This was a collaborative project between the University of Dar es Salaam and the UK based Society for Environmental Exploration, who took hundreds of young volunteer researchers to several Tanzanian forests, including some in the East Usambara and Udzungwa Mountains. The surveys collected a wealth of specimens (botanical and zoological) that were subsequently sent for identification and study in museums in South Africa, Europe, and USA (Poynton and Clarke, 1999; Menegon et al., 2004; Broadley et al., 2006). In Tanzania, some areas such as the Usambara and Uluguru Mountains have received more attention and both became among the best-studied sites across east Africa for reptiles and amphibians, and localities of outstanding herpetological diversity (Howell, 1993). Conversely, most of the available data for other areas of Tanzania, like the southern highlands or several forest fragments in the Udzungwa Mountains, were still based on Loveridge’s work, and more remote mountain ranges, like the Nguu, Nguru, Ukaguru, Rungwe and Livingstone Mountains remained extremely poorly known (Menegon et al., 2008; Rovero et al., 2014).

Specimens from Ethiopia were first collected around the mid-1800s (Largen, 2001) during early expeditions by British and Italian explorers, but the Highlands of Ethiopia remained largely unexplored scientifically until the middle of the twentieth century. In 1926-27, W.H. Osgood, a mammalogist based at the Chicago Field Museum, collected a few amphibians and reptiles during an expedition in the Bale Mountains. More attention to the frogs of the region dates from fieldwork conducted by M.J. Largen during brief visits to Bale in 1971, 1972 and 1974, plus two extended trips with D.W. Yalden, P.A. Morris and others. Following the completion of a new highway was only in 1983 that reaching the Harenna Forest on the southern side of the Bale massif, became feasible. A preliminary survey of the Harenna Forest was carried out from 3-23 August 1986 by M.J. Largen and D.W. Yalden, during which an impressive number of new amphibian and reptile species were acquired. During the 1990s, Malcolm Largen and colleagues published several species description of endemic Ethiopian amphibian, as result of the expeditions in the Bale Mountains carried out during the 1980s, remarkable new genera and species of frog from the Bale Mountains (e.g. Largen and Spawls, 2010). Large areas of the country, especially in the highlands on the western side of the Great Rift Valley, remain biologically unexplored. Most recent information on biological exploration on Bale Mountains are summarized by Gower et al. (2013).

The mountain archipelago of central and northern Mozambique is widely scattered and still is a relatively unexplored part of the Eastern Afromontane, but a potentially biologically important region. Montane isolates in this vast region have rarely been studied untill relatively recently (Branch et al., 2014). *Ad hoc* surveys for some taxonomic groups in this region suggest the montane islands (Mt. Gorongosa, Mt. Mabu, Mt. Chiperone and Mt. Namuli) contain high levels of biodiversity particularly in terms of single site endemics (Schneider et al., 2005; Branch and Bayliss, 2009; Branch and Tolley, 2010; Branch et al., 2014; Bayliss et al., 2014) but still very little information on amphibian and reptile diversity is available or published.

Post-1998 herpetofaunal discoveries by MM and others

Between 1998 and 2014, MM carried out 53 individual surveys in 39 sites across five East African countries for an estimated total of approximately 340 days of active searching. Most of the material collected, about five thousands specimens, have been deposited in the collection of the Science Museum of Trento where it's available for study, duplicates were deposited in the herpetological collection of the University of Dar es Salaam, in Tanzania and Natural History Museum, London. The appendices detailed below offer a window onto the varied outputs and findings that have been generated from biological exploration of these areas. These are arranged as case studies, where the various outputs are summarized. A complete and comprehensive study of the material awaits further work where taxonomic clarification, understanding of biogeographic patterns and the conservation of EA amphibians and reptiles can be evaluated.

A total of 101 (62 amphibians and 38 reptiles) putative new species have been found by MM and colleagues from 1998 to date (Table 2-1). Of these, 27 have been formally described. Over 80 % of the discoveries were made in the Eastern Arc Mountains of Tanzania, while more recently, several new reptiles, especially chameleons, were found in the isolated inselbergs of northern Mozambique (see Table. 2).

Appendix 1 is a case study detailing the discovery and description of the distinctive and charismatic new bush viper *Atheris matildae*, and a strategy aimed both to avoid collection of wild animals for the pet trade, and to establish a captive insurance population to aid its conservation. Appendix 2 shows a taxonomic description paper, including genetic and bioacoustic features, detailing a new species of amphibians in the genus *Callulina*. Appendix 3 then gives an assessment, based on species inventory complemented by preliminary diagnostic identification of taxa from the Mahenge Mountains, along with implications for the biogeography and conservation of its forest fragments. Appendix 4 lists the main overlooked areas across the Eastern Afromontane where to carry out future herpetological investigation.

Table 2-1 Chronology of MM's surveys and discoveries. The great majority of sites and associated discoveries are in Tanzania and span over 15 years. In two sites, namely the Uzungwa Scarp and the Nguru Mountains, both in Tanzania, the number of species new to science that were discovered, and in part described, is outstanding. Colour coding: green = Tanzania; pink = DRC; brown = Rwanda; purple = Ethiopia; blue = Mozambique.

Survey	Amphibians (Total n = 62)	Reptiles (Total n = 38)
1. 1998-2000: Several herpetological surveys in the Uzungwa Scarp Forest Reserve	*Afrixalus sp. 1* *Afrixalus sp. 2* *Arthroleptis sp. 1* *Arthroleptis sp. 2* *Arthroleptis sp. 3* *Arthroleptis sp. 4* *Leptopelis sp. 1* *Leptopelis sp. 2* *Leptopelis sp. 3* *Leptopelis sp. 4* *Nectophrynoides poyntoni* *Nectophrynoides sp.* *Probreviceps sp.*	*Rhampholeon moyeri* *Rhampholeon sp.* *Urocotyledon sp.* *Dipsadoboa sp.* *Crotaphopeltis cf. tornieri*
2. 2002: Surveys in the montane grasslands in southern Udzungwa	*Phrynobatrachus sp.*	*Tetradactylus udzungwensis* *Tetradactylus cf. ellenbergeri*
3. 2002: First herpetological survey of the Nguu Mountains (Nguu North Forest Reserve)	*Hoplophyne cf. rogersi*	*Crotaphopeltis cf. tornieri* *Rhampholeon sp.*

4. January 2003: Survey in the Mamiwa Kisara North FR, Ukaguru Mts.	*Afrixalus cf. uluguruensis* *Leptopelis cf. grandiceps* *Nectophrynoides paulae* *Nectophrynoides laticeps* *Probreviceps durirostris*	*Trioceros cf. werneri*
5. November 2003: Uzungwa Scarp		*Dipsadoboa sp.*
6. March 2003: Brief survey in the Mwanihana forest in the Udzungwa NP		*Lygosoma sp.*
7. September 2004: Survey on the western side of Northen Udzungwa Mts.	*Nectophrynoides sp.*	*Urocotyledon rasmusseni*
8. October 2004: First survey on the Nguru South FR at high elevation	*Arthroleptides sp.* *Arthroleptis nguruensis* *Arthroleptis sp.* *Callulina hanseni* *Callulina meteora* *Hyperolius burgessi* *Nectophrynoides sp. 1* *Nectophrynoides sp. 2* *Nectophrynoides sp. 3* *Hoplophryne cf. uluguruensis*	*Crotaphopeltis cf. tornieri* *Trioceros sp.* *Kinyongia sp.* *Rhampholeon sp.*
9. November 2004: First herpetological survey on Kanga FR	*Arthroleptides sp.* *Callulina kanga* *Leptopelis cf. grandiceps*	*Crotaphopeltis cf. tornieri* *Dipsadoboa sp.* *Urocotyledon sp.*
10. December 2005: Surveyed three forest reserves in the North Pare Mountains (Kindoroko, Minja and Mramba FRs)	*Callulina laphami*	
11. 2005: Brief survey on the western side of Mt. Kilimanjaro between 3000 and 4500 m asl.	*Strongylopus sp.*	

12. February 2005: First herpetological survey of the Kilindi FR in the Nguu Mts.	*Nectophrynoides sp.* *Hoplophryne cf. rogersi*	*Crotaphopeltis cf. tornieri*
13. June 2005: Nguru South FR		
14. March 2006: Kigogo FR, southern Udzungwa Mts.	*Afrixalus sp. 1* *Probreviceps sp.*	*Kinyongia sp.*
15. April 2006: Kigogo FR		
16. September 2006: Other survey in the Nguru South FR (high altitude)		
17. October 2006: First survey ever in Ilole Forest Rubeho Mountains		
18. November 2006: Herpetological survey in Sali FR, Mahenge Mts.	*Hoplophryne sp.*	*Crotaphopeltis cf. tornieri* *Cnemaspis sp.* *Kinyongia sp.*
19. December 2006: Survey of Mahale NP (low altitude) and the first herpetological survey of Ntakata forest in the Mahale Mts.		*Leptosiaphos sp.*
20. March 2007: Additional survey in Ilole FR, Rubeho Mountains	*Probreviceps sp.*	*Crotaphopeltis cf. tornieri*
21. In May 2007: Survey of Mafwomero FR in the Rubeho Mts.	*Nectophrynoides sp.*	
22. February 2008: Other survey in the Nguru South FR (Low) and Nguu North FR		

23. In March 2008: Survey of Chome FR in the South Pare Mountains.	*Callulina stanleyi* *Callulina shengena* *Leptopelis cf. parkeri*	*Urocotyledon sp.*
24. October 2008: First survey in Kahuzi-Biega NP, DRC		
25. November 2008: Herpetological survey in the Nyungwe Forest NP, Rwanda	*Amietophrynus sp. 1*	
26. January 2009: Rungwe		
27. June 2009: Herpetological surveys in the Bale Mountains and Western forests of Ethiopia		
28. February 2010: Kitulo NP		
29. February/March 2010: Poroto Mts.		
30. March 2010: Madehani forest		*Dipsadoboa sp.*
31. March 2010: Rungwe	*Leptopelis sp.* *Arthroleptis sp.*	
32. December 2010: Nyungwe NP, Rwanda		
33. January 2011: Three forest fragments in the Livingstone Mountains + Mbeya area	*Arthroleptis sp. 1* *Arthroleptis sp. 2* *Afrixalus sp.* *Hyperolius davenporti* *Leptopelis sp.*	*Atheris matildae* *Kinyongia sp.*
34. February 2011: Poroto Mts.		

35. November 2011: Extensive survey in Kahuzi-Biega, DRC	*Afrixalus sp.* *Amietophrynus sp. 1* *Hyperolius sp.*	*Trioceros sp.*
36. December 2011: Mikuvi FR, Ukaguru Mts.	*Leptopelis sp.*	
37. December 2011: East Usambara Mts.		
38. December 2012: Kimboza FR, Uluguru North and Uluguru South FR	*Leptopelis sp.* *Nectophrynoides sp.* *Nectophrynoides pseudotornieri**	*Rieppeleon sp.*
39. November 2013: Brief visit to Uzungwa Scarp (N. poyntoni)		*Urocotyledon sp.*
40. November and December 2014: Three mountains of northern Mozambique	*Notophryne sp.*	*Dipsadoboa sp.* *Nadzikambia sp.* *Rhampholeon sp.1* *Rhampholeon sp. 2*

Discussion

Herpetological exploration and its implications for taxonomic approaches to conservation

Between 1928 and the present, the number of species discovered and described from the Eastern Afromontane has increased substantially but not constantly. The patterns of species discovery and description have varied among taxonomic groups dependent upon individuals who made particularly large contributions. For example, Arthur Loveridge who worked on amphibians in East Africa was prolific with new descriptions (Howell, 2000) but this was followed by a hiatus until 1990-2000s, when Kim Howell and colleagues (Schiøtz, Poynton, Menegon, and Loader) re-invigorated research on East African amphibians – particularly in areas such as the Udzungwa Mountains.

Similarly across the same time frame (1920s - present), both species concepts and methods used to define species boundaries from an operational point of view have increased in number and complexity (Minelli, 1993; De Queiroz, 2007). Different methods of diagnosing species can lead to different values in term of species richness per site, number of endemics and size of species ranges (Vane-Wright et al., 1991). It is in fact widely thought that use of a phylogenetic species concept versus a non-phylogenetic species concepts, may lead to recognition of a greater number of much less inclusive units, therefore a greater number of entities (Winter et al., 2012).

Table 2-2 Species richness and numbers of endemic amphibians (and total amphibian species recorded) known at different times, and using different taxonomic approaches at five key sites in the Eastern Arc Mountains. PS = phylogenetic species; NPS = non-phylogenetic species.

	Uzungwa Scarp	Nguru South	S. Pare	Mahenge	E. Usambara
Sp. richness 1928 EAM	0	0	0	0	23
Sp. richness 2004 EAM	7 (7)	5 (11)	1	7 (28)	18 (46)
Sp. richness 2015 EAM (described)	26 (32)	16 (28)	4 (7)	10 (40)	19 (47)
Sp. richness 2015 EAM (PS)	33 (40)	28 (42)	4 (7)	15 (40)	19 (47)
Site endemics 2004 (NPS)	4	0	0	0	5
Site endemics 2015 (NPS)	4	3	2	0	5
Site endemics (PSC) 2015	17	19	4	7	14

Effects on species richness and endemism

The phylogenetic species approach led to an increase in species richness, species endemism and, as a consequence, the number of endangered species as assessed using the current IUCN categories (Brooks, 2011; Rivers et al., 2014). This increase in numbers of threatened species is due to the fact that the new species are often the result of splitting of previously recognised ones, with the result that the areas occupied by each is usually a subset of the original species' range (Brooks et al., 2004; Brooks, 2011). This is particularly well exemplified by the increase of the number of site endemics (see Table 2-2) In the Uzungwa Scarp Forest Reserve, for example, both in 2004 and 2015 there are four site endemics identified by using a traditional morphological approach while, based on phylogenetic reconstruction, complemented in many cases with species delimiting approaches (the Generalized Mixed Yule Coalescent method (GMYC) and Bayesian species delimitation methods), the number of site endemic species increases to 17 (Rovero et al., 2014; Loader and Menegon unpublished). Such a difference in species numbers is explained by the splitting of several Eastern Arc endemics, previously grouped together on the ground of similar morphologies, into single site endemic species. For example, there has recently been a radical change in our concept of the genus *Callulina*. Prior to 2003, the genus was considered monotypic with the single species found throughout the Eastern Arc (Howell, 1993). Now, seven species have been described, of course reducing the range of each new species (Loader et al., 2010; Menegon et al., 2011).

Geographically, this has had a large impact on the richness of localities. For example, the Nguru Mountains, where the lack of field investigations and a purely morphological approach to species definition, led to the recognition of just eleven species, of which five were Eastern Arc endemics (Emmrich, 1994). Intensive field surveys carried out between 2004 and 2006 and the application of phylogenetic methods to species boundary definition, led to the recognition of 19 site endemics, making Nguru an outstanding site at continental level for amphibian conservation (Table 2-2; Menegon et al., 2008; 2009; Rovero et al., 2014).

Effect on conservation site ranking

The criteria underlying the identification of Biodiversity Hotspots assume species (especially endemic species) to be the fundamental element of conservation efforts (Myers et al., 2000). Similarly, evaluation of the effectiveness of existing conserved areas often focuses on taxonomic diversity (Fleishman et al., 2006). Key goals of conservation are to protect both species and the functional and genetic diversity they represent (Tucker et al., 2012). In fact, in recent times, phylogenetic diversity has become a key component of biodiversity, as it reflects evolutionary heritage, functional diversity and the potential ability of organisms to adapt to future conditions (Vane-Wright et al., 1991; Cadotte et al., 2010) discussed further in Chapter 5) Conservation efforts should therefore be aimed at protecting not just species, but also the underlying functional and genetic diversity they represent (Tucker et al., 2012).

The biological importance of the Eastern Arc Mountains of Kenya and Tanzania has been summarized and highlighted by (Burgess et al., 2007) and updated by Rovero et al. (2014) by the inclusion of the results of targeted faunal surveys carried out between 2005 and 2009. These surveys raised the number of endemic and regional endemic vertebrates by 24% (from 170 to 211 species), including 27 new species of which 23 are amphibians and reptiles, showing the biological value of the EAM to have been underestimated (Rovero et al., 2014). If recent targeted surveys in the area have addressed the underestimation of the actual biological diversity by increasing the number of species recorded, the lack of sufficient taxonomic studies using phylogenetic approaches aimed to detect cryptic diversity across taxa, remains a major concern (Mendoza and Arita, 2014). In fact, Rovero et al. (2014) cites only one paper on mammals, two on birds, two on reptiles (chameleon only), two on general herpetofauna (site-based) and one on amphibian (taxon based), which are based on the use of phylogenetic methods. These cover just a very small part of animal families and genera occurring in the area. Ranking the five sites in Table 3 based on site endemic species recognized by non-phylogenetic species delimitation approaches, the ranked order is as follow: (1) Usambara Mts., (2) Uzungwa Scarp, (3) Nguru South, (4) Pare Mts., (5) Sali FR. If the ranking takes into account the proportion of cryptic diversity detected through the use of phylogenetic methods, the ranked order becomes: (1) Nguru South, (2) Uzungwa Scarp, (3) Usambara Mts., (4) Sali FR, (5) Pare Mts.

Recommendations for future sampling in the Eastern Afromontane Region

In spite of 250 years of taxonomic classification and over 1.8 million species already catalogued in a central database (Pimm et al., 2014), results suggest that some 86% of existing species on Earth and 91% of species in the ocean remain undescribed (Mora et al., 2011). There are a number of factors that can influence our knowledge on the global diversity of life, including species definitions, changes in taxonomy or in taxonomic efforts and the lack of completeness of biological inventories (Mora et al., 2011).

The current understanding of species occurrence and distribution across the Eastern Afromontane, as in many other areas, is based on the actual pattern of species distribution, and on the level of accuracy with which the occurrence data have been collected. The distribution of the latter is generally biased by environmental and anthropogenic factors like geographical barrier and geopolitical issues, that has reduced or currently reduce accessibility to certain areas (Pyke and Ehrlich, 2010). Biases are also related to the historical process of inventory (Hortal and Lobo, 2006), such as taxonomists' home range, or the proximities of roads or research stations (see Fig 2-3; Graham et al., 2004; Beck and Kitching, 2007). For For example, the highest apparent numbers of vertebrate species in South America are in areas close to research stations, as are many GBIF (Global Biodiversity Information facility) records (Pimm et al., 2014). This could results in patterns of recorded taxa, which reflect unnatural spatial structures (Baselga et al., 2007; Lobo et al., 2010; Diniz-Filho et al., 2013).

Figure 2-3 spatial distribution of Global Biodiversity Information Facility database records of all organisms in the U.K. and Ireland and in East Africa. In the latter image, records are still concentrated along geographic and infrastructural features.

Non-systematic sampling also results in biased descriptions of species' geographic ranges, and leads to major errors in the distribution of endangered or conservation target species (Dennis and Hardy 1999, Dennis 2001). The countries included in the Eastern Afromontane have received different amount of sampling intensity and efforts both during early and recent attempts to document the diversity of the region, because of areas accessibility, political problems and civil unrest.

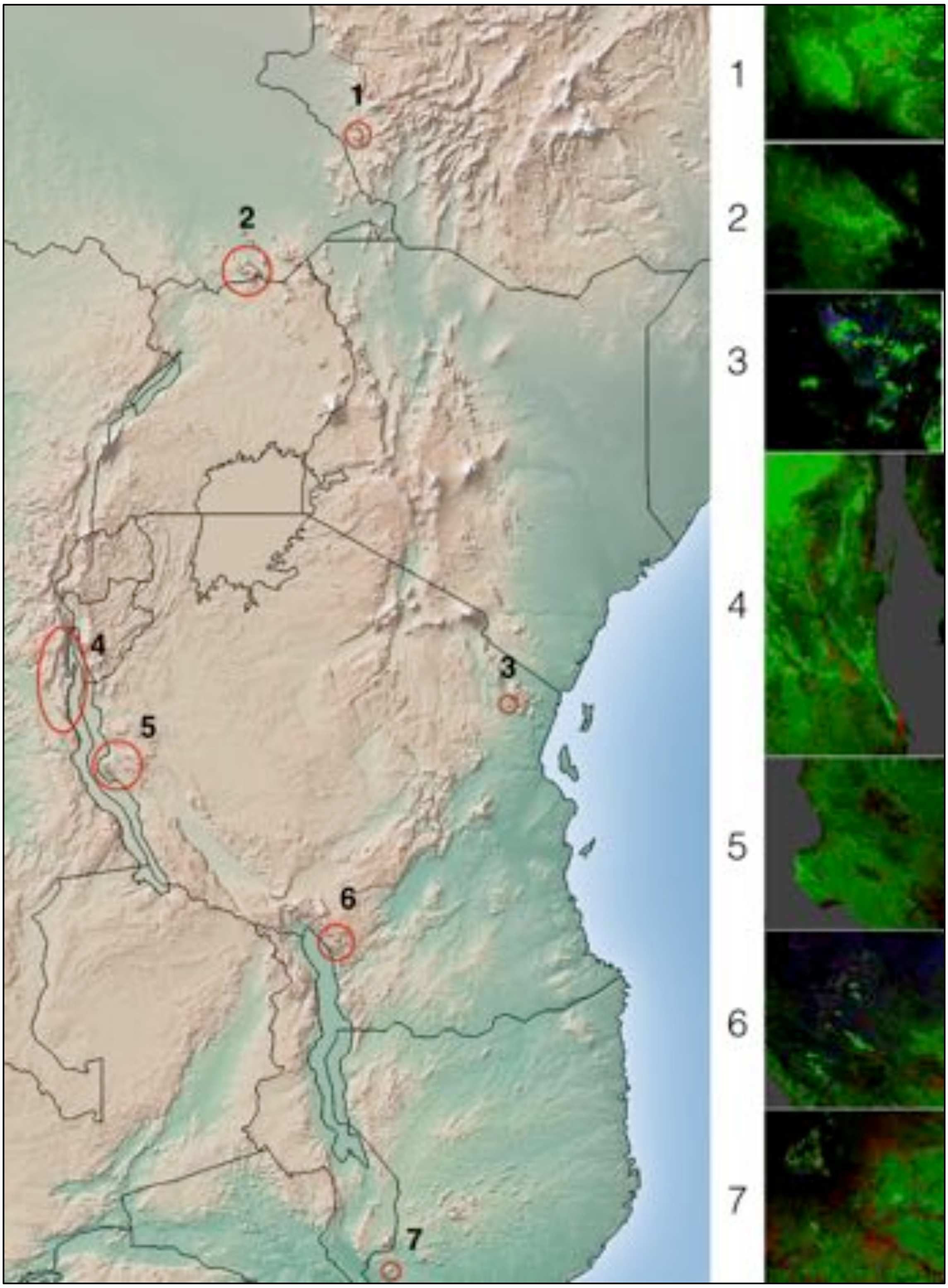


Figure 2-4 Map showing seven particularly poorly known sites across the Eastrn Afromontane from an herpetological point of view. Gura Ferda (1), Imatong Mountains (2), Mefi Hill (3), proposed Ngamikka Park (4), Mahale Mountains (5), Livingstone Mountains (6), Mount Chiperone (7)

Among the least known areas of the Eastern Afromontane, the Imatong Mountains in South Sudan and the Gura Ferda Plateau are particularly poorly known, furthermore they are geographically isolated and still have extensive forest cover across a range of altitudes. These features make the sites among the most interesting areas to investigate for mountain-associated amphibian and reptiles, as well other organisms, in the northern sector of the Eastern Afromontane (see Appendix 4) The presence of known endemic species in the Imatong, suggest that a wealth of range restricted, endemic species could be to be discovered in the future. The Gura Ferda plateau is, perhaps, even more interesting in that it lies in an area that could have affinities with Albertine Rift mountains species and the highly peculiar Ethiopian highland fauna, often characterized by species living at high altitude with restricted ranges (Largen and Spawls, 2010).

The Misotshi-Kabogo range, along with Imatong and Gura Ferda, represents one of the largest overlooked area in the Eastern Afromontane. In contrast to the other two and on the basis of the available records, the Misotshi-Kabogo is probably a southern continuation of the Albertine Rift mountain range. What makes the Misotshi-Kabogo range particularly interesting is its size, altitudinal gradient, isolation and habitat diversity (see Appendix 4). Molecular investigation on the few samples obtained from Misotshi-Kabogo show a large genetic divergence between congeneric species from Misotshi-Kabogo and from more northern massifs, suggesting a high level of isolation and the presence of a peculiar amphibian fauna.

The wealth of field investigation and taxonomic descriptions carried out in the last two decades by several herpetologists have greatly improved the understanding of the biological diversity of amphibians and reptiles in the Eastern Afromontane. Despite this there are still large areas of the region where very little is known, the exploration of these areas could further, significantly, increase the number of total species in the Eastern Afromontane region and improve our understanding of the underlying evolutionary and biogeographical patterns.

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Appendix 1 Description of a highly endangered forest Viper in the genus *Atheris*

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Article

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Description of a new and critically endangered species of *Atheris* (Serpentes: Viperidae) from the Southern Highlands of Tanzania, with an overview of the country's tree viper fauna

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Abstract

A new species of arboreal forest viper (Serpentes: Viperidae: *Atheris*) from a forest fragment in the Southern Highlands of Tanzania is described and named *Atheris matildae* **sp. nov.** The species resembles the forest horned viper, *Atheris ceratophora* Werner, by bearing horn-like supraciliary scales but it differs in size, body proportions, scalation, scale ultrastructure, and distribution. Genetic divergence is also assessed and the two species have an estimated divergence time of approximately 2.2 million years. An overview of the genus *Atheris* in Tanzania, including new distribution data, is presented and the conservation status of the new taxon is discussed.

Key words: Viperidae, *Atheris*, new species, Southern Highlands, Tanzania, Conservation

Introduction

Until recently Tanzania's Southern Highlands were largely unexplored biologically, the assumption being that they were of comparatively little significance. However, extensive work over the last decade by the Wildlife Conservation Society has shown that not only are the montane forests and grasslands north of Lake Nyasa extremely species rich, but they also demonstrate a high endemism and thus are as important for conservation as the Eastern Arc chain to the northeast (Davenport, 2005; 2006). New taxa discovered from this area include the chameleon *Kinyongia vanheygeni* (Necas, 2009) and most notably Africa's first new genus of monkey for 83 years, the kipunji *Rungwecebus kipunji* (Davenport *et al.*, 2006). Furthermore, a number of novel vertebrates are currently being described of which the distinctive forest viper that is the subject of this work, is one.

The genus *Atheris* Cope, 1862 occurs across tropical Africa, south to Mozambique. Of the 14 recognised species, five occur in Tanzania. With one exception, all *Atheris* species are to some degree, arboreal and share a similar morphology; a relatively slender body, large broad head, and a prehensile tail. The only leaf litter-dwelling species, *Atheris barbouri* Loveridge, 1930 is a small heavy-bodied snake, with a short, blunt head, lacking the prehensile tail; and little is known about its biology (see Fig. 7). The conservative morphology of the arboreal *Atheris* species has, over the decades, led to a poor resolution of their taxonomy (Ernst & Rödel, 2002). Despite this and although the genus is still in need of full taxonomic revision (Broadley, 1998), several recent contributions have improved our understanding of its taxonomy, usually prompted by the discovery of new species (Broadley, 1998; Lawson & Ustach, 2000; Ernst & Rödel, 2002; Branch & Bayliss, 2009). Recent investigations in Tanzania's Southern Highlands have brought to light a new species of forest viper. The type series of this new taxon includes one subadult individual and three adult male specimens sufficiently distinctive to be taxonomically assessed and named. Along with *A. ceratophora*, it is the only *Atheris* species bearing horn-like scales above the eyes. Little is known yet about its ecology but it is likely to occur in only one or a very few isolated forest fragments and be of very considerable conservation concern.

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Material and methods

Ventral and subcaudal scales were counted using standard techniques (Dowling, 1951). Scale rows were recorded at midbody (MSR). Interrictals are the scales on the dorsal surface of the head between the posterior supralabials, at the corners of the mouth (ricti). Supra- and infralabials were counted for both sides. Suprarostrals were defined as the only scales in contact with the upper edge of the rostral, excluding the bordering nasals; Total length (TL), snout-vent length (SVL) and tail length were recorded to the nearest millimetre. Comparative scale counts and character states for other *Atheris* species were based on data provided in Broadley (1998), supplemented with recent revisions (e.g. Lawson & Ustach, 2000; Lawson *et al*., 2001) and new species descriptions (*A. broadleyi* Lawson, 1999; *A. hirsuta*, Ernst & Rödel, 2002) and specimens available in the herpetological collection of the Museo Tridentino di Scienze Naturali, Trento, Italy and University of Dar es Salaam, Tanzania. Total genomic DNA was extracted and a portion (about 1100 bp) of the cytochrome b was sequenced using the protocol described in Ursenbacher et al. (2006). SEM observations and photographs were performed at the Museo Tridentino di Scienze Naturali using a LEO XVP (Carl Zeiss SMT Ltd., Cambridge, U.K.) at high vacuum on gold-coated prepared material. GeneBank accession numbers for the specimens sequenced are as follow: sample MTSN 9344, accession number: JF825389; sample MNHG 2667.45, accession number: JF825388.

Label acronyms are as follow: MTSN: Museo Tridentino di Scienze Naturali, Trento, Italy; KMH: Kim M. Howell field tags; SHCP: Southern Highlands Conservation Programme herpetological Collection, Mbeya, Tanzania; MNHG: Museum d'histoire naturelle, Geneve; ZMB: Museum für Naturkunde, Berlin. Comparative material is listed in the cited publication and in the appendix.

Taxonomy

Within the Atherini tribe, the snakes found in the Southern Highlands are referred to the genus *Atheris* Cope, on the basis of the following diagnostic features: no enlarged supraocular shield (present in *Proatheris* Broadley, 1966), tail prehensile and subcaudals single (tail non-prehensile and subcaudals paired in *Montatheris* Broadley, 1996).

Atheris matildae Menegon, Davenport & Howell sp. nov.

(Fig. 1–3)

Holotype. Adult male, MTSN 9344, collected in a forest fragment in Southern Tanzania, at about 1995 m by Omari Kibure and Obadia Mwaipungu in February 2009; fixed in 70% EtOH, tissue fixed in 90% EtOH.

Paratypes. 2 adult males, MTSN 9399 and MTSN 9418 and an immature MTSN 9417 collected in February 2011 at the same locality as the holotype, by Michele Menegon, Tim Davenport and Sophy Machaga.

Additional material. 10 specimens collected at the type locality between March and April 2011 and being kept alive for conservation purposes. 4 are males, 5 are females and 2 are immatures. Among them there is the adult female individual shown in Fig. 3.

Type locality. Remote fragmented montane forest in Tanzania's Southern Highlands. Precise locality withheld until conservation insurance population secure. Additional information on the locality can be obtained for scientific purposes from the authors upon request. (www.atherismatildae.org)

Diagnosis. *Atheris matildae* **sp. nov.** is distinguished from all other members of the genus except *A. ceratophora* by the presence of two to three very enlarged erect, hornlike, supraciliary scales. It is distinguished from *A. ceratophora* by the combination of the following morphological and molecular features, based on the data from 69 specimens from all over the known range of *A. ceratophora*: (1) its larger size, TL of *A. matildae* type is 643mm (the biggest male *A. ceratophora* ever recorded does not exceed 510mm TL), (2) higher count of maximum transverse head scales (max. 20 in *A. ceratophora*, 28 in *A. matildae*), (3) four subequal suprarostral scales in *A. matildae,* the two central ones of the same size and the outer ones double in size in *A. ceratophora*, (4) marked difference in dorsal scale microdermatoglyphic pattern (irregular smooth surface in *A. ceratophora*, presence of papillae-like ridges in *A. matildae*), (5) in *A. matildae*, an extensive black marking across the frontal part of the mouth, including part of nasal, rostral, mental and few infralabial scales is often present, the above described colour feature has not been recorded in the examined *A. ceratophora* specimens and photographs. Genetic divergence of mitochondrial gene cytochrome b between *A. ceratophora* collected at type locality and *A. matildae* expressed as actual substitution difference is 3.18% based on uncorrected p-distance of 0.03180.

FIGURE 1. Comparison of general shape, size and body proportion between *A. matildae* holotype (left) and a fully grown specimen of *A. ceratophora* from Udzungwa (MTSN 7506, SVL=54.2) (right) and an illustration of lateral head scalation.

Paratypes and additional material variation. Details and meristics for the type series are summarized in Table 1. A total of 13 specimens have been observed, three of them are paratypes. The most significant differences between holotype and paratype specimens are in body colouration (see Fig. 2). The young specimen MTSN 9417 tends to be more greenish, the zigzag ornamentation is more conspicuous and the top of the head is marbled in green/yellow. The two adult individuals, both males, are similar in colouration to holotype, with a back dorsum and a bright yellow zigzag dorsolateral pattern. Ten additional specimens have been recently collected and are being kept alive for conservation purposes. 5 are males, 5 are females, and 2 are young. A black patch around nasal, rostral, mental and first infralabials is present in most of the observed males but also in few females and immature individuals. Males in general tend to be darker with belly suffused with black. Adult females tend to be more yellow, in some cases with immaculate throat and belly; horn-like scales are yellow with black outer edges. Side of the head can be completely yellow or with black patches at the tip of the scales. In preservation the specimen retains the original colouration (see Fig. 1).

FIGURE 2. *A. matildae* paratypes showing body shape, colouration and head details. Top - down: MTSN 9399; MTSN 9418 and MTSN 9417.

FIGURE 3. Illustration showing the variation in colour of the rostral and chin area, The black patches are present in many individuals of both sexes.

TABLE 1. Measurements and scalation of type-series of *A. matildae*, with data of *A. ceratophora* for comparison.

	A. matildae	*A. matildae*	*A. matildae*	*A. matildae*	*A. ceratophora*
Specimen number	MTSN 9344	MTSN 9417	MTSN 9418	MTSN 9399	n = 53
Type	Holytype	Paratype	Paratype	Paratype	N/A
Sex	Male	Inmmature	Male	Male	Males only
Head Lepidosis					
Suprarostrals (SRO)	4	4	4	4	5–9
Interorbitals (IOS)	12	12	121	12	7–11
Maximum transverse head scales (MTHS)	28	28	27	28	19–20
Circumorbital scales (COS)	16	16	16	162	13–19
Interoculabials (IOL)	1	1–2	1	1–2	0–1
Interocunasals (ION)	3–4	4	4	3–4	2–4
Supralabials (SL)	10	10	10	10	7–11
Infralabials (IL)	11	11	11	11	8–12
Body scalation					
Midbody Scale Rows (MSR)	26	27	26	26	19–25
Ventrals	150	142	149	150	136–150
Subcaudals	49	44	50	49	49–58
Max length (SVL+Tail) in mm	643 (547+96)	391 (334+57)	625 (532+93)	631 (538+93)	510 (416+94)
SVL/Tail	5.7	5.85	5.7	5.78	5.4 – 5.8 range

Description of holotype. Adult male preserved in 70% EtOH. Snout-vent length (SVL) 540.7 mm, tail 96.0 mm, rostral width 2.9 mm, rostral height 0.8 mm; eye diameter (vertical) 3.2 mm; snout to eye 3.3 mm. A heavy-bodied forest viper, sub-quadrangular in cross-section, with a rather thick prehensile tail (SVL/Tail approximately 5.7 times); head pear-shaped, with a very distinct neck, rounded snout and swollen supraorbital region that does bear two/three elongated, horn-like scales; eyes relatively large, laterally placed, and with a horizontal diameter approximately 3/4 of the snout length. Crown of head covered in small scales, slightly larger over the temporal region (maximum transverse head scales—28); they bear a prominent keel and become mucronate over the head; the rostral is flattened, rectangular, about 3.5 times broader than high, contacting first supralabials and four small; unkeeled, roundish, subequal suprarostrals, nasal wider than high, with raised, embossed posterior edge, nostril circular and approximately in the centre of the nasal; internasals 5, all strongly keeled; interorbitals 9, keeled; circumoculars 16–16, not keeled but terminating in black blunt knobs; 1 row of suboculars present; circumoculars separated from nasals by two to three rows of feebly knobbed scales; a row of three irregular scales, bordering supralabials between nasal and lower circumoculars; supralabials 10–10, the first three smaller, and 6–8 with a swollen lower edge; infralabials 10–10, posteriormost with swollen upper edge and first in contact at the midline behind the mental; mental triangular, approximately twice as wide as deep; gulars bordering chin shields feebly-keeled, but prominently keeled towards the rictus; 2 preventrals, first largest; 150 ventrals; 49 undivided subcaudals (including spine); anal entire; 25 rows dorsal scales anteriorly, 26 rows at midbody, 19 rows posteriorly.

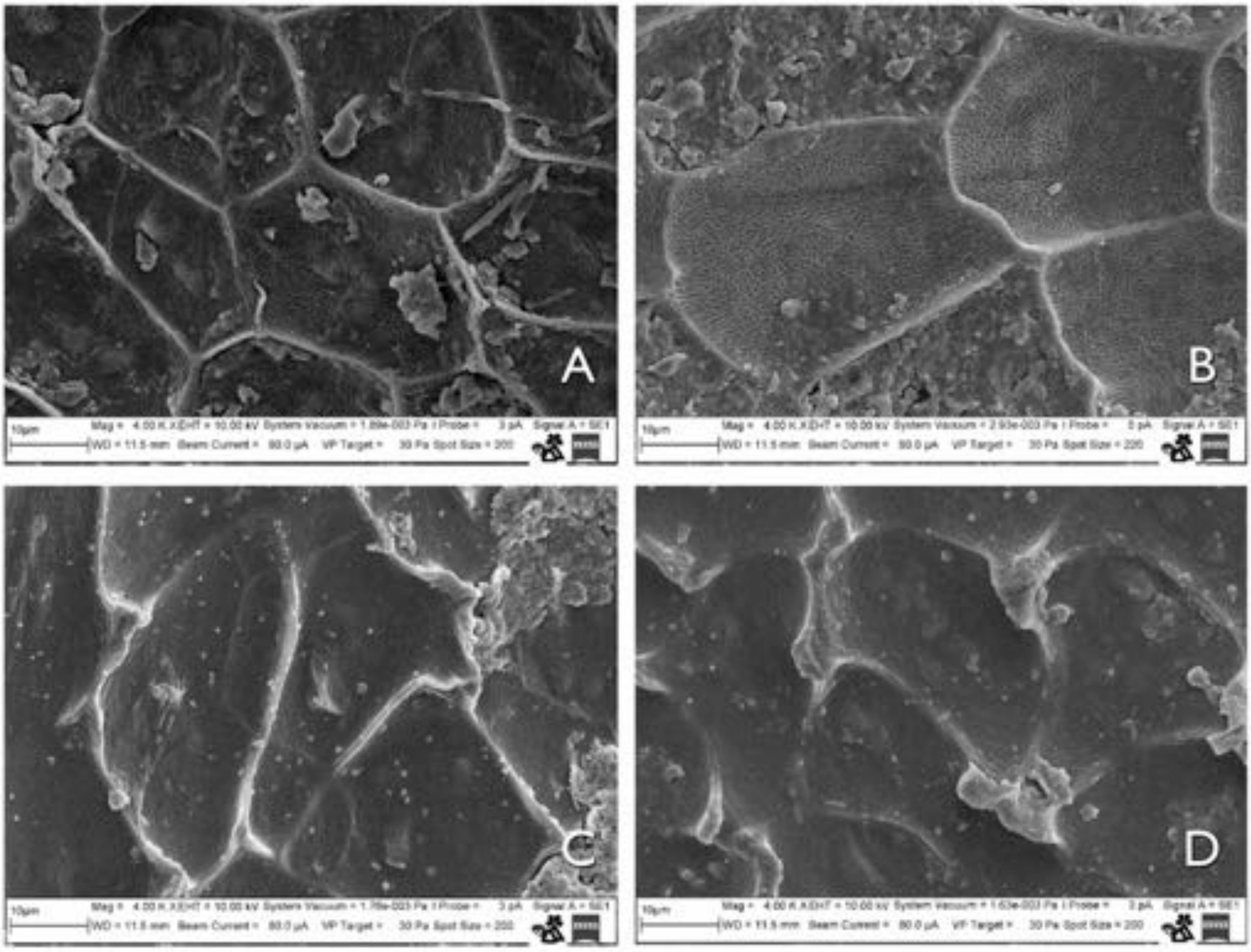

FIGURE 4. Dorsal scale microdermatoglyphic pattern of *A. matildae* (A–B) and *A. ceratophora*: (C–D). Note in B the papillae-like ridges covering the keratin layers on the scale of *A. matildae*.

Hemipenes. Both hemipenes are only partially everted. They resemble the *A. ceratophora* one as described by Emmrich (1997). The sulcus is bifurcate on a typically divided organ. The extreme basal area is naked, followed by an area with enlarged proximal spines, most prominently on the outer side of each lobe. Towards the apex, on the inner side of the sulcus, there are a few smaller scattered spines, while the distal area seems to be characterized by the lack of clearly differentiated ornamentation. A more detailed description of hemipenial morphology will be possible when a fully everted hemipenis becomes available for examination.

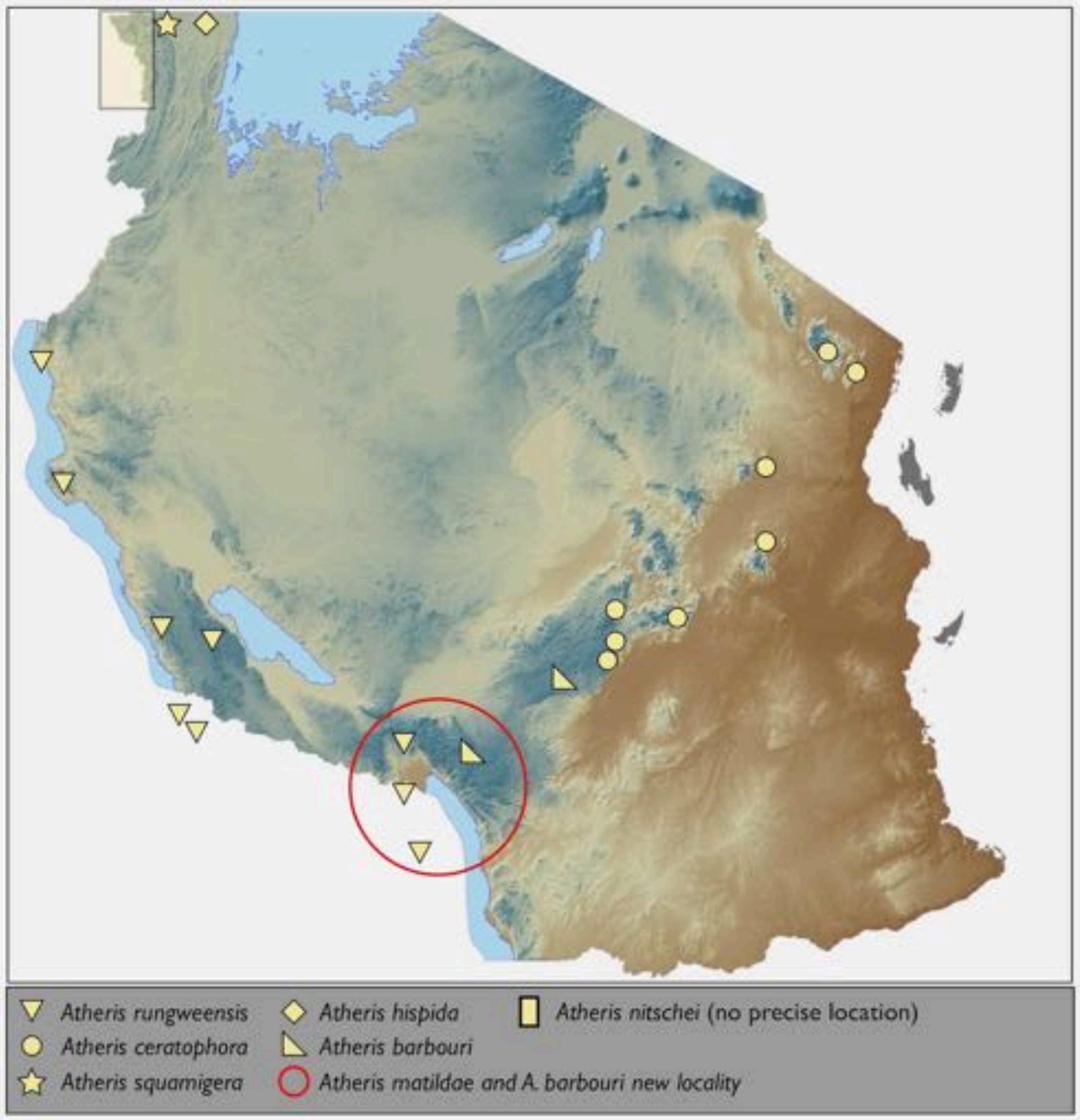

FIGURE 5. Map of the *Atheris* species occurring in Tanzania (the occurrence of *A. nitschei* within Tanzania borders still need confirmation).

FIGURE 6. The forest fragment in the Southern Highlands of Tanzania where *A. matildae* was collected, and a detail of the forest canopy.

Colouration. Dorsally it appears as a black snake with bright yellow dorso-lateral zig-zag lines. Flanks are marbled in yellow. Dorsum of the head is almost entirely black with scattered yellow scales or groups of scales, sides of the head are mainly yellow with an irregular longitudinal black marking. An extensive black marking across the frontal part of the mouth, including part of nasal, rostral, mental and few infralabial scales is present, by contrast it delimitates an inverted pale triangle. Eyes are light olive green (in life). Throat is yellow; belly is pale yellow to greyish-green, suffused by black speckling; horn-like scales are yellow with black outer edges. In preservation the specimen retains the original colouration (see Fig. 1).

Dorsal scale microdermatoglyphics. The surface microstructure of several scales from midbody and the last third of the body of two specimens of *A. matildae* (MTSN 9344, 9417) and three specimens of *A. ceratophora* from Usambara Mountains (MTSN 5117, 5118, 5121) were examined by scanning electron microscopy, in order to evaluate the intra- and inter-specific differences. Two-dimensional classes of microdermatoglyphics were identified; the coarser one consists of juxtaposed or imbricated layers of keratin with a raised edge, forming a ‘scaly background’. This layer is shared by both *A. ceratophora* and *A. matildae*. At greater magnification (4000x) a further pattern of microdermatoglyphics is visible in *A. matildae*, where papillae-like ridges cover the entire surface of the keratin layer. The latter ornamentation is absent in *A. ceratophora* specimens from type locality.

Distribution and conservation. *Atheris matildae* is currently known only from the type series and a few other individuals of both sexes, all collected in a remote montane forest fragment in the Southern Highlands. The site probably represents the remnants of a wider forested landscape, interspersed with plateau grasslands and possibly naturally isolated from other Southern Highland forest blocks. For this reason the forests are of great bio- logical value and now the focus of further exploration and conservation intervention. During the last decade the Southern Highlands have been the subject of extensive biological investigation by the Wildlife Conservation Society. However, this species has not been detected in any other areas. It is therefore probable that *A. matildae* is a range-restricted forest species, now relying on just a few forest fragments. *A. matildae* has an extent of occurrence smaller than 100 km^2 with extent of occurrence, area of occupancy and quality of habitat in continuing decline. According to IUCN guidelines (IUCN 2010) therefore, we propose to list *A. matildae* as ‘Critically Endangered’ CR B1b(i,ii,iii). Further investigations are being carried out in order to collect more information on this magnificent snake, and a small breeding programme has been established (see www.atherismatildae.org).

Etymology. *Atheris matildae* is named for TRBD's daughter Matilda Davenport, one of the next generation of herpetologists. We suggest the common name 'Matilda's Horned Viper'.

Discussion

The genus *Atheris* includes 14 recognised species and occurs across tropical Africa, south to Mozambique. It is part of the Atherini tribe as morphologically defined by Broadley (1996). This originally included four genera: the genus *Atheris*, the two monotypic genera *Proatheris* and *Montatheris* and the enigmatic genus *Adenorhinos*. Recent molecular investigations on vipers revealed the presence of five major monophyletic groups within the family (with *Causus* used as outgroup), where the Atherini tribe represents a major monophyletic group along with the genera *Bitis*, *Echis*, *Cerastes* and the Eurasian viperines (Lenk *et al*., 2001). Within the tribe, *Proatheris* is basal and the genus *Adenorhinos* clusters with *Atheris* and is here considered belonging to the latter genus, agreeing with Lenk *et al*. (2001) and Branch & Bayliss (2009).

Atheris squamigera (Hallowell, 1854) is the most wide-ranging and variable species within the genus, and occurs from Nigeria in the West, across the Congolian forest belt to Kakamega forest in western Kenya in the East (Phelps, 2008). A few species have relatively wide distributions within a specific region, two species occur in the Upper Guinea forests *A. chlorechis* (Pel, 1851) and *A. hirsuta* Ernst & Rödel, 2002. *A. broadleyi* Lawson, 1999, once confused with *A. squamigera* and only recently recognised as a separate species by Lawson (1999) is known from the forested parts of Cameroon, Congo, Central African Republic and Gabon (Chirio & LeBreton, 2007). Several species are apparently restricted to a few localities and further investigations are needed to assess their actual distribution and conservation status. *A. subocularis* Fischer, 1888 is known only from two localities in western Cameroon (Lawson *et al*., 2001). *A katangensis* De Witte, 1953 is known from the northeast sector of Upemba National Park in the Shaba Province of southeastern Democratic Republic of Congo. *A. hispida* Laurent, 1955 is known to occur in isolated populations in western Kenya, western Tanzania, southwestern Uganda, and eastern

Democratic Republic of Congo (Spawls *et al*., 2002). The Kenyan endemic *A. desaixi* Ashe, 1968 is known from only a few localities on Mt. Kenya and the Nyambeni Hills (Broadley, 1998; Spawls *et al*., 2002). The poorly known *A. acuminata* Broadley, 1998 is known only from the Kyambura (also known as Chambura) Game Reserve, in Uganda (Broadley, 1998). The recently described *A. mabuensis* Branch & Bayliss, 2009 is known from two isolated mountains in northern Mozambique, Mt. Mabu and Mt. Namuli (Branch & Bayliss, 2009). The latter species represents the southernmost limit of the entire genus.

The genus *Atheris* in Tanzania. Recent collections throughout the country, together with an examination of material deposited in international institutions, have provided new data on the occurrence and distribution of the genus *Atheris* in Tanzania. Along with *A. squamigera* and *A. hispida* in the northwestern part of the country, Tanzanian forests and woodlands harbour another four *Atheris* species. The Eastern Arc Endemic *Atheris ceratophora* was described by Werner (1896) for the East Usambara mountains, to which it had been considered endemic until new records extended the known range of the species to the West Usambara, Udzungwa and possibly Uluguru mountains (Rasmussen & Howell, 1982). The presence of the species in the Ulugurus was based on a specimen donated by F. Werner to the Museum of Gothenburg, and the label of this specimen was reported to read "Ukami, East Africa". Ukami is identified as a geographical area in East Tanzania from app. 6°30'–7"20'S and 37°50' to 38°30'E, and the Uluguru Mountains are the only large areas of rain forest in this area, another possibility is that the location refers to the Ukami village in the Udzungwa and to the neighbouring forest patches. In 2005, subsequent field collections carried out in the Ulugurus by Frontier Tanzania, included two specimens of *A. ceratophora* identified by D. G. Broadley. The two specimens (field labels KMH 26230 and 26631) were found in the Uluguru North Forest reserve at two different sites, at 1300 and 1700 m respectively. Menegon *et al*. (2008) further expanded the known range of the species on the basis of two specimens collected in the Kanga Forest Reserve in the Nguru South Mountains (see Fig. 5 for the current distribution).

The rare and enigmatic *Atheris barbouri*, probably one of the world’s least known snakes, was originally collected and described by Loveridge (1930) from two widely separated localities in the Udzungwa and Ukinga ranges. The eight specimens originally collected have been the only ones known for over 65 years, until Rasmussen & Howell (1998) published a review of the species based on a further 13 specimens collected by local residents in the Southern Udzungwa Mountains. Basic ecological information was also provided. More recently a gravid female was collected in March 2007 by L. Mahler in the vicinity of fields of agricultural crops in the Bomalang’ombe village. This represents a range extension within the southern Udzungwa plateau. Between 2009 and 2010 two other specimens were found. One adult male (Fig. 7), by D. Moyer at Kidabaga Forest Reserve (the type locality) and one adult female in Madehani Forest (Fig. 7) in a clearing surrounded by bamboo forest, representing the first specimen in the Ukinga mountains since A. Loveridge’s collection (see appendix for details). Four additional specimens were also recently collected by MM and TRBD in the same forest as *A. matildae*, in a *Hagenia abissinica* dominated forest habitat. This new record represents a major range extension southward and indicates that *A. barbouri* and *A. matildae* are sympatric.

The type of the Rungwe bush viper, *Atheris rungweensis* Bogert, 1940 was found on Mt. Rungwe and described as a southern race of *A. nitschei* (Bogert, 1940). It was subsequently elevated to a full species due to differences in lepidosis, colour pattern and because of the lack of signs of intergradation between the two forms (Broadley, 1998). Recent findings obtained during fieldwork by WCS in the Southern Highlands have added a few localities for *A. rungweensis* to those reported by Broadley (1998). A specimen collected in Mahale National Park and two more specimens from the eastern shore of the southern half of Lake Tanganyika at Nkasi/Ntantwa in Rukwa region, suggest that the species probably occurs in woodland and forest patches, from Gombe Stream National Park in the North, all the way along the eastern shore of the lake, down to Mbizi Forest in Sumbawanga and Mt. Rungwe in the South (see Fig. 5 for the updated distribution).

The occurrence of *A. nitschei* Tornier, 1902 in Tanzania is still debated. The only verifiable records are represented by the type specimens (syntypes ZMB 16834, ZMB 17669), collected from a locality originally given as: ‘Mpororosumpf, zwischen Papyrusstauden, Deutsch-Ost-Afrika”. During colonial times the Mpororo region was situated in the so-called “Zwischenseengebiet” (in-between lakes region), between Lake Victoria, Lake Kivu and Lake Tanganyika. Today this region comprises parts of northwest Tanzania, Rwanda and Burundi. The collection locality could have been situated in the region of today's border between Tanzania and Rwanda (as mentioned by McDiarmid *et al*., 1999). Suitable habitat for the species occurs in the system of swamps along Kagera River that borders the two countries; however, the presence of *A. nitschei* within Tanzania has not been confirmed.

FIGURE 7. *Atheris* species in Tanzania (from left to right): *Atheris barbouri* from Udzungwa Mts.; *Atheris barbouri* from Ukinga Mts.; *Atheris rungweensis* from Mt. Rungwe; *Atheris rungweensis* from Mbizi Forest in the Ufipa range; *Atheris ceratophora* from Udzungwa Mts.; *Atheris nitschei* from Nyungwe National Park, Rwanda (the occurrence of the latter species in Tanzania still needs confirmation).

Tanzania's biodiversity is unparalleled on mainland Africa, and nowhere is this more evident than in its forest herpetofauna. However, the endemics for which the nation is so renowned are seriously threatened by habitat loss, disease and overexploitation for the wildlife trade. The latter is largely unmanaged, often illegal and increasingly pervasive. Collection from the wild is mostly unsustainable and has reached a level whereby it represents perhaps the biggest threat to Tanzania's amphibians and reptiles. And yet with political will, a scientifically-derived quota system and trade that focuses on captive breeding rather than wild capture, the threat could be turned into a conservation opportunity.

Unfortunately however, that scenario is for the future and in the meantime great care is needed to avoid the loss of many of Tanzania's rarest and most charismatic animals, especially those most recently discovered. Against this background, we have initiated a breeding programme for *A. matildae* in Tanzania. This is intended not only as an 'insurance population' to protect the new species from overexploitation, but also to begin the conservation of its threatened habitat so that this unique animal can persist in the wild (see www.atherismatildae.org).

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APPENDIX

Additional material included. In addition to the records obtained from literature the following material was included in the analysis.

Atheris ceratophora: KMH 26230 and 26631, Uluguru North Forest Reserve; MTSN 8874, Kigogo Forest Reserve, Udzungwa Mountains; MTSN 5032 and 5033, Udzungwa Scarp forest Reserve, Udzungwa Mountains; KMH 23977 and 35468 Kanga Forest Reserve – Nguru Mountains, MTSN 5117, 5118 and 5121 from Usambara Mountains, MNHG 2667.45 from Usambara Mountains.

Atheris barbouri: MTSN7311 Edge of New Dabaga Forest Reserve, Udzungwa Mountains; MTSN 9177 Bomalang'ombe, Udzungwa Mountains; MTSN 7299 Madehani forest, Ukinga Mountains; MTSN 9500, 7400 and 7399 from Livingstone Mountains.

Atheris rungweensis: MTSN 8993 Mahale National Park; SHCP 07-R-02 Nkasi/Ntantwa, Rukwa Region.

Atheris hispida: KMH 7940 Minziro Forest Reserve.

Atheris squamigera KMH 7757 and 7758 Rumanyika Forest Reserve.

Appendix 2 Description of a range restricted frog in the genus *Callulina*

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A remarkable new species of *Callulina* (Amphibia: Anura: Brevicipitidae) with massive, boldly coloured limb glands

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Abstract

A large new species of *Callulina* is described from a series of 22 specimens from the montane and upper montane forest of the Nguru Mountains, Tanzania. The most striking features of *Callulina meteora* **sp. nov.** are the massive and boldly coloured glands on the arms and legs and a metallic sheen to the skin. The new species is distinguished further on the basis of acoustic and molecular data. The position, size and conspicuousness of the enlarged glands in the new species are strikingly similar to those of *Nectophrynoides viviparus*, a toad found also in the Eastern Arc Mountains of Tanzania. The new species is known from a single forest reserve and is of high conservation concern.

Key words: aposematism, Eastern Arc, glands, *Nectophrynoides*, Nguru, Tanzania

Introduction

Patterns of species distributions in the Eastern Arc Mountains of Tanzania and Kenya are characterised by high levels of endemism (Myers *et al*., 2000; Burgess *et al*., 2007). However, much of the documented endemism is patchy, with greatest diversity being found in the East Usambara, Uluguru and Udzungwa Mountains (Fig. 6). This patchiness can, in part, be attributed to uneven sampling (Burgess *et al*., 2007; Ahrends *et al*., 2011). Increasing understanding of other mountain blocks in the Eastern Arc suggest that high levels of endemism are more widely distributed throughout the region, but many species remain to be described (Menegon & Davenport, 2008; Menegon *et al*., 2008, 2011; Loader *et al*., 2011a). The Nguru Mountains is one part of the Eastern Arc from where a number of new amphibians, with highly restricted distribution, have been described recently (Poynton *et al*., 2009; Loader *et al*., 2010a,b, 2011b) and more reported to await description (Menegon *et al*., 2008; Loader *et al*., 2009b). Potentially this area might be considered as one of the richest areas for biodiversity in the Eastern Arc Mountains and therefore globally.

The eight nominal species in the genus *Callulina* Nieden 1911, are almost entirely restricted to submontane and montane forest of the Eastern Arc Mountains (Loader *et al*., 2010b). A hotspot of species richness for this genus is the Nguru Mountains, with two species recently described (Loader *et al*., 2010b), one from the Nguru South Forest Reserve (*C. hanseni*) and another from Kanga Forest Reserve (*C. kanga*). We describe here the third new species endemic to the Nguru Mountains. A fourth probably new species ("*Callulina* sp. 3") was mentioned by Menegon *et al*. (2008) but is not described here because it has a more complex distribution, is less easily diagnosed, and is the subject of ongoing research.

Material and methods

Specimens were collected from two separate surveys conducted in the Nguru South Forest Reserve in 2004 and 2008. Specimens and tissues collected in 2004 are deposited in the collection of Museo Tridentino di Scienze Nat-

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urali, Trento, Italy (MTSN) and were fixed and preserved in 70% ethanol. Specimens collected in 2008 are deposited in the collection of the Natural History Museum, London, UK (BM), were fixed in 5% formalin and are preserved in 70% ethanol. Corresponding tissues are held at the Institute of Biogeography, University of Basel, Switzerland. Photographs and sound recordings are deposited in the Museo Tridentino di Scienze Naturali, Trento, Italy, and in the collections of the Department of Zoology and Marine Biology, University of Dar es Salaam, Dar es Salaam, Tanzania.

All specimens were measured using a Mitutoyo CD-6"CS electronic caliper. Measurements were taken to the nearest 0.1 mm by SPL. Following Loader *et al*. (2009a, 2010a, b), the measurements taken and abbreviations used here are: ED, horizontal diameter of eye; ETD, eye–tympanum distance; HL, upper arm length; HW, head width at level of jaw articulation; IOD, interorbital distance; LF3, length of finger 3, measured from the distal edge of the basal subarticular tubercle; LT4, length of toe 4, measured from the proximal edge of the basal subarticular tubercle; ND, nostril diameter; NED, nostril–eye distance; NLD, nostril–lip distance; SUL, snout–urostyle length; TD, horizontal diameter of tympanum; TL, tibiofibula length; TSL, length of tarsus; WDF3, width of disc of finger 3; WDTF3, width of finger 3 at level of distal subarticular tubercle. Summary statistics are provided in Table 1. The data were compared with previous *Callulina* morphometric datasets (de Sá *et al*., 2004; Loader *et al*., 2009a, 2010a,b). Specimens examined and locality data are provided in the species account.

TABLE 1. Morphometric data for all *Callulina meteora* **sp. nov.** specimens (n= 22), all measurements in mm. See Materials and Methods for explanation of abbreviations.

	SUL	TL	TD	ETD	ED	ND	NED	HW	LF3	LT4	TSL	HL	NLD	WDF3	WDTF3	IOD
Min.	13.9	4.6	0.5	1.1	1.9	1.1	1.3	4.8	1.7	1.9	3	3.7	0.7	0.5	0.5	2.5
Max.	40.6	14.6	2	2.8	4.5	2.6	3.2	15.7	5.6	7.3	11.3	12.8	1.9	1.4	1.6	6.8
Mean	31.7	11.5	1.3	2	3.6	2.1	2.4	11.8	4.2	5.5	8.8	9.9	1.5	1.2	1.1	5.6

Skeletal characters were examined from radiographs and cleared and stained specimens. Two specimens (MTSN 8132, BMNH 2008.464) were cleared and double-stained for bone and cartilage based on the method of Taylor & Van Dyke (1985). Radiographs were made for MTSN 8129, 8131, and 8133-34 to check characters revealed in the cleared and stained specimens. Comparative material examined is listed in previous publications (Loader *et al*. 2010a, b). Calls were recorded using a Sharp MT 877 mini-disc recorder, and analysed using Raven (Charif *et al*. 2004). Methods for DNA extraction (from ethanol preserved tissue samples), PCR, sequencing and subsequent analyses exactly follow the approach outlined by Loader *et al*. (2010a). Sequences were generated for parts of the mitochondrial (mt) genes 12S, 16S and cytochrome b (cytb) for new samples of the new species, complementing previous datasets (Loader et al. 2009, 2010a,b). New sequences were deposited in Genbank, FN563060 – 3065.

Callulina meteora sp. nov.

(Figs. 1–3, 5–7; Table 1)

Callulina sp. 1 Menegon *et al*. (2008: p.114, appendix 1, tables 3, 4).

Holotype. BMNH 2008.450 (Field tag MW 6825) a mature female (Fig. 1). Collected from the Maskati side of the Nguru South Forest Reserve, Tanzania, 6.069027778 S - 37.50066667 E, 1980 m (Fig. 6) by David Gower, Roy Hinde, Simon Loader, Hendrik Müller, Maria Müller, and Mark Wilkinson in January 2008.

Paratypes. MTSN 8129-8134 (MTSN 8134, 39 ova in vitellogenesis; MTSN 8130 cleared and stained), MTSN 8141, collected by Michele Menegon between October 26 and November 02, 2004 in the Nguru South Forest Reserve, 6.06630176 S - 37.49802743 E, Nguru Mountains, Morogoro Region. Tanzania. BMNH 2008.118-451-452-453-454-455-456-457-458-459-460-461-462-463-464 same collection data as holotype.

Diagnosis. The species is assigned to *Callulina* within Brevicipitidae based on the following morphological features: Moderately sized wedge-shaped lobes on the mentomecklian elements, posteroventrally directed (variably reduced/enlarged in *Probreviceps, Balebreviceps*, and *Breviceps*, see Largen & Drewes 1989); cultriform process of the parasphenoid with broad base but narrow alary processes, tapering laterally (cultriform process of the parasphenoids widely variable in breviciptids, Largen & Drewes 1989); nasals almost meet at midline (broadly separated in *Breviceps* and *Balebreviceps*); clavicle well-developed and straight though slightly curved anteriorly at the point of contact between coracoid and scapulae (clavicle straight in *Breviceps*, *Probreviceps*, *Spelaeophryne*); omosternum large (rudimentary or small in *Breviceps, Probreviceps*, moderate in *Balebreviceps*); tympanum present and usually well-differentiated (absent in *Balebreviceps* and *Probreviceps uluguruensis*); double condylar articulation between the urostyle and the sacral vertebrae (fused in *Balebreviceps, Breviceps,* and *Probreviceps*); truncated terminal phalanges (simple in *Spelaeophryne, Probreviceps, Breviceps,* and *Balebreviceps*); single posterior denticulated row in the palate of *Callulina* (two denticulated rows in *Probreviceps*, glandular mass in *Breviceps*).

FIGURE 1. Dorsal, ventral and lateral views of the female holotype (BMNH 2008.450) of *Callulina meteora* **sp. nov.**

FIGURE 2. *Callulina meteora* **sp. nov.** in life from the type locality, showing some of the species variability.

Callulina meteora is morphologically distinct from most other species of *Callulina* (*C. kreffti, C. kisiwamsitu, C. dawida, C. kanga, C. laphami* and *C. stanleyi*) in having large glands on the limbs. The new species is distinguished from *C. shengena* and *C. hanseni* (also with enlarged limb glands) by the presence of a tympanum (absent in *C. shengena*) and limb glands that are distinctly differently coloured to the rest of the limbs (no distinctive gland colour in *C. hanseni*).

Description of holotype. Female. Body stout, head short but as wide as body. Snout truncate in lateral view, snout-tip extending slightly beyond upper and lower jaws. Snout tip rounded at edges, flattened not pointed at apex. *Canthus rostralis* rounded. Dorsal aspect of head covered by small, rounded, irregular-shaped warts. Ventral region with larger, granular like warts on chin and underside. Eyelids smooth with very small irregular shaped warts. Pupil was horizontal before preservation. Tympanum distinct, suboval (not as tall as long), smooth, with granular warts on slightly raised rim around edge of disc. Dorsum of body with small, irregular glandular masses giving warty appearance. Ventral surface with larger irregular shaped glandular masses, slightly larger and more granular on flanks. Forelimb slender. Massive continuous glands covering dorsal and ventral aspects of forearm. Surface of massive arm gland with smaller, irregular, slightly smoother bumps. Webbing almost absent on hand, only marginal rudimentary skin joining each finger. Distal phalanges moderately long, thick, truncate, expanded only slightly, rounded at edges. Inner tubercle smaller than outer tubercle, separated by a mid-palmar tubercle. First finger shortest, followed by second, fourth, third. Tubercles darker than silvery/metallic background of hand. Hind limbs stout, tibia, metatarsus and carpal area of tarsus covered by an enlarged glandular mass. Distal phalanges of feet moderately long, thick, truncate, expanded only slightly, rounded at edges. First toe marginally the shortest, followed by second, third, fifth, fourth. On toes and fingers, terminal phalanx darker, with a fold of skin, marked by a white line at the dorsal junction between the penultimate and ultimate phalanges. Webbing almost absent on foot, only marginal rudimentary skin joining each toe. Inner and outer tubercle in contact, equal in size. Vent ventro-posteriorly positioned.

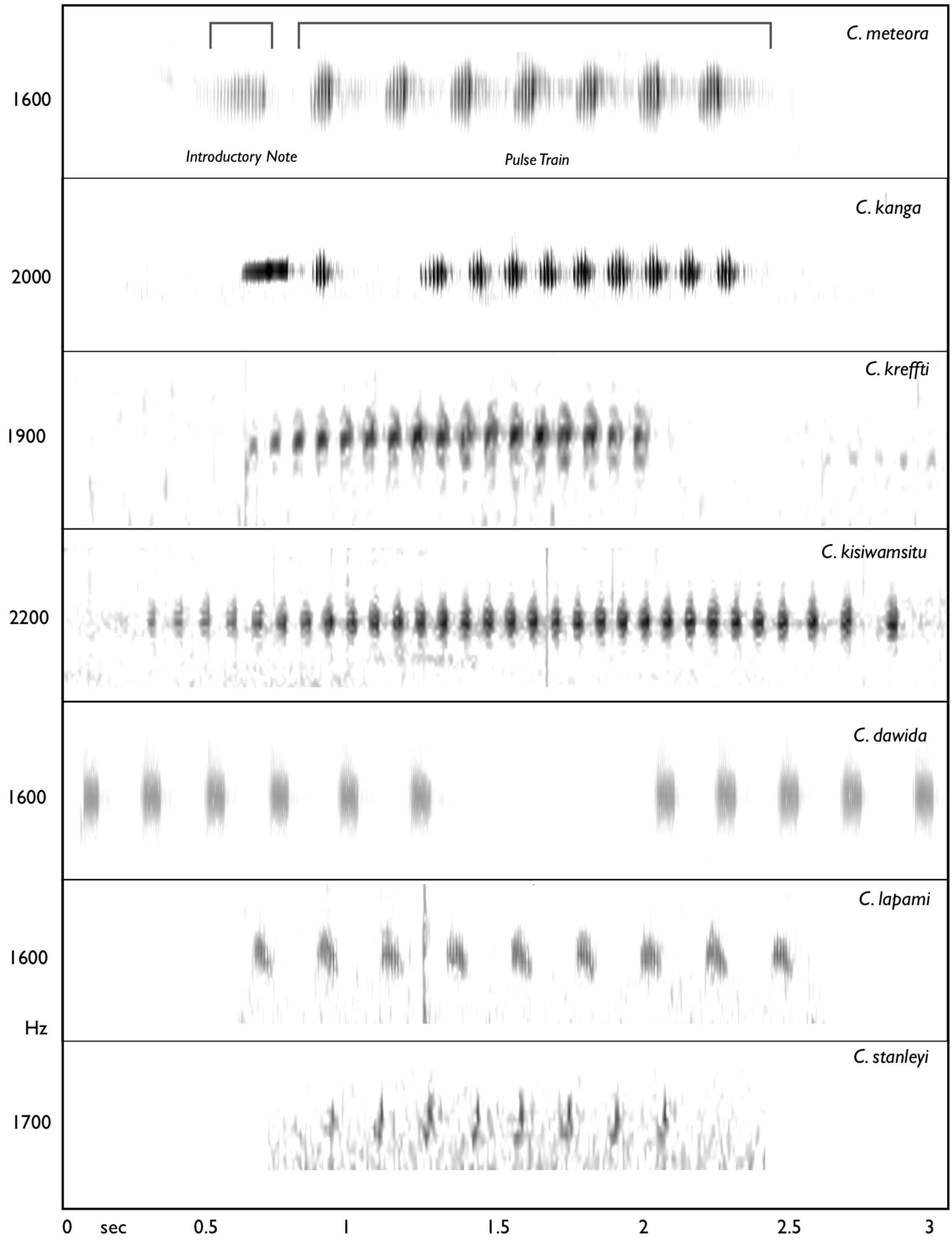

FIGURE 3. Sonograms of *Callulina* species.

Measurement of holotype. SUL = 38.2; TL = 13.5; ED = 4.2; TD = 2.0; ETD = 2.2; ND = 2.5; NED =2.8; JW = 13.8; LF3 = 4.4; LT4 = 5.7, TSL = 11; HL = 12.1; NLD =1.7; WDF3 = 1.3; WDTF3 = 1.2; IOD = 6.1.

Colour. In preservative, the holotype dorsal ground colour is pale grey/brown, with darker brown patterning as irregular lateral dorsal markings. The flanks are a paler grey/brown. The dorsal and ventral surfaces of the thighs are dark brown, contrasting strongly with the silvery/metallic colouration of the glands. The ventral surface of the body is cream, with a dark brown colouration on the lateral edges. In life, the holotype has the same patterning as in preservative but with more vivid colours. The massive glands on the limbs are silvery, giving a striking metallic sheen to the surface (see Fig. 2).

Variation. The tympanum is usually distinct in *Callulina meteora*, but in some paratypes (BMNH 2008.464, BMNH 2008.453-455, BMNH 2008.461, BMNH 2008.451) it is poorly demarcated and was measured by dissecting the skin around the region. Otherwise there is little notable (non-colour) morphological variation among individuals apart from between the sexes. Males are significantly smaller in body length (T-test: <0.05, females, SUL= 34.8–40.6 mm, x= 41.55 mm, number= 8; males, 26.5–35.4 mm, x= 30.1 mm, number= 9), and head width (T-test: <0.05). All other morphological characters analysed are not significantly different between the sexes.

Colour variation. *Callulina meteora* individuals show wide colour variability with some rather constant patterns. In all examined individuals, the massive limb glands are paler than surrounding areas, often whitish or silvery with, as in the rest of the body, a metallic sheen (see Fig. 2). The body can be almost completely white or coppery brown, with darker areas on the dorsum, inguinal, axillary and tympanic zones and on limbs. In some specimens (e.g., MTSN 8129), darker areas on the dorsum are extensive and almost black. The entire animal has a metallic sheen that persists after preservation. Eye colour in life is bright yellow to orange.

Call. Advertisement calls of two males *Callulina meteora* were recorded at the collecting site by M.M. between October 26 and November 2 2004 both during the day and the night. Calling males were seen at the base of trees. The call is composed of a single periodic pulse train, introductory notes, and repeated notes. These three elements are sometimes arranged in different ways: a single periodic pulse train could be heard with or without introductory notes. Full calls in rainy weather were heard to comprise three or four modules of introductory notes followed by a single pulse train and a final introductory note followed by a group of similar pulse trains. The presence of the introductory note was previously known for the recently described *C. kanga* only (Loader *et al.*, 2010b). The last call element of *C. meteora* considered alone shows temporal properties similar to the call of the other *Callulina* species. The single periodic pulse train is composed of six pulses and has a length ranging from 0.067 to 0.078 sec, but the temporal properties of repeated note remains the same. The introductory notes usually rising in intensity are composed of 16 to 21 pulses and have a length ranging from 0.203 to 0.238 sec. All sound emissions have an average intensity maximum around 1.6 kHz (see Fig. 3).

FIGURE 4. Views of the habitat at the type locality of *Callulina meteora* **sp. nov.**

Habitat and natural history. *Callulina meteora* is partly sympatric with *C. hanseni* (Loader *et al*., 2010b) and seems to be restricted to the montane and upper montane forest of the Nguru South Forest Reserve (Menegon *et al*., 2008). All specimens were collected between 1950–2100 m asl but calls were heard up to 2200 m. This distribution is similar to that reported for *Arthroleptis nguruensis* (Poynton *et al*., 2009). Some specimens were found during the day by digging in soft soil and leaf litter accumulating at the base of large trees. This microhabitat together with the presence of strongly keratinized and well-raised metatarsal tubercles is suggestive of semifossoriality. On the basis of the presence of large eggs found in the oviduct of a dissected female, we presume that the species is oviparous with direct development. Although no direct evidence is known for the genus *Callulina*, oviparity with direct development is regarded as the most likely reproductive mode for this genus of breviciptids (Müller *et al*., 2007).

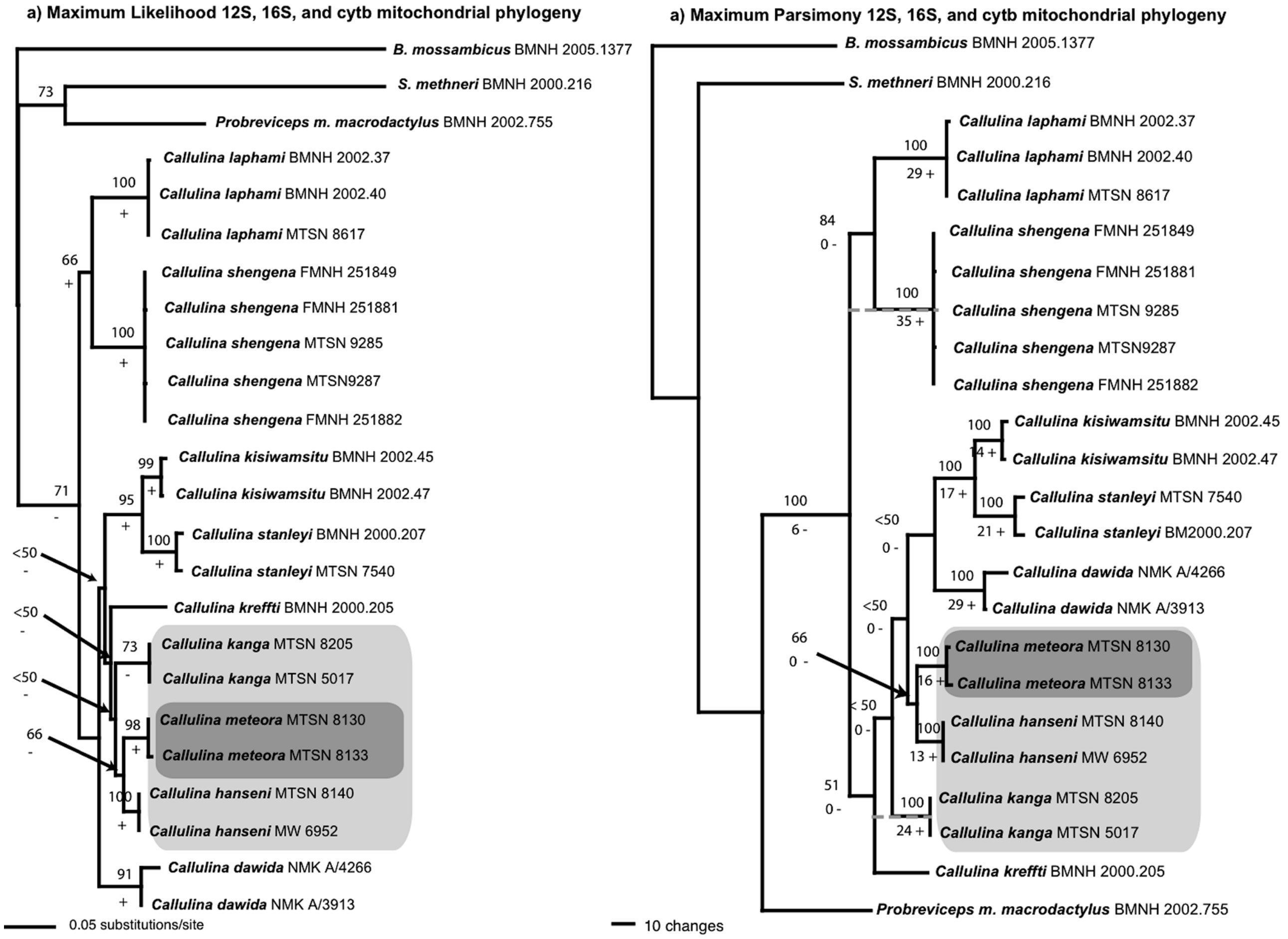

FIGURE 5. Phylogenetic tree of all nominal species of *Callulina,* using a) Maximum likelihood, with bootstrap results given above and symbols below show the results of Kishino–Hasegawa tests of differences in log likelihoods between optimal and the best suboptimal trees obtained from converse topological constraints. Presence (+) or lack (-) of support at the $p \leq 0.05$ level, b) Maximum Parsimony, numbers above branches are bootstrap proportions, numbers below internal branches are decay indices. The symbols following the decay index values show the results of Templeton tests of differences in length between the MPTs and the best suboptimal trees obtained from converse topological constraints. Presence (+) or lack (-) of support at the $p \leq 0.05$ level.

Conservation status. Based on current knowledge of the species' distribution and habitat preference, the estimated extent of occurrence of *Callulina meteora* is equal to or less than 42 km^2, and the estimated area of occupancy not larger than 26 km^2; these are respectively the area including the elevational distribution (1980–2100 m) of this species in the Nguru South Forest Reserve and the area included in the polygon obtained by linking the localities where the presence of the species was recorded (Fig. 6). Therefore, according to Red List (IUCN 2009) categories based on the criterion of an extent of occurrence estimated to be less than 100 km^2, the presence of one population at only a single location, compounded with an observed decline in area, extent and quality of the habitat (IUCN, 2010), we suggest that

C. meteora qualifies as critically endangered or, more technically, CR B1b (iii). The proposed conservation status would need to be re-evaluated if specimens are recovered below 1980 m, but herpetological surveys examining species turnover between 800–2200 m have been conducted in the area over the past seven years (S.P.L. and M.M. unpublished data; Owen *et al*., 2008) and *C. meteora* has never been recovered below 1980 m. Currently the population density appears to be locally high but with increasing pressure due to land-use changes in the region (pers. obs.), and predicted climate-mediated changes that could affect the high montane zone, the species is clearly facing threats.

Etymology. The specific epithet is used as an adjective and derives from the greek word *meteoron,* meaning "thing high up," in reference to the type locality of the species, situated close to the top of the Nguru Mountains.

Molecular analyses. To examine the distinctiveness of and relationships among *Callulina* species, we analysed sequence data for 12S, 16S, and cytb mt genes for all nine nominal species, including *C. meteora*. The dataset comprised 24 individuals and 1124 unambiguously aligned characters, of which 707 were constant, 140 variable and uninformative, and 277 informative under parsimony. *Breviceps mossambicus* was used to root trees and *Probreviceps m. macrodactylus* and *Spelaeophryne methneri* were used as additional outgroups (see Loader et al. 2010a).

Parsimony analysis yielded two most parsimonious (MP) trees of 915 steps (Fig. 5b). These topologies differ from the optimal likelihood tree (Fig. 5a). The two MP trees differ in the position of *C. shengena* and *C. kanga*. In one MP tree, *C. shengena* is sister to *C. laphami*, and in the other it is sister to all other (except *C. laphami*) *Callulina* species. *Callulina kanga* is recovered as sister to a clade comprising a Nguru radiation (*C. meteora, C. hanseni*), *C. dawida, C. stanleyi* and *C. kisiwamsitu* in the former MP tree. In the second, *C. kanga* is sister to *C. kreffti*. Neither of the alternative topologies is well supported. Most analyses supported the monophyly of *Callulina* (best trees with a non-monophyletic *Callulina* are significantly suboptimal in Templeton but not likelihood topology tests). All nominal species are robustly monophyletic (Fig. 5). Likelihood analysis recovered a tree similar to that presented by Loader *et al*. (2010a: fig. 8b) differing only in the position of *C. shengena*. In Loader *et al*. (2010a) *C. shengena* was sister to all other *Callulina* species (but not well supported) whereas here *C. shengena* is sister to *C. laphami*, also only very weakly supported. The new species, *C. meteora*, forms a clade with *C. hanseni* in all analyses. Pairwise distances values highlight the genetic distinctiveness of all named *Callulina* species (as previously shown in Loader *et al*., 2010b), including the new species *C. meteora* (3.8–4.0 % different to its sister group *C. hanseni*). Despite the genetic distinctiveness of the nominal species, the phylogenetic signal in the available mt sequence data is insufficient to provide a compelling resolution of their interrelationships using the methods employed here.

Revised key to the species of *Callulina*

Externally, *Callulina* species are distinguished from other breviciptids by their truncate to expanded toe and finger tips.

1a.	Tympanum present, though often obscured superficially by granular skin	2
1b.	Tympanum absent	8
2a.	Finger tips expanded, wider than the distal subarticular tubercle	3
2b.	Finger tips truncated, not expanded beyond the width of the first subarticular tubercle	*C. dawida*
3a.	Ratio between the width of Finger 3 at the level of the distal subarticular tubercle and the width of its finger tip > 0.75	5
3b.	Ratio between the width of Finger 3 at the level of the distal subarticular tubercle and the width of its finger tip < 0.75	4
4a.	Distance between tympanum and eye usually less than tympanum diameter. Distinctive call, known only from East Usambara Mountains	*C. kreffti*
4b.	Distance between tympanum and eye usually greater than tympanum diameter. Distinctive call, known only from Nguru Mountains	*C. kanga*
5a.	Large, distinctive and continuous glands on arms and legs	7
5b.	No large, distinctive and continuous glands on arms and legs	6
6a.	Large, robust head. Distinctive call, known only from South Pare Mountains	*C. stanylei*
6b.	Less robust head. Distinctive call, known only from West Usambara Mountains	*C. kisiwamsitu*
7a.	Prominent, distinctly coloured glandular mass on arms and feet	*C. meteora*
7b.	Prominent, but not distinctly coloured glandular mass on arms and feet	*C. hanseni*
8a.	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*
8b.	Prominent, relatively pale glandular mass on arms and feet. A less distinct and less continuous interocular band sometimes present. South Pare Mountains	*C. shengena*

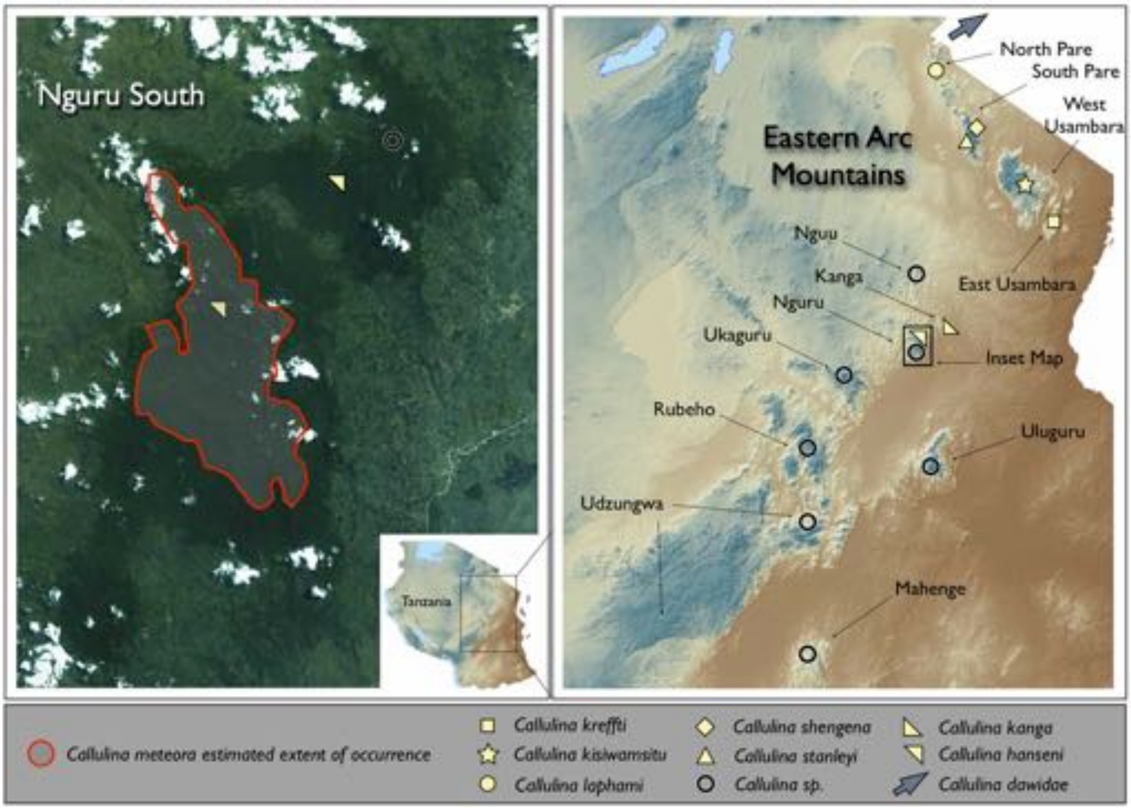

FIGURE 6. Map of the Eastern Arc Mountains of Tanzania showing the distribution of the species in the genus *Callulina* and distribution of *C. meteora* **sp. nov.** (red outlined shaded area) in the Nguru Mountains.

Discussion

Making confident evolutionary inferences from the available phylogenies is hampered by the lack of robust resolution of relationships. Although we can draw confidence on some areas of agreement between the results from different methods, we generally have a preference for Maxmimum Likelihood over parsimony analyses of DNA sequence data. Taking the topologies at face value, we identify two patterns of interest. First, the *Callulina* from the southern part of the Eastern Arc (South of the Taita, Pare and Usambara Mountains) are nested relatively deeply within the tree, indicative of a more northerly origin of the extant *Callulina* lineage as a whole. Although tentative, this topology is also supported by morphology, with the southern *Callulina* (including probably additionally undescribed species from Mountain blocks not yet sampled in molecular phylogenies) having more expanded toes. Second, although most sister species are allopatrically distributed on isolated mountain blocks, suggesting a possibly vicariant mode of speciation, the Nguru species (and at least *C. meteora* and *C. hanseni*) are each other's closest relatives. At least the sister pair of *C. meteora* and *C. hanseni* are syntopic in the montane forests of the Nguru Mountains, indicating a potentially different mode of speciation from most other *Callulina*. The former pattern of allopatric/geographic speciation is thought to characterise many Eastern Arc lineages, with high diversity possibly explained by expanding and contracting cycles of moist forest cover, connecting and disconnecting populations (Lovett *et al*., 2005; Burgess *et al*., 2007). Given that the pattern of one species of *Callulina* per each mountain block does not hold, the recent taxonomic and molecular phylogenetic work, while indicative of interesting patterns, raises questions that will only be answered by sampling *Callulina* from the southernmost mountains in the Arc.

A particularly remarkable and distinctive feature of the new species is the strongly contrastingly coloured and large glandular masses on the arms, legs and feet. The detailed function of these conspicuous glandular masses is unknown, but their similarity in position, size and conspicuousness to glandular masses of the Tanzanian endemic toad *Nectophrynoides viviparus* is striking (Fig. 7). *Nectophrynoides viviparus* was described in 1905 by Tornier but the function of the glands has not been examined in detail (as noted by Barbour & Loveridge, 1928). The convergence of these atypical features in two distantly related species from different families (Bufonidae and Brevicipitidae) raises questions of function and origin.

FIGURE 7. Similarity in enlarged limb glands in *Callulina meteora* **sp. nov.** (above) and *Nectophrynoides viviparus* (below).

Both species exude a sticky white substance from these glands (Menegon & Loader pers. obs.) that we assume is toxic. We suggest that the combination of bold colour and conspicuousness of the glands relative to the limbs may act as a warning signal, although we note that most aposematic organisms are more colourful. The two species share a similar habitat and habit, in being found often within leaf litter in moist montane forest, though the degree to which they might be sympatric or syntopic is not currently clear. The most obvious explanations that come to mind are random (non-causal) similarity; similarity through aposematism in poisonous species that are relatively drably coloured because of somewhat cryptic habits; similarity through display of toxicity to the same or similar predators (at least when the features first evolved); similarity caused by mimicry. Clearly, much further research is required to test these and other possible hypotheses.

In addition to the presence of massive limb glands, a further interesting characteristic of *Callulina meteora* is the metallic sheen of the skin. Among African amphibians, a similar feature is known in the terrestrial hyperoliid *Chrysobatrachus cupreonitens* Laurent 1951, a species from the high altitude grassland of the Itombwe Mountains, in eastern Democratic Republic of Congo (Schiøtz, 1999) and in the arboreal bufonid *Churamiti maridadi* Channing & Stanley 2002 from Mamiwa-Kisara forest in the Ukaguru Mountains of Tanzania. Further investigations might assess whether there is any causal relationship between the presence of this peculiar feature and any environmental variables, such as altitude.

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Appendix 3 Herpetological inventory of the Mahenge Mts. of Tanzania, with comments on biogeography

Herpetofauna of Montane Areas of Tanzania. 4. Amphibians and Reptiles of Mahenge Mountains, with Comments on Biogeography, Diversity, and Conservation

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Chapter 8

Herpetofauna of Montane Areas of Tanzania. 4. Amphibians and Reptiles of Mahenge Mountains, with Comments on Biogeography, Diversity, and Conservation

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Abstract

The Mahenge Mountains are located in the Eastern Arc Mountains, part of a global biodiversity hotspot. Few amphibian and reptile surveys have been conducted in this area, with only one study of the submontane forest zone. This previous study revealed a characteristic endemic Eastern Arc amphibian and reptile forest fauna. New studies outlined here extend the sampling time considerably and the geographical coverage (including Mselezi Forest Reserve). These studies reveal a remarkable diversity of amphibians and reptiles increasing known faunal diversity considerably, including several putative new species that await further taxonomic verification. Biogeographical analyses using Bray-Curtis indices show Mahenge clusters with the geographically close Udzungwa Mountains and Southern Highlands (non–Eastern Arc highland fragment including Mt. Rungwe). This clustering of areas suggests an interesting biogeographical history and deserves special attention in the future. In light of the remarkable amphibian and reptile diversity, conservation of Mahenge habitats, in particular Sali Forest Reserve, is desperately required.

Introduction

The Mahenge Mountains form the southernmost part of the Eastern Arc Mountains (EAM), a recognized biodiversity hotspot (Myers et al., 2000). These mountains constitute several relatively poorly known forest fragments. To the west, the Mahenge Mountains are bordered by the Kilombero floodplain, separating them from the Udzungwa Mountain range. To the south the mountains form an almost continuous mountain block with the Mbarika Mountains, which are linked to the Livingstone Mountains and the Southern Highlands north of Lake Nyasa (Fig. 1).

The Mahenge Mountains are estimated to have lost 89% of their original forest cover (Newmark, 1998), although the rate of loss appears to have declined in the last few decades. However, most of the forest fragments that remain in the Mahenge Mountains are small and heavily encroached upon due to accessibility and proximity to humans (Mbilini & Kashaigili, 2005). The forests consist of a number of fragments with different degrees of disturbance and natural habitats remaining. However, the two southernmost forests, Sali and Muhulu Forest Reserves (FR), have remained relatively intact due to their isolation.

Knowledge of the distribution of species across the fragmented forests of Mahenge is very incomplete. Currently, assessments of the Mahenge Mountains indicate that the area has one endemic vertebrate species (Mariaux & Tilbury, 2006), 11 Eastern Arc near-endemic vertebrates, and five Eastern Arc near-endemic trees (Burgess et al., 2007). Because of the relatively low species diversity and endemism in the Mahenge Mountains, it is ranked low on the list of conservation priorities for the Eastern Arc (Critical Ecosystem Partnership Fund, 2005).

Historically, biological research in the Mahenge Mountains has been limited. Early exploration surveyed the herpetofauna and small mammals in the 1960s in Mahenge (Rees, 1964; with material examined and described by Poynton, 1977, 1991, 2003). Parts of the Mahenge Mountains were the focus of research by Frontier Tanzania, including surveys of amphibians and reptiles. Research focused on Mahenge Scarp Forest Reserve, Nawenge Forest Reserve, and Nambiga Forest Reserve (outlined in the reports of Frontier Tanzania, 2001–2004). However, these areas constitute mainly lowland habitats (Hinde et al., 2001), including miombo-type habitats, and not submontane or montane forest habitats. A botanical appraisal of catchment forest reserves (Lovett & Pócs, 1993) provided information on the flora of the region and each

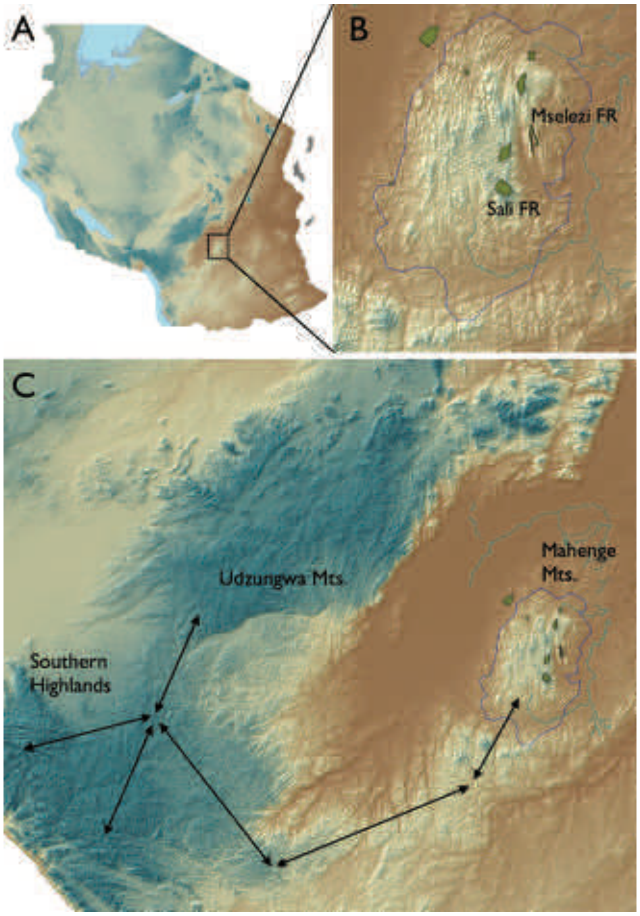

FIG. 1. Map of Tanzania showing (**A**) the position of the Mahenge Mountains within the Eastern Arc Mountains. (**B**) Position of surveyed forest fragments within the Mahenge Mountains landscape. (**C**) Possible faunal dispersal routes across Udzungwa, Southern Highlands, and Mahenge Mountains.

forest reserve. Several plant species with restricted distributions have been recorded (Lovett & Pócs, 1993). These include two endemic *Impatiens* spp. and the Eastern Arc endemic *Allanblackia stuhlmannii* (Lovett & Pócs, 1993). The presence of such species links the forest habitats to the biologically rich forests of the rest of the Eastern Arc Mountains.

Sali FR was the subject of one previous focused survey of the herpetofauna, updating the species inventory for amphibians and chameleons (Loader et al., 2004). The three-day survey provided the first glimpse of the affinities to other Eastern Arc submontane/montane forest herpetological assemblages. The presence of particular species strongly suggested Eastern Arc affinities, including *Scolecomorphus kirkii, Petropedetes yakusini, Leptopelis vermiculatus, Nectophrynoides tornieri,* and *Afrixalus uluguruensis*. Overall, given the lack of surveys in Mahenge, new field research continues to be required to make a proper assessment of the diversity of amphibians and reptiles in this Eastern Arc Mountain fragment.

This paper documents surveys recently conducted in Mahenge Mountains across two separate forest reserves. The surveys constitute the most intensive survey in this region and forest habitat, totaling 59 survey days. The work builds considerably on the three-day survey of Loader et al. (2004), which is the only known survey restricted to submontane forest habitats in the region.

Methodology

Study Area

The Mahenge Mountains are an Eastern Arc outlier of small, biologically poorly known forest patches. Covering 2,802.29 km^2, they are located at 8°37′–8°38′S and 36°42′–36°44′E, and rise from 460 to 1500 m above sea level (masl). To the west, the Mahenge Mountains are bordered by the Kilombero floodplain that separates them from the Udzungwa Mountain range and the Southern Highlands, and to the south, the Mahenge Mountains form a continuous series of ridges with the Mbarika Mountains, which are attached to the great mountain ranges north of Lake Nyasa. The forest fragments investigated are Sali and Mselezi Forest Reserves, both located in Ulanga District in Morogoro Region (Table 1; Fig. 1).

Field Survey Methods

Several techniques were used to collect amphibians and reptiles. Pitfall bucket lines were constructed at each field site for eight days to sample terrestrial herpetofauna, with two sites in Sali FR and one site in Mselezi FR. Three 50-m lines of drift fencing with 33 pitfall buckets were used for a total period of 792 trap-nights. Pitfalls were checked both in the morning and evening, with specimens collected from the buckets. Visual Encounter Surveys (VESs) of a total duration of 96 man-hours were used to sample quantitatively amphibians and reptiles through both quadrats and transects. VESs sample diurnal fossorial, arboreal, and water-associated species. Nocturnal acoustic monitoring techniques were used to detect the frog species. Furthermore, opportunistic sampling was made of all taxa throughout the survey. Material collected is listed in Appendix 1 by K. M. Howell collector numbers. The surveys were conducted between 12 Oct and 29 Nov 2005 in Sali FR and 25 Nov and 9 Dec 2005 in Mselezi FR. The taxonomy largely follows that of Frost et al. (2006). Tables 2 and 3 give the list of species for the two forest reserves surveyed.

The species collected were identified on the grounds of morphological, molecular, and bioacoustic analysis and through comparison with material held in the herpetological collections of the University of Dar es Salaam, Tanzania; the Natural Sciences Museum of Trento, Italy; and the Natural History Museum, London, United Kingdom, and are deposited in the Natural Sciences Museum of Trento. Molecular analyses were carried out at the Institute of Biogeography of the University of Basel, Switzerland. Analysis of similarity was performed with the software PAST—Palaeontological Statistics. A dendrogram was constructed based on a matrix of endemic and near-endemic Eastern Arc Mountain amphibian and reptile species by site, using the Bray–Curtis (Sørensen) similarity index.

Results

Species Diversity and Endemism

Tables 1 and 2 list the species recorded for the Mahenge Mountains across the two fragments surveyed in this study,

TABLE 1. History and geography of two Forest Reserves in the Mahenge Mountains. Information from Lovett and Pócs (1993) with updates from observations during the Frontier-Tanzania survey of Forest Reserves in the Mahenge Mountains (Frontier-Tanzania, 2004–2005).

	Sali FR	Mselezi FR
Year of establishment	1954	1954
Coordinates	8°54′–8°57′S, 36°37′–36°41′E	8°46′S, 36°43′E
Size (ha)	1890	2245
Altitude (masl)	1050–1500	500–900
Rainfall (mm/year)	1700	1500
Vegetation type	Primary submontane and montane forest; dry grassland, wetlands, rocky outcrops	Riverine lowland forest along the stream in the valley bottom; semi-evergreen drier lowland forest

and Figure 2 illustrates some of these taxa. The Mahenge Mountain herpetofauna, based on previous works (Hinde et al., 2001; Loader et al., 2004) and on the results presented here, comprises at least 41 species of amphibians in 13 families and 25 species of reptiles in eight families. The present study recorded seven amphibian taxa and one reptile taxon that are sufficiently distinct from the other known taxa that they may be considered new species based on the available data. Furthermore, the taxonomic status of all the species collected during the surveys has been assessed in the context of more extensive work on the genera, involving molecular, osteological, bioacoustic, and morphological analysis conducted from 2005 to date (S. Loader, unpubl. data; M. Menegon, unpubl. data; J. Poynton, unpubl. data). Specific determination is still in doubt for several taxa and will require further taxonomic work over the coming years, but all new putative species have minimally shown distinct genetic divergence (>3% difference using mitochondrial markers). For several putative species there is often supporting evidence for distinctiveness based on either vocalization and morphology or both. A similar case is shown in the Nguru Mountains (Menegon et al., 2008), and new species continue to be described from this area (e.g., Blackburn, 2008; Poynton et al. 2008; Loader et al., 2010).

Faunal composition includes typical elements of the Tanzanian lowlands, due to the close proximity to the Kilombero River valley (e.g., *Hyperolius tuberilinguis*, *Pyxyicephalus edulis*, *Phrynomantis bifasciatus*), and a majority of forest and highlands elements showing a strong link with the other forested areas in the Eastern Arc Mountains (e.g., species belonging to the genera *Callulina, Hoplophryne, Petropedetes, Probreviceps,* and *Nectophrynoides*). These EAM endemic or near-endemic amphibian genera are all represented by putative new species, highlighting the taxonomic distinctiveness of the Mahenge highland herpetofauna and further stressing the need for continued taxonomic assessment.

The forest-associated reptile assemblage is also distinctive, as exhibited in amphibians. A few putative new species are likely endemic to the Mahenge Mountains. A single female of the forest chameleon genus *Kinyongia* was collected, and it appears to be a distinctive species, closely related to the *K. oxyrhina* (Krystal Tolley, pers. comm.). *Kinyongia oxyrhina* is a species widely distributed across several of the Eastern Arc Mountain blocks, including Udzungwa, Uluguru, Nguu, Nguru, and Rubeho (Krystal Tolley, pers. comm.; Menegon, unpubl. data). The forest gecko *Cnemaspis* sp. is another putative endemic species new to science (Bauer & Menegon, in prep.). The Mahenge endemic dwarf chameleon *Rhampholeon beraduccii* collected and described from the previous survey in Sali FR already suggested the distinctiveness of the Mahenge reptile fauna. *Rhampholeon beraduccii* was again collected in the surveys outlined here. Other enigmatic findings are shown by the collection of two snake species. One specimen is tentatively identified as *Xyeledontophis* cf. *uluguruensis*, a poorly known species described from the Uluguru Mountains (Broadley and Wallach, 2002) and recently recorded in the Nguru Mountains (Menegon et al., 2008). Another interesting record is the burrowing asp *Atractaspis aterrima*, a Guineo-Congolian species, known also from a few localities within the Eastern Arc (Spawls et al., 2004; Menegon et al., 2008). Like amphibians, the occurrence of coastal/lowland elements is due to the close proximity of the Kilombero Valley, which contains lowland species and links to coastal areas along the Kilombero river valley (e.g., *Rieppeleon brevicaudatus*, *Dendroaspis angusticeps*, *Naja melanoleuca*).

Zoogeographical Patterns

Bray–Curtis Similarity Index values were computed for each Eastern Arc mountain fragment using non-transformed amphibian and reptile species occurrence data, based on Burgess et al. (2007), updated by recent publications (Blackburn, 2009), and unpublished data by the authors (Fig. 3). The pattern obtained shows an interesting clustering of sites reflecting similarities in species assemblages, which in general can be correlated to geographic proximity. The Taita Hills are separated from all the other sites, probably because of the paucity of Eastern Arc endemic and near-endemic species. The northernmost Tanzanian sites (North and South Pare Mountains) cluster together and are distinct from all other EAM blocks. The Pare and Taita differences reflect the distinctiveness of these assemblages (e.g., Müller et al., 2005; Loader et al. 2009, 2010). These results contrast with comparisons of amphibians given in Loader et al. (this volume) that identified closer links between West Usambara and South Pare to the exclusion of the North Pare Mountains. Clearly the biogeographical history of these areas remains uncertain and requires further detailed analyses using appropriate methodologies (e.g., historical biogeographical approaches).

Three other clusters are identified in the dendrograms constructed using the Bray–Curtis Similarity Index: (1) a cluster that includes the north-central Eastern Arc fragments (East and West Usambara, Nguru, and Uluguru); interestingly, the East and West Usambara do not form a single grouping; (2) the Central Eastern Arc region (Malundwe, Nguu, Rubeho, and Ukaguru), areas with relatively low species diversity but poorly surveyed; and (3) a southern Eastern Arc cluster, (Udzungwa, Mahenge, and Rungwe [Southern Highlands], which is a non–Eastern Arc Mountain fragment). We note the close relationship shared between Udzungwa and Rungwe, to the exclusion of Mahenge.

Table 2. List of the amphibians of Mahenge Mountains.

Taxon	Sali FR	Mselezi FR	Data source (if not present study)
Arthroleptidae			
Arthroleptis lonnbergi	X	X	
Arthroleptis sp. 1	X	X	
Arthroleptis cf. *reichei*	X	X	
Arthroleptis xenodactyloides	X	X	
Leptopelis flavomaculatus	X		
Leptopelis uluguruensis	X		
Leptopelis vermiculatus	X		
Bufonidae			
Amietophrynus gutturalis			Loader et al., 2004
Amietophrynus maculatus			Loader et al., 2004
Nectophrynoides cf. *tornieri*	X		
Nectophrynoides sp. 1	X		
Nectophrynoides sp. 2	X		
Mertensophryne loveridgei		X	
Hemisotidae			
Hemisus marmoratus			Loader et al., 2004
Hyperolidae			
Afrixalus cf. *uluguruensis*	X		
Afrixalus fornasinii	X	X	
Afrixalus sp. 1	X		
Afrixalus sp. (cf. brachycnemis)			Loader et al., 2004
Hyperolius puncticulatus	X		
Hyperolius mitchelli			Loader et al., 2004
Hyperolius tuberilinguis			Loader et al., 2004
Hyperolius nasutus			Loader et al., 2004
Hyperolius sp. 1	X		
Kassina senegalensis			Loader et al., 2004
Microhylidae			
Hoplophryne sp.	X		
Phrynomantis bifasciatus			Loader et al., 2004
Brevicipitidae			
Callulina sp.	X		
Probreviceps cf. *rungwensis*	X		
Breviceps mossambicus			Loader et al., 2004
Spelaeophryne metheneri	X	X	
Petropedetidae			
Petropedetes sp.	X	X	
Phrynobatrachidae			
Phrynobatrachus acridoides		X	
Phrynobatrachus natalensis	X	X	
Phrynobatrachus uzungwensis	X		
Ptychadenidae			
Ptychadena anchietae		X	
Pyxicephalidae			
Amietia angolensis	X		
Pyxicephalus edulis			Loader et al., 2004
Pipidae			
Xenopus muelleri			Loader et al., 2004
Rhacophoridae			
Chiromantis xerampelina			Loader et al., 2004
Scolecomorphidae			
Scolecomorphus cf. *kirkii*	X		Loader et al., 2004

Discussion

Loader et al. (2004) outlined the differentiation of the highland herpetofauna of the Mahenge Mountains from the lowland area of the Kilombero Valley. The assessment, based on amphibians, showed high turnover patterns across this transect, with the presence of key species only in the montane zone. These key species were associated with other highland areas of the Eastern Arc. The study by Loader et al. (2004) supported what was well understood by geologists and some biologists previously (Lovett & Pócs, 1993); these Eastern Arc highland regions have a shared biogeographic history, as implied by the species they share. However, based on the rather limited sampling and taxonomic understanding, no endemics

TABLE 3. List of the reptiles of Mahenge Mountains.

Taxon	Sali FR	Mselezi FR	Data source (if not present study)
Gekkonidae			
Cnemaspis sp.	X	X	Loader et al., 2004[1]
Chamaeleonidae			
Chamaeleo dilepis	X		
Trioceros melleri	X		
Kinyongia cf. *oxyrhina*	X		
Rhampholeon beraduccii	X		Loader et al., 2004[1]
Rieppeleon brevicaudatus	X	X	Loader et al., 2004
Scincidae			
Melanospes cf. *loveridgei*	X	X	
Trachylepis striata	X	X	
Typhlopidae			
Rhinotyphlops mucruso	X		Loader et al., 2004
Colubridae			
Crotaphopeltis tornieri	X		
Crotaphopeltis hotamboeia	X		
Dasypeltis medici		X	
Lycodonomorphus whitey	X		
Philothamnus hoplogaster	X		
Philothamnus angolensis	X		
Philothamnus semivariegatus		X	
Psammophis orientalis	X		
Natriciteres sylvatica	X		
Thelotornis mossambicanus	X	X	
Xyeledontophis cf. *uluguruensis*	X		
Lamprophiidae			
Lamphophis cf. *fuliginosus*	X		
Atractaspis aterrima	X		
Elapidae			
Dendroaspis angusticeps		X	
Naja melanoleuca	X	X	
Viperidae			
Causus defilippii	X		

[1] Data source shows different taxonomic identity.

were identified, but rather, species with seemingly larger distributions across southern Eastern Arc areas (e.g., *Scolecomorphus kirkii*) and in some cases potentially northern areas (e.g., *Nectophrynoides tornieri*). Closer scrutiny of the region was encouraged despite Mahenge's relatively low diversity. Mahenge's apparent close relationship to other hyperdiverse Eastern Arc areas suggested it might also be diverse, but lack of research had precluded a proper assessment.

In this study, we outlined new discoveries revealing a remarkable diversity of amphibians and reptiles of the Mahenge Mountains. Numerous putative new species have been identified but await further taxonomic work. These results improve substantially our understanding of the Mahenge Mountain herpetofauna. Surveys across a wider geographical area in Mahenge have identified putative new endemic species belonging to characteristic endemic Eastern Arc genera. The only previous survey conducted in this area (Loader et al., 2004), above 850 m in Sali FR, documented only seven montane Eastern Arc endemics or near-endemic species: *Scolecomorphus kirkii, Nectophrynoides tornieri, Speleaeophryne methneri, Petropedetes yakusini, Leptopelis vermiculatus, Afrixalus uluguruensis,* and *Hyperolius puncticulatus*. This study increased the total numbers substantially to 41 species, with radiations of putative endemic species belonging to genera previously recorded (e.g., *Nectophrynoides,* three species; *Leptopelis,* two species; *Afrixalus,* two species; *Arthroleptis,* two species). In addition, genera previously not recorded for this area include *Callulina, Hoplophryne,* and *Probreviceps*. Overall the increase in species diversity is remarkable and requires that remaining forest reserves in these regions to be assessed to understand how widely distributed these species are.

The documented diversity further exemplifies the hyperdiverse Eastern Arc amphibian fauna. Recent surveys, including those documented in this volume (Lawson & Moyer, 2008; Menegon et al., 2008; Loader et al., 2010), have started to establish that, beyond the well-surveyed forests (Uluguru, Udzungwa, and Usambara), areas previously considered less diverse often contain highly diverse assemblages after more extensive surveys are conducted. Surveys across all the fragmented forest areas of the Eastern Arc Mountains are required before anything can be presumed about species diversity.

The reptile fauna, even more poorly understood, is also a comparatively diverse assemblage in Mahenge. Prior to this study, only seven taxa were recorded; our surveys raised this total to 24 species, including one recently described endemic species (*Rhampholeon beraduccii*; Mariaux & Tilbury, 2006). Several reptiles are recorded as being putative new species, which await taxonomic verification (*Kinyongia* cf. *oxyrhina*, *Melanoseps* cf. *loveridgei, Crotaphopeltis* cf. *tornieri, Xyele-*

FIG. 2. Left column, descending: *Probreviceps* cf. *rungwensis*, *Callulina* sp., *Nectophrynoides* sp.; right column, descending: *Kinyongia* cf. *oxyrhina*, *Rhampholeon beraduccii*, *Cnemaspis* sp.

dontophis sp., and *Cnemaspis* sp.). The surveys also include the first records in this mountain fragment of the following genera: *Atractaspis*, *Kinyongia, Melanoseps, Crotaphopeltis,* and *Xyeledontophis*. This addition to the number of known reptile species in the area suggests that the region is both diverse with species characteristic of the Eastern Arc and contains numerous endemic species.

The presence of several Eastern Arc endemic or near-endemic genera and species suggests a strong link between the Mahenge Mountains and other EAM forested areas. Mahenge Mountains are geographically adjacent to the Udzungwa Mountains, and this proximity is also reflected in the close biogeographical relationships (see Fig. 3). Between these two areas is the deep and wide Kilombero Valley, which currently acts as a barrier to species dispersal. Evidence of this potential barrier has been provisionally outlined in the genetic differences exhibited by populations on either side of the Kilombero Valley. For example, populations of *Kinyongia oxyrhina* in Mahenge and Udzungwa show sufficient genetic differentiation to suggest long-term separation (Krystal Tolley, pers. comm.).

To the south, where the Mahenge Mountains are joined to the Southern Highlands by the Mbarika Mountains, dispersal might have also occurred across this southwestern corridor.

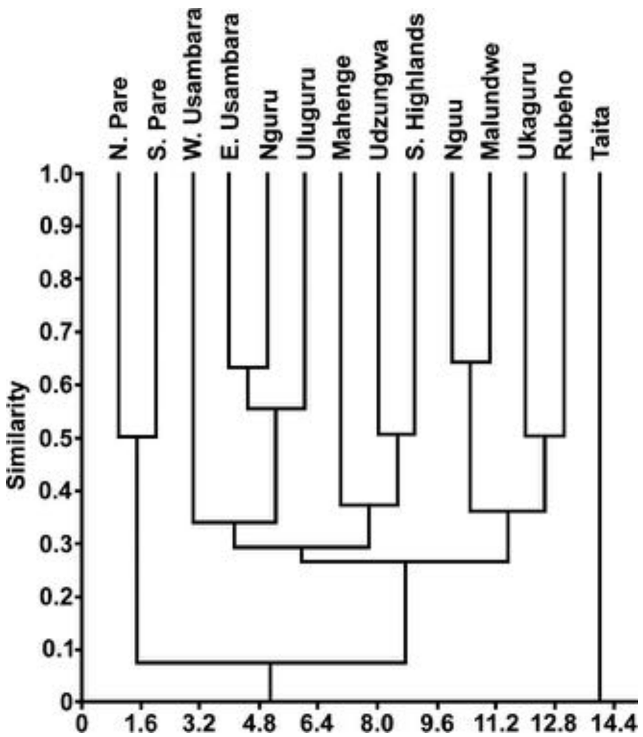

FIG. 3. Dendrogram based on a matrix of endemic or near-endemic Eastern Arc Mountain amphibian and reptiles species (n = 132) by site, using the Bray–Curtis (Sørensen) similarity index.

This is suggested by the clustering of the Southern Highland site, Rungwe, with Mahenge and Udzungwa. No previous studies exist that suggest the likelihood of this corridor as a potential route for amphibian and reptile species dispersal, but our evidence supports a close faunal similarity between the Mahenge Mountains, Southern Highlands, and Udzungwa Mountains. Interestingly, Rungwe groups with Udzungwa, and this might suggest barriers to dispersal are sufficiently weak between Udzungwa and the Southern Highlands to generate similarity between these faunas. More significant barriers to dispersal are assumed for both the Kilombero Valley (Mahenge-Udzungwa) and the Mbarika Mountains (Southern Highlands–Mahenge) as evidenced by Mahenge's more distinct assemblage in similarity measures. Further testing of these barriers would contribute to our understanding of the biogeographic history of this region of Tanzania. There is some evidence from phylogenetic studies, but this appears to show contrasting patterns. For example Mariaux and Tilbury's (2006) recent study of dwarf chameleons of the genus *Rhampholeon* showed that the nearest relative of *Rhampholeon beraduccii* is *Rhampholeon nchisiensis* of the Southern Highlands. Similarly, populations of *Probreviceps rungwensis* in Mahenge and the Southern Highlands cluster might potentially indicate that this pattern is a general trend. Whether Mahenge, Udzungwa, and Southern Highland faunal similarities can be explained by either dispersal, vicariance, or both patterns will require more comprehensive phylogenetic evidence.

The discovery of a highly diverse forest amphibian and reptile assemblage in a forest fragment of the Eastern Arc Mountains is not unremarkable. Focused survey effort has repeatedly revealed diverse assemblages in the Eastern Arc (e.g., Barbour & Loveridge, 1928; Menegon et al., 2008). Despite the small size of habitats, and often the level of disturbance, these small, ancient forest remnants harbor unique faunal communities (Lovett, 1993). Recent evidence has also suggested that many lineages have long evolutionary histories, presumably due to isolation and long-term persistence of habitats. This finding has further elevated the conservation value of the Eastern Arc Mountains (Burgess et al., 2007). Mahenge can be added as another example of a location showing high amphibian diversity and of high conservation importance.

Comparisons across the Eastern Arc suggest the total forest area is relatively small in Mahenge (see Burgess et al., 2007). Despite the small size of the natural vegetation remnants in Mahenge, species diversity is relatively high. In particular Sali FR, with its broad altitudinal coverage across the montane forest elements, has particularly diverse elements, including several possibly endemic species. The typical montane endemic element associated with the Eastern Arc is mainly restricted to Sali forests above 900 m, including species in the genera *Crotaphopeltis, Hoplophryne, Callulina, Nectophrynoides, Probreviceps, Scolecomorphus*, and *Xyeledontophis,* but absent from Mselezi forests. A taxonomic assessment of the species collected here is required to confirm their taxonomic status, together with focused conservation attention on Sali FR. Assessment of the remaining forest fragments in the Mahenge Mountains might reveal other areas that are equally diverse, and this remains a priority for future work in this area (particularly submontane and montane elements). If current species estimates are correct, Sali FR could rank among the most important forest reserve sites for the conservation of the forest herpetofauna in Eastern Africa. However, further voucher-based research is needed to test this hypothesis.

Acknowledgments

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Appendix 1

List of Material Collected with K. M. Howell (KMH) Collector Numbers

Amphibians

Arthroleptidae: *Arthroleptis* sp. 1—KMH 26255, 26238–40, 26265, 26267, 26268, 26463, 26636, 26655, 26656, 26983, 26985, 26987; *Arthroleptis lonnbergi*—KMH 26908; *Arthroleptis* cf. *reichei*—KMH 26632, 26633; *Arthroleptis xenodactyloides*—KMH 26253, 26423, 26460, 26642, 26657–60, 26982, 26984, 26988, 26989, 26995; *Leptopelis flavomaculatus*—KMH 26645; *Leptopelis uluguruensis*—KMH 26251, 26258, 26259; *Leptopelis vermiculatus*—KMH 26635, 26964, 26965; **Bufonidae:** *Nectophrynoides* cf. *tornieri*—KMH 26254, 26261, 26262, 26467, 26648, 26652, 26986; *Nectophrynoides* sp. 1—KMH 26252, 26259, 26260, 26263, 26264, 26649, 26650, 26912; *Nectophrynoides* sp. 2—KMH 26637–39, 26641, 26643, 26644, 26988, 26999; *Mertensophryne loveridgei*—KMH 26653; **Hyperolidae:** *Afrixalus* cf. *uluguruensis*—KMH 26269, 26646, 26950, 26957, 26966, 26992, 26993; *Afrixalus fornasinii*—KMH 26462, 26634, 26952; *Afrixalus* sp. 1—KMH 26958, 26962, 26970, 26971, 26974; *Afrixalus* sp. 2—KMH 26651, 26968, 26980; *Hyperolius* cf. *puncticulatus*—KMH 26956, 26961; *Hyperolius* sp. 1—KMH 26955, 26959, 26960, 26967, 26972, 26976, 26977, 26979, 26996; *Hyperolius* sp. 2—KMH 26951, 26954, 26973, 26977, 26978; **Brevicipitidae:** *Callulina* sp.—KMH 26266, 26911, 26963, 26975; *Probreviceps* sp.—KMH 26256, 26640, 26969, 26991; *Spaleophryne methneri*—KMH 26461, 26990; **Microhylidae:** *Hoplophryne* sp.—KMH 26981; **Petropedetidae:** *Petropedetes* sp.—KMH 26242–50, 26419, 26420, 26907; **Phrynobatrachidae:** *Phrynobatrachus natalensis*—KMH 26422, 26459, 26654; *Phrynobatrachus acridoides*—KMH 26421, 26458; *Phrynobatrachus uzungwensis*—KMH 26241, 26953, 26994.

Additional specimen records in Loader et al., 2004.

Reptiles

Gekkonidae: *Cnemaspis* sp.—KMH 23945, 23946, 26756, 26757, 26762, 26767, 26768, 26772; **Scincidae:** *Melanoseps loveridgei*—KMH 23948, 26765, 26771, 26774; Trachylepis striata—KMH 26769; **Chamaeleonidae:** *Rhampholeon beraduccii*—KMH 26752, 26754, 26758, 26759, 26775; *Rieppeleon brevicaudatus*—KMH 23947, 26753, 26760; **Colubridae:** *Crotaphopeltis tornieri*—KMH 26781, 26763; *Crotaphopeltis hotamboeia*—KMH 26782, 26784; *Dasypeltis medici*—KMH 26780; *Lycodonomorphus whytei*—KMH 26773; *Natriciteres sylvatica*—KMH 26418, 26783; *Philothamnus semivariegatus*—KMH 23949; *Philothamnus* cf. *angolensis*—KMH 26417, 26779; *Philothamnus hoplogaster*—KMH 26416; *Psammophis orientalis*—KMH 26785; *Thelotornis mossambicanus*—KMH 26778; *Xyeledontophis* cf. *uluguruensis*—KMH 26755; **Lamprophiidae:** *Atractaspis aterrima*—KMH 26764; *Lamprophis* cf. *fuliginosus*—KMH 26766; **Viperidae:** *Causus defilippii*—KMH 26776, 26777.

Additional specimen records in Loader et al., 2004.

Appendix 4. Priority areas for future herpetological researches

1. Gura Ferda plateau, Ethiopia

Gura Ferda is a National Forest Priority Area in Ethiopia, it does not have any IUCN category assigned and seems that to date there's no available biological data for the montane forest. Gura Ferda plateau covers an area of about 450 km^2 of dense montane rainforest with an altitudinal range that spans from 648 to 2330 m (APAAT, 2010). The plateau represents an isolated, vast outcrop surrounded by drier lowlands at an average altitude of about 800 m. Aerial and satellite imagery show an untouched dense forest cover, with little or no sign of human disturbance (Fig. 2-7).

Figure 2-5 Gura Ferda plateau in SW Ethiopia, satellite and aerial photographs.

2. Imatong Mountains, South Sudan

The Imatong Mountains are located on the South Sudan – Uganda border and covers about 8400 km^2, including the Imatong Central Forest Reserve (1032 km^2) and the Dongotana Hills (21 km^2) in South Sudan and the Agoro-Agu Forest Reserve (236 km^2) in Uganda (Sommerlatte and Sommerlatte, 1990, Davenport and Howard, 1996) Loveridge (1956) provided the first herpetological account of the Imatong Mountains. Among the 244 snakes representing 44 taxa, only few were from the montane forest of the Imatong, among them, interestingly *Toxycodryas blandingii* and *Bitis Nasicornis* (Loveridge, 1956), two species occurring in the Congo river lowland forest and the Albertine Rift forests at medium altitude. In 1943 the Field Museum of Natural History of Chicago acquired a small series of Amphibians and Reptiles, collected on Imatong Mountains by Dr. N. Weber. The specimens represent only seven species but they were the only glimpse of the herpetofauna of the area. In the series there was one unknown chameleon species, described by Schmidt as *Trioceros kinetensis* (Schmidt, 1943). Subsequently Colin Tilbury, during an examination of the reptile collection in the Natural History Museum of Nairobi, found another unknown chameleon collected by Parker and Stubbs in 1982 at Lomoriti, south west Imatong Mountains, he described it as *Trioceros conirostratus* (Tilbury, 1998). To date no endemic amphibians are know to occur in South Sudan or in the Imatong mountains.

3. Itombwe Massif, DRC

Since its earliest exploration in the first decade of this century by Emil Grauer (Matschie, 1914), the Itombwe Massif, a 6000 sq km area of forested mountains and high grassland plateaux in eastern DRC, has been recognized as one of the most biologically distinctive regions in Africa (Omari et al., 1999). Most of the biological exploration of Itombwe was undertaken before the independence of the country in 1960, and was capped by Alexander Prigogine's collections of the region's avifauna made between 1950 and 1967, totalling 565 species (Stubbs, 1988; Omari et al., 1999) The Itombwe massif herpetofauna was partly investigated by Laurent (1964) who described two endemic genera, *Laurentophryne* and *Chrysobatrachus*, while the former was not recorded since its description in description in 1950, the latter was rediscovered in 2011 during a brief herpetofaunal survey of several sites (Greenbaum, 2012).

4. Misotshi-Kabogo range, DRC

The Belgian ornithologist Alexander Prigogine sent a field team to the Misotshi-Kabogo range to collect bird specimens in mid 1950s (Prigogine, 1960), while the Belgian herpetologist Raymond collected amphibians in the forest (Laurent, 1952). Since then, and for over 50 years, the Misotshi-Kabogo region has been impossible to visit because of rebel activity, and it is only recently that a survey has become feasible. In the last few years WCS has started surveying the Kabogo massif, following its identification as the largest block of forest on the shores of Lake Tanganyika on Google Earth. In particular, they had the opportunity to undertake aerial surveys of the area, from which it was clear that the site was of remarkable biological and conservation interest, with presence of large mammals like chimpanzee (*Pan troglodytes*) and elephants (*Loxodonta cyclotis*) (WCS, 2007). From the aerial survey, the forest appeared large, intact and completely isolated, and stretches over ca. 100 km along the escarpment on the western edge of Lake Tanganyika. Of particular interest was the fact that forest exists down to the lakeshore at several sites along the lake and therefore ranges in altitude between about 770 and 2,750 metres. There are few places in Africa where continuous forest spans this elevation range. From the few specimens available in museums, a glimpse of the species that inhabit Kabogo montane forests has been possible (this study). Preliminary analyses suggest there are many as yet undescribed species unique to the massif (this study).

5. Mt. Chiperone, Mozambique

Very little published information is available on Mt Chiperone and the surrounding area (Timberlake et al., 2007). The first recorded biological survey on Mount Chiperone was carried out from 25–31 July 1950 by Jali Makawa, a collector for the ornithologist C.W. Benson (Benson, 1950; Spottiswoode et al. 2006). He reported that there was extensive evergreen forest on the eastern slopes (around 780 ha), more extensive than anything in southern Malawi at the time. There are reports of small mammals and birds being collected around 2002 by persons from the Chicago Field Museum and Department of Biology of the University of Maputo, but results are not yet available. In 2005, a Darwin Initiative expedition collected forest birds and, opportunistically, reptiles and amphibians. Among the reptiles recorded, one has been subsequently described as species new to science and is endemic to the mountain

(*Rhampholeon nebulauctor*; Branch et al., 2014). To date no systematic herpetological surveys have been carried out on the forest of Mt. Chiperone.

Appendix 5. The eField Guide: A key resource for Eastern Afromontane herpetology

Field Guides are an essential resource for experts, and laypersons for understanding the diversity of species in an area. They inform and provide valuable insights and are often used towards protection and conservation of species. However, in the age of rapid species discovery whereby taxonomists have a number of tools at their disposal for detecting species (e.g. genetic, acoustic, and morphological data) field guides rapidly become obsolete and in some cases can be positively misleading. New solutions need to accompany such rapid changes in the scientific work that is being undertaken. A rapidly updatable eField Guide is such a solution, as it is a resource that provides both rich informative information (including calls, and clickable evolutionary trees) but crucially can be made up-to-date rapidly. Furthermore, the eField Guide will provide key data for the conservation management of species and communities to be used by experts and policy makers alike (Figure 2-5; 2-6).

For each described and putative species, the e-book contains the following information

- Description

  Including details on the external morphology and colour pattern aimed to facilitate the morphological identification of species in the field. This will include multiple photos including species variation according to gender and geography – often not included in field guides due to space limitations.

- Remarks

  Including current taxonomic or conservation issues regarding the species.

- Distribution with enlarged map

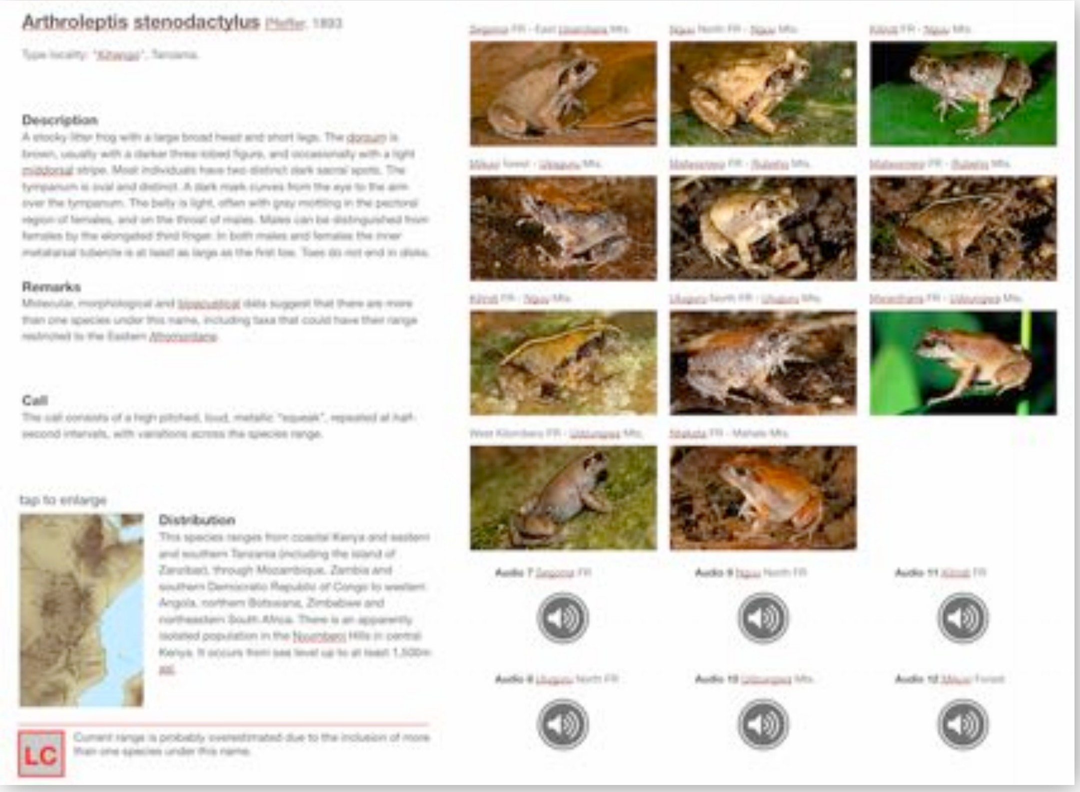
- Call with information on the recording localities

- Videos showing behaviour of species

- IUCN Red List category

The e-book will be regularly updated and will therefore be ‘progressive’ and up-to-date resource of the current situation of the species assemblage occurring across the Eastern Afromontane region.

Figure 2-6 layout of the e-guide cover



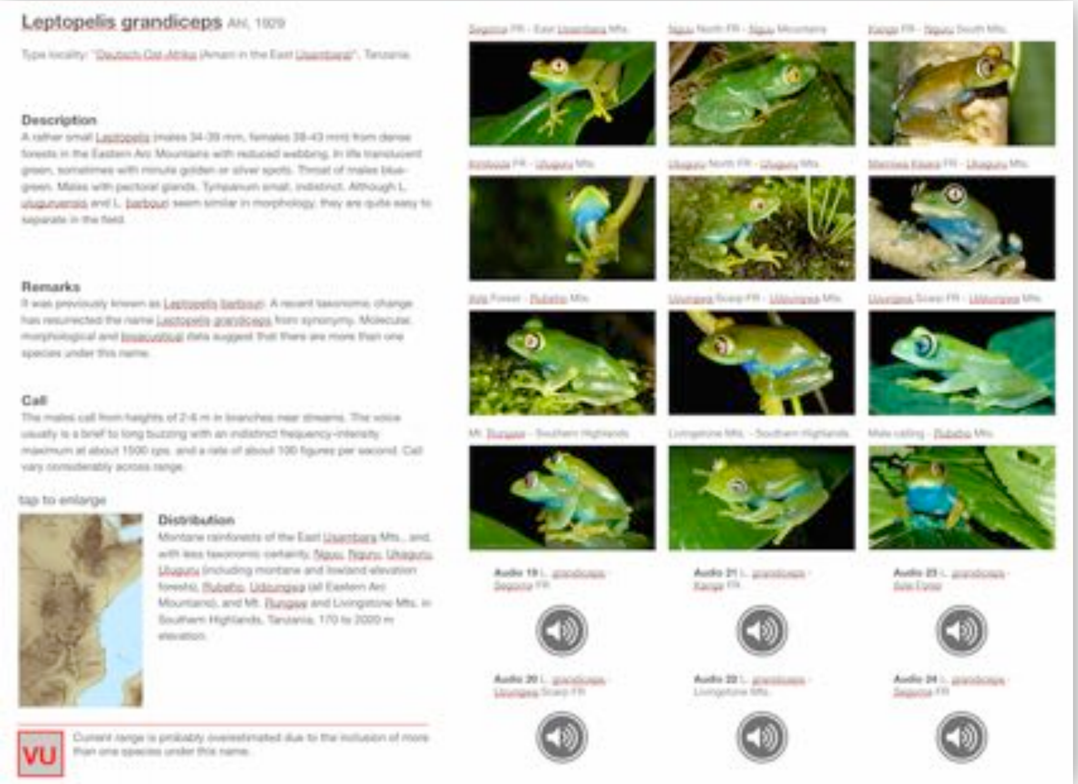


Figure 2-7 layout of two pages of the e-guide

Chapter 3 : Biogeography of Amphibians

Patterns and drivers of diversity in Eastern Afromontane amphibians

Figure 3-1 Exampe of habitat and three amphibian species (from top) *Leptopelis grandiceps* (Tanzania), *Paracassina kounhiensis* (Ethiopia) and *Boulengerula fisheri* (Rwanda)

Abstract

The massifs and isolated mountains of the Eastern Afromontane Hotspot contain some of the highest densities of endemic vertebrate species in the world. Using a newly compiled dataset comprising 3,488 geo-referenced records of 274 taxa, I have mapped the distributions of mountain-restricted amphibians across the region to examine zoogeographical partitions, and to identify likely environmental drivers of richness and endemism. Underlying this work are interests in comparing the amphibian faunas of the region's mountain ranges and more isolated 'sky islands', and identification of key sites for species conservation. I have used βsim dissimilarity cluster analyses to identify biogeographical partitions, and random slope GLMMs to explore relationships between species richness/endemism in the different mountain ranges and environmental predictors including altitudinal measures, EVI and cloud cover. There was strong geographical congruence between richness and endemism, and between these measures at the species and genus level, but not across different amphibian families. Richness and endemism were highest in a belt between 2^0 and 10^0 S, and especially in the Albertine Rift and Eastern Arc. Relationships between richness/endemism and environmental predictors, especially EVI and minimum altitude of surrounding land, were different across mountain ranges. The Albertine Rift and Eastern Arc mountains appear to have accumulated high amphibian diversity in different ways, with the latter having exceptional diversity despite their small sizes, driven by low altitude barriers between them. There was almost complete species turnover between mountain ranges. Our results do not support the idea that the Eastern Afromontane is a discrete zoogeographic region, rather what holds it together is not a sharing of species, but a common dynamism in the spatial and altitudinal turnover of taxa. I rank sites according to importance in terms of taxon richness and endemism and highlight their protection status.

Introduction

Species richness and endemism patterns are important precursors in the delimitation of biogeographical units and in setting area-based conservation priorities (Slatyer et al. 2007, Tucker et al. 2012, Fritz and Rahbek, 2012). Species with restricted ranges are, for obvious reasons, a focus for efforts to conserve the world's biological richness (Myers et al. 2000, Tucker et al. 2012). Generally, areas with high species endemism are characterized by complex or isolated habitats and climates, and are often at high altitudes, with cool temperature conditions (Fjeldså and Lovett 1997, Fjeldsa et al. 1999, Moir et al. 2009). Species with small ranges tend also to occur in climatically heterogeneous regions, because these areas often provide pockets of suitable habitat that act as refugia, buffering species from extinction during large-scale climatic shifts (Ohlemüller et al. 2008, Tolley et al. 2011, Keppel et al., 2011, Murienne et al. 2013). Amphibians, which tend to have small ranges, niches tightly constrained by environmental conditions, and limited dispersal ability (Smith and Green 2005) represent a valuable proxy group for investigating patterns of organism distribution and richness, and ultimately, for identifying areas for conservation (Buckley and Jetz 2007).

In mainland Africa, the rain forests of the Congo River basin are surprisingly relatively species-poor in vertebrates generally, and forest amphibians in particular (Linder et al. 2012). In contrast, the Eastern Afromontane Biodiversity Hotspot (EAH) forests include remarkably species-rich areas (Lamoreux et al. 2006, Plumptre et al. 2007, Rovero et al. 2014). In fact, East Africa contains one of the highest known concentrations of endemic plants and vertebrates on Earth (Myers et al. 2000, Plumptre et al. 2007, Rovero et al. 2014), due in part to the highly diverse montane floras and faunas of the Eastern Arc Mountains of Tanzania and Kenya (EA), Albertine Rift (AR) (Plumptre et al. 2007, Rovero et al. 2014) and Ethiopian Highlands (EH) (Bussmann, 2006). Hypotheses explaining the extraordinary biodiversity of East African mountains generally point to post-Oligocene climatic shifts as triggers for lineage diversification in the presence of refugia, with major lineage diversification in the Pleistocene (Bowie et al. 2006, Couvreur et al. 2008).

The forests of EAH are relicts of a once widespread pan-African forest, which became fragmented due to aridification starting in the early Oligocene. They have persisted through Pleistocene glaciation cycles, due to Indian Ocean circulation bringing reliable orographic rainfall (Murienne et al. 2004, Lovett and Wasser 2008, Tolley et al. 2011). In general, the forests, as well the mosaic of montane forest and grasslands, are confined to the summits and upper slopes of mountain blocks and volcanic cones, with the exception of few sites in the Eastern Arc, where forest still covers the full altitudinal gradient from valley bottoms to the highest peaks, at over 2500 m in the Udzungwa and Uluguru Mts. (Lovett and Wasser 1993, Lovett 1996).

Knowledge of amphibian species richness and endemism patterns across East Africa is fragmented, with no overall synthesis yet attempted. This has mainly been due to a lack of distributional data across the region, much of which is difficult to access due to many areas being remote and politically unstable (Branch et al. 2012, Conradie et al. 2012). In the last twenty years, however, biological exploration of poorly known areas has produced a large volume of new distributional and molecular data refining our understanding of forest amphibians and making it possible to investigate biogeographical patterns across the region.

The first aim was to map spatial patterns of richness and endemism in mountain-restricted amphibians of the Eastern Afromontane Hotspot (EAH) I then examine geographical congruence in amphibian-importance metrics, measure turnover rates across and within mountain ranges to identify zoogeographical partitions, and identify environmental correlates previously (EV1, altitude and cloud cover e.g. Buckley and Jetz 2007, Qian 2010) used to explain richness and endemism patterns. Finally, I identify key sites for the conservation of mountain-associated amphibians across the region.

Material and Methods

The study area

The Eastern Afromontane Biodiversity Hotspot (EAH; Fig. 3-2) covers more than one million km2 from the Asir Mountains of southwest Saudi Arabia, south to the Chimanimani Highlands of eastern Zimbabwe. It includes four main mountain ranges: Eastern Arc Mountains (EA) and Southern Highlands of Tanzania and mountains of northern Malawi, here considered as one biogeographic unit, despite the latter two are formally not included in the Eastern Arc; Albertine Rift (AR) that includes parts of Rwanda, Burundi, Uganda, Tanzania and the Democratic Republic of Congo (henceforth D. R. Congo); the Ethiopian Highlands (EH) covering much of Ethiopia, along with small parts of Eritrea, Djibouti, and Sudan; and Kenyan Central Highlands (KH) including Mt. Kenya, Mt. Elgon, Mau range and Aberdare Mountains (Conservation International 2012). In addition, EAH includes a number of outlying mountains, notably the northern Tanzanian neogene volcanic Highlands (Mt. Kilimanjaro, Mt. Meru, Mt. Kitumbeine and Mt. Hanang; (Brooks et al. 2002), and scattered forested inselbergs in northern Mozambique and the eastern highlands of Zimbabwe (Wild 1964, Beasley 1995). The Asir Mountains of southwest Saudi Arabia and highlands of Yemen, lie outside the African continent, and so were excluded from the analysis.

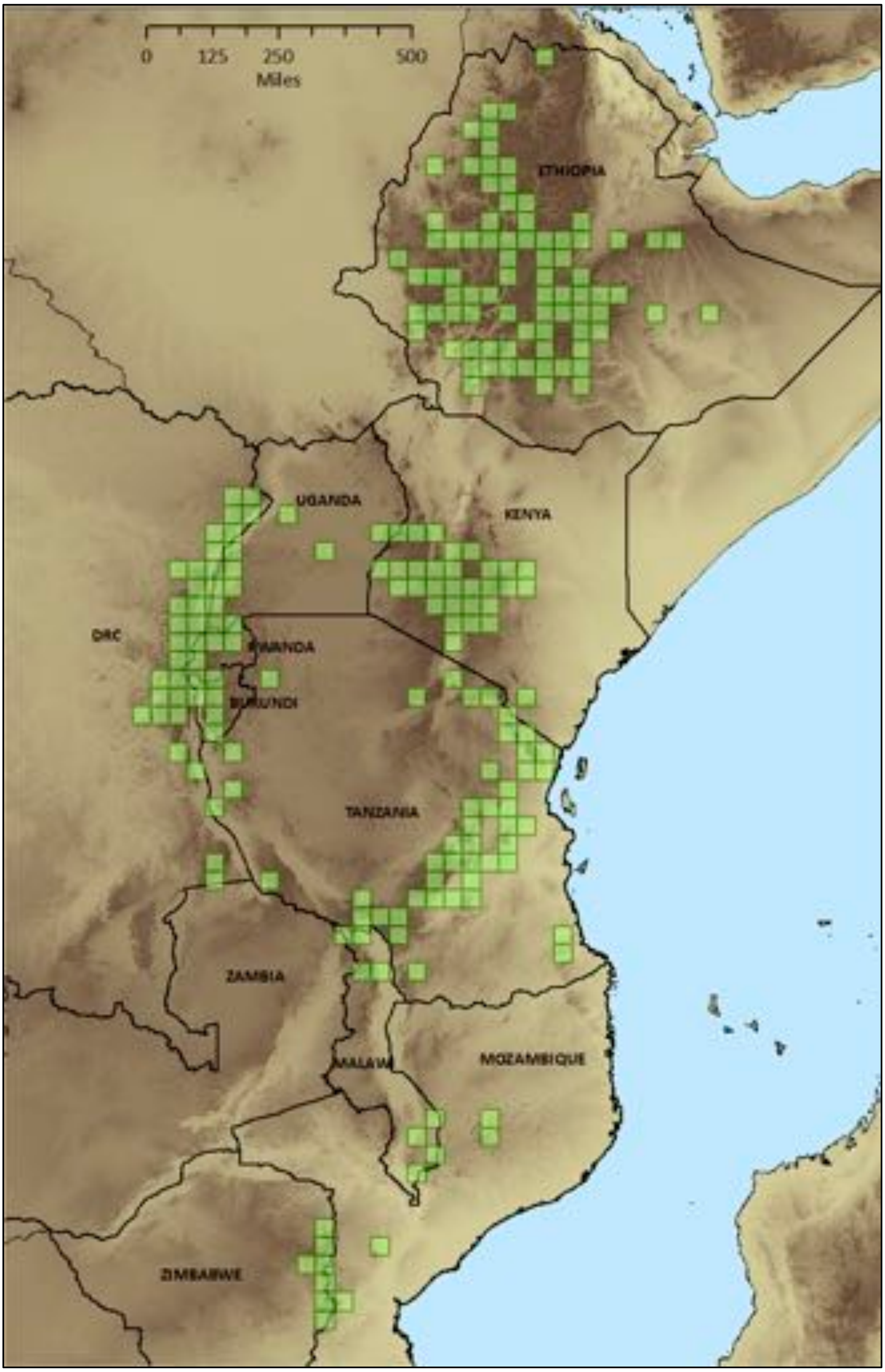


Figure 3-2 The Eastern Afromontane with the sampling grid. The 222 0.5 degree grid cells encompass the area with available occurrence data.

Mountain-restricted amphibian species and the data set

The region was subdivided into sampling units made up of a superimposed grid of 0.5° cells (Fig. 3-2). The Hotspot itself centres upon montane areas dominated by forests and grasslands at altitudes of 1000-3000 m a.s.l. (highest point is Mt. Kilimanjaro at 5895 m). The lower bound of the hotspot is variable, dependent on local geography, with 800-1000 m as a typical lower altitudinal limit in EH, KH and AR, but with areas as low as 300 m included within EA (Platts et al. 2011). I've included only those amphibians whose entire geographical range is known to fall within the EAH.

Amphibian occurrence data were compiled from the following herpetological collections or databases: Trento Science Museum, Natural History Museum, London; Dept. Zoology and Wildlife Conservation at the University of Dar es Salaam, Tanzania; and the WCS' Southern Highlands Conservation Programme collection. The extensive online GBIF (Global Biodiversity Information Facility; http://www.gbif.org) and Herpnet (www.herpnet.org) databases were interrogated, and data from the following US herpetological collections gathered: American Museum of Natural History, New York; California Academy of Science, San Francisco; Field Museum, Chicago; and Museum of Comparative Zoology at Harvard University. Recent unpublished data from several years of collecting across the D. R. Congo sector of the AR forests, especially in Itombwe, Kahuzi-Biega and Marungu were provided by Eli Greenbaum, University of Texas at El Paso. Data from Chimanimani Highlands were made available by James Harvey.

Our studies, along with recent published and unpublished work from Tanzania (Menegon et al. 2008, Loader et al. 2011b), Ethiopia, Rwanda and D. R. Congo (S. Loader, E. Greenbaum pers. comm. 2012) indicate the presence of several distinct lineages/taxa not yet formally described (assessed genetically, or both genetically and morphologically) within the study area. These phylogenetically assessed undescribed species were included in the analysis, because they represent an accurate and up-to-date indication of diversity across the various mountain ranges. Unpublished data on 'undescribed' lineages are available in the following works: Dürrenberger (2012); Mengistu (2012); Rudolf (2012); Hugli (2013); and Liedtke (2014) I acknowledge that there may be issues associated with including these taxa, so I mapped species richness using both databases including undescribed taxa (3,488 geo-referenced records representing 274 taxa) and including described species only (3,403 geo-referenced records representing 170 species). The latter sample includes many specimens that, despite being included in currently described species, have been subsequently found to represent distinct taxa, soon to be described as separate species. Records were assembled and aggregated to a 0.5° grid cell resolution and I was careful to exclude records where locality was poorly/erroneously defined or where species identity was uncertain.

Patterns of diversity

For datasets including and excluding ‘undescribed’ taxa, I mapped species distribution per cell using Biodiverse 0.17 (Laffan et al. 2010). Species richness was estimated for each 0.5° grid cell by counting the number of species recorded in that cell, plus additional species recorded from the four grid cells to the north, south, east and west of the cell, if it was surrounded by cells with data. This ‘neighbourhood’ analysis helped to minimize the effect of arbitrary boundaries between grid cells (Prendergast 1997). An alternative would be to simply calculate the richness within each individual cell, but I follow Slatyer et al. (2007) and other studies in considering neighbourhood values. There was, in any case, a significant positive correlation between richness of cells using the neighbourhood measure and that using only the cell value ($r = 0.74$, $df = 220$, $p < 0.0001$; database including both described and undescribed taxa).

A weighted endemism index was calculated (Crisp et al. 2001, Slatyer et al. 2007), using a procedure that avoids the assignment of problematic arbitrary region or range-size thresholds for defining endemic species. This involves applying a continuous weighting function, assigning high weights to species with small ranges, and progressively smaller weights to species with larger ranges (Slatyer et al. 2007). The values, therefore, do not represent the number of endemic species by sub-region (e.g. AR or EA endemics) but the incidence of species with small range sizes within each cell.

Correlations between α diversity measures at different taxonomic levels and across different families were examined using Pearson’s Product Moment correlation analyses. The βsim dissimilarity metric (Lennon et al. 2001) was used to run a cluster analysis using an unweighted pair-group method with link average. Moving window, cluster and neighbourhood segregation analyses were conducted in Biodiverse 0.17 (Laffan et al. 2010).

To examine environmental predictors of species richness and endemism scores, I used random slope generalized linear mixed models (GLMMs;) Only cells in the four main mountain ranges identified in the cluster analysis (AR, EA, EH and KH) were included as a fixed factor, as I was particularly interested in how the relationship between the response variables and predictors varied across these mountain ranges.

While differences in survey effort across sites is a general issue in studies of this nature (Rocchini et al. 2011, Ficetola et al. 2014, Rovero et al. 2014), I felt it especially important to consider these potential biases in our GLMMs relating species richness/endemism and environmental predictors. The relationship between the number of species found within a cell and survey effort is not straightforward for several reasons (e.g. differences in expertise/search techniques, geographical coverage of search effort within cells, different sizes of sites). Foremost among these, however, is the likely positive feedback produced where cells with a track record of yielding new discoveries are likely to attract further attention. Given these complexities, and mindful that some of these issues may be influenced by the environmental predictors themselves, I opted not to include survey effort as a covariate in the analyses, but rather to screen out those sites where lack of survey effort was most likely to have deflated species lists. This was done by MM, based on a literature search and personal knowledge of sites. Of the original 200 cells, 119 were retained following exclusion of: 54 of 79 cells from EH where surveying has been sparse; 12 from 30 in the KH; 13 from 48 cells in ARM; and just two from 43 in EA where surveying has been more systematic. While species richness and endemism scores were strongly correlated with survey effort values across the original dataset (richness: $r_s = +0.55$, $p < 0.001$, $n = 200$, endemism: $r_s = +0.58$, $p < 0.001$), among the subset of well-surveyed cells, richness was weakly correlated with effort in ARM ($r_s = +0.34$, $p = 0.047$, $n = 35$), but not significantly correlated with effort at the other sites ($r_{smax} = +0.14$, pmin = 0.50) Similarly, endemism score was weakly correlated with effort in ARM ($r_s = +0.33$, $p = 0.05$, $n = 35$), but not at the other sites ($r_{smax} = +0.26$, $p_{min} = 0.21$).

I developed GLMM models for two response variables: species richness (Poisson error), and species endemism score (Gaussian error). Each predictor variable was entered into the analysis separately. Analyses were run using the ‘lme4’ package in R Studio v.0.94.84 (R Development Core Team 2014). I label the GLMM analyses as exploratory, and as such, I’m interested more in the direction and relative strength of predictor effects (across mountain ranges) than predictions of their actual values.

I considered six predictor variables. Minimum and maximum altitudes within a 9-cell neighbourhood centred on each 0.5 degree cell were calculated from Shuttle Radar Topography Mission (SRTM) version 4.1 (Jarvis et. al., 2008) at 90 m spatial resolution. A measure of altitudinal heterogeneity within cells was calculated as follows: the grid cell was subdivided into 100 equally-sized squares and the mean altitude of pixels in each of the 100 squares recorded. I then subtracted the mean altitude of the 10th lowest lying square from the 10th highest altitude square, unless the 10th highest square lay above 3000 m. In these cases, we took the higher altitude to be 3000 m (areas above that altitude were deemed to be largely unsuitable for amphibians. Mean daily temperatures for the period 1901–2009 at 0.5^0 resolution were obtained from the University of East Anglia CRU-TS 3.1 Climate Database (University of East Anglia CRU, 2008). Mean percentage daily cloud cover for the period 1901–2009 at 0.5^0 resolution were obtained from the University of East Anglia CRU-TS 3.1 Climate Database (Harris et al. 2014). Finally, mean Enhanced Vegetation Index (EVI) data from MODIS MOD13Q1 product were acquired at 16 day intervals for the period 2001–2005 at 250 m resolution (NASA Land Processes Distributed Active Archive Center, 2012. Data are distributed by the Land Processes Distributed Active Archive Center (LP DAAC), located at USGS/EROS, Sioux Falls, SD; http://lpdaac.usgs.gov). Pixel quality data, indicating the presence of cloud and snow, which detract from the quality of the EVI measurement, was used on a pixel-by-pixel basis to select only those measurements of EVI with the greatest likelihood of accuracy.

Results

Geographical patterns of α diversity

Species richness and endemism

The median number of amphibian species per neighbourhood was seven (lower and upper quartiles 3-14; min/max 1-46 species). Thirteen of the 222 grid cells contained more than 30 species. Median number of genera per cell was five (quartiles 3-8; min/max 1-18). Richness values using described species only (Fig. 3-3_b) were of course lower, but cell values using described species only were significantly correlated with those including both described and undescribed taxa (Fig 3-3_a; $r = +0.70$, $df = 220$, $p < 0.0001$). Discrepancies between the two values were strongest in some areas of the EA and AR, where recent collecting in previously poorly sampled areas has been most prevalent (Doggart et al. 2006, Menegon et al. 2008, Rovero et al. 2014). Subsequent analyses use both described and undescribed species.

Large portions of the Eastern Afromontane had comparatively low species and genus richness – no cell north of 2° S contained more than 20 amphibian species or eight genera (Fig. 3-3). There was also low richness across all grid cells south of 10° S (Southern Tanzania). There are four main areas of elevated species richness (Fig. 3-3_a). Three are located in EA: South Nguru, Kanga and Uluguru mountains at 6° S; Udzungwas and the Uzungwa Scarp Forest Reserve at 8° S; and Usambara and Nguu Mountains at 5° S. The other lies in AR, on the border between Rwanda and D. R. Congo at around 3° S. The pattern of genus richness mirrors that of species, although there is high richness across much of the Eastern Arc Mountains, with particularly high values in Udzungwa, Nguru, Uluguru and Usambara Mountains. Genus richness in AR (Fig. 3-3_c) is proportionally lower than its species richness, although there remains a second hotspot of genus richness in the NE corner of the Itombwe massif.

Patterns of richness and endemism across families

The families *Hyperoliidae* and *Phrynobatrachidae* have particularly high diversity values across AR (Appendix 1, Fig 3-7 and 3-9). The families Pipidae and Ptychadenidae are best represented in the northern part of the region, the latter with remarkable species richness in the Eastern part of the Ethiopian Rift valley, while completely lacking mountain-associated species south of Lake Victoria (Supplementary material Appendix 1, Fig 3-8 and 3-9). Areas with both high species richness and endemism for the families *Bufonidae*, *Caeciliidae* and *Brevicipitidae*, are centred in EA but are poorly represented outside this region (Appendix 1, Fig 3-7 and 3-8). The family Arthroleptidae is widespread, but with centres of diversity around the northern part of EA and around Itombwe in AR (Appendix 1, Fig 3-7). The family Pyxicephalidae is widespread but with high richness around Nyungwe/Itombwe in D. R. Congo (Appendix 1, Fig 3-9), and with range-restricted species prevalent in the neogene volcanoes of northern Tanzania, and in the highlands of southern Malawi.

Congruence between alpha diversity measures

There was strong congruence between cells with high species, genus and family richness (r_{min} = +0.82; Table 1). Species level endemism values were also highly correlated with those for species richness, but correlations were generally weaker between genus endemism and the other measures, and particularly between family richness and endemism at species and genus levels. Congruence (correlations) between species richness in the different amphibian families were far weaker (Table 1) with 12 of the 36 pairwise correlations being non-significant and 11 actually having negative coefficients. Species richness in Arthroleptidae (mean r = +0.37 ± 0.24 SD), *Brevicipitidae* (mean r = +0.25 ± 0.38) and Bufonidae (mean r = +0.32 ± 0.30) were strongly positively correlated with most other families. Notable cross-family positive correlations were between Brevicipitidae and both Caeciliidae and Bufonidae, three families with large species diversity in the Eastern Arc (Appendix 1, Fig 3-7 and 3-8).

Table 3-1 Pearson's correlation coefficients between α richness measures for a) all families, and b) across the main amphibian families. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns non-significant. df = 220 in all cases.

a)	Genus endemism	Species endemism	Family richness	Genus richness
Species richness	+0.70 ***	+0.86***	+0.82***	+0.93 ***
Genus richness	+0.72***	+0.78***	+0.93***	
Family richness	+0.60***	+0.60***		
Species endemism	+0.74***			

b)

	Pyxicephalidae	Ptychadenidae	Pipidae	Phrynobatrachidae	Hyperoliidae	Caeciliidae	Bufonidae	Brevicipitidae
Arthroleptidae	+0.31***	-<0.019 ns	+0.14*	+0.21**	+0.67***	+0.5***	+0.57***	+0.57***
Brevicipitidae	+0.06 ns	-0.17*	-0.15*	-0.05 ns	+0.26***	+0.79***	+0.67***	
Bufonidae	+0.25***	-0.09 ns	-0.04 ns	+0.18**	+0.4***	+0.57***		
Caeciliidae	+0.02 ns	-0.17*	-0.13 ns	-0.01 ns	+0.2**			
Hyperoliidae	+0.48***	-0.05 ns	+0.34***	+0.4***				
Phrynobatrachidae	+0.37***	+0.04 ns	+0.38***					
Pipidae	+0.18**	+0.08 ns						
Ptychadenidae	-0.12 ns							

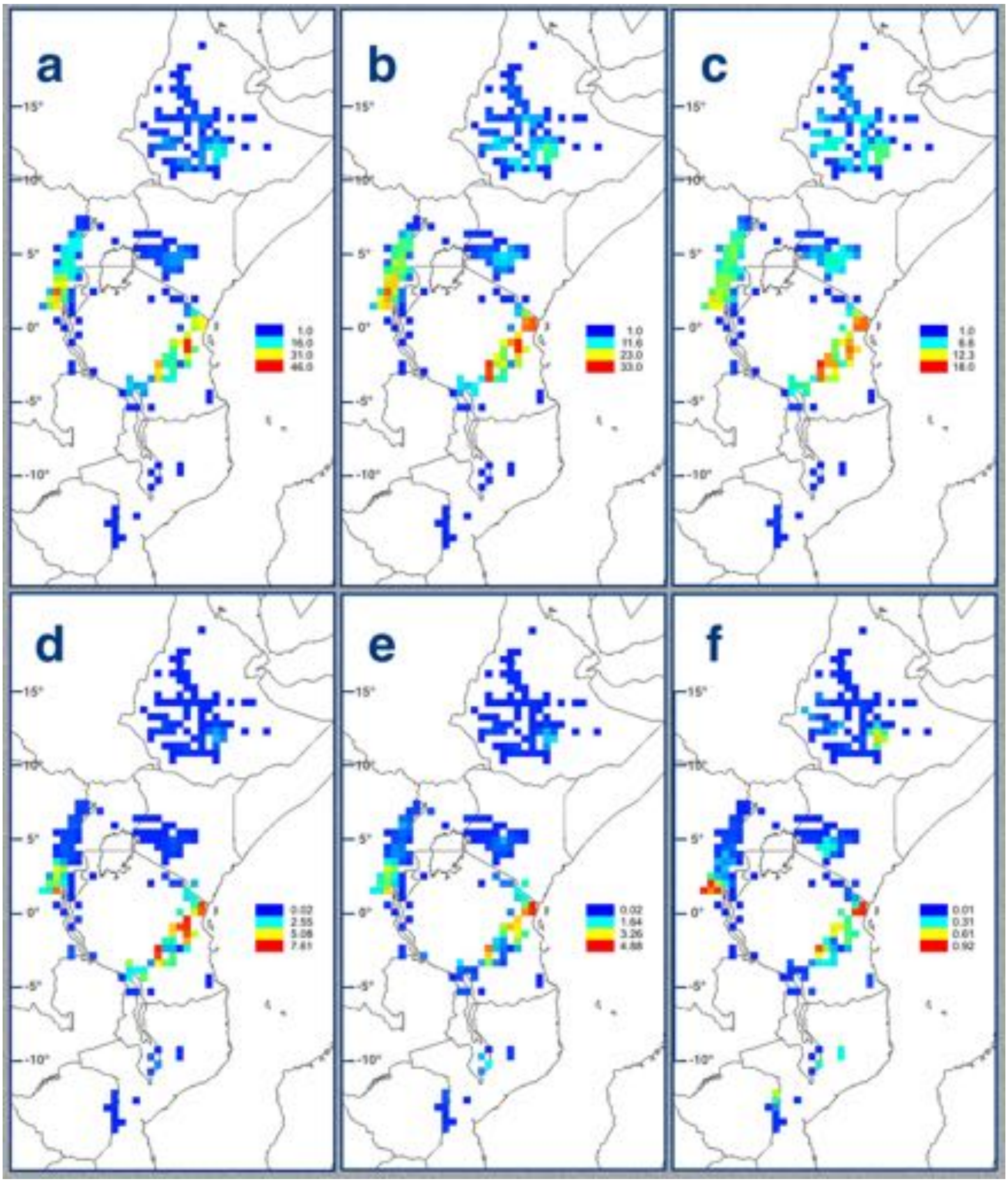


Figure 3-3 Map showing species richness of: (A) full dataset including undescribed species; (B) dataset with described species only; (C) genera. Species endemism of (D) full dataset including undescribed species; (E) dataset with described species only; (F) genera. Colors and relative numbers indicate species richness by neighborhood.

Patterns of β diversity

The rate of species turnover between mountain ranges was very high (Supplementary material Appendix 1, Fig 3-10), and, with few exceptions (i.e. western KH and Northern AR), the vast majority of species were confined to individual mountain ranges (Fig. 3-4). Cluster analysis produced strong geographical grouping of cells at all three taxonomic levels. At species level, five well-defined clusters were identified (Fig. 3-4), representing: a) EA Mountains of Tanzania, including the Southern Highlands, and outliers such as Mulanje and Zomba Mts.; b) southern Malawi and highlands in northern Mozambique; c) EH; d) AR and KH; and e) two smaller clusters of cells including the Chimanimani highlands of eastern Zimbabwe and mountains in southern Malawi and northern Mozambique. The isolated neogene volcanoes of northern Tanzania (e.g. Mount Kilimanjaro, Mount Meru), Marungu Mountains at the southern tip of the AR, and the Mahale Mountains, did not group with any of the major mountain ranges.

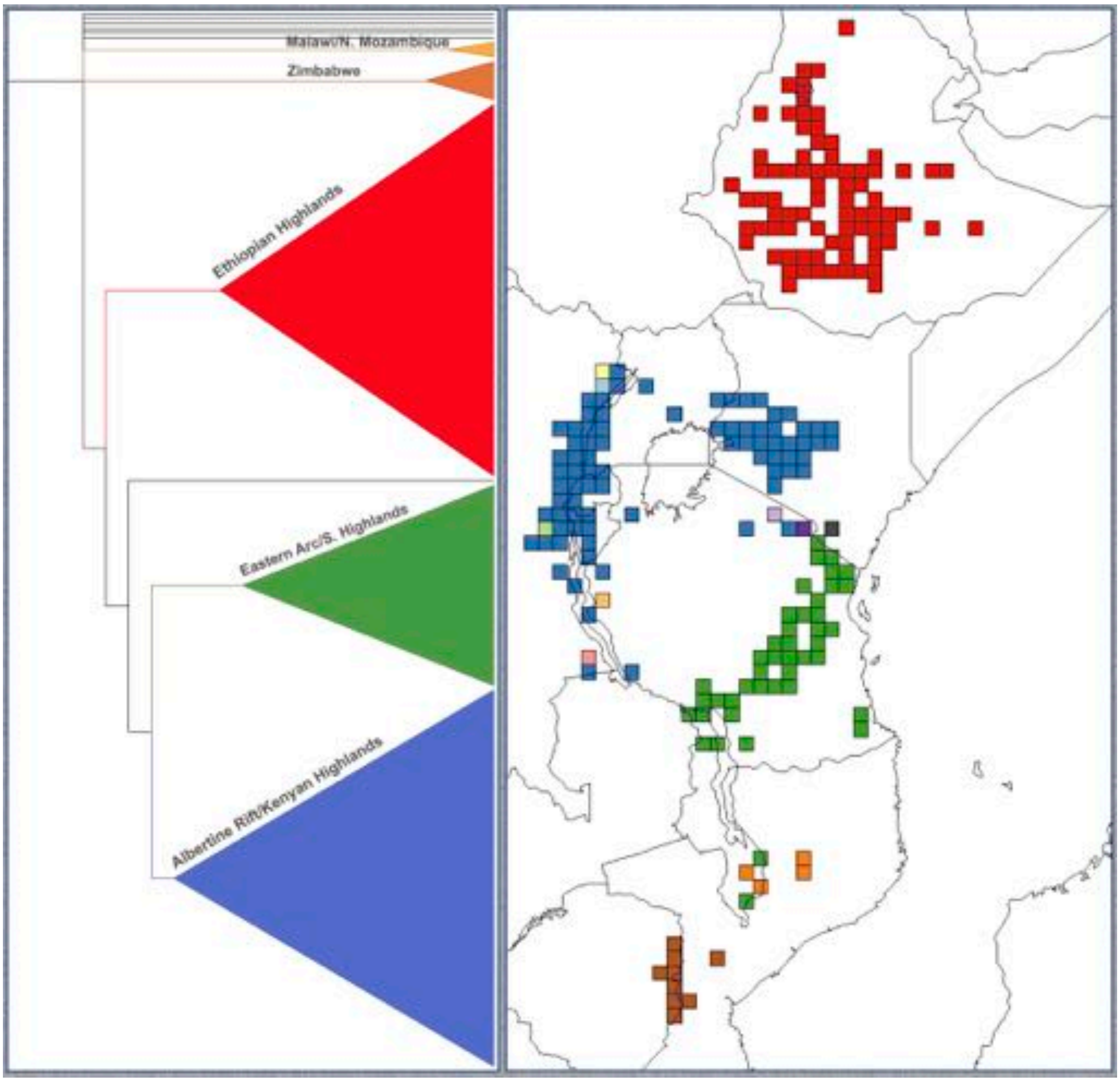


Figure 3-4 Biogeographical zonation and phenogram based on mountain associated amphibian species distribution obtained through cluster analysis. Note the high congruence between phenogram clustering and geographic areas at species level.

EVI, cloud cover and minimum altitude were stronger predictors of amphibian species richness and endemism across the four mountain ranges than were maximum altitude or altitudinal range (Table 2; Fig. 3-5). The relationships between cloud cover and both richness and endemism were fairly consistent across mountain ranges (gradients of relationship lines in Fig. 3-5 tend to be similar across mountain ranges). However, relationships for EVI, and especially for minimum altitude, were markedly different between the mountain ranges. In both of these cases, it is EA that is ‘exceptional’. For example, species richness and endemism increased much more steeply with increases in EVI at EA than at the other mountain ranges. Most striking was that the relationship between species richness and minimum altitude was strongly negative at EA, a pattern unlike that in any of the other ranges. Finally, endemism score decreased most strongly with increasing minimum altitude at EA, but this relationship was also stronger at AR than at ET or KH.

Table 3-2 Results of random slope GLMMs explaining relationship between Species richness/endemism score and a series of environmental variables. Shown for each model are the intercepts (α) and coefficients (β) ± standard errors for each mountain range. EAM Eastern Arc Mountains (n = 42 cells); ARM Albertine Rift (n = 35); ET Ethiopian Highlands (n = 25); KH Kenyan highlands (n = 18) As an indication of model fit, AIC values and ΔAIC for each model is shown, although number of parameters is constant across models. Models are ordered according to ΔAIC.

	EAM		ARM		ETH		KH		
richness	**α**	**β**	**α**	**β**	**α**	**β**	**α**	**β**	**AIC/ΔAIC**
EVI	-1.51	0.007	3.65	0.004	8.02	0.001	10.7	-0.001	1000/0
Minalt	25.0	-0.017	15.6	0.002	11.1	<-0.001	-0.001	1021/21	
Cloud	-26.5	0.711	-51.3	0.909	0.973	0.207	-17.9	0.375	1029/29
Maxalt	26.1	-0.004	4.15	0.005	2.18	0.003	5.05	0.001	1042/42
Alt range	20.0	-0.002	18.2	-0.002	10.5	0.001	7.40	0.002	1078/78
Endemism	**α**	**β**	**α**	**β**	**α**	**β**	**α**	**β**	**AIC/ΔAIC**
Cloud	-5.01	0.135	-2.29	0.054	-3.48	0.089	-1.51	0.031	457/0
Minalt	4.50	-0.002	3.07	-0.002	1.09	<-0.001	<0.001	462/5	
EVI	0.755	0.001	1.44	<0.001	0.704	<0.001	0.796	<-0.001	
Alt range	4.41	-0.002	1.91	<-0.001	0.001	0.378	0.001	469/12	
Maxalt	4.34	<-0.001	<0.001	-0.046	<0.001	-0.244	<0.001	472/15	

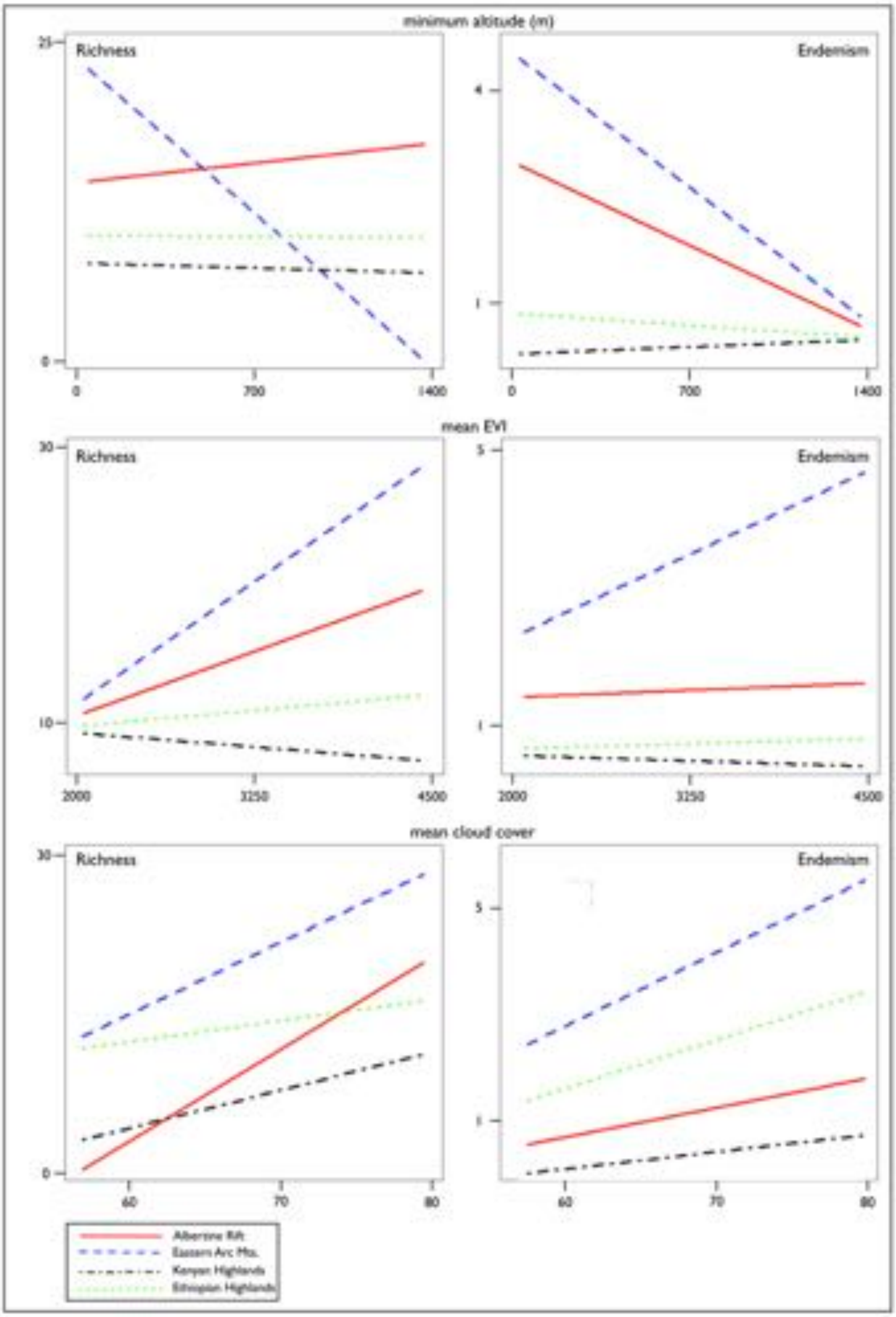


Figure 3-5 The relationships between species richness (left) and endemism score (right) with EVI, cloud cover and minimum altitude across four mountain ranges derived from random slope generalized linear mixed models (GLMMs) AR Albertine Rift Mountains (n = 35 cells); EAM Eastern Arc Mountains (n = 42); ET Ethiopian highlands (n = 25); KH Kenyan Highlands (n = 18).

Discussion

Richness and endemism across the Eastern Afromontane

There are areas with disproportionately high levels of amphibian richness and endemism across the EAH and most of these centres were located in EA. This is despite the fact that the area of habitat suitable for montane amphibians within EA is tiny compared to that in the more extensive montane habitats in EH or KH. I suggest that high richness and endemism within EA is due to the 'island effect' (Stuart et al. 2012) driving an exceptional level of species turnover. In EA, richness was highest on relatively low mountains with foothills at very low altitudes (down to 250 m), while the other main mountain ranges, minimum altitude did not influence richness values. Driving both species richness and endemism is the isolation between individual mountains, an isolation that does not occur in the extensive and well-connected highlands elsewhere in the hotspot (Fig. 3-6).

Each of the EA blocks has a large altitudinal range (up to 300-2600 m) and covers a relatively small area, resulting in steep environmental/thermal gradients characterised by high habitat heterogeneity (Lovett 1996). Despite such altitudinal ranges, the highest altitudes are always well within physiological tolerable limits for amphibians, meaning that the entire area is suitable for them (Hu et al. 2011, Wells 2007, Duellman and Trueb 1994). In contrast, large portions of land area in the other highlands are towards or beyond the upper tolerable limits (>3000 m), effectively excluding amphibians. Also, EA lacks the connecting plateaux and land bridges present in the AR, EH and KH. Such land connections at medium/high altitude may provide suitable habitat for dispersal between peaks and ranges while allowing forests to reconnect more easily during wetter and warmer periods, resulting in increased dispersal and gene flow across these expanses.

The high species richness and endemism of tropical moist forest in the East Africa highlands has been linked to habitat persistence during glacial/dry periods (Lovett and Wasser 1993, Marchant 2006, Fjeldså et al. 2011, Tolley et al. 2011, Loader et al. 2014). However, persistence/stability alone does not explain the disproportionate richness and endemism across the different mountain ranges in the Eastern Afromontane, unless there are significant differences between them. I suggest that differences in diversity across areas with comparable habitat stability, relate to differences in their degree of insularization, driven by the topographic complexity within individual mountain ranges, especially lowland barriers between peaks. Such differences are exemplified in the isolated steep peaks of the EA versus the vast highlands with scattered peaks of the EH (e.g. Watson 2002). Mountain ranges with high landscape heterogeneity and higher temperature in their foothills act as true sky islands (Warshall, 1995), offering a much greater landscape resistance to gene flow. This isolation, coupled with long-term habitat stability, has led to exceptional levels of amphibian diversity/importance in parts of the hotspot. The positive correlation between topographic complexity and genetic distance was recently reported for Andean frogs (Guarnizo and Cannatella 2013), where potential barriers that restrict gene flow within species are closely related to factors associated with temperature and topography.

The Eastern Arc Mountains of Tanzania harbour, both collectively and at individual block level, a higher diversity than the rest of the Eastern Afromontane. Recent discoveries have suggested that the numerous, isolated granite massifs capped with forests in the interior of Mozambique, north of the Zambezi River, may also act as sky islands for several faunal groups. This includes high levels of species richness and endemism in groups such as butterflies (Congdon et al. 2010), freshwater crabs (Daniels and Bayliss 2012), reptiles (e.g. Branch and Bayliss 2009, Tilbury 2010, Branch et al. 2014), and bats (Taylor et al. 2012) However, recent herpetological surveys in the area (MM and SL) found no strict forest-associated amphibians (*Unpublished data*).

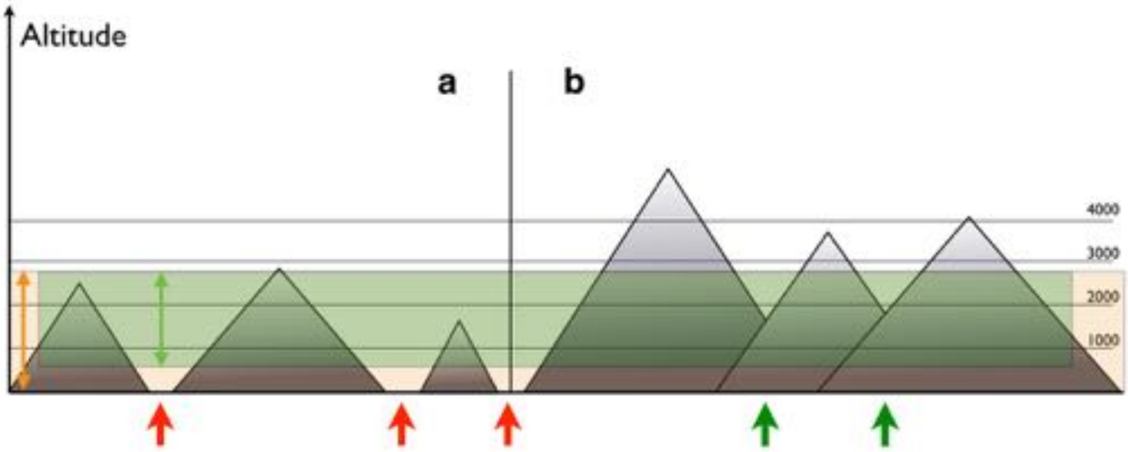


Figure 3-6 Different degree of insularization across the EAH. EA type mountain blocks (A) have a large altitudinal range resulting in steep gradients characterised by high habitat heterogeneity, entirely within physiological tolerable limits for amphibians. Minimum altitudes are much lower than in other regions, resulting in inhospitable expanses between the foothills, exacerbating isolation. Forest fragments in the AR, EH, and KH (B) are often lying on plateaux at high altitude that may be more suitable for dispersal between peaks and mountain ranges during climatic fluctuation than do low-lying areas. Also large areas are toward or beyond the upper tolerable limits for amphibians, effectively eliminating large portions of available habitat. Orange double arrow represents the optimal altitudinal range for amphibians. Red bold arrows indicate intervening areas at low altitude corresponding to hot and dry savanna. Green bold arrows indicate intervening areas on plateaux and high land bridges.

Is the Eastern Afromontane a discrete zoogeographic region?

There is almost complete turnover in amphibian species across the main mountain ranges. Such high β diversity is difficult to reconcile with the notion that this is a discreet biogeographical region as previously proposed from work on plants (White 1978, 1983). Rather, our results concur with those of Linder et al. (2012) who looked across multiple taxonomic groups and showed that EAH is characterized by exceptional species turnover rate. In effect, what makes it distinct from the surrounding regions is the dynamic nature of the faunal assembly processes acting on its isolated mountains, rather than sharing of species. If this pattern is congruent across other montane taxa, the EAH system would either stand out as multiple independent regions, or not stand out as a distinct biogeographical entity, because each montane region would be subsumed into the surrounding regions (Linder et al. 2012). Given this, the recognition of the Eastern Afromontane as a discrete biogeographic unit is difficult, and our analysis can help explain its uncertain definition historically and in previous analyses (Linder et al. 2012, Holt et al. 2013).

Fine scale biogeographical patterns and congruence

The sites identified as having high richness largely correspond to those previously identified as being hotspots of amphibian diversity (Plumptre et al. 2007, Menegon et al. 2008, Rovero et al. 2014), and other taxonomic groups (Plumptre et al., 2007, Davenport et al. 2013, Rovero et al. 2014). Higher taxonomic richness and endemism (at the genus level) generally mirror those of species richness/endemism. Some notable exceptions include Bale Mountains of Ethiopia and Chimanimani Highlands of Zimbabwe, where amphibians show high genus richness, but comparatively low species richness (Gower et al. 2012). High importance at the genus level in these areas has also been noted in other taxonomic groups such as birds (Burgess et al. 2000) and mammals (Yalden et al. 1996). Recent phylogenetic studies of amphibians in EAH further highlight the distinctive lineages found in Ethiopia (Sui Ting et al. 2014, Loader et al. 2014).

There were marked differences in distribution and richness across the different amphibian families, and explanations for these differences deserve further research. Some differences could be a product of niche conservatism, where the retention of niche-related ecological traits within clades leads to occupation of 'traditional' habitats and avoidance of areas with unsuitable conditions (Wiens et al. 2010). This could, in part, explain the distribution and species richness within the families *Ptychadenidae* or *Pipidae* (Supplementary material Appendix 1, Fig 3-9), for which the stream-dominated steep forests of EA could represent a less suitable habitat than the flat, open plateaux of AR, KH and EH. The ancestral areas of the various families could also account for the occurrence of the three mountain-associated genera of brevicipitids in East Africa (Appendix 1, Fig 3-8), having likely originated in East Africa (Loader et al., 2014), with limited possibilities to disperse further west because of the barrier represented by the Rift valley and the vast expanses of dry savanna.

Conservation implications

With so many range-restricted and threatened species, EAH represents the most important area of mainland Africa for conservation of amphibians. Range-restricted species in tropical montane areas have been sometimes described as 'the living dead', deemed to be of low conservation value (Fjeldså et al. 2011). However, some of these species are, in fact, among the most abundant amphibians in a given area and presumably are well adapted to their local environment (Channing et al. 2005;

Menegon et al. 2011). Also, they often represent lineages that have persisted for millions of years in spite of dramatic shifts in global climate (Loader et al. 2014; Siu Ting et al. 2014). This said, the hotspot contains 97 threatened amphibian species, more than any other region in Africa (IUCN, 2015).

As an aid to site prioritisation, I present a ranked list of the sites that fall within the 25 highest scoring cells for amphibian species richness and endemism scores (Table 3-3). ‘Top-ranking’ sites are in the Nguru, East Usambara and Southern Udzungwa Mountains within EA, and in the Nyungwe/Itombwe landscape in AR. Because species turnover is almost complete across mountain ranges and also very high across sites (Menegon et al. 2008, 2011, Loader et al. 2011a, Gower et al. 2013), a small number of protected areas, however well-managed and resourced, will never fully capture the diversity of the region’s amphibians. Thus, a specific strategy aimed at identification and implementation of conservation initiatives at forest fragment scale must be considered. Of the 19 priority amphibian areas identified in the 25 top ranking cells, only a few (Itombwe in D. R. Congo, some of the Livingstone forests fragments in Tanzania, and some fragments in the Western EH) lack any official protection status. However, across the Eastern Afromontane, very few protected areas, besides National Parks, are suitably resourced, resulting in very weak levels of protection (Plumptre et al. 2007, Hall et al. 2009, Rovero et al. 2012, Laurance et al. 2012, Gower et al. 2013). Success in maintaining amphibian diversity is linked not just to protected area status but to well-resourced, managed and monitored protected areas with law enforcement, control of access, resource management, monitoring and evaluation, maintenance of equipment, budget management and existence of annual work plans (Struhsaker et al. 2005, Dudley et al. 2007, Le Saout et al. 2013).

Table 3-3 Twenty-five top ranking sites, according to amphibian species, genus, and family level richness, and endemism at the species and genus level. Sites that are fully protected are shown in green, sites that are partially protected are shown in blue and those without formal protection are shown in red (according to respective country legislation and IUCN guidelines).

	Taxon richness			**Endemism score**	
	Species	***Genus***	***Family***	***Species***	***Genus***
1	Nguru South	Udzungwa Ib/II	Udzungwa Ib/II	S Udzungwa Ib	Itombwe
2	Uluguru North IV	Uluguru North IV	Uluguru North IV	Mahenge IV	E Usambara IV
3	Udzungwa Ib/II	S Udzungwa Ib	Uluguru South IV	Uluguru North IV	Bale Mts. II
4	Udzungwa Ib/II	S Udzungwa Ib	Rubeho/Udzungwa II/IV	Nguru South IV	Ukaguru IV
5	S Udzungwa Ib	Nguru South IV	Udzungwa Ib/II	E Usambara IV	Bale Mts. II
6	E Usambara IV	E Usambara IV	Udzungwa Ib/II	Livingstone Forests	Itombwe
7	E Usambara IV	E Usambara IV	S Udzungwa Ib	East Usambara IV	S Udzungwa Ib
8	Ukaguru/Nguru IV	Uluguru South IV	Nguru South	Uluguru South IV	Ethiopia W Forest
9	Uluguru South IV	Usambara IV	E Usambara IV	Rungwe/Poroto II	Itombwe
10	Nguru IV/Nguu IV	Rubeho/Udzungwa II/IV	E Usambara IV	Ukaguru IV	Udzungwa II
11	West Usambara IV	Udzungwa Ib/II	Usambara IV	Nyungwe IV	Addis Ababa
12	Ukaguru IV	W Usambara IV	W Usambara IV	Itombwe	Uluguru North IV
13	Virunga/Nyungwe II/IV	Uluguru IV	Uluguru IV	Nguru IV/Nguu IV	S Udzungwa Ib
14	Usambara IV	S Udzungwa Ib	Udzungwa Ib/II	Ukaguru/Rubeho IV	Uluguru S IV
15	Uluguru IV	Ukaguru/Nguru IV	Udzungwa/Uluguru II/IV	Kahuzi-Biega II	Virunga/Nyungwe II/IV
16	Rubeho/Udzungwa II/IV	S Udzungwa Ib	Udzungwa Ib/II	Udzungwa II	Mahenge IV
17	Ukaguru/Rubeho IV	Udzungwa/Uluguru II/IV	Itombwe	South Pare IV	E Usambara IV
18	Kahuzi-Biega II	Itombwe	Udzungwa/Mahenge II	Bale Mts. II	Addis Ababa
19	Nyungwe IV	Nguru IV/Nguu IV	Mahenge IV	Rubeho IV	Usambara IV
20	Udzungwa II	Udzungwa/Mahenge II	Nyungwe/Itombwe II	Nyungwe/Itombwe II	Bale Mts. II
21	Nyungwe/Itombwe II	Mahenge IV	Ruwenzori II	Ukaguru/Nguru IV	Nguru IV/Nguu IV
22	Udzungwa II	Itombwe	Itombwe	West Usambara IV	Nguu Mts. IV
23	Udzungwa/Uluguru II/IV	Ukaguru IV	Kahuzi-Biega II	Bale Mts. II	Nguru South IV
24	Udzungwa II	Virunga/Nyungwe II/IV	Nyungwe IV	Nguu Mts. IV	West Usambara IV
25	Rubeho IV	Ukaguru/Rubeho IV	Bwindi/Virunga II	Virunga II	Livingstone Forests

Appendix 1

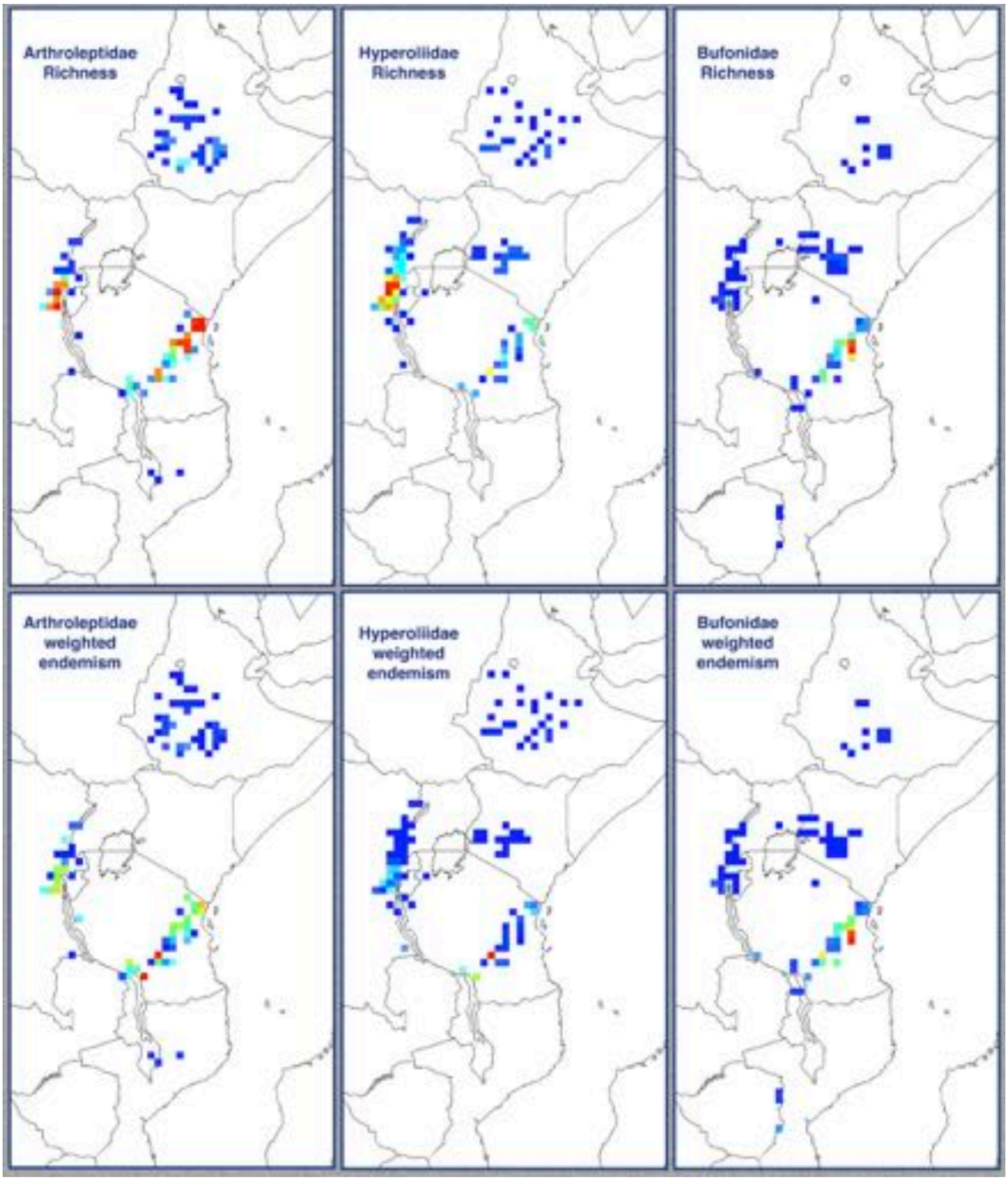


Figure 3-7 Map showing cells with recorded occurrence and relative species richness for 9 relevant families across the Eastern Afromontane. The scale ranges from red (high species richness) to dark blue (low species richness).

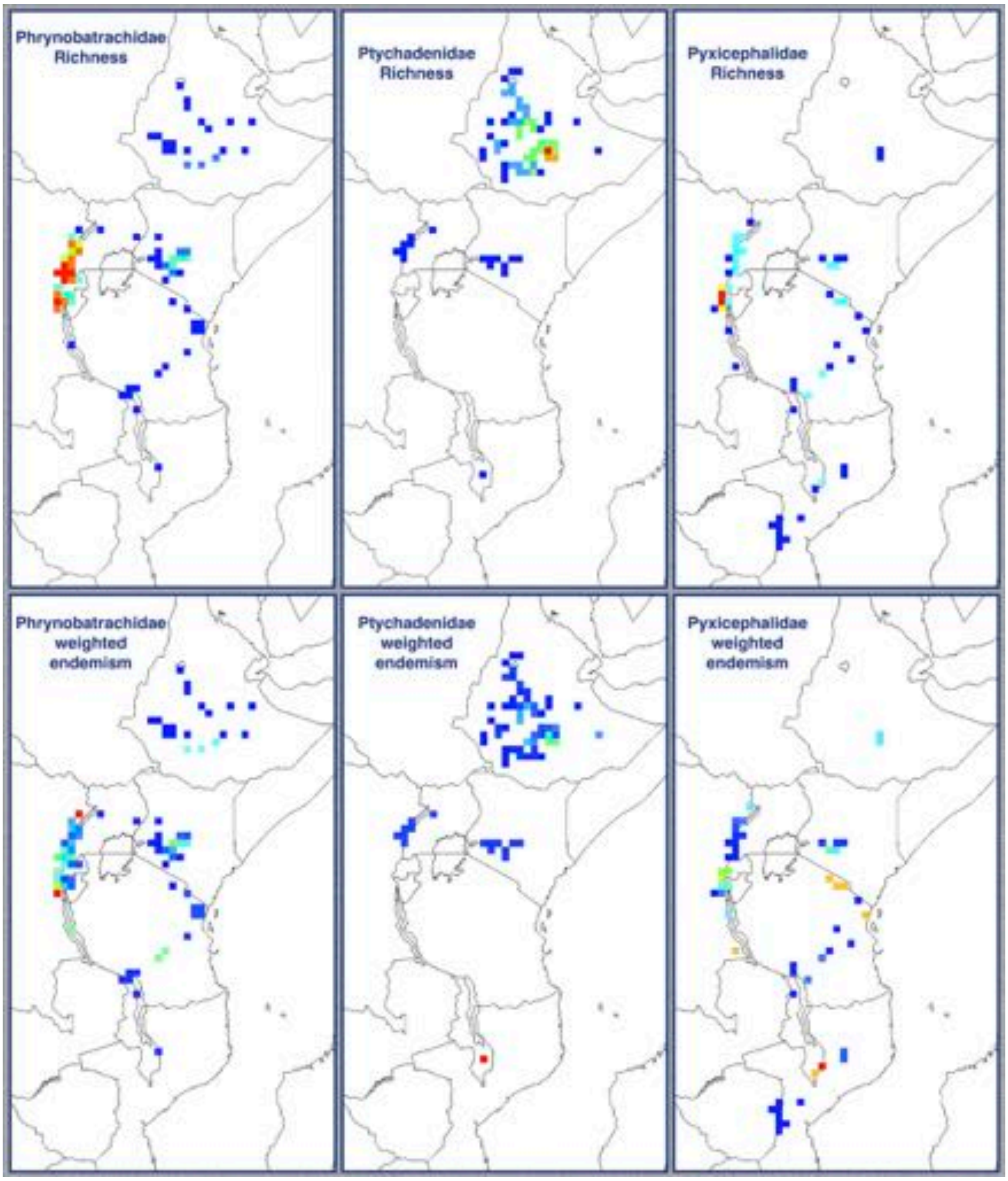


Figure 3-8 Map showing cells with recorded occurrence and relative species richness for 9 relevant families across the Eastern Afromontane. The scale ranges from red (high species richness) to dark blue (low species richness)

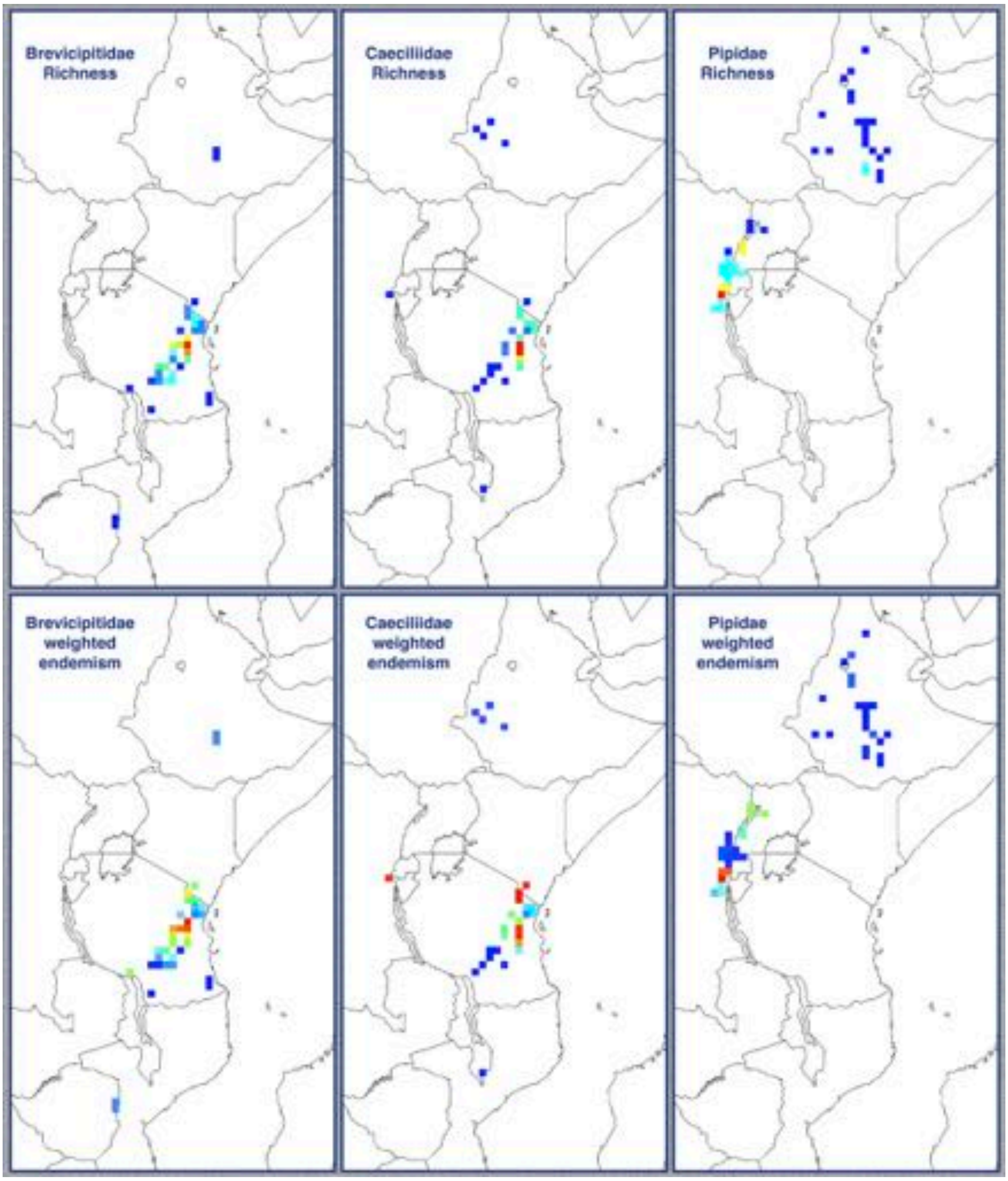


Figure 3-9 Map showing cells with recorded occurrence and relative species richness for 9 relevant families across the Eastern Afromontane. The scale ranges from red (high species richness) to dark blue (low species richness).

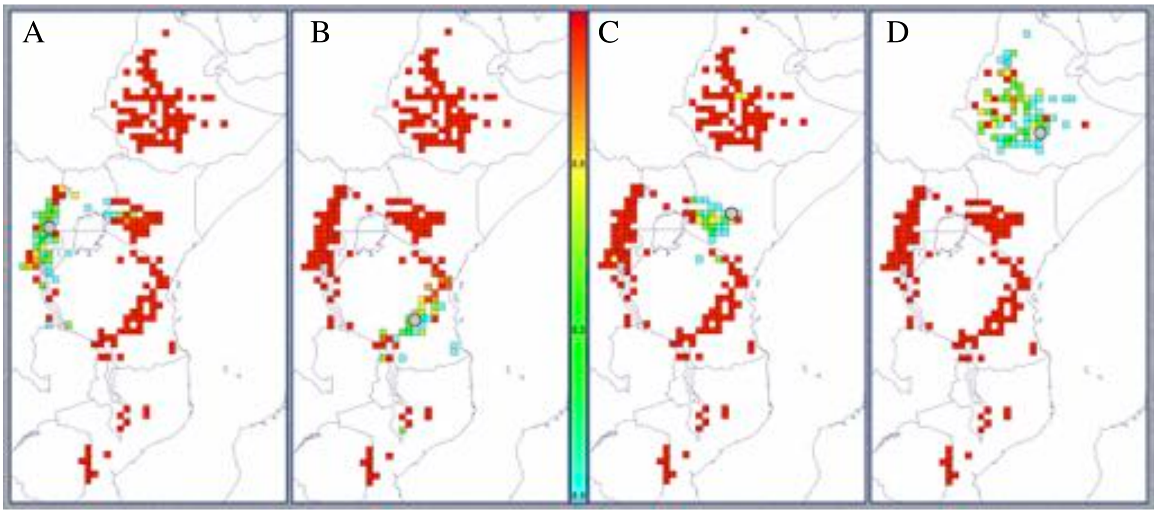


Figure 3-10 Four examples of species turnover patterns across cells. Turnover was measured using the S2 dissimilarity metric. Index cells are randomly placed and denoted as grey circles. The scale ranges from 0 (complete similarity) to 1 (complete dissimilarity) A: the index cell placed in the Albertine Rift; B: index cell in the Eastern Arc Mountains; C: index cell in the Kenyan Highlands; D: index cell the Ethiopian Highlands.

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Chapter 4 : Origin of montane Herpetofauna

The genus *Atheris* (Serpentes: Viperidae) in East Africa: Phylogeny and the role of rifting and climate in shaping the current pattern of species diversity

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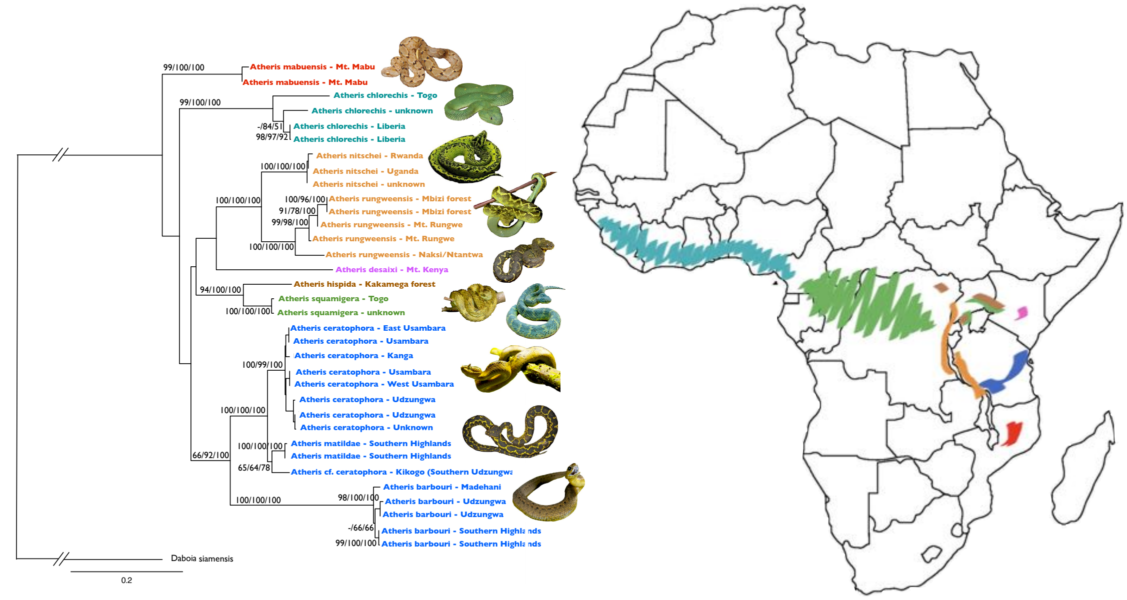

Figure 4-1 The phylogenetic tree of *Atheris* with mapped distribution color coded by clade

Abstract

Past climatic and tectonic events are believed to have strongly influenced species diversity in the Eastern Afromontane Biodiversity Hotspot. I've investigated the phylogenetic relationships and historical biogeography of the East African genus *Atheris* (Serpentes: Viperidae), and explored temporal and spatial relationships between *Atheris* species across Africa, and the impact of palaeoclimatic fluctuations and tectonic movements on cladogenesis of the genus. Using mitochondrial sequence data, the phylogeny of East African species of *Atheris* shows congruent temporal patterns that link diversification to major tectonic and aridification events within East Africa over the last 15 million years (my). Our results are consistent with a scenario of a delayed direct West-East colonisation of the Eastern Arc Mountains of *Atheris* by the formation of the western rift. Based on the phylogenetic patterns, this terrestrial, forest-associated genus has dispersed into East Africa across a divided route, on both west-southeasterly and west-northeasterly directions (a C-shaped route). Cladogenesis in the Eastern Arc Mountains and Southern Highlands of Tanzania corresponds to late Miocene and Plio-Pleistocene climatic shifts. Taxonomically, our data confirmed the monophyly of *Atheris* as currently defined, and reveal four major East African clades, three of which occur in discrete mountain ranges. Possible cryptic taxa are identified in the *Atheris rungweensis* and *A. ceratophora* clades.

Introduction

The contemporary pattern of species diversity and distribution in African forests is thought to have been influenced by a combination of past climatic events, tectonic movements, geological activities and stochastic dispersal events (see Moritz 2000 for a review of speciation models and Kingdon, 2013 for speciation in Africa. The influence of these geographic events are expected to leave 'signatures' in the patterns of diversification in extant lineages of animals and plants. Recently, there have been several studies looking at contemporary species to reveal patterns of taxon diversification to reveal temporal patterns of forest dynamics in Africa (Bowie et al. 2006; Lawson 2010, 2013; Blackburn and Measey 2009; Loader et al. 2011). These studies have begun to clarify the importance of specific historical events in shaping Africa's fauna and flora.

The montane forests of East Africa, collectively called the Eastern Afromontane, are relicts of a past, more pan-African forest, which became fragmented during periods of climatic aridification and subsequent climatic fluctuations starting from the early Oligocene (Lovett and Wasser, 1993). The forested surface is thought to have been highly dynamic through the Pliocene and Pleistocene, with forests restricted to montane areas during warm dry periods, but expanding to lower elevations during cool, wet periods (Lovett et al. 2008). Underlying these changes in forest distribution are the geological changes that have occurred in the areas. Some regions are volcanic (old or more recent events), while others are much older and geologically stable (Griffiths, 1993) and still others (such as the Southern Highlands, are a combination of the two). Consequently, the region represents an interesting system for understanding the effects of abiotic (climate and geology) processes on forest-dependent biodiversity.

The viperid snake genus *Atheris* is an appropriate group for studying the biogeography of the pan-African forest and the role of plate tectonics and mountains in shaping the current pattern of species diversity and distribution. The genus is distributed across most of tropical Africa, with a number of endemic species in montane regions (e.g. Branch et al, 2009; Menegon et al. 2011). Of the 15 recognised species, only one (*Atheris squamigera* Hallowell, 1854) has a particularly wide distribution ranging from Nigeria in the west, across the Congolian forest belt to western Kenya in the east and Angola in the south (Phelps, 2010). All other *Atheris* species have small known ranges. For example, *A. desaixi* is found in just two localities in the Kenyan highlands, *A. mabuensis* in two mountain blocks in Mozambique, *A. acuminata* is known for a single locality in Uganda and the newly described *A. matildae* in a single forest fragment in the Southern Highlands of Tanzania (Ashe 1968, Branch et al., 2009; Menegon et al. 2011). Current *Atheris* distribution includes areas that have been suggested to be forest refugia during the last glacial maxima. These refugia include forests from west of the Dahomey gap, across the Guineo-Congolian rainforest to the Eastern Afromontane in the east, and southward into central Mozambique. In the west, *Atheris* species generally occur at low or medium altitude (Chirio and LeBreton, 2007; Phelps, 2010; Penner et al. 2013) while in the east, there are ten species that are restricted to specific mountainous regions or isolated mountain blocks in the Eastern Afromontane Ecosystem (Menegon et al., 2011).

Using a reconstructed phylogeny, I aim to address a number of hypotheses concerning the taxonomy and biogeography of *Atheris*. First, I assess the taxonomic status of species using genetic samples from species across the genus' range. Second, I use molecular clock estimates to investigate whether the timing of the divergence within this clade corresponds to specific geographic events. As a complement to the time-tree, I use ancestral reconstructions of areas to examine spatial relationships within the genus and how they are related to timing and origin of clades. I hypothesize that the forest viper genus *Atheris*, distributed in both west and east African forests, will show a west-east discontinuity. This pattern would link to the initial fragmentation of the pan-African forest, and subsequent cladogenetic events that correspond to the major geological or climatic event changes in each region.

Materials and Method

Sampling and DNA extraction

Samples of *Atheris* species were collected in the field and from preserved material in several museums (Table 1). Genomic DNA was extracted from 29 tissue samples using QIAamp DNA Mini Kit (Qiagen, Hombrechtikon, Switzerland) following the manufacturer's protocol. Additionally, all cytochrome b (*cytb*) and NADH dehydrogenase sub-unit 4 (*ND4*) sequences of *Atheris* species available on GenBank were included in the dataset (Table 1).

Table 4-1 Details of the samples analysed, with locality and GenBank accession numbers.

Taxon	Voucher	Location	Country	cytb	ND4
Atheris barbouri	MTSN 9177	Bomalang'ombe	Tanzania	x	x
Atheris barbouri	MTSN 7299	Madehani FR	Tanzania	x	x
Atheris barbouri	MTSN 7311	New Dabaga FR	Tanzania	x	x
Atheris barbouri	MTSN 7399	Livingstone Mountains	Tanzania	x	x
Atheris barbouri	MTSN 7400	Livingstone Mountains	Tanzania	x	x
Atheris ceratophora	KMH 35468	Kanga FR	Tanzania	x	x
Atheris ceratophora	AJ275682	Usambara Mts.	Tanzania	AJ275682	-
Atheris ceratophora	CAS 168976	West Usambara Mts.	Tanzania	x	x
Atheris ceratophora	MNHG 2667.45	East Usambara Mts.	Tanzania	JF825388	-
Atheris cf. ceratophora	MTSN 8874	Kigogo FR	Tanzania	x	x
Atheris ceratophora	MTSN 5032	Udzungwa Mts.	Tanzania	x	x
Atheris ceratophora	MTSN 5033	Udzungwa Mts.	Tanzania	x	x
Atheris ceratophora	MTSN 7558	Udzungwa Mts.	Tanzania	x	x
Atheris ceratophora	MNHG 2667.45	Usambara Mts.	Tanzania	x	x
Atheris ceratophora	MTSN 8947	Udzungwa Mts.	Tanzania	x	x
Atheris ceratophora	DQ305456	unknown	unknown	DQ305456	DQ305474
Atheris chlorechis	isolate 1579	unknown	unknown	-	EU624211
Atheris chlorechis	AT819	Nimba County	Liberia	x	x
Atheris chlorechis	AT820	Nimba County	Liberia	x	x
Atheris chlorechis	AJ275679		Togo	AJ275679	-
Atheris desaixi	AJ275680	Mt. Kenya	Kenya	AJ275680	-
Atheris hispida	AJ275681	Kakamega Forest	Kenya	AJ275681	-
Atheris mabuensis	AT821	Mount Mabu	Mozambique	-	x
Atheris mabuensis	AT822	Mount Mabu	Mozambique	x	x
Atheris matildae	MTSN 7570	Southern Highlands	Tanzania	-	x
Atheris matildae	MTSN 9344	Southern Highlands	Tanzania	x	x
Atheris nitschei	T1032	Nyungwe Forest NP	Rwanda	x	x
Atheris nitschei	AJ275683		Rwanda	AJ275683	-
Atheris nitschei	CAS 201708	Kabale District	Uganda	AF471070	-
Atheris rungweensis	MB121	Mount Rungwe	Tanzania	x	x

Atheris rungweensis	MTSN 7569	Mbizi FR	Tanzania	-	x
Atheris rungweensis	MTSN 7312	Mount Rungwe	Tanzania	x	x
Atheris rungweensis	AY223557		Tanzania	AY223557	AY223618
Atheris rungweensis	SHCP 03-R-24	Mt. Rungwe	Tanzania	x	x
Atheris rungweensis	SHCP 07-R-02	Nkasi/Ntantwa	Tanzania	x	x
Atheris rungweensis	SHCP-07-R-02	Nkasi/Ntantwa	Tanzania	x	x
Atheris squamigera	AJ275684	unknown	Togo	AJ275684	-
Atheris squamigera	EU624303	unknown	Tanzania	EU624303	EU624212
Daboia russelii				NC011391	
Sphenodon punctatus				NC 004815	
Sceloporus occidentalis				NC 005960	
Pogona vitticeps				NC 006922	
Varanus salvator				NC 010974	
Lacerta viridis				NC 008328	
Acrochordus granulatus				NC 007400	
Boa constictor				NC 007398	
Python molurus				NC 015812	
Ramphotyphlops braminus				NC 010196	
Typhlops reticulatus				NC 010971	
Naja naja				NC 010225	
Micrurus fulvius				NC 013481	
Dinodon semicarinatus				NC 001945	
Hypsiglena torquata				NC 013992	
Enydris plumbea				NC 010200	
Agkistrodon piscivorus				NC 009768	
Atropoides olmec				*AY223585*	*AY223632*
Bothrops asper				*EU624301*	*EU624210*
Crotalus horridus				NC 014400	
Crotalus ravus				*AY223609*	*AY223647*
Gloydius brevicaudus				NC 011390	
Hypnale hypnale				*AY352750*	*AY352812*
Lachesis muta				*AY223604*	*AY223644*
Ovophis okinavensis				NC 007397	
Porthidium arcosae				*AF292575*	*AF292613*
Porthidium lansbergii				*AY713375*	*AF393623*
Porthidium nasutum			*Costa-Rica*	*AY223579*	*U41887*
Porthidium ophryomegas				*AY223580*	*U41888*
Porthidium nasutum			*Ecuador*	*AF292574*	*AF292612*
Sistrurus catenatus				*AY223610*	*AY223648*
Sistrurus miliarius				*AY223611*	*U41889*

Viridovipera stejnegeri				NC 012146	
Bitis arietans				*EU624304*	*EU624213*
Bitis nasicornis				*DQ305457*	*DQ305475*
Bitis peringueyi				*DQ305458*	*DQ305476*
Bitis worthingtoni				*AJ275692*	*EU624220*
Bitis xeropaga				*EU624307*	*EU624221*
Causus defilippi				NC 013479	
Cerastes cerastes				*EU624308*	*EU624222*
Daboia mauritanica				*EU624313*	*EU624229*
Echis ocellatus				*EU624311*	*EU624225*
Eristicophis macmahonii				*AJ275711*	*EU624227*
Macrovipera albizona				*AJ275727*	*EU624231*
Macrovipera lebetina			*Turkey*	x	x
Protoatheris superciliaris				*AJ275685*	*EU624230*
Vipera ammodytes			*Serbia and Montenegro*	x	x
Vipera aspis			*France*	x	x
Vipera berus			*Switzerland*	x	x
Vipera latastei			*Spain*	x	x
Vipera ursinii			*France*	x	x
Vipera monticola			*Morocco*	x	x

Genetic analyses

Fragments of the *cytb* were amplified by Polymerase Chain Reaction (PCR) using the primers L14724Vb and H115914Vb (Ursenbacher et al., 2006). For some problematic samples the amplification of the *cytb* was conducted in two parts, using internal primers (first set: L14724Vb and H15548Vb; second set: L15162Vb and H15914Vb) Additionally, a portion of the ND4 was amplified using the primers *ND4* and LEU described by (Arevalo et al., 1994). All amplifications were conducted in 30μl volumes with 2-4μl of DNA template (depending on the DNA concentration), 1xPCR buffer (Qiagen), 2mg/ml of Q solution (Qiagen), 2mM for the *cytb* and 3mM for the ND4 of $MgCl_2$, 0.2mM dNTPs, 0.5μM of each primer and 0.5 units of Taq polymerase (Qiagen). Amplification conditions consisted of 37-45 cycles of denaturation for 45 sec at 94°C, annealing for 60 sec at 50°C and extension for 90 sec at 72°C conducted with a Eppendorf MasterCycler (Eppendorf, Schönenbuch/Basel, Switzerland) PCR products and sequencing were outsourced to Macrogen Inc. (Seoul, Korea) Sequences were deposited in GenBank (see Table 4-1).

Phylogenetic reconstruction

The sequences were checked and edited using CODONCODE ALIGNER v4.02 (CodonCode Corporation, Dedham, US). The mtDNA sequence of *Daboia siamensis* from GenBank (NC_011391) was included in the dataset as an outgroup. For both genes, separate alignments were conducted using CLUSTALX v2.0 (Thompson et al., 1997). We conducted a partition homogeneity test with PAUP* v4.10 (Swofford, 2002) using 1,000 replicates in order to determine whether both regions could be combined in a unique data matrix (Farris et al., 1995). For all *Atheris* haplotypes, the numbers of base differences per site were calculated from the net average between groups of sequences. Analyses were conducted in MEGA v5.1 (Tamura et al., 2011). The model of sequence evolution was selected with the software JMODELTEST v2.1.1 (Darriba et al. 2012). Maximum likelihood (ML) estimates of the phylogeny were calculated using PHYML v3.0 (Guindon and Gascuel, 2003) using the previously selected model. Maximum parsimony (MP) analyses were performed using PAUP* with heuristic searches and random stepwise addition, and the robustness of MP reconstructions were tested with bootstrap resampling (1,000 repetitions). Additionally, Bayesian inference analysis was performed in MRBAYES v3.2 (Huelsenbeck and Ronquist, 2001) using the GTR+I+G substitution model with four chains of $1x10^6$ generations, and sampling performed every 100 generations. After controlling the log values with TRACER 1.5 (Rambaut and Drummond, 2003), the first 10% was discarded as burn-in and the analysis was conducted twice and compared to avoid local optima (Huelsenbeck and Imennov, 2002).

Divergence time estimation

To estimate the divergence time between each clade without any molecular clock constraint, a Bayesian approach with a MCMC integration was used to date the most recent common ancestor (MRCA) using BEAST 1.7.4 (Drummond et al. 2002; Drummond and Rambaut 2003). The analysis was run for 20 millions generations, with sampling each 1,000 generations and a 10% burn-in. A Yule branching process with a log-normal relaxed clock for estimating posterior probability density of divergence time was implemented to perform the simulations. Due to the lack of calibration points within the *Atheris* genus, GenBank sequences of *Sphenodon punctatus*, four saurians, five primitive snakes, two Elapidae, three Colubridae, 17 Crotalinae and 19 Viperinae were added to this analysis (See Table 1 for details). The complete alignment regrouping the 81 specimens was conducted using CLUSTALX for each gene separately, and finally combined. The calibration points were the following: (1) fossil evidence suggests that the divergence between saurians and snakes occurred 130-150 million years ago (mya; Carroll, 1988). I've used a normal prior with a mean set at 140 mya and a standard deviation of 6 mya (95% CI: 130-150 my); (2) the oldest *Viperidae* is known from western Europe in the lowermost Miocene (23.8 mya; Szyndlar and Rage, 1999). A log-normal prior with a zero offset of 23.8 my, with a log-normal mean of 2 and standard deviation of 1 (95% CI: 25-62 my) was set up; (3). The oldest *Crotalinae* fossil (the sister taxa of the *Viperinae*) was present in sediments of the early Miocene (17.8 mya) (Holman, 1981) a log-normal prior was consequently used, with a zero offset of 17.8 my, a log-normal mean of 2 (SD=1; 95% CI: 19-56 my); (4) referring to the first fossil vertebra of *Sistrurus* found (Parmley and Holman, 2007), the divergence between the genus *Crotalus* and *Sistrurus* can be estimated to be older than 9 mya. I've implemented a log-normal prior with a zero offset of 9 my, a lognormal mean of 1 (SD = 1; 95%CI: 9.5-23.1 my); (5) The first fossil observed of the 'Vipera berus' group was dated to the Late Miocene (MN12, 7.5-6.8 mya; Szyndlar and Rage, 2002). Thus, I've implemented a lognormal prior with a zero offset of 7 my, a lognormal mean of 1 (SD=1;95% CI: 7.5-21.1my); (6) I've considered that the split between *V. latastei* from Spain and *V. monticola*, inhabiting the Atlas Mountains (Morocco) and formerly recognized as a subspecies of *V. latastei* (Beerli et al. 1996; Saint Girons, 1953), predate the Messinian salinity crisis about 5.3-5.6 mya, (Krijgsman et al., 1999) as demonstrated by Velon-Antón et al. (2012). I've applied a log-normal prior with a zero offset of 5.4 my, a lognormal mean of 1 (SD=1;95% CI: 5.9-19.5 my); (7) finally, as the most recent calibration point, I've used the invasion of South America by the snake genus *Porthidium* after the uplift of the Isthmus of Panamá about 3.5 mya (Wüster et al., 2002). I've consequently modelled the divergence between Central American and Southern American *Porthidium* species with a normal distribution with a mean of 3.5 my (SD=0.61; 95% CI: 2.5-4.5 mya) All the time intervals are according to the cited publications.

Inference of Ancestral Area

Multiple ancestral area analyses were carried out to infer the geographic origins of the genus *Atheris*, and to establish main events and direction of species radiation. Dispersal-extinction-cladogenesis (DEC) analysis and Bayesian binary MCMC

(BBM) in RASP 2.1 (Ronquist, 1997; Yu et al., 2013) using the ultrametric tree generated in BEAST. The analyses were run under the JC69 model for 2,000,000 generations, with ten chains, a sampling frequency every 100 generations and a 10% burn-in.

Results

Phylogeny and Dating

Our phylogeny reconstructs evolutionary relationships among *Atheris* representing ten of the 15 known species. The haplotype analysis involved 22 unique nucleotide sequences. The analysis of 1084 bp of the *cytb* revealed 362 (33.4%) variable sites of which 261 (24.1%) were phylogenetically informative under MP criteria. For *ND4*, 248 (38.0%) sites were variable, of which 180 (25.6%) were phylogenetically informative.

The dataset of both genes were combined into one dataset for the phylogenetic analyses (partition homogeneity test; p = 0.077). The best model of substitution selected by JMODELTEST was TVM+I+G (freq. A = 0.318; freq. C = 0.297; freq. G = 0.094, freq. T = 0.291; R(a)=1.097; R(b) = R(e) = 18.31; R(c) = 2.346; R(d) = 0.983; proportion of invariable sites = 0.550; gamma distribution shape parameter = 2.452). The heuristic parsimony analysis provided eight equally parsimonious trees of 1,133 steps (Consistency Index = 0.646; R Index = 0.813) ML, MP and Bayesian analyses produced generally similar topologies (Fig. 4-2). Marked genetic divergences were observed between all species. All representatives of Atheris species in the analysis were shown to be monophyletic with strong support and the analysis recovered five well-supported subclades within the main *Atheris* phylogeny (Fig. 4-2).

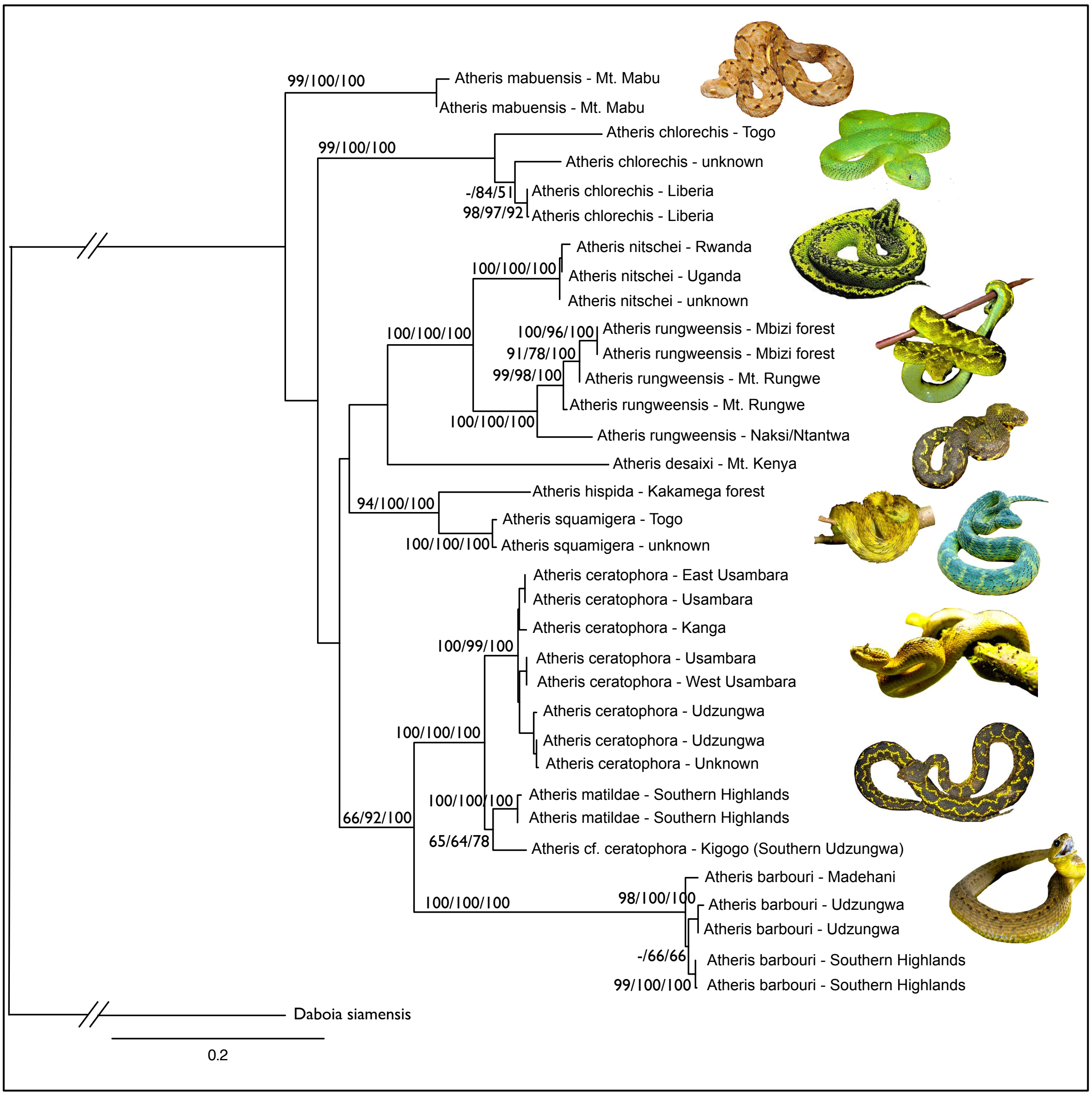

Figure 4-2 Maximum Likelihood tree with ML, MP bootstrap supports and Bayesian posterior probabilities (only when >50%).

Differences between analyses were noted in the position of A. *mabuensis* from Mozambique, which is in a basal position and the sister to the rest of the genus in the ML analysis, whereas A. *chlorechis* is basal in the MP and BEAST analyses (MRBAYES analysis did not support one or the other relationships) (Fig. 4-3). The West African A. *chlorechis* is sister to the larger clade representing the East African radiation of *Atheris*, within which three main clades are evident. Within this east African clade, a well-supported clade includes the Albertine Rift species A. *rungweensis* and A. *nitschei*, and the Kenyan endemic A. *desaixi*. A. *squamigera* and A. *hispida* are sister taxa, A. *barbouri* is sister to the clade that includes A. *matildae* and A. *ceratophora*.

Uncorrected p-distances were estimated pairwise for all the species and these ranged from 3.2% (between *A. ceratophora* and *A. matildae*) to 14.8% (between *A. desaixi* and *A. barbouri*; Table 3).

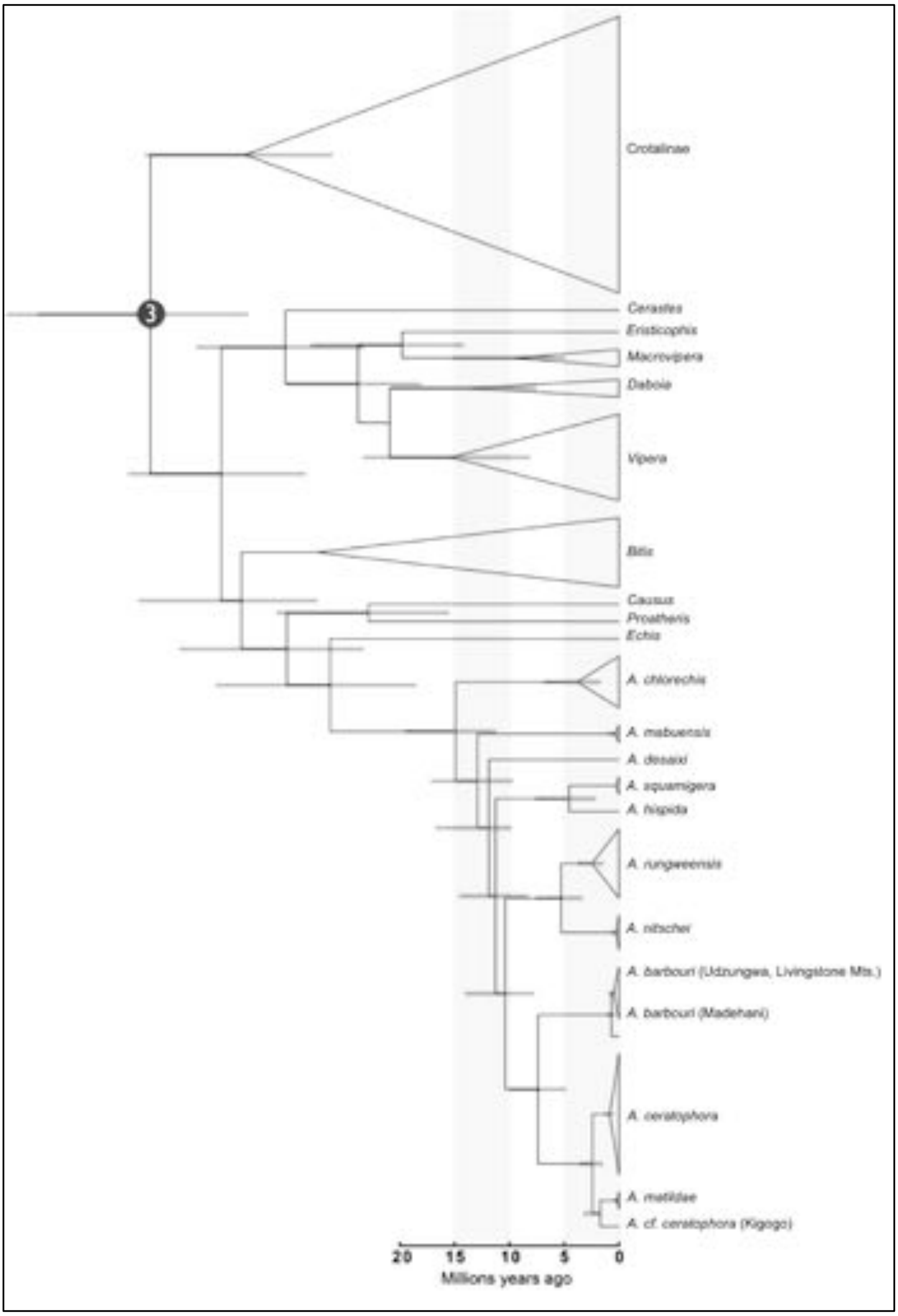

Figure 4-3 Beast (Drummond and Rambaut 2003) maximum credibility ultrametric tree showing the timing of the evolution of the *Atheris* species and other *Viperidae*. Horizontal grey bars indicate 95% confidence intervals. Number 3 refers to the 3rd calibration point in the text. The other used calibration points fall within the collapsed clades of *Crotalinae* and *Vipera* and are not displayed in the figure.

Ancestral areas and current distribution

The most recent common ancestor (MRCA) for the *Atheris* genus was estimated to occur approximately 15 mya (95% CI: 11.2-19.6 mya; Table 4-2, Fig. 4-4) The Bayesian binary MCMC (BBM) analyses and dispersal-extinction-cladogenesis (DEC) results concerning the ancestral state of the genus are equivocal (Table 2) with low likelihood values both for a western or an eastern origin. *Atheris chlorechis* in the West, *A. desaixi* in Kenya and *A. mabuensis* in Mozambique, represent old lineages at the limits of a Miocenic early radiation, which have reached the current area of generic distribution between 15 and 11 mya (nodes A, B and C). Most of the subsequent lineages appear to have originated in the mountains of East Africa, with two major radiations from a common ancestor that inhabited the Albertine Rift around 11 mya. About 7.5 mya, the common ancestor of *A. barbouri*, *A. matildae* and *A. ceratophora* complex occurred in the Southern Highlands of Tanzania. Both clades have their ancestral area in the Southern Highlands and they gave origin to two highly morphologically diverging snake species. The ancestral area of the *A. ceratophora*/*matildae* complex, including the still unassigned lineage sister to *A. matildae*, is the Southern Highlands of Tanzania, where *A. matildae* occurs. The unassigned lineage, herein called *A.* cf. *ceratophora*, occurs in the forest fragments of the southern plateau of the Udzungwa Mountains. Interestingly, the MRCA within A. ceratophora sensu stricto seems to have originated in the Usambara Mountains, suggesting a pattern of multiple colonisations/extinctions of the Eastern Arc Mountains.

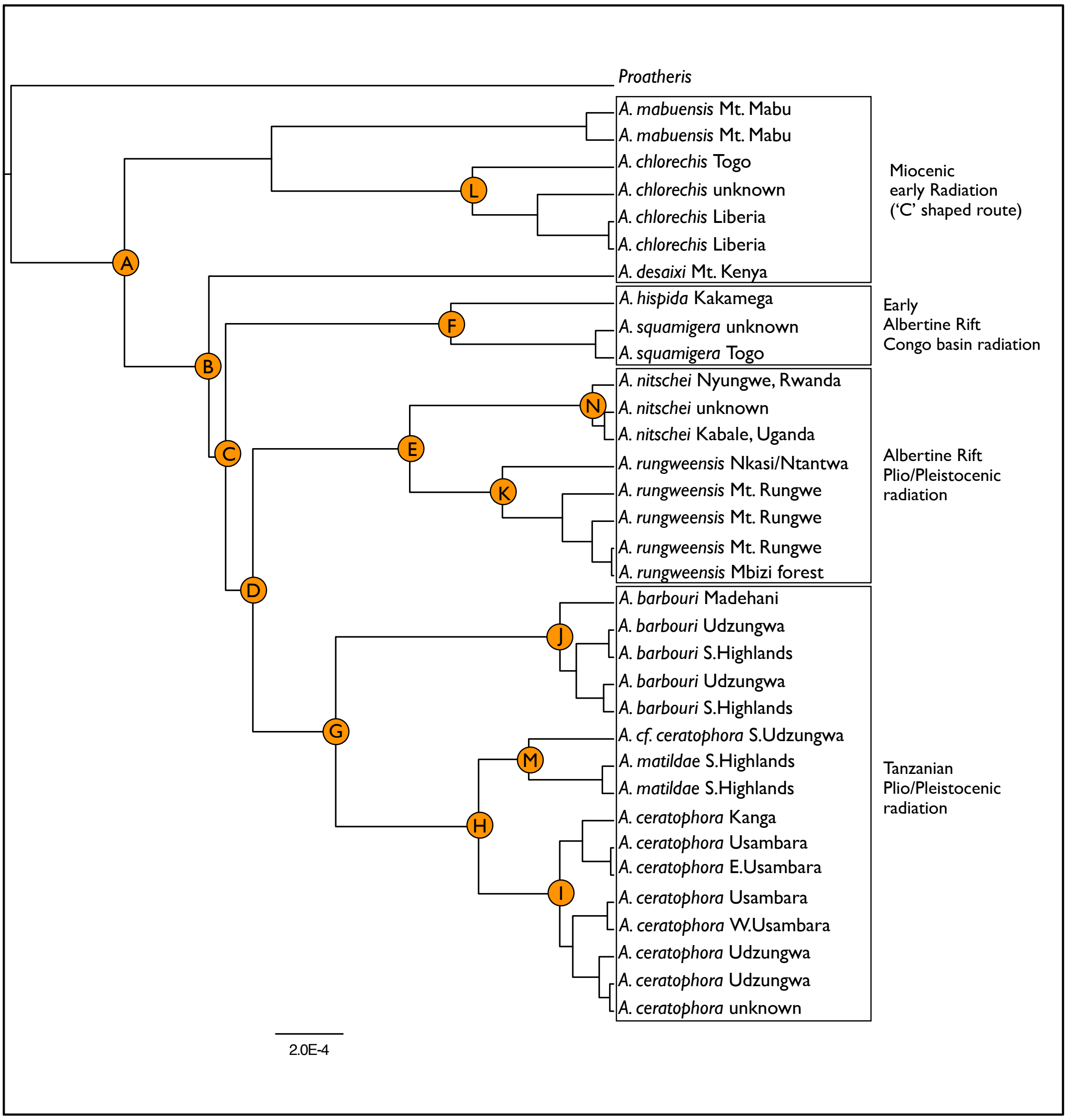

Figure 4-4 Likelihood optimization probabilities for the ancestral areas represented by letters at each node in the *Atheris* ultrametric tree. The legend for the nodes is in Table 4-2. Terminal taxa are grouped according to the time and area of radiation.

Table 4-2 Estimated date and 95% confidence interval (CI) of main nodes (MRCA = Most recent common ancestor) with associated inferred Ancestral Area from the Bayesian binary MCMC (BBM) analysis. The node labels correspond to those in Fig. 3.

Node	Event	Estimated age in mya (95% CI)	Inferred Ancestral Area (ML value)
A	MRCA within the genus	15.1 (11.2-19.6)	West Africa (0.3) Mount Kenya (0.2)
B	Split between *A. desaixi* and the rest of the genus (without *A.chlorechis* and *A. mabuensis)*	12.6 (9.1-16.8)	Mount Kenya (0.53)
C	Split between *A. squamigera* + *A. hispida* group and the rest of East Africa species	11.4 (8.3-14.6)	Albertine Rift (0.73)
D	Split between the Albertine Rift species and the Eastern Arc species	10.8 (7.7-14.1)	Albertine Rift (0.65)
E	Split between *A. rungweensis* and *A. nitschei*	5.4 (3.3-7.6)	Albertine Rift (0.63)
F	Split between *A. squamigera* and *A. hispida*	4.8 (2.1-7.7)	Albertine Rift (0.89)
G	Split between *A. barbouri* and *A. ceratophora* / *A. matildae* group	7.4 (4.8-10.1)	Southern Highlands (0.94)
H	Split between *A. matildae* and *A. ceratophora*	2.5 (1.5-3.6)	Southern Highlands (0.9)
I	MRCA within *A. ceratophora*	0.9 (0.5-1.3)	East Usambara (0.45) Southern Highlands (0.35)
J	MRCA within *A. barbouri*	0.7 (0.4-1.1)	
K	MRCA within *A. rungweensis*	2.5 (1.4-3.8)	
L	MRCA within *A. chlorechis*	4.1 (1.7-6.9)	
M	MRCA within *A. matildae*	0.2 (0.0-0.5)	
N	MRCA within *A. nitschei*	0.3 (0.0-0.7)	

Discussion

Molecular phylogeny and taxonomic implications

In previous molecular phylogenetic analyses of *Viperidae* the number of nominate *Atheris* species sampled was particularly limited with only a single representative per species (Lenk, 2001 Wüster et al. 2008). Our phylogenetic reconstruction includes nine out of the ten known *Atheris* species occurring in East Africa, (for the single known specimen of *A. acuminata* DNA samples was not available). In many cases, several specimens from different localities for each species were sequenced, allowing a more thorough estimation of phylogenetic and geographic structure within these taxa. Our phylogenetic results extract five well supported main clades of *Atheris* species, consistent with geographic distribution (Figure 4-2): (1) *Atheris mabuensis*, (2) a clade representing the Albertine Rift/Mt. Kenya radiation including *A. nitschei*, *A. rungweensis* (though *A. desaixi* position is not strongly supported) (3) The Albertine Rift/lowland forest clade including *A. hispida* and *A. squamigera* (4) *A. chlorechis* clade, and (5) a Tanzanian mountain radiation, including *A. barbouri*, *A. ceratophora* and *A. matildae*. The sister pairing of *A. squamigera* and *A. hispida* is consistent with previous phylogenetic reconstructions (Lenk 2001; Wüster et al. 2008) and with morphological and immunological studies (Groombridge, 1987; Hermann and Joger, 1997). The recently discovered and geographically isolated A. mabuensis forms a clade separated from the rest of the East African species.

The analysis recovered two distinct clades among *Atheris rungweensis*, with each clade representing distinct geographic locations. The first clade includes samples from Southern montane forest localities, e.g. Nkuka forest on Mount Rungwe, and Mbizi forest on the Ufipa Plateau, and the second comprises a single sample from Central areas, Nkasi/Ntantwa, a more open area, at lower altitude with a different type of forest, close to Lake Rukwa, with scattered small forest fragments. The two clades have a p-distance 3.4% and deserve closer morphological analysis to see if they should be recognized taxonomically. The type locality for *Atheris rungweensis* corresponds to the areas sampled on Mount Rungwe, and therefore the Nkasi/Ntantwa would likely represent a new species if this population is shown to be distinct. Populations of *Atheris rungweensis* are also known both from Mahale and Gombe Stream National Parks, along the eastern shore of Lake Tanganyika (Menegon et al., 2011) but no sample from this region was successfully amplified.

Our phylogenetic analyses show that the Eastern Arc endemic *Atheris ceratophora*, displayed genetic differences across its range, with populations between Usambara, Kanga, and Udzungwa (Kigogo population excluded) showing variation in p-distance of 0.2–1.0%, the Udzungwa population being the most different. Within this clade, Emmrich (1997) found some differences in body scalation, for instance between Udzungwa and the other populations sampled. Thus, further analyses across this range are required to assess the taxonomic variability, but our genetic analyses suggest that the differences are likely not great enough to warrant taxonomic distinction. Interestingly, the morphologically distinct and newly described species *Atheris matildae* was regrouped with a population that, even if only based on a single specimen, seems morphologically similar to *Atheris ceratophora* (herein called *A*. cf. *ceratophora* from Kigogo on Figures 4-2 and 4-3). Even if the genetic difference is lower between this single individual and A. matildae (p-distance: 2.4%) than with *A. ceratophora* (p-distance 2.9-3.1%), the support from our data is limited. I cannot be sure that this *A*. cf. *ceratophora* sample does not form the sister group to the Eastern Arc ceratophora clade – seemingly more in line with its general morphology. Further sampling, morphological and genetic analyses are required to resolve the grouping among these populations. However, regardless of its precise phylogenetic position, the Kigogo population appears to be distinct from all previously known taxa and consequently requires further morphological study.

Atheris barbouri, formerly placed in the genus *Adenorhinos*, showed limited genetic variation across its range, with populations in Udzungwa and Southern Highlands (principally southern Livingstone Mountains) forming a clade but with the exclusion of populations of Madehani in the northern Livingstone Mountains. Overall, the molecular analysis provided evidence for some cryptic genetic diversity, such as with the sample of *A. rungwensis* from Nkasi/Ntantwa, and an *Atheris* cf. *ceratophora* population from Kigogo. Both examples of cryptic diversity would require further molecular and morphological analyses to determine if new *Atheris* species can be supported.

Table 4-3 Estimates of p-distance between major clades of *Atheris* species. The analysis involved 22 haplotypes of 1737 bp (combining parts of both cytochrome b and ND4 genes) Evolutionary analyses were conducted in MEGA v5.1 (Tamura et al., 2011).

	A. matildae	*A. squamigera*	*A. rungweensis*	*A. nitschei*	*A. chlorechis*	*A. mabuensis*	*A. barbouri*	*A. ceratophora*	*A. desaixi*
A. matildae									
A. squamigera	0.111								
A. rungweensis	0.094	0.11							
A. nitschei	0.109	0.119	0.056						
A. chlorechis	0.104	0.09	0.096	0.1					
A. mabuensis	0.116	0.108	0.098	0.109	0.089				
A. barbouri	0.105	0.133	0.127	0.128	0.124	0.137			
A. ceratophora	0.032	0.119	0.094	0.104	0.105	0.117	0.104		
A. desaixi	0.129	0.114	0.107	0.118	0.124	0.132	0.148	0.119	
A. hispida	0.122	0.054	0.109	0.121	0.094	0.107	0.145	0.121	0.116

Biogeography

Atheris has a wide distribution across Africa and the restriction of species to forest habitats makes this group an ideal candidate for exploring the historical fluctuations of forests. Ancestral reconstruction of the genus *Atheris* shows an equivocal result – with similar likelihoods for both a western and an eastern African origin. The uncertainty may be due to several reasons, including extinction or under-sampling of species diversity, which may have provided a more conclusive result, for instance the inclusion of the other true West African species, *A. hirsuta*, could provide additional information on the overall biogeographic scenario of the genus. However, an alternative explanation would be that the pattern reflects a widespread distribution of the *Atheris* ancestor. It is notable that the apparent timing of the early diversification of the lineage (estimated between 15 and 18 mya) coincides with a period when a pan-African forest belt was present (Zachos, 2001; Böhme, 2003).

This pan tropical forest would have harboured forest species over a much larger area than now. The basal part of the *Atheris* tree includes the taxa *A. mabuensis*, *A. chlorechis* and *A. desaixi*, which are species that cover almost the full geographic extremities of the genus' extent of occurrence across Africa. Both the timing of speciation events and the widespread distribution of ancestral *Atheris* species is consistent with more widespread forest habitats in the Miocene. The initial split (ca. 15 mya) within the *Atheris* genus was observed between the clade including *A. mabuensis* and *A. chlorechis*, and the rest of the genus, and shortly thereafter, the lineage containing *A. desaixi* also split from the rest of the genus. Subsequently (12.6-13.3 mya), there was a split between *A. chlorechis* and *A. mabuensis*, which today occurs in West Africa and Mozambique, respectively. These splits are timed within a period with pronounced aridification (Plana, 2012), resulting in the reduction and fragmentation of the tropical forest. This fragmentation could have contributed to the separation of biogeographic regions (such as Central, West and East African forests), resulting in the isolation of several *Atheris* lineages as observed in other reptiles, e.g. chameleons (Tolley et al., 2011) and amphibians (Loader et al., 2013).

The *Atheris* forest vipers appear to have been present in East Africa in the mid-Miocene (*A. desaixi* in Kenyan Highlands and *A. mabuensis* in Mozambique), but extant lineages in the Eastern Arc are much more recent. Ancestral reconstructions showed that the Albertine Rift is the likeliest origin for the recent East African radiation at various nodes (D-G) prior to the radiation in the Eastern Arc and Southern Highlands (*A. ceratophora, A. matildae*, *A. barbouri*) (Node H). The dated phylogeny suggests that *Atheris* was present in the Southern Highlands of Tanzania around 7 mya, and subsequently diversified across the Eastern Arc Mountains (*A. ceratophora*) with species penetrating the southern margins of the Eastern Arc (*A.* cf. *ceratophora*/*matildae*, *A. barbouri* in Udzungwa) from the Southern Highlands (Fig. 4-5). A pattern of old and new origins of the East African forest lineages has also been revealed in other taxa (Couvreur et al., 2008; Fjeldså and Bowie, 2008).

The origins of the East African *Atheris* lineages (ca. 15 and 7 my) coincide with known periods of aridification and geological activity in Africa that isolated the rainforests of Central/West African from East Africa. Our results point to a role for aridification in the structuring of biodiversity in African forests, and suggest that repeated reconnections between rain forest blocks allowed for biotic exchange at specific times, while the break-ups might have induced speciation via isolation and vicariance (Couvreur et al., 2008).

The presence of *Atheris* lineages (primarily *A. ceratophora*) only relatively recently in the Eastern Arc Mountains, indicates an important biogeographical pattern. The Eastern Arc Mountains are among the oldest and climatically most stable mountain ranges in Africa (Schlüter 1997; Burgess et al. 2007) and harbour lineages of forest-associated species predating the Miocene climatic optimum (Fjeldså and Bowie 2008; Tolley et al. 2011). Despite this, and the fact that *Atheris* species were already present (*A. desaixi* from Kenya and *A. mabuensis* from Mozambique) across East Africa, only a recent radiation within *Atheris* is present in the Eastern Arc, A possible cause of the absence of old lineages is extinction of once present taxa. Fossil data would be required to evaluate the likelihood of this hypothesis. An alternative explanation is that the genus was never distributed in the Eastern Arc prior to its recent dispersal (Fig. 4-5). From our ancestral reconstructions, a west to east colonization (nodes D to G) pattern is suggested.

According to our time-tree, the timing of the speciation event between west/central areas and the Albertine Rift corresponds to large geological perturbations, including rifting and the formation of Lake Tanganyika. The rift-associated lakes and mountains are traditionally believed to be composed of two distinct segments: an older, volcanically active eastern branch and a younger, volcanic western branch (McConnell, 1972). However, recent studies have shown that initiation of rifting in the western branch began more than 20 mya, much earlier than previously thought, and contemporaneously with the eastern branch of the *Atheris* genus (Roberts et al. 2012). These geographical changes could have constituted a barrier to the movement of forest-dependent species. Consequently, these barriers might have contributed to a "C-shaped" (Fig. 4-5; areas 2 and 3) dispersal route into East Africa and the subsequent, more recent, radiation into the Tanzanian Mountains, from south of lake Tanganyika. A possible inferred scenario of *Atheris* dispersal in the Tanzanian mountains considers an early radiation from the south, across the Eastern Arc Mountains, north to the Usambaras. There followed a subsequent wave of extinctions and a re-colonisation of Mount Kanga, in the Nguru Mountains and Udzungwa Mountains (and possibly Uluguru Mountains) from relict populations that had survived in the Usambaras.

Our results are consistent with some reports that show that montane forest faunas are a mixture of old, paleo-endemic lineages, and recent *in-situ* speciation (Fjeldså and Bowie, 2008; Demos, et al. 2013). Such patterns have been shown in avian and mammal assemblages, which are a combination of relict taxa (since the mid-Tertiary thermal optimum) and recently speciated clades (Fjeldså and Bowie, 2008; Demos et al. 2013). Contrasting this, are other forest groups, like chameleons in the genus *Kinyongia*, showed relictual distributions, with diversification substantially pre-dating the Miocene and Plio-Pleistocene climatic shifts associated with cladogenesis and little evidence of recent immigration (e.g. Tolley et al., 2011). The reasons for such contrasting phylogenetic patterns are not well understood but are likely to represent a combination of differences in the taxon's ability to disperse, historical events excluding or facilitating dispersal, and local extinctions (Dobrovolski et al., 2012). Further comparative studies are necessary to untangle the causal reasons for differences that might provide a more general explanation of the biodiversity patterns in this region.

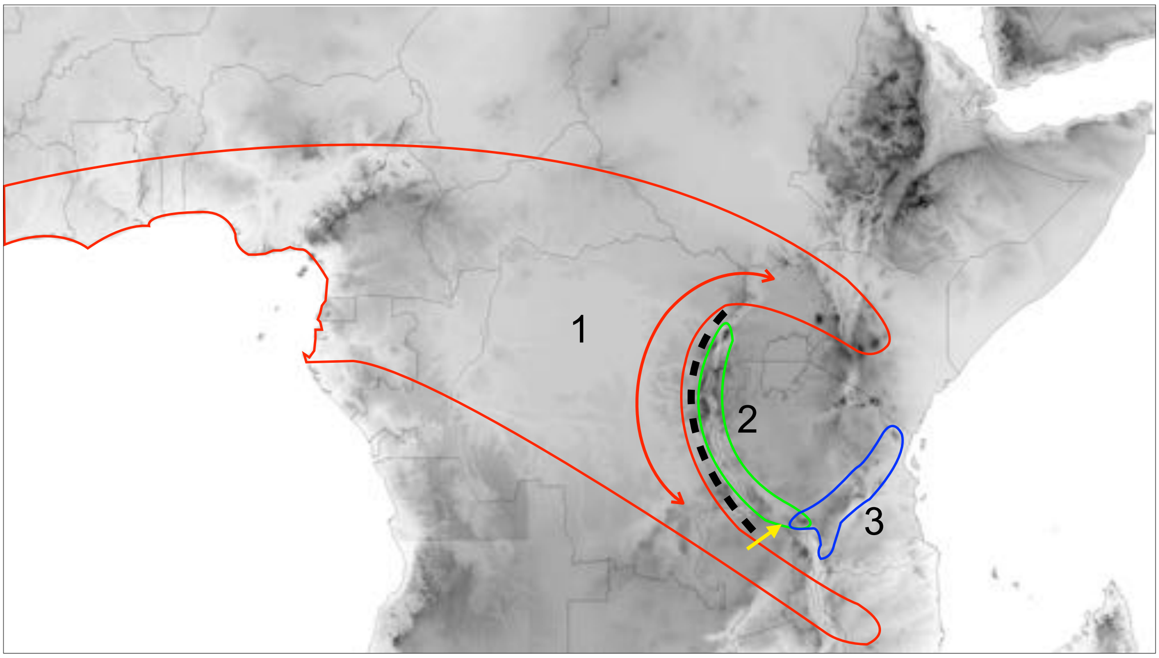

Figure 4-5 The pattern of distribution of *Atheris* since the origin of the genus. (1) Possible minimum extent of range of the genus about 11 million years ago (mya), when the lineages of A. chlorechis in the west, A. desaixi in the northeast and A. mabuensis in the southeast were already split. (2) Albertine Rift radiation initiated 5.4 mya. (3) Southern Highlands of Tanzania colonization and subsequent radiation in the Eastern Arc Mountains initiated 7.4 mya. The yellow arrow represents the possible way of penetration of the genus into East Africa. The dashed line represents the barrier constituted by the early stages of the Albertine Rift depression 20 mya that may have forced the dispersing lineages to move north or south of the barrier (the 'C-shaped' route, represented by the red arrow).

Conclusions

I provide a molecular phylogenetic tree for the forest viper genus *Atheris*. The tree generally provides strong supports for the relationships amongst each species. Our study also reveals potentially new cryptic species. Our *Atheris* time-tree and area reconstruction showed that during the Miocene Climatic Optimum (18-16 mya) the genus was likely to be widespread within the rainforests of tropical Africa. Early differentiation in the genus corresponds to the Middle Miocene Disruption, around 15 mya, a period characterized by a wave of animal and plant extinctions, due to a global cooling (Bohme, 2003). The more recent cooling events appear to have restricted species to forest refugia (e.g. Central Africa, Kenyan Highlands, Albertine Rift).

Despite two *Atheris* lineages being present in East Africa soon after the formation of the genus (*A. mabuensis* and *A. desaixi*), the lack of occurrence of any old Eastern Arc lineage before late Miocene/early Pliocene suggests a recent dispersal into this region. A possible reason for this might have been a barrier to forest species, with both the formation of the western branch of the Great Rift Valley and associated local climate perturbations. This event would have split the previously contiguous tropical forests of East and Central Africa. This could have resulted in blocking the direct west-east dispersal routes of forest-associated organisms, promoting a 'C-shaped' dispersal route (Figure 4-5), with a northern and a southern front. Our study underlines the potential role of the Rift Valley in shaping dispersal routes of forest-associated organisms. More broadly this study shows the biogeographical relationships among regions, and why there are such distinctive faunal assemblages in the Eastern Arc, Kenyan Highlands and Albertine Rift.

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Chapter 5 : Measuring and mapping diversity

Measuring and mapping East Africa's forest bufonids: implications for the conservation of a major amphibian radiation

Abstract

Among several important radiations of forest-associated amphibians in the Eastern Afromontane (EA), the most speciose concerns the ovoviviparous forest toad genus *Nectophrynoides*. Although just six described species were known in 1998, the genus now potentially numbers over 30 species. The increase in diversity is due to exploration in poorly known areas, and identification of new taxa (both cryptic and overlooked) with use of molecular methods. Here the phylogeny of the genus is reconstructed in order to describe relationships between taxa, and to identify key conservation sites for the group using different metrics, including species and endemic richness, phylogenetic diversity/endemism, and evolutionary distinctiveness. Finally, congruence between importance for *Nectophrynoides* and that in amphibians generally, and forest-associated chameleons and snakes, is examined.

Within EA, there are both very ancient paleo-endemic bufonid species, which may be monotypic, along with more recent radiations, indicating both old and recent diversification patterns within the genus. There were strong correlations between the different measures, e.g. species and endemic richness, phylogenetic diversity/endemism. Some sites had low species richness and yet contained one or two distinctive taxa that increased their importance in terms of evolutionary distinctiveness. There was strong congruence between richness of *Nectophrynoides* and patterns of amphibian diversity in the region, suggesting that this well-known and conspicuous group could act as a proxy for the importance of sites for amphibians generally. Congruence with chameleons and snakes was weak, but these two groups were themselves strongly congruent. Not a single forest bufonid species occurs at more than one site, and most had known ranges smaller than 100 km^2.

Already, a high proportion of *Nectophrynoides* are 'Endangered' or 'Critically Endangered' and this proportion will rise steeply if all cryptic species are distinguished taxonomically. A conservation strategy aimed to protect *Nectophrynoides* cannot solely be based on species richness, but must also consider

those sites such as West Usambara and Ukaguru, which have relatively low numbers of species, but important paleo-endemic taxa.

Introduction

Global patterns of biodiversity are the result of processes that operate over both space and time (Mittelbach et al., 2007). Time-calibrated phylogenetic reconstructions have improved our understanding of the tempo and mode of species' diversifications and have helped in identifying both adaptive and, in some cases, non-adaptive radiations across the tree of life (Losos and Mahler, 2010; Van Bocxlaer et al., 2010). According to the theory of adaptive radiation many of the dominant patterns in the evolutionary history of life are due to ecological processes (Lack, 1947, Schluter, 2000), where key innovations (Hunter and Jernvall 1995; Jønsson et al. 2012) and the availability of new resources allow organisms to exploit a novel ecological opportunity (Simpson, 1953) that become a driver of a rapid lineages diversification process that initiate to decrease as the ecological opportunity declines (Nee et al. 1992; Rabosky 2009, Mahler et al., 2010).

Much of the literature on evolutionary radiations equates species richness with adaptive radiation, and many of the world's best known and most remarkable radiations are rich in both species and adaptations. These include the African Rift Lake cichlids (Seehausen, 2006), the Hawaiian honeycreepers (Lerner et al., 2011) or the Caribbean lizards (Losos and Mahler, 2010). Such lineages undoubtedly represent adaptive radiations, and suggest that species proliferation and ecological radiation occur together. On the other hand, other clades contain a great number of species, but little ecological or phenotypic disparity, and result in species distributed almost entirely allopatrically, and ecologically similar, like the North American salamanders of the genus *Plethodon* (Kozak and Wiens, 2006) or the pygmy chameleons Rhampholeon (Fisseha et al., 2013).

With 580 described species, the family *Bufonidae* is the third most speciose among amphibians, after the American family *Craugastoridae* with over 700 known species and the almost cosmopolitan family *Hylidae* with over 900 known species (Frost, 2014). Unlike most other amphibians, bufonids have been able to colonize most parts of the world in a relatively short period of time (1999; Van Bocxlaer et al., 2010). Van Bocxlaer et al. (2010) proposed the evolution of an 'optimal range-expansion phenotype', a generalist phenotype that was also characteristic of the first lineage that has colonized Africa, as crucial for their success. The genera *Churamiti* and *Nectophrynoides* represent a remarkable amphibian radiation, occurring across most of the Tanzanian portion of the Eastern Afromontane Biodiversity Hotspot and characterized by specialized forest dependent and ovoviviparous species (Menegon et al., 2004; 2007). Although just six *Nectophrynoides* species had been described by 1998, the genus now numbers 29 species (described and not formally described), both due to exploration in poorly-known areas, and identification of new taxa with use of molecular methods. This undescribed diversity has relevance both for a better understanding of the actual species richness across the Biodiversity Hotspot, and to inform conservation strategies at the regional scale (Vieites et al., 2009).

The Eastern Arc Mountains and Southern Highlands of Tanzania (herein, the Tanzanian Highlands (TH); Figure 5-1) are part of the Eastern Afromontane Hotspot and are renowned for their high diversity (Rovero et al., 2014). Various studies have supported the theory that its long history of persistence and stability has driven this high diversity (Burgess et al., 2007; Loader et al., 2014). However, because the area is highly fragmented and contains isolated areas which are highly heterogeneous in size, topology and climate (Burgess et al., 2007). It is likely that diversity is not equally distributed within the region (Loader et al., 2011; Tolley et al., 2011; Loader et al., 2014).

Further understanding of the distribution of bufonid diversity might provide important information on specific areas to prioritize for conservation. Based on current estimates, such hyper-diverse areas still require decades of taxonomic work, a taxonomic lag dubbed the ‘Darwinian shortfall’ (Diniz-Filho et al., 2013). Solutions for speeding up taxonomic descriptions (e.g. Clarke et al. 2001) have been suggested, but there remains an urgent need for biodiversity data to underpin formulation and evaluation of conservation strategies (Diniz-Filho et al., 2013). A correct understanding of the spatial distribution of biodiversity is essential in conservation planning and one of the main challenges is represented by the uneven spatial distribution of species and associated biological values (Lamoreux et al., 2005), that coupled with the data constraints, especially across the tropics, make the identification of suitable surrogate measures of the overall diversity an essential tool for conservation.

Despite conservation efforts being traditionally focused on species, they should seek to protect not only species, but also the underlying functional and genetic diversity they represent (Tucker et al., 2012). A strictly species-based approach may underrepresent rare, threatened, or genetically distinct species, and over-represent widespread species (Faith, 1992; Moritz et al., 2000; Tucker et al., 2012).

By using phylogenetic biodiversity measures (PBMs) we can now establish the amount of evolutionary history and where it is located, and therefore, where habitat loss/destruction and other environmental issues might threaten evolutionary history most. PBMs can also provide insights into more general patterns of diversity given the relationships between high PBM between organisms and habitat stability. These might explain why diversity is high in certain locations, areas which deserve particular conservation attention (Loader et al., 2014). The first introduced phylogenetically based index of diversity was Phylogenetic Diversity (PD), a measure that represents the sum of branch lengths within a tree connecting all species of interest at a site, commonly measured with Faith's index (Faith, 1992) Two more indices have been subsequently identified: Phylogenetic endemism (a site-based index of the amount of evolutionary history uniquely represented within a given area); and Evolutionary Distinctiveness (a species-based measure that identifies species with less redundancy for the unique genetic information they contain) (Faith, 1992; Faith and Baker, 2006; Cadotte et al., 2010).

The aim of this chapter is to investigate and map the diversity/importance within the forest bufonid genera *Nectophrynoides* and *Churamiti* using a range of biodiversity metrics (e.g. species richness and phylogenetic metrics), to examine congruence between these metrics, and to explore the potential for using bufonid richness as a proxy for overall amphibian richness at sites, or richness in other herpetofaunal groups. Finally, I use the above results to identify important sites for conservation across the TH, and to discuss implications for the conservation of the group.

Material and Methods

Taxon sampling, Phylogenetics, Time Tree Construction and Ancestral areas

Specimens of East African dwarf bufonids and outgroup taxa were obtained through extensive fieldwork in various localities between 1998 and 2011 by MM, SPL, KMH, RK, TBD, and DG (Supplementary Table 1). Genetic data were acquired using protocols outlined in) Data were processed as outlined in Liedtke et al. (submitted) with an alignment of concatenated partial 12S, 16S, cytochrome oxidase (*cox1*), recombination-activating gene 1 (*rag1*) and chemokine (C-X-C motif) receptor 4 (*cxcr*4) sequences assembled, based on previously published data (e.g.). Extraction, amplification and sequencing followed standard protocols (see Liedtke et al. submitted) The bioinformatics program Geneious 6.1.4 was used to assemble data, and MUSCLE (Edgar, 2004) with default settings for sequence alignment. Alignment ambiguities and gaps from 12S and 16S data partitions were excluded from phylogenetic analyses using GBLOCKS version 0.91b (Castresana, 2000). *Cox1*, *cxcr4*, and *rag1* codon positions were determined using TranslatorX (Abascal et al., 2010). For all fourteen partitions, best-fit models of nucleotide substitution for each gene partition were identified using Akaike information criterion (AIC; Akaike, 1973) minimisation implemented in jModeltest 2.1.3 (Posada, 2008). We examined saturation of data by examining patristic distances (JC and HKY models of molecular evolution) for each gene partition.

The dataset, which included all *Nectophyrnoides* and *Churamiti* and representatives of the genera *Altiphrynoides* and *Mertensophryne* along with outgroups, was analysed by Bayesian inference (BI). We included outgroup taxa that were used for the calibration of our timetree (see Liedtke et al. submitted). The reconstructed chronogram was used for estimating diversity metrics (see below). We carried out DEC analyses using the program Lagrange v. 20120508 (Ree and Smith, 2008) on the time-calibrated tree obtained and DIVA (Yu et al, 2010). The input files for Lagrange were assembled using the web-based configurator (http://www.reelab.net/lagrange/configurator/index).

Diversity indices

A spatial analysis of diversity was conducted on a grid, with results summarized for each pixel, often associated to a specifically recognized and named mountain block/fragment. Each taxon record was represented as a point within a grid cell 0.25° latitude and 0.25° longitude (each cell has a size of approximately 756 km^2). For each grid, other than calculating the number of currently described species (SR), we calculated the following metrics, using the software Biodiverse version 0.18 (Laffan et al., 2010): 1. Weighted Endemism Index (WE) was calculated, where we assigned high weights to species with small ranges, and progressively smaller weights to species with larger ranges (Crisp et al., 2001; Slatyer et al., 2007); 2 Phylogenetic Species Richness (PSR), a count of described and undescribed species as calculated by GYMC, bGYMC and BPD; 3. Phylogenetic Diversity (PD; see FAITH, 1992; Rosauer et al., 2009); 4. Phylogenetic Endemism (PE; Faith, 1992); and 5. Evolutionary Distinctiveness (ED), used to estimate the amount of unique evolutionary history represented by each terminal taxon of the reconstructed phylogeny.

To assess congruence across these diversity measures, and the degree of congruence between importance measures for bufonids and montane amphibians as a whole (including bufonids), forest chameleons (genera *Rhampholeon*, *Kinyongia* and *Trioceros*), and forest snakes (Genera *Atheris*, *Dipsadoboa*, *Crotaphopeltis*, *Prosymna*, *Elapsoidea*, *Buhoma*, *Telothornis*), I used Spearman's rank correlation analyses.

Results

Phylogenetics, species delimitation, and ancestral areas

The genetic sampling presented provides an almost comprehensive geographic coverage of all known and undescribed species or populations of mountain-restricted dwarf bufonids of Eastern Afromontane Region with the exception of the Congolian Dwarf toad *Laurentophryne parkeri* for which there were no samples available. In the genus *Nectophrynoides*, only *N. cryptus*, and *N. laevis,* both from the Uluguru Mountains, were not sampled. The phylogenetic tree shows all genera are recovered as monophyletic. Based on the genetic dataset, the single threshold GMYC and bGMYC species delimitation model revealed 34 distinct evolutionary candidate species in the genus *Nectophrynoides* (of which 13 are described and 21 remain undescribed), and two within the genus *Churamiti* (see Figure 5-1).

In addition, a Bayes Factor species Delimitation (BFD, Grummer et al., 2014) was used to compare two alternative scenarios of species diversity based on GYMC results and expert opinion. These were: 1. Thirty-six separate species (matching the GYMC/bGYMC results; and 2. Thirty separate species, where the two individuals of *Churamiti* from Ukaguru are placed within one species, two individuals of *Nectophrynoides* sp. from North Udzungwa are placed into a single species, and five individuals of *Nectophrynoides* sp. from Mahenge are placed into a single species (Figure 5-1). To assess the consistency of GYMC and bGYMC and Bayes Factor delimitation, other available taxonomic evidence including genetics (P-distances), morphology, geography and acoustic data was considered. From this, we compiled Figure 5-1 (note the yellow line by the branches tips show conflicting GYMC and BFD arrangements). In summary, the GYMC and bGYMC estimation of lineages strongly reflects the distinctiveness of most taxa detected on the basis of morphology, genetic distinctiveness, acoustic information, and geography, but with some over-estimation. For the purposes of this study, we used conservative estimates of species units as a composite of both GYMC/bGYMC and BFD approaches which represents our 'best estimate' of actual species numbers, summarized in Table 1.

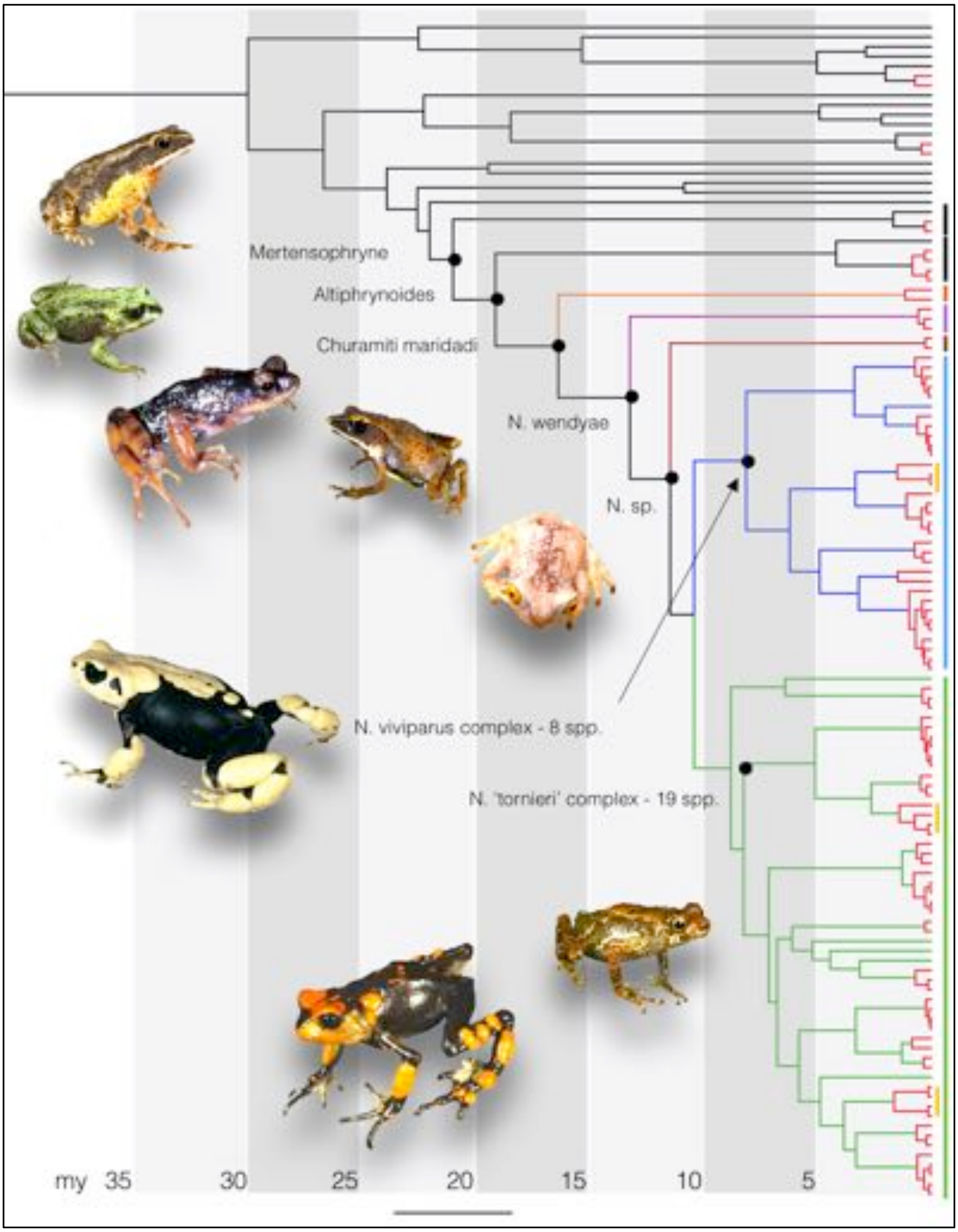

Figure 5-1 Time-calibrated phylogeny of dwarf bufonids of East Africa including the genera *Mertensophryne*, *Altiphrynoides*, *Churamiti* and *Nectophrynoides*, terminal branches colored red represent maximum likelihood clusters (i.e. individuals belonging to the same species) as delimited by the bGMYC and BFD algorithm.

Lineage diversification rate and ancestral area reconstruction in TH

The net accumulation of lineages over time is shown in the LTT plots (Figure 5-2) and it is clear that the rates of diversification have varied through time. The net diversification rate for *Nectophrynoides* was estimated to be 0.183 per myr with no extinction, and 0.092 per myr assuming high rates of extinction ($y = 0.9$). Based on the likelihood ratio tests, the data fit a pure-birth model more closely than a birth-death model, and are better explained by models with variable extinction and speciation (BOTHVAR) rather than either variable extinction (EXVAR) or variable speciation (SPVAR) rates on their own (see Supplementary Table 2). When data were compared to 10,000 Yule-process-simulated trees, the difference was significant ($p < 0.05$). Based on the gamma statistic (calculated gamma = -1.76; $p < 0.05$) diversification rates of *Nectophrynoides* appear to have decreased over time at certain stages. Liedtke et al. (submitted) also detected rate variation in *Nectophrynoides* through overall rate was generally homogenous across African bufonids.

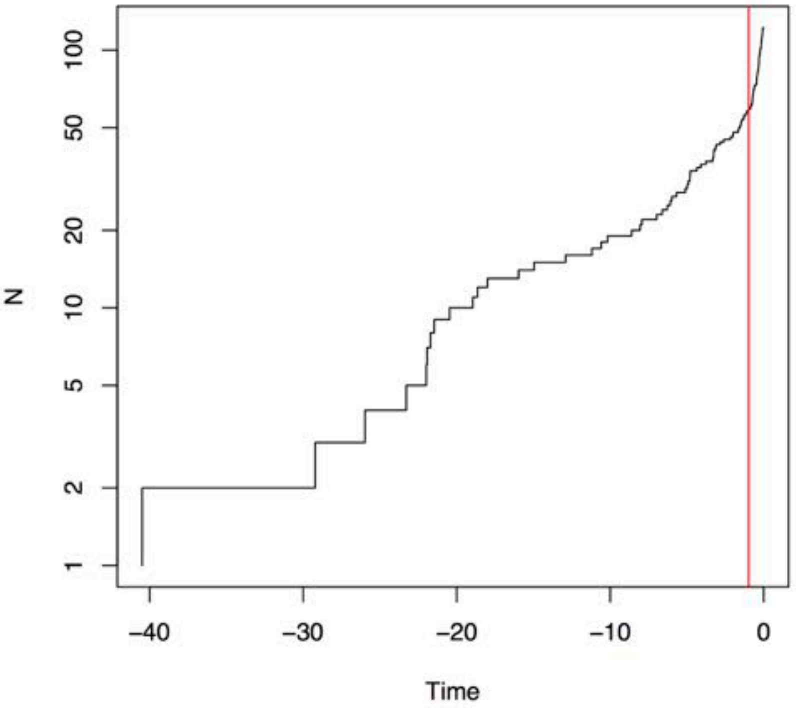

Figure 5-2 Lineages through time plots for the bGMYC tree of dwarf bufonids showing as the rates of diversification have varied through time, with a boost of diversification between 20 and 10 million years ago.

The phylogeny of the *Nectophrynoides*-*Churamiti* radiation can be split into some major groups: *i*. early basal splits (e.g. *Churamiti, N. wendyae,* and *N* sp. (Nguru); *ii*. *N. viviparus* complex; and, *iii*. N. “dwarf” complex (Figure 5-1). An ancestral reconstruction of this phylogeny shows in both Bayesian and DIVA reconstructions equivocal areas, with low probabilities given for any of these. It is therefore not possible to reconstruct the ancestral area for the complete phylogeny. For the major clades, there is a similar pattern, though for each radiation and ancestral reconstruction there are some general patterns that can be seen, for all radiations, Central and Southern eastern arc regions are ancestral, with more recent dispersal to Southern (Livingstone, Southern Highlands and Mahenge) and Northern areas (Usambaras). Specifically for each radiation: *i*. the Central and Southern eastern arc region (Udzungwa, Uluguru, Rubeho, Ukaguru, Nguru) is reconstructed as being ancestral; *ii*. the Central and Southern eastern arc regions (Udzungwa, Uluguru, Rubeho, Ukaguru, Nguru) are reconstructed as being ancestral, though the type of event and probability is low; and, *iii*. the same uncertainty and geographical placement of central and southern eastern arc region is (Udzungwa, Nguru) is reconstructed as being an important node.

Diversity measures: congruence and bufonid distribution across Tanzanian Highlands

There were very strong positive correlations between the following measures: PSR, PD, and PE across the 11 sites containing bufonids (PSR vs. PD: r_s = +0.96, p < 0.0001; PSR vs. PE: r_s = +0.96, p < 0.0001; PD vs. PE: r_s > +0.99, p < 0.0001). The relationship, however, between PSR and maximum ED (that of the species with the highest ED value) was not strong (r_s = +0.61, p = 0.06; Figure 5-3). Sites with relatively low PSR but which contained at least one species with very high ED were Ukaguru and Usambara. ED patterns show lineage-specific patterns that provide information on which species are distinctive. Figure 5-5 shows that certain species to have a disproportionate amount of evolutionary history. The species shown to have high ED include: *Churamiti maridadi, N.* sp. (Uluguru), *N. vestergaardi*, *N.* sp. (Nguru), and *N. asperginis*.

The diversity of dwarf bufonids species across TH is distributed unevenly (Table 1). Species richness is shown to be strongly concentrated in two main locations: Uluguru (North and South) and Udzungwa Scarp Forests. The pattern remains consistent for species richness based on phylogenetic estimates (PSR: combined GYMC/bGYMC and Bayesian Species delimitation of units) though the relative proportion is shown to be more highly concentrated in the Uluguru, in particular Uluguru North. It should also be noted that this area also has two phylogenetic units missing from the dataset (*N. cryptus* and *N. laevis*). Phylogenetic metrics, both PD and PE, as expected due to the strong autocorrelations, show similar patterns of diversity, highlighting the richness of Uluguru (North and South) and Udzungwa Scarp Forest, while also indicating the diversity of Ukaguru Mountains.

Table 5-1 Summary of phylogenetic indexes per site and relative number of species as delimited by the bGMYC and BFD algorithm (*note that two described species, both from the Ulugurus are missing in the phylogeny and related phylogenetic indexes, but included in the total number of species).

Site	PD	PE	ED	Total species
Uluguru	89.64	52.75	58.88	10*
Udzungwa	88.76	51.77	57.70	6
Nguru	60.18	25.81	27.89	4
Ukaguru	70.01	35.03	35.06	3
East Usambara	49.35	16.18	15.41	2
Mahenge	53.50	15.28	15.58	2
Livingstone	40.56	6.84	4.44	1
Nguu	40.48	7.65	4.19	1
Rubeho	41.57	9.14	8.28	1
SH	40.48	6.76	2.92	1
West Usambara	40.48	8.28	5.14	1

There was a significant positive correlation between species richness of forest bufonids and that of mountain-associated amphibians as a whole (r_s = +0.85, p = 0.001, n = 11 in all correlations), a weaker correlation with forest snakes (r_s = +0.60, p = 0.05), and no significant correlation between bufonid and Chameleon richness (r_s = +0.43, p = 0.19) Amphibian richness (including bufonids) was positively correlated with both Chameleon richness (r_s = +0.65, p = 0.03) and forest snakes richness (r_s = +0.71, p = 0.01) but not as strongly as the latter groups were linked (r_s = +0.77, p = 0.006).

Discussion

The bufonid radiation across the Tanzanian Highlands

Current taxonomic diversity of the genus *Nectophrynoides* in East Africa is thirteen species (Menegon, et al. 2007). The phylogenetic species richness estimates outlined in this study (ca. >30 spp) clearly demonstrates shows that dwarf bufonid diversity across Tanzanian mountains is underestimated, probably by at least 40%. Such an underestimation might suggest a large mismatch in our more broader understanding of amphibian diversity in the Tanzanian highlands – particularly given the recent taxonomic focus on this group (e.g. Poynton, et al. 1999; Menegon et al. 2004; Menegon, 2007). An underestimation in other amphibian groups from the Tanzanian highlands based on new phylogenetic data has been shown (e.g. brevicipitids: Loader et al. 2014) which presumably indicates a common shortfall in taxonomic knowledge in the region. Further taxonomic work is required to overcome this taxonomic impediment and which molecular approaches will continue to facilitate.

The cryptic diversity reported in this study highlights a remarkable feature of species richness within the dwarf bufonid radiation. For the continent of Africa the radiation stands out for the exceptional numbers of species in a single genus. The numbers are comparable, if not greater, to other speciose areas such as the Cameroonian mountains (Burgess et al., 2007b). For example, as is currently known and according to our PSR, nowhere else in Africa (probably across the globe) does a single amphibian genus have ten species occupying the same small mountain forest fragment, as is seen in the Uluguru Mountains (308.6 km^2). Here, four species are endemic to Uluguru South only, and six species to Uluguru North only (Platts et al., 2011). Large numbers of species in one genus are known from Cameroon highlands (e.g. Blackburn et al. 2008) but not as many as outlined in this study. Although it should be stated it is unclear whether differences reflect actual differences or due to sampling differences. Some bufonid radiations across the planet are known to be diverse, including *Atelopus* and *Ansonia* radiations in the Neotropics and SE Asia (Frost, 2014). However based on current estimates, the relationship between the number of species and the area in which *Nectophrynoides* occurs, represent an unmatched example of remarkably high species density across the globe.

Eastern Arc as both a museum and cradle of bufonid diversity

Phylogenetic results, dating estimates and LTT plots suggest that, despite slight variation in the species accumulation rate, forest bufonid lineages have been steadily accumulating in East African mountains over the last 30 Myr, with an increase of diversification rate, at about 15 Myr, concomitant with the origin of the genus *Nectophrynoides*. This supports the hypothesis that forest habitats across the EA have persisted for a long time (Lovett et al., 2005) and represent an area of both persistence of old lineages (museum) and recent diversification (cradle of diversity). Phylogenetic endemism results point to heterogeneous patterns of *Nectophyrnoides* evolutionary history across the mountains, with some showing large differences. The high amount of evolutionary history stored in the Uluguru's and in the southern Udzungwa, fits well with previous understanding of the high diversity of biota (Rovero et al., 2014). Potentially the results outlined here and before might suggest this area as being a more stable and persisting habitat (e.g. museum like) – as also shown in palaecological reconstructions (Mumbi, et al. 2008). Geologically, the area is thought to be old, and both the age and habitat stability might be the causal factor explaining higher diversity in *Nectophrynoides* than in other areas. In contrast to persisting habitats, there are examples of areas that might be less stable and persisting. The phylogenetic results indicate that the single extant *Nectophrynoides* species in the Southern Highlands region (*N. cf. viviparus*) is the product of a recent divergence and dispersal from an EAM ancestor. The Southern Highlands of Tanzania have a different history to that of the nearby EAM, being affected, in parts, by recent periods of volcanism (Msanya et al., 2007) that could have affected habitat stability. As for brevicipitids (Loader et al., 2014), there is no evidence for forest bufonids having been present in the Southern Highlands for more than 1.4 Myr, although it is not clear whether this can be attributed to a lack of habitat persistence, or to restricted dispersal opportunities from the more central parts of EAM. Along with the occurrence of old, persisting lineages, there's clear signs of more recent cladogenesis associated with Plio-Pleistocene climatic shifts. Species of recent origin generally occur at lower elevation and show a remarkable morphological similarity.

Congruence across diversity metrics

Reliable representations of the distribution of biodiversity are pre-requisites for assessing potential conservation prioritization strategies (Whittaker et al., 2005).

However, the standard measure, species richness, is often absent or incomplete for many areas and this hampers formulating, without biases, conservation strategies. A number of surrogate measures have been developed, being both cost effective, relatively quick to obtain, and potentially better estimates of diversity (Wiens et al., 2008). For forest bufonids, sites with high SR correspond closely with those with high PSR, PD, PE, and WE.

That SR is so highly correlated with the other metrics might seem surprising given the number of taxa that are undescribed in SR estimates (sixteen undescribed species, with only 42% of diversity recognized taxonomically) Omission of this cryptic diversity in SR estimates does not much alter spatial patterns of biodiversity significantly, when compared to other metrics. Potentially the presence of a similar signal in the SR metric compared to other surrogate metrics is linked to biases in recent species descriptions that emphasize the importance of Uluguru and Udzungwa – and are therefore not under-represented (Clarke, 1988; Poynton et al., 1998; Menegon et al., 2004). So, although relative proportions might differ between metrics, such as SR and PSR, the general spatial arrangement of diversity is relatively similar. Importantly, we see one notable difference in site importance using the different metrics. Using PE, PD, and WE highlights Ukaguru as having high importance, which is not recovered with SR or PSR (see Figure 5-3). This distinction is linked to the amount of phylogenetic divergence exhibited in Ukaguru taxa (PE and PD) and their ranges (WE) compared to the other areas. One ‘stand out’ taxa is the divergent species *Churamiti maridadi* which is scaled highly in ED measures (see Figure 5-5) and has an extremely narrow range of occurrence (IUCN, 2014).

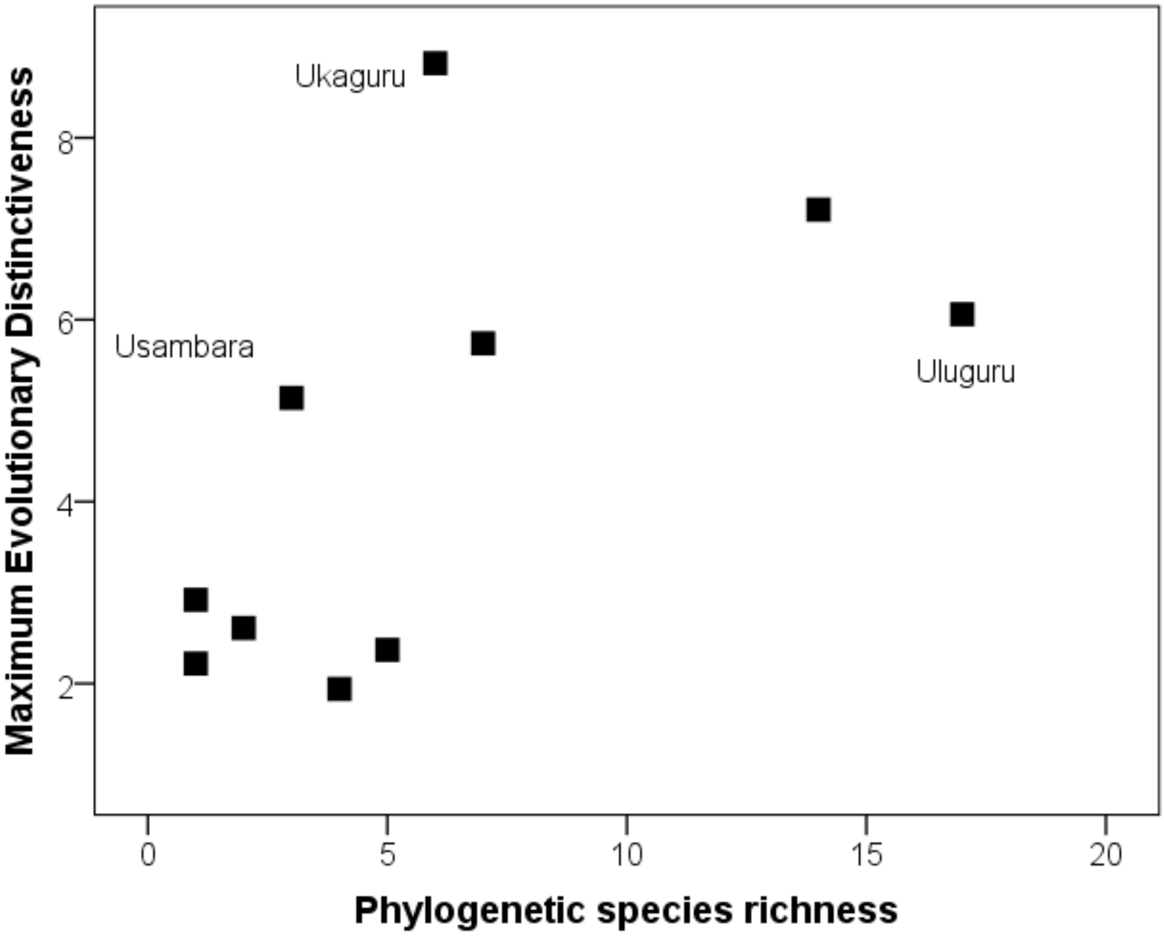

Figure 5-3 The relationship between phylogenetic species richness (PSR) and maximum evolutionary distinctiveness (EDmax) at eleven sites across the Tanzanian Highlands. Marked are Ukaguru and Usambara (relatively low PSR but high EDmax) and Uluguru (high PSR but relatively low EDmax)

As expected, ED values tend to identify taxa in ancient, species-poor, phylogenetically isolated clades, as highly ranked taxa in conservation assessments (Isaac et al., 2007). The species with higher ED are often, but not always, species that belong to areas highlighted as being rich using other metrics (SR, SPR, PD, PE and WE; see also below). Biogeographical analyses that aim to understand the likely scenario for ancestral areas, and, potentially, areas whereby species speciated, also generally show qualitatively similar spatial patterns to those revealed with diversity metrics.

Although some uncertainty remains in the statistical support for many nodes, the general pattern of area relationship reconstruction reveals Central (e.g. Uluguru) and Southern (e.g. Udzungwa) sites as being ancestral. The logical link between these patterns is that areas that contain highest diversity (both species numbers and phylogenetic diversity) also share a long evolutionary history. Such a link between evolutionary history and species diversity has also been made in other recent studies (Tolley et al., 2011; Dimitrov et al., 2012; Loader et al., 2014). Studies looking at the same region and applying similar approaches have highlighted central areas as being ancestral (Tolley et al., 2011; Loader et al., 2014) though contrasting in the areas where the distribution of evolutionary history was highest. Tolley et al. (2011) noted, as in this study, that the Ulugurus had a disproportionate amount of evolutionary history (PD), whereas Loader et al. (2014) noted high PD for the Ukagurus. Given the varying patterns of the distribution of PD within lineages and across geographical areas in TH, a multi-taxonomic study of evolutionary history of the area will be required before any clear resolution of the centres of highest diversity can be more precisely quantified (Grenyer et al., 2006).

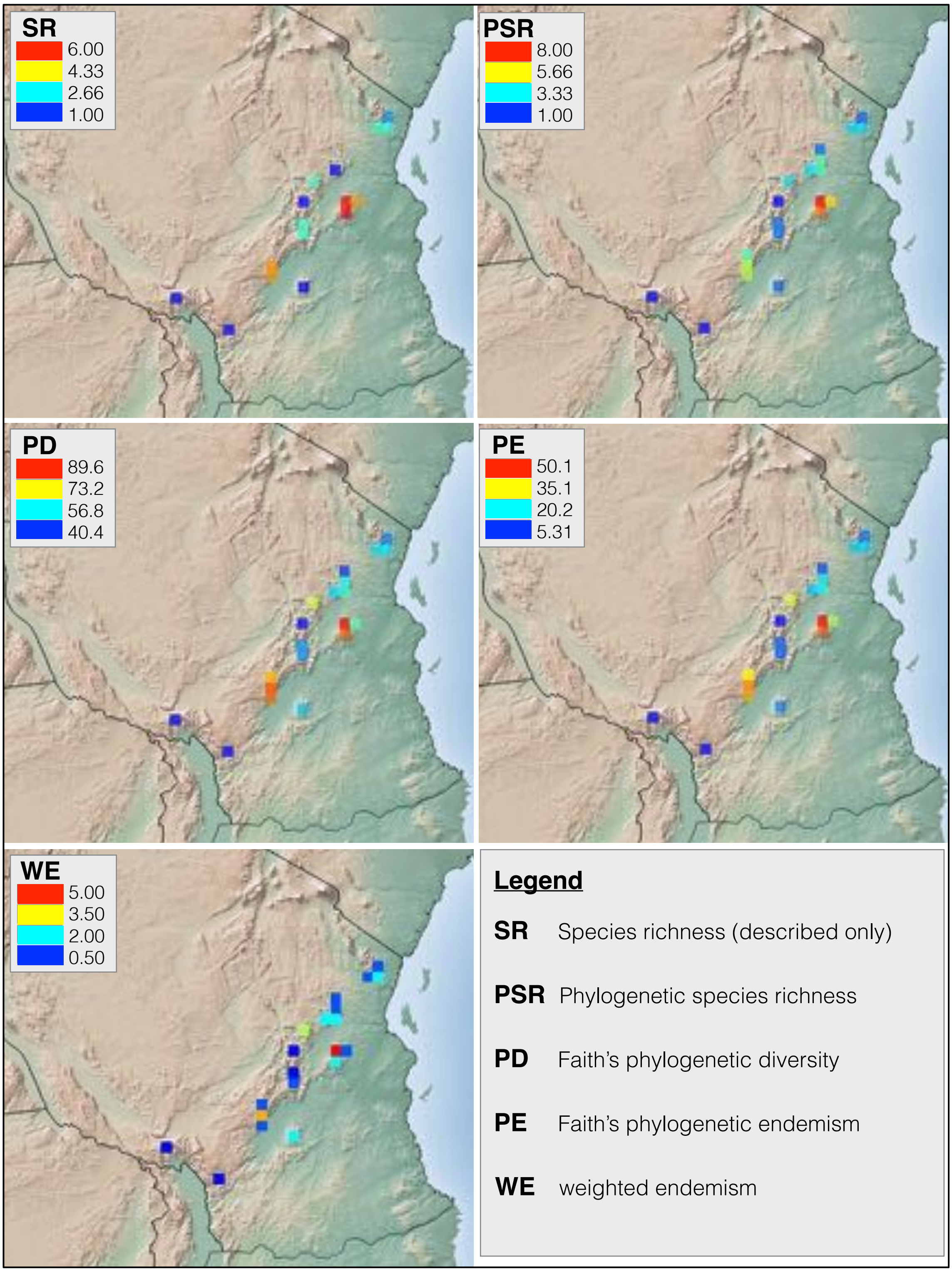

Figure 5-4 Map showing species richness, phylogenetic species richness, Faith's phylogenetic diversity, Faith's phylogenetic endemism and weighted endemism of species in the genera *Nectophrynoides* and *Churamiti* across the Eastern Arc Mountains of Tanzania.

Can dwarf forest bufonids act as an indicator for less-well known groups?

Across montane East Africa, distribution data is incomplete for virtually all species groups, as detailed surveys over the entire region are not available, due to the remoteness of areas and consequent lack of field surveys (CEPF, 2010) This means that conservation decisions have to be made based on studies of just a subset of taxonomic groups (Howard et al. 1998), use of higher taxonomies (Balmford et al., 1998), incomplete distribution knowledge, or incomplete species lists (Polasky et al., 2000). Forest bufonids, and the genus *Nectophrynoides* in particular, represent one of the most widespread and speciose groups of forest associated amphibians across Tanzanian mountains. They occur across most of the EA mountain blocks, covering both the submontane and montane forest belt, and they are usually easy to detect due to their loud calls and exposed calling behaviour. This makes them a potentially ideal candidate as a surrogate for overall amphibian diversity.

We found a significant positive correlation between species richness of dwarf forest bufonids and that of mountain-associated amphibians as a whole, suggesting that dwarf bufonids could indicate areas with high amphibian species richness and therefore to be used as representative of entire species pools across the EA. Congruence with forest snakes and forest chameleons was weaker, probably reflecting differences in evolutionary history and pattern of diversification (Caro and O'Doherty, 1999. A study by Rodrigues and Brooks (2007) showed taxonomic surrogates are more effective than surrogates based on environmental data, and surrogate efficiency was higher for taxa belonging to the same realm, suggesting that the use of dwarf bufonids as a surrogate of amphibians as a whole could help informing on capturing richness in less well known taxa (Rodrigues and Brooks, 2007).

The identification of surrogate species or groups of species in conservation planning is important because of inadequate funding in relation to the number of species that require attention and limited understanding of the full extent of ecological requirements of species (Caro et al., 1999). In order to address the need of reducing the many dimensions of species requirements occurring in an area to a workable and representative subset of species, various surrogate approaches have been identified in the past on the basis of taxonomy, habitat preferences, life-history characteristics, or more broad ecological functions to represents broader species pools (Wiens et al., 2008).

Identifying important sites for bufonid conservation

As elsewhere in the tropics, deforestation in the Tanzanian Highlands has been disproportionately severe in the lower and middle elevations of the mountains (Hall et al., 2009; Platts et al., 2011). Forest cover on some mountain blocks has been reduced much more than others (Table 3), with the Pares and West Usambara, Malundwe, and Mahenge affected more than other areas (Hall et al., 2009; Platts et al., 2011). Our analyses for dwarf bufonids consistently identify two centres of diversity: Uluguru (North and South) and Udzungwa Scarp. Inclusion of phylogenetic measures and weighted endemism further include the Ukaguru region. Fortunately, these three areas have experienced limited deforestation in recent years (Hall et al., 2009) compared to other areas within the Eastern Arc. Reducing future forest loss should, of course, be a continued focus of conservation activities in the region. The Udzungwa has received relatively high levels of protection, such as having parts designated as a national park (Burgess et al., 2007). However, the main area of amphibian diversity is located in southern Udzungwa Scarp area, an area outside of the national park, and currently given only the status of nature reserve, that represents the highest grade of protection offered by the Forest and Beekeeping Division of the Ministry of Natural Resources and Tourism of Tanzania, therefore an upgrade compared to its previous designation of Forest Reserve, but still with no clear indication of its obligations in terms of practical conservation measures.

A recent work by Rovero and colleagues (2014) further highlighted the 'Outstanding Universal Value' of the EA. In particular, the comparison of richness of endemic vertebrates in the EA versus six tropical forest sites that are already World Heritage sites shows that the EA rank among the top sites, and for amphibians and reptiles the EA is the top site for endemism (United Republic of Tanzania, 2011). There still remains a lack of an effective strategy and related investments for conservation actions on the ground assuring the long term conservation of the many, often small, forest fragments across the EA. However, some effective steps have been made with the most valuable forest fragments being upgraded to National Park and Nature Reserve status, and the current efforts to recognize the most important sites under the 'Outstanding Universal Value' criteria of the World Heritage Convention,

ED measures indicate that certain taxa, and hence the sites within which they occur, deserve specific conservation attention. Firstly, within the Udzungwa Scarp, *N. wendyae, N. asperginis* and *N. poyntoni,* have extremely narrow distributions, estimated at < $0.5km^2$ (IUCN, 2014; Seki et al., 2011). One species is currently being re-introduced which was extinct in the wild ten years previously (for *N. asperginis*; see Lee et al., 2006; IUCN, 2014) while *N. poyntoni* has not been observed since 2003, despite dedicated searches (Seki et al., 2011; Tonelli, pers. obs.). The loss of habitat in this area and extinction of these key species would be a considerable loss of evolutionary history. Again, raising the protection status of the Udzungwa Scarp is key to their survival, but it is imperative that alongside the upgrade in its status, an effective strategy of habitat protection on the ground needs to be implemented.

For the Uluguru, two species, both undescribed but genetically and morphologically distinct (Supplementary Table 3), are identified as being particularly evolutionarily distinct. Interestingly, the species occur in quite different areas that are also subject to different conservation protection. One is seemingly restricted to the grassland forest mosaic of the Lukwangule plateau, in Uluguru South region, and part of the relatively well-protected Uluguru Nature Reserve (Ngalason, 2010). The second, however, occurs at significantly lower elevations in the Mkungwe Forest Reserve, which is poorly protected, and subject to greater human impact (Hall et al., 2009). It is imperative that these taxa are described and their distribution fully understood so appropriate conservation measures can be put in place.

Although not surprising given its morphological divergence, the identification of the endemic forest toad *Churamiti maridadi*, as evolutionarily distinctive, places further emphasis on the need to understand and protect this species better. It is currently known only from one locality, placing high value on the protection of the Mamiwa Kisara Forests. Further research is required to understand the ecology of this species so suitable management strategies can be formulated. Beyond these sites, there are species found outside the areas of high diversity that deserve close attention. These include species in the Nguru, Nguu and West Usambara mountains. *Nectophrynoides vestergaardi* might be of particular concern given the high levels of deforestation identified in recent years within its range (Hall et al., 2009). Lastly, as demonstrated in this study the assessment of the biological value of an area or in identifying species of conservation concern can be rapidly expedited by phylogenetic indices.

The fact that the evolutionary distinctiveness of a species can be calculated on species awaiting formal description or lacking a IUCN red list evaluation due to data deficiency, as samples just need to be included in a phylogenetic tree is particularly favourable – particularly given the drive to barcode so many of the planets species

(Isaac et al., 2012). This study provide a comprehensive indication about evolutionary distinctiveness of taxa and biological importance of sites, despite the large proportion of undescribed, cryptic species in the genus *Nectophrynoides*, and despite the gap represented by the so called Linnaean shortfall.

With so many globally rare, range-restricted and threatened species, the EAR clearly represents one of the most important areas on mainland Africa for conservation. A lack of expertise is hampering the discovery of species and identification of site importance. Although areas within the Tanzanian Highlands remain poorly surveyed, a situation which must be remedied, a priority must now be placed on the description of the TH biodiversity with investment required in developing programme and infrastructure that will promote the ecological understanding of the biological diversity of this area.

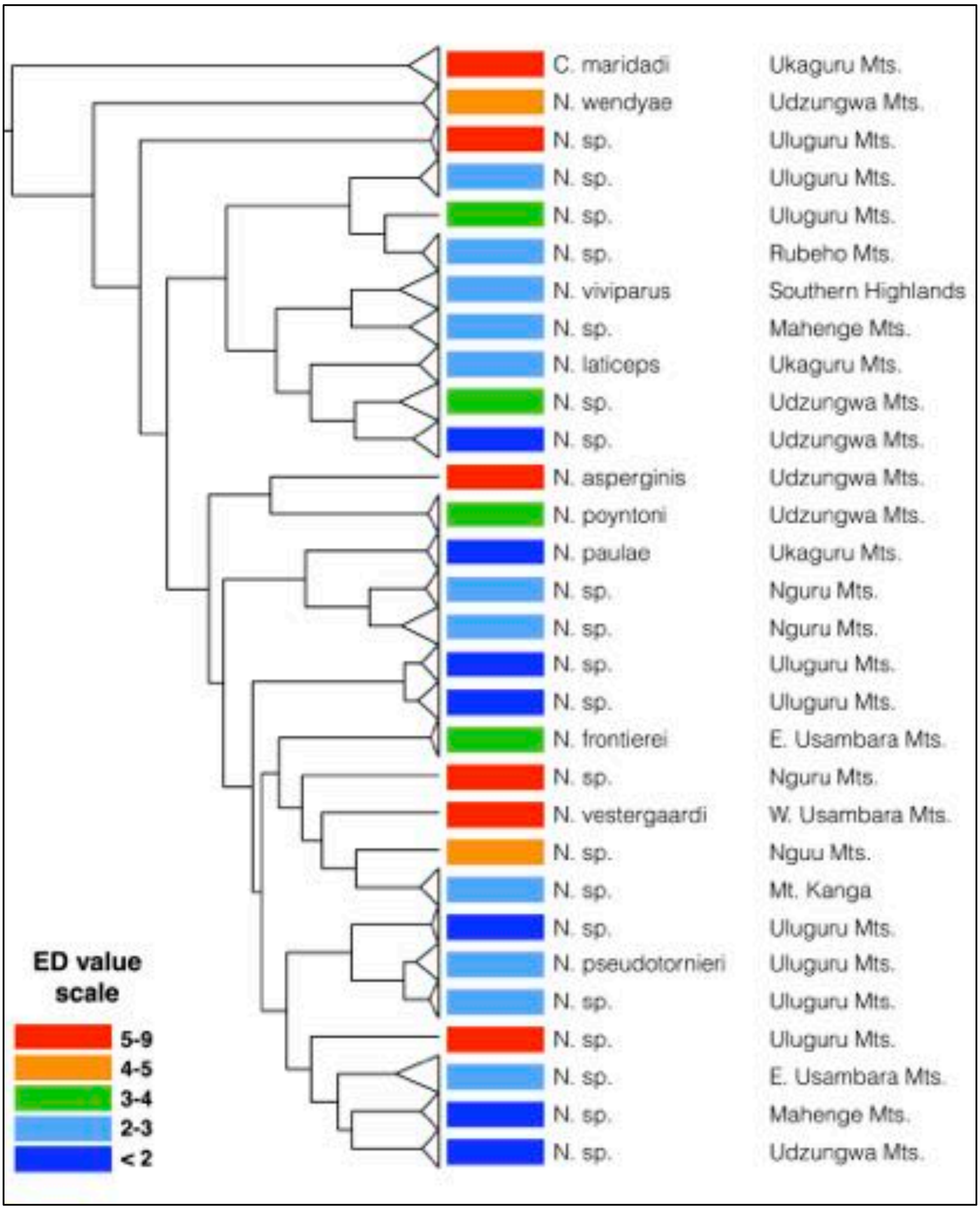

Figure 5-5 Conservation priority per species according to ED (evolutionary distinctiveness), mapped on phylogenetic trees of *Nectophrynoides* and *Churamiti*. Warmer colours represent higher priority.

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Chapter 6 : Conclusions and future research

Conclusions

The main aim of this PhD was to investigate historical and current biogeography of the herpetofauna of the Eastern Afromontane, describes pattern of species richness and endemism across the region and identify some of the main environmental drivers. Focused analyses on a genus of forest vipers and on dwarf forest bufonids were carried out in order to understand elements of evolutionary history of the herpetofauna across the region and to prioritize sites for conservation.

Chapter 2 documented the past and present of herpetological discovery in the Eastern Afromontane region and identified priority areas where future research could lead to significant herpetofaunal discoveries. The chapter described and summarized the history of herpetological contributions in the last 150 years and more recently since 1998 and the present, chronicle discoveries of 101 new species at 39 East African sites. It then discussed how different taxonomic approaches and the use of biodiversity indexes have had important implications for our understanding of biological values of areas and lead to differences in site prioritization. Finally, it introduced a work-in-progress to publish an e-guide on the region's amphibians and reptiles, which will aid inventory and ecological work across the region.

Chapter 3 investigated patterns of species richness and endemism of mountain-restricted amphibians across the Eastern Afromontane region, and explored relationships between species richness/endemism in the different mountain ranges and environmental predictors, with EVI and minimum altitude of surrounding land, being different across mountain ranges and pointing to scenario where different mountain ranges appear to have accumulated high amphibian diversity in different ways. It also examined zoogeographical partitions through the use of dissimilarity cluster analyses and the results did not support the idea that the Eastern Afromontane is a discrete zoogeographic region, rather what holds it together is not a sharing of species, but a common dynamism in the spatial and altitudinal turnover of taxa. Finally, it ranks sites according to importance in terms of taxon richness and endemism and highlight their protection status.

Chapter 4 studied the phylogenetic relationships and historical biogeography of the East African vipers in the genus *Atheris* and explored temporal and spatial relationships between the different species species across Africa. It then investigated the impact of palaeoclimatic fluctuations and tectonic movements on the cladogenesis of the genus. The reconstructed phylogeny of East African species of *Atheris* showed congruent temporal patterns that link diversification to major tectonic and aridification events within East Africa over the last 15 million years. This points to a diversification pattern of the East African species consistent with a scenario of a delayed direct West-East colonization of the Eastern Arc Mountains, influenced by the formation of the western rift. It then identifies possible cryptic taxa in the *Atheris rungweensis* and *A. ceratophora* clades.

Chapter 5 focused on the radiations and evolution of forest-associated toad in the genus *Nectophyrnoides,* through phylogenetic reconstruction, finding that the Eastern Arc Mountains are characterized by the presence of both very ancient paleo-endemic species, along with more recent radiations. The chapter then identified key conservation sites using different importance metrics. These included species and endemic richness, phylogenetic diversity/endemism, and evolutionary distinctiveness. There were strong correlations between the different measures, but some sites with low species richness contained one or two distinctive taxa characterized by long, unique evolutionary history that increased their importance in terms of site-specific evolutionary distinctiveness. Congruence between richness of *Nectophrynoides* and amphibians as a whole was strongly positive, suggesting that this now better known and conspicuous group could act as a proxy for assessing relative importance of sites for amphibians generally. A high proportion of *Nectophrynoides* are ‘Endangered’ or ‘Critically Endangered’ and the number of threatened species will rise steeply under the new taxonomic assessment. The high spatial and elevational turnover of *Nectophrynoides* species indicates the importance of conserving forest at all altitudes and across the entire fragments, in order to address the differences that exist between sites at different altitudes within the same fragment and at similar altitudes in different fragments on the same mountain block. An appropriated conservation strategy should aim to preserve forest habitat both along the entire elevational gradient as well fragments in geographically complex terrains, where isolated populations and therefore potentially new species may exist.

How hot is the Eastern Afromontane biodiversity hotspot for herps?

During the last two decades, the forest area in Africa has decreased by an estimated 34-41,000 km^2 per year (FAO, 2010). Tropical moist forests are amongst the most species-rich terrestrial habitats on Earth, making deforestation a crucial issue for biodiversity conservation (Joppa & Pfaff, 2011; Laurance et al., 2012b) and a recent study by Laurance et al. (2012) showed that half of all the protected areas that have been established to counteract the current loss are experiencing a loss of biodiversity that is alarmingly widespread both in terms of taxonomic and functional diversity (Laurance et al., 2012). African rainforests have been dubbed the 'odd man out' because of their lower species richness when compared with the other two main rainforest areas of the planet, the Neotropics and South East Asia (Richard, 1973; Couvreur, 2014). Despite being still understudied, the 'odd man out' pattern in tropical plants of the African rainforests is supposed to be due to macro-scale climate differences between the three regions (Parmentier et al., 2007) and, especially in East Africa, by the tectonic turbulence that has characterized the history of the African continent across the last 30 million years (Chorowicz, 2005). The same climatic instability that has led to generally low plant richness seems to have produced similar results in the current pattern of amphibian and reptile species (Loader et al., 2014). In fact, the overall herpetofaunal richness of east Africa in not comparable in absolute numbers to its Neotropical or Asian counterparts, but it does have a remarkable level of species richness almost entirely packed in small, scattered, highly stable fragments of ancient forests, resulting in a 'density' of vertebrate endemism among the highest on Earth (Meyer, 2000). In fact, East Africa contains one of the highest known concentrations of endemic plants and vertebrates on Earth (Myers et al. 2000, Plumptre et al. 2007, Rovero et al. 2014). Hypotheses explaining the extraordinary biodiversity of East African mountains generally point to post-Oligocene climatic shifts as triggers for lineage diversification in the presence of refugia, with major lineage diversification in the Pleistocene (Bowie et al., 2006; Couvreur et al., 2008). The system of montane forest fragments of the Eastern Arc Mountains of Tanzania alone is particularly hot, it collectively covers about 5000 km2 only (Platts et al., 2010) of forest arranged in small to very small fragments (median fragment size of 10.0 km2, and a mean fragment size is. 58.0 km2; (Newmark, 1998) and it harbours more than half of the mountain-restricted amphibian species of Africa (http://amphibiaweb.org, this work).

The current extent of forest cover across the Eastern Afromontane is 11% of the estimated original cover (CEPF, 2012) and is the result of thousands of years of human pressure (Green et al., 2013). But it is only in the last 300 years that the Eastern Arc has suffered an estimated 80% total loss in forest area, and has lost 25% of forest area since 1955 (Hall et al., 2009), with an additional 5% loss (210 km^2) of evergreen forest between 1975 and 2000 (Green et al., 2013). The current situation is exacerbated by the strong positive correlations between human density and both species richness, and endemism across the tropical African mountain ranges (Burgess et al., 2007). Despite the recognized importance of the Eastern Afromontane biodiversity value and more than a century of biological investigations, the region is still poorly studied for various taxa, and new species are frequently discovered and described (Rovero et al., 2014). The recent, sharp increase in species description boosted by new DNA-based approaches for delimiting species (Ceccarelli et al., 2014), reflects the general underestimation of biodiversity in this region (Loader et al., 2011a; Menegon et al., 2011a; Loader et al., 2014; Ceccarelli et al., 2014).

Why is it this hot?

Dwarf forest bufonids of the Eastern Arc Mountains are emblematic of the endemic amphibian fauna across the entire Eastern Afromontane as they are the kind of species that make the EA hotpot so hot. In fact, not a single species occurs at more than one site, and most had known ranges smaller than 100 km^2 (this study). In most sites, both ancient lineages and more recently speciated taxa co-occur and more than half of the currently known species are formally undescribed. A similar situation is shared by many other groups of amphibians and reptiles and, more broadly, forest-associated vertebrates (Rovero et al., 2014) and contributes to the general pattern of species richness and endemism of the area (Menegon et al., 2008, Menegon et al., 2011, Loader et al., 2014). The combination of the age of the forest fragment, their geographic and ecological isolation and the habitat stability and persistence (Loader et al., 2014), in a general context characterized by pronounced climatic and geological turbulence (Lovett & Wasser, 1993) makes the vast complex of forest fragments an ancient and stable archipelago of individually evolving sites, especially for the less vagile organisms (Ceccarelli et al., 2014, Loader et al., 2014). The extremely high levels of amphibian richness and endemism of some areas across the region, is almost certainly due to the exceptional level of species turnover both between mountains, between forest fragments and between areas of the same forests fragment (Linder et al., 2012, this study). While differences in diversity across areas with comparable habitat stability, could relate to differences in their amount of available energy and degree of insularization, driven by the topographic complexity within individual mountain ranges, especially when lowland barriers between peaks are present.

How to maintain the current level of herpetological biodiversity?

Biodiversity conservation is proven not to be an easy task (Marchese, 2015) and there are serious concerns about the effectiveness of existing strategies for its protection. Since it was introduced by Myers and colleagues (2000), the concept of hotspots was used as a key strategy for global conservation action, attracting over $1 billion in conservation investment (Sloan et al., 2014) and is based on the argument that biodiversity conservation requires prioritization to be effective, because funds are limited and must be allocated carefully (Myers, 2003). Several criticisms have been made of the hotspot concept, especially because ignoring coldspots could mean the loss of large, natural, and ecologically important areas that contribute to many ecosystem services (Dalton, 2000; Kareiva & Marvier, 2003).

The Eastern Afromontane is both an area of many range-restricted and threatened species, and lineages that have persisted for millions of years in spite of dramatic shifts in global climate (Siu-Ting et al., 2014; Loader et al., 2014). It represents the most important area of mainland Africa for conservation of amphibians and reptiles and it offers an extraordinary conservation challenge to face. Because of the extreme species turnover across mountain ranges and individual forest fragments (Menegon et al., 2008; 2011; Loader et al., 2011; Gower et al., 2013), a small number of protected areas, however well-managed and resourced, will never fully capture the diversity of the region's amphibians. Thus, a specific strategy aimed at identification and implementation of conservation initiatives at forest fragment scale must be considered. Across the Eastern Afromontane, very few protected areas, besides National Parks, are suitably resourced, resulting in very weak levels of protection (Plumptre et al., 2007; Hall et al., 2009; Rovero et al., 2012; Laurance et al., 2012; Gower et al., 2013). Thus, success in maintaining long-term amphibian diversity is linked not just to protected area legal status, which is proven not to be sufficient (Laurance et al., 2012, Rovero et al., 2012) but to a situation where protected areas are well-resourced, managed and monitored, with actual law enforcement, effective control of access, resource management, monitoring and evaluation activities and existence of annual work plans (Struhsaker et al., 2005; Dudley et al., 2007; Le Saout et al., 2013). These must include a plan aimed to restore connectivity between forest patches recently fragmented because of human impact.

The current situation is the result of million of years of evolution of life and at least 8000 years of human impact (Ellis, 2011), but is just in the last century that the human pressure have increased at an unprecedented rate with the majority of the biosphere transformed into intensively used anthromes (Ellis, 2011). Between 2015 and 2100, the populations of Burundi, Malawi, Uganda, United Republic of Tanzania and Zambia are projected to increase at least five-fold, making some parts of the Eastern Afromontane one of the most densely populated region of the world (United Nations, 2013). The projected increase in population will present an unprecedented challenge to habitat and species conservation across most of the Eastern Afromontane. Several studies in recent years have examined the impact of forecasted urbanization growth on biodiversity and found that the amount of urban land near protected areas and within biodiversity hotspots is expected to increase on average by more than three times and about four times respectively between the year 2000 and 2030 (Güneralp et al., 2013). As a reaction to a lucid assessment of global trends in population growth and associated habitat and species loss and increasing resource demands, there is an urgent need to try new conservation approaches (Kareiva et al., 2014). In particular the 'new conservation' approach assert that protected areas alone are unlikely to be sufficient to accomplish major conservation goals in the light of the current global rates of biodiversity loss and ecological systems changes, rather it promotes an active management of the surrounding matrix, aimed to minimize damage to biodiversity also for unprotected lands where resource extraction, agriculture, and other productive activities occur, by granting more attention to the environment outside protected areas (Kareiva et al., 2014).

In vast homogeneous areas like savannahs or extensive woodlands, where there are comparably fewer range restricted species, the impact of the forecasted urbanization and land conversion can be controlled and mitigated by the realization of a network of protected areas in order to assure the long-term survival of most valuable and threatened species and habitats. This can be achieved without necessarily excluding human activities as with the Natura 2000 network in Europe (Gantioler et al., 2010). On the other hand, in the case of the forest network of the Eastern Afromontane, where an exceptionally high amount of biodiversity is packed in a system of small, isolated forest fragments characterized by high ratio of locally endemic species, a totally different approach should be taken into consideration. This should be aimed at ensuring full protection of all the forest fragments that collectively represent a very small proportion of the productive land across the entire area (CEPF, 2012).

Over the last 25 years, the number of protected areas has grown exponentially, especially in developing countries where most of the biodiversity is present with a significant shift in their mission which has moved from biodiversity conservation alone to allowing local resource use in order to improve human welfare (Naughton-Treves et al., 2005). Despite this tendency, high biodiversity forest fragments across the Eastern Afromontane are of extreme biological value at global scale and represent a tiny fraction of the total available land, often in poorly exploitable situation, they should be granted full protection, without permission of any human activity within their boundaries other than collection of medicinal plants, scientific research and monitoring. The surrounding matrix, where productive activities and urbanization take place, needs a long-term strategic plan aimed ensure connectivity between protected areas and, at the same time, livelihoods for the human population by improving land use efficiency, restore ecological heterogeneity and sustain biodiversity in agricultural systems through the development of appropriate policy frame-works and management solutions (Benton et al., 2003). Given the forecasted human population growth in the Eastern Afromontane area in the coming decades and the related increase of human pressure on natural environment, a more broad conservation strategy should include:

- Full and effective protection to the already gazetted protected areas.
- Identification and provision of legal status to all the high biodiversity fragments not yet included in the protected area network.
- Development of appropriate policy frameworks aimed to act on the surrounding agricultural and urbanized matrix, in order to dissipate the increasing pressure due to the forecasted population growth and to sustain higher level of biodiversity outside protected areas.
- Promotion, when possible, of the participation of the private sector in forest protection, through long-term leases of forest fragments as part of the caveats included in land concessions granted for productive activities.

The conservation of the extraordinary amount of biodiversity packed into the small, scattered forest fragments of the Eastern Afromontane is something that Eastern African countries cannot afford *not* to conserve, because of the relatively small amount of land where it is encased and because the lost biological values will never be replaced.

Future researches and directions

In the last two decades across the Eastern Afromontane, there has been a boost of biodiversity studies with a sharp increase in species description that reflects the general underestimation of biodiversity in this region on one hand and represent significant advancement in geographic sampling and on the effectiveness of new methods for delimiting species (Dimitrov et al., 2012; Ceccarelli et al., 2014) on the other. The use of phylogenetic reconstruction has boosted the recognition of description of cryptic species, helping in the assessment of the actual biological value of areas. The increasing availability of genetic information on taxa should promote the use of phylogenetic indexes in order to move from a conservation approach solely based on species richness to a more inclusive one. This would utilize the extensive information provided by the use of phylogenetic diversity metrics, and can inform conservation on the underlying functional diversity and evolutionary potential both at species and site level (Winter et al., 2012).

Compared to the Neotropics and South East Asia, where the increase in geographic inventories, taxonomic and phylogenetic works has been accompanied by an increasing number of ecological studies (Duellman and Trueb, 1986, Wells, 2007), these are still almost completely missing for the forest associated African amphibian and reptiles species (Howell, 2000). Detailed ecological studies are essential in assessing forest-associated amphibians and reptiles tolerance to various threats including habitat alteration, climate change and emerging diseases (Hopkins, 2007) and, along with the need of complete inventories for still overlooked areas, is the area of research that most needs a boost. If the use of amphibians as models in ecological research has a remarkable history because of their sensitivity to environmental changes (Hopkins, 2007), the use of East African species as subjects for ecological studies aimed to both address their needs in terms of conservation actions and as models for studying more broad applied ecological issues such as habitat loss, pollution, disease, and global climate change, has yet to be initiated. Ecological studies usually require long-term commitments and the remoteness of many areas coupled with the shortage of appropriated facilities *in situ*, could be one of the limiting factors. A possible solution would be the establishment of field stations in relevant areas, which can provide the needed facilities for long-term ecological field studies and would promote the building of conservation capacity through participation of targeted local scientists, with tailored training in order to provide them the skills, inspiration and confidence needed. The need for conservation career development in emerging countries is also considered a crucial step forward both in the Convention on Biological Diversity, in the Kyoto Protocol and the Millennium Development Goals.

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The genus *Atheris* (Serpentes: Viperidae) in East Africa: Phylogeny and the role of rifting and climate in shaping the current pattern of species diversity

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ABSTRACT

Past climatic and tectonic events are believed to have strongly influenced species diversity in the Eastern Afromontane Biodiversity Hotspot. We investigated the phylogenetic relationships and historical biogeography of the East African genus *Atheris* (Serpentes: Viperidae), and explored temporal and spatial relationships between *Atheris* species across Africa, and the impact of palaeoclimatic fluctuations and tectonic movements on cladogenesis of the genus. Using mitochondrial sequence data, the phylogeny of East African species of *Atheris* shows congruent temporal patterns that link diversification to major tectonic and aridification events within East Africa over the last 15 million years (my). Our results are consistent with a scenario of a delayed direct west–east colonisation of the Eastern Arc Mountains of *Atheris* by the formation of the western rift. Based on the phylogenetic patterns, this terrestrial, forest-associated genus has dispersed into East Africa across a divided route, on both west-southeasterly and west-northeasterly directions (a C-shaped route). Cladogenesis in the Eastern Arc Mountains and Southern Highlands of Tanzania corresponds to late Miocene and Plio-Pleistocene climatic shifts. Taxonomically, our data confirmed the monophyly of *Atheris* as currently defined, and reveal four major East African clades, three of which occur in discrete mountain ranges. Possible cryptic taxa are identified in the *Atheris rungweensis* and *A. ceratophora* clades.

1. Introduction

The contemporary pattern of species diversity and distribution in African forests is thought to have been influenced by a combination of past climatic events, tectonic movements, geological activities and stochastic dispersal events (see Moritz et al., 2000 for a review of speciation models and Kingdon, 2013 for speciation in Africa). The influence of these geographic events are expected to leave 'signatures' in the patterns of diversification in extant lineages of animals and plants. Recently, there have been several studies looking at contemporary species to reveal patterns of taxon diversification to reveal temporal patterns of forest dynamics in Africa (Bowie et al., 2006; Lawson, 2013; Blackburn and Measey, 2009; Loader et al., 2011). These studies have begun to clarify the importance of specific historical events in shaping Africa's fauna and flora.

The montane forests of East Africa, collectively called the Eastern Afromontane, are relicts of a past, more pan-African forest, which became fragmented during periods of climatic aridification and subsequent climatic fluctuations starting from the early Oligocene (Lovett, 1993; Lovett et al., 2000). The forested surface is thought to have been highly dynamic through the Pliocene and Pleistocene, with forests restricted to montane areas during warm dry periods, but expanding to lower elevations during cool, wet periods (Lovett et al., 2000). Underlying these changes in forest distribution are the geological changes that have occurred in the areas. Some regions are volcanic (old or more recent events), while others are much older and geologically stable (Griffiths, 1993) and still others (such as the Southern Highlands, are a combination of the two). Consequently, the region represents an interesting

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system for understanding the effects of abiotic (climate and geology) processes on forest-dependent biodiversity.

The viperid snake genus *Atheris* is an appropriate group for studying the biogeography of the pan-African forest and the role of plate tectonics and mountains in shaping the current pattern of species diversity and distribution. The genus is distributed across most of tropical Africa, with a number of endemic species in montane regions e.g. Branch and Bayliss, 2009; Menegon et al., 2011). Of the 15 recognised species, only one (*Atheris squamigera* Hallowell, 1854) has a particularly wide distribution ranging from Nigeria in the west, across the Congolian forest belt to western Kenya in the east and Angola in the south (Phelps, 2010). All other *Atheris* species have small known ranges. For example, *A. desaixi* is found in just two localities in the Kenyan highlands, *A. mabuensis* in two mountain blocks in Mozambique, *A. acuminata* is known for a single locality in Uganda and the newly described *A. matildae* in a single forest fragment in the Southern Highlands of Tanzania (Ashe, 1968; Branch and Bayliss, 2009; Menegon et al., 2011). Current *Atheris* distribution includes areas that have been suggested to be forest refugia during the last glacial maxima. These refugia include forests from west of the Dahomey gap, across the Guineo–Congolian rainforest to the Eastern Afromontane in the east, and southward into central Mozambique. In the west, *Atheris* species generally occur at low or medium altitude (Chirio and LeBreton, 2007; Phelps, 2010; Penner et al., 2013) while in the east, there are ten species that are restricted to specific mountainous regions or isolated mountain blocks in the Eastern Afromontane Ecosystem (Menegon et al., 2011).

Using a reconstructed phylogeny, we aim to address a number of hypotheses concerning the taxonomy and biogeography of *Atheris*. First, we assess the taxonomic status of species using genetic samples from species across the genus' range. Second, we use molecular clock estimates to investigate whether the timing of the divergence within this clade corresponds to specific geographic events. As a complement to the time-tree, we use ancestral reconstructions of areas to examine spatial relationships within the genus and how they are related to timing and origin of clades. We hypothesize that the forest viper genus *Atheris*, distributed in both west and east African forests, will show a west–east discontinuity. This pattern would link to the initial fragmentation of the pan-African forest, and subsequent cladogenetic events that correspond to the major geological or climatic event changes in each region.

2. Material and methods

2.1. Sampling and DNA extraction

Samples of *Atheris* species were collected in the field and from preserved material in several museums (Table 1). Total genomic DNA was extracted from 29 tissue samples using QIAamp DNA Mini Kit (Qiagen, Hombrechtikon, Switzerland) following the manufacturer's protocol. Additionally, all cytochrome b (*cytb*) and NADH dehydrogenase sub-unit 4 (*ND4*) sequences of *Atheris* species available on GenBank were included in the dataset (Table 1).

2.2. Genetic analyses

Fragments of the *cytb* were amplified by Polymerase Chain Reaction (PCR) using the primers L14724Vb and H115914Vb (Ursenbacher et al., 2006). For some problematic samples the amplification of the *cytb* was conducted in two parts, using internal primers (first set: L14724Vb and H15548Vb; second set: L15162Vb and H15914Vb). Additionally, a portion of the ND4 was amplified using the primers *ND4* and LEU described by (Arevalo et al., 1994). All amplifications were conducted in 30 μl volumes with 2–4 μl of DNA template (depending on the DNA concentration), 1xPCR buffer (Qiagen), 2 mg/ml of Q solution (Qiagen), 2 mM for the *cytb* and 3 mM for the ND4 of $MgCl_2$, 0.2 mM dNTPs, 0.5 μM of each primer and 0.5 units of Taq polymerase (Qiagen). Amplification conditions consisted of 37–45 cycles of denaturation for 45 s at 94 °C, annealing for 60 s at 50 °C and extension for 90 s at 72 °C conducted with a Eppendorf MasterCycler (Eppendorf, Schönenbuch/Basel, Switzerland). PCR products and sequencing were outsourced to Macrogen Inc. (Seoul, Korea). Sequences were deposited in GenBank (see Table 1).

2.3. Phylogenetic reconstruction

The sequences were checked and edited using CODONCODE ALIGNER v4.02 (CodonCode Corporation, Dedham, US). The mtDNA sequence of Daboia siamensis from GenBank (NC_011391) was included in the dataset as outgroup. For both genes, separate alignments were conducted using CLUSTALX v2.0 (Thompson et al., 1997). We conducted a partition homogeneity test with PAUP* v4.10 (Swofford, 2002) using 1000 replicates in order to determine whether both regions could be combined in a unique data matrix (Farris et al., 1995). For all *Atheris* haplotypes, the numbers of base differences per site were calculated from the net average between groups of sequences. Analyses were conducted in MEGA v5.1 (Tamura et al., 2011). The model of sequence evolution was selected with the software JMODELTEST v2.1.1 (Darriba et al., 2012). Maximum likelihood (ML) estimates of the phylogeny were calculated using PHYML v3.0 (Guindon and Gascue, 2003) using the previously selected model. Maximum parsimony (MP) analyses were performed using PAUP* with heuristic searches and random stepwise addition, and the robustness of MP reconstructions were tested with bootstrap resampling (1000 repetitions). Additionally, Bayesian inference analysis was performed in MRBAYES v3.2 (Huelsenbeck and Ronquist, 2001) using the GTR + I + G substitution model with four chains of 1×10^6 generations, and sampling performed every 100 generations. After controlling the log values with TRACER 1.5 (Rambaut and Drummond, 2003), the first 10% was discarded as burn-in and the analysis was conducted twice and compared to avoid local optima (Huelsenbeck and Imennov, 2002).

2.4. Divergence time estimation

To estimate the divergence time between each clade without any molecular clock constraint, a Bayesian approach with a MCMC integration was used to date the most recent common ancestor (MRCA) using BEAST 1.7.4 (Drummond et al., 2002; Drummond and Rambaut, 2003). The analysis was run for 20 millions generations, with sampling each 1000 generations and a 10% burn-in. A Yule branching process with a log-normal relaxed clock for estimating posterior probability density of divergence time was implemented to perform the simulations. Due to the lack of calibration points within the *Atheris* genus, GenBank sequences of *Sphenodon punctatus*, four saurians, five primitive snakes, two Elapidae, three Colubridae, 17 Crotalinae and 19 Viperinae were added to this analysis (see Table 1 for details). The complete alignment regrouping the 81 specimens was conducted using CLUSTALX for each gene separately, and finally combined. The calibration points were the following: (1) fossil evidence suggests that the divergence between saurians and snakes occurred 130–150 million years ago (mya; Carroll, 1988). We used a normal prior with a mean set at 140 mya and a standard deviation of 6 mya (95% CI: 130–150 my); (2) the oldest Viperidae is known from western Europe in the lowermost Miocene (23.8 mya; Szyndlar and Rage, 1999). A log-normal prior with a zero offset of 23.8 my, with a log-normal

Table 1
Details of the samples analyzed, with locality and GenBank accession numbers.

Taxon	Voucher	Location	Country	*cytb*	ND4
Atheris barbouri	MTSN 9177	Bomalang'ombe	Tanzania	×	×
Atheris barbouri	MTSN 7299	Madehani FR	Tanzania	×	×
Atheris barbouri	MTSN 7311	New Dabaga FR	Tanzania	×	×
Atheris barbouri	MTSN 7399	Livingstone Mountains	Tanzania	×	×
Atheris barbouri	MTSN 7400	Livingstone Mountains	Tanzania	×	×
Atheris ceratophora	KMH 35468	Kanga FR	Tanzania	×	×
Atheris ceratophora	AJ275682	Usambara Mts.	Tanzania	AJ275682	–
Atheris ceratophora	CAS 168976	West Usambara Mts.	Tanzania	×	×
Atheris ceratophora	MNHG 2667.45	East Usambara Mts.	Tanzania	JF825388	–
Atheris cf. ceratophora	MTSN 8874	Kigogo FR	Tanzania	×	×
Atheris ceratophora	MTSN 5032	Udzungwa Mts.	Tanzania	×	×
Atheris ceratophora	MTSN 5033	Udzungwa Mts.	Tanzania	×	×
Atheris ceratophora	MTSN 7558	Udzungwa Mts.	Tanzania	×	×
Atheris ceratophora	MNHG 2667.45	Usambara Mts.	Tanzania	×	×
Atheris ceratophora	MTSN 8947	Udzungwa Mts.	Tanzania	×	×
Atheris ceratophora	DQ305456	Unknown	Unknown	DQ305456	DQ305474
Atheris chlorechis	Isolate 1579	Unknown	Unknown	–	EU624211
Atheris chlorechis	AT819	Nimba County	Liberia	×	×
Atheris chlorechis	AT820	Nimba County	Liberia	×	×
Atheris chlorechis	AJ275679		Togo	AJ275679	–
Atheris desaixi	AJ275680	Mt. Kenya	Kenya	AJ275680	–
Atheris hispida	AJ275681	Kakamega Forest	Kenya	AJ275681	–
Atheris mabuensis	AT821	Mount Mabu	Mozambique	–	×
Atheris mabuensis	AT822	Mount Mabu	Mozambique	×	×
Atheris matildae	MTSN 7570	Southern Highlands	Tanzania	–	×
Atheris matildae	MTSN 9344	Southern Highlands	Tanzania	×	×
Atheris nitschei	T1032	Nyungwe Forest NP	Rwanda	×	×
Atheris nitschei	AJ275683		Rwanda	AJ275683	–
Atheris nitschei	CAS 201708	Kabale District	Uganda	AF471070	–
Atheris rungweensis	MB121	Mount Rungwe	Tanzania	×	×
Atheris rungweensis	MTSN 7569	Mbizi FR	Tanzania	–	×
Atheris rungweensis	MTSN 7312	Mount Rungwe	Tanzania	×	×
Atheris rungweensis	AY223557		Tanzania	AY223557	AY223618
Atheris rungweensis	SHCP 03-R-24	Mt. Rungwe	Tanzania	×	×
Atheris rungweensis	SHCP 07-R-02	Nkasi/Ntantwa	Tanzania	×	×
Atheris rungweensis	SHCP-07-R-02	Nkasi/Ntantwa	Tanzania	×	×
Atheris squamigera	AJ275684	Unknown	Togo	AJ275684	–
Atheris squamigera	EU624303	Unknown	Tanzania	EU624303	EU624212
Daboia russelii				NC011391	
Sphenodon punctatus				NC 004815	
Sceloporus occidentalis				NC 005960	
Pogona vitticeps				NC 006922	
Varanus salvator				NC 010974	
Lacerta viridis				NC 008328	
Acrochordus granulatus				NC 007400	
Boa constictor				NC 007398	
Python molurus				NC 015812	
Ramphotyphlops braminus				NC 010196	
Typhlops reticulatus				NC 010971	
Naja naja				NC 010225	
Micrurus fulvius				NC 013481	
Dinodon semicarinatus				NC 001945	
Hypsiglena torquata				NC 013992	
Enydris plumbea				NC 010200	
Agkistrodon piscivorus				NC 009768	
Atropoides olmec				*AY223585*	*AY223632*
Bothrops asper				*EU624301*	*EU624210*
Crotalus horridus				NC 014400	
Crotalus ravus				*AY223609*	*AY223647*
Gloydius brevicaudus				NC 011390	
Hypnale hypnale				*AY352750*	*AY352812*
Lachesis muta				*AY223604*	*AY223644*
Ovophis okinavensis				NC 007397	
Porthidium arcosae				*AF292575*	*AF292613*
Porthidium lansbergii				*AY713375*	*AF393623*
Porthidium nasutum			*Costa-Rica*	*AY223579*	*U41887*
Porthidium ophryomegas				*AY223580*	*U41888*
Porthidium nasutum			*Ecuador*	*AF292574*	*AF292612*
Sistrurus catenatus				*AY223610*	*AY223648*
Sistrurus miliarius				*AY223611*	*U41889*
Viridovipera stejnegeri				NC 012146	
Bitis arietans				*EU624304*	*EU624213*
Bitis nasicornis				*DQ305457*	*DQ305475*
Bitis peringueyi				*DQ305458*	*DQ305476*

Table 1 (*continued*)

Taxon	Voucher	Location	Country	*cytb*	ND4
Bitis worthingtoni				*AJ275692*	*EU624220*
Bitis xeropaga				*EU624307*	*EU624221*
Causus defilippi				NC 013479	
Cerastes cerastes				*EU624308*	*EU624222*
Daboia mauritanica				*EU624313*	*EU624229*
Echis ocellatus				*EU624311*	*EU624225*
Eristicophis macmahonii				*AJ275711*	*EU624227*
Macrovipera albizona				*AJ275727*	*EU624231*
Macrovipera lebetina			*Turkey*	×	×
Protoatheris superciliaris				*AJ275685*	*EU624230*
Vipera ammodytes			*Serbia & Montenegro*	×	×
Vipera aspis			*France*	×	×
Vipera berus			*Switzerland*	×	×
Vipera latastei			*Spain*	×	×
Vipera ursinii			*France*	×	×
Vipera monticola			*Morocco*	×	×

mean of 2 and standard deviation of 1 (95% CI: 25–62 my) was set up; (3) The oldest Crotalinae fossil (the sister taxa of the Viperinae) was present in sediments of the early Miocene (17.8 mya) (Holman, 1981). a log-normal prior was consequently used, with a zero offset of 17.8 my, a log-normal mean of 2 (SD = 1; 95% CI: 19–56 my); (4) referring to the first fossil vertebra of Sistrurus found (Parmley and Holman, 2007), the divergence between the genus Crotalus and Sistrurus can be estimated to be older than 9 mya. We implemented a log-normal prior with a zero offset of 9 my, a lognormal mean of 1 (SD = 1; 95%CI: 9.5–23.1 my); (5) The first fossil observed of the 'Vipera berus' group was dated to the Late Miocene (MN12, 7.5–6.8 mya; Szyndlar and Rage, 2002). Thus, we implemented a lognormal prior with a zero offset of 7 my, a lognormal mean of 1 (SD = 1;95% CI: 7.5–21.1my); (6) we considered that the split between *V. latastei* from Spain and *V. monticola*, inhabiting the Atlas Mountains (Morocco) and formerly recognized as a subspecies of *V. latastei* (Beerli et al., 1996; Saint Girons, 1953), predate the Messinian salinity crisis about 5.3–5.6 mya, (Krijgsman et al., 1999) as demonstrated by Velon-Antón et al. (2012). We applied a log-normal prior with a zero offset of 5.4 my, a lognormal mean of 1 (SD = 1;95% CI: 5.9–19.5 my); (7) finally, as the most recent calibration point, we used the invasion of South America by the snake genus Porthidium after the uplift of the Isthmus of Panamá about 3.5 mya (Wüster et al., 2002). We consequently modelled the divergence between Central American and Southern American Porthidium species with a normal distribution with a mean of 3.5 my (SD = 0.61; 95% CI: 2.5–4.5 mya). All the time intervals are according to the cited publications.

2.5. Inference of ancestral area

Multiple ancestral area analyses were carried out to infer the geographic origins of the genus *Atheris*, and to establish main events and direction of species radiation. Dispersal–extinction–cladogenesis (DEC) analysis and Bayesian binary MCMC (BBM) in RASP 2.1 (Ronquist, 1997; Yu et al., 2013) using the ultrametric tree generated in BEAST. The analyses were run under the JC69 model for 2,000,000 generations, with ten chains, a sampling frequency every 100 generations and a 10% burn-in.

3. Results

3.1. Phylogeny and dating

Our phylogeny reconstructs evolutionary relationships among *Atheris* representing ten of the 15 known species. The haplotype analysis involved 22 unique nucleotide sequences. The analysis of 1084 bp of the *cytb* revealed 362 (33.4%) variable sites of which 261 (24.1%) were phylogenetically informative under MP criteria. For *ND4*, 248 (38.0%) sites were variable, of which 180 (25.6%) were phylogenetically informative.

The dataset of both genes were combined into one dataset for the phylogenetic analyses (partition homogeneity test; $p = 0.077$). The best model of substitution selected by jModelTest was TVM + I + G (freq. $A = 0.318$; freq. $C = 0.297$; freq. $G = 0.094$, freq. $T = 0.291$; $R(a) = 1.097$; $R(b) = R(e) = 18.31$; $R(c) = 2.346$; $R(d) = 0.983$; proportion of invariable sites = 0.550; gamma distribution shape parameter = 2.452). The heuristic parsimony analysis provided eight equally parsimonious trees of 1,133 steps (Consistency Index = 0.646; R Index = 0.813). ML, MP and Bayesian analyses produced generally similar topologies (Fig. 1). Marked genetic divergences were observed between all species. All representatives of *Atheris* species in the analysis were shown to be monophyletic with strong support and the analysis recovered five well-supported subclades within the main *Atheris* phylogeny (Fig. 1).

Differences between analyses were noted in the position of *A. mabuensis* from Mozambique, which is in a basal position and the sister to the rest of the genus in the ML analysis, whereas A. chlorechis is basal in the MP and BEAST analyses (MrBayes analysis did not support one or the other relationships) (Fig. 2). The West African A. chlorechis is sister to the larger clade representing the East African radiation of *Atheris*, within which three main clades are evident. Within this east African clade, a well-supported clade includes the Albertine Rift species A. rungweensis and A. nitschei, and the Kenyan endemic *A. desaixi*. *A. squamigera* and *A. hispida* are sister taxa, *A. barbouri* is sister to the clade that includes *A. matildae* and *A. ceratophora*. Uncorrected p-distances were estimated pairwise for all the species and these ranged from 3.2% (between *A. ceratophora* and *A. matildae*) to 14.8% (between *A. desaixi* and *A. barbouri*; Table 3).

3.2. Ancestral areas and current distribution

The most recent common ancestor (MRCA) for the *Atheris* genus was estimated to occur approximately 15 mya (95% CI: 11.2–19.6 mya; Table 2, Fig. 3). The Bayesian binary MCMC (BBM) analyses and dispersal–extinction–cladogenesis (DEC) results concerning the ancestral state of the genus are equivocal (Table 2) with low likelihood values both for a western or an eastern origin. *Atheris chlorechis* in the West, *A. desaixi* in Kenya and *A. mabuensis* in Mozambique, represent old lineages at the limits of a Miocenic early radiation, which have reached the current area of generic distribution between 15 and 11 mya (nodes A, B and C). Most of the

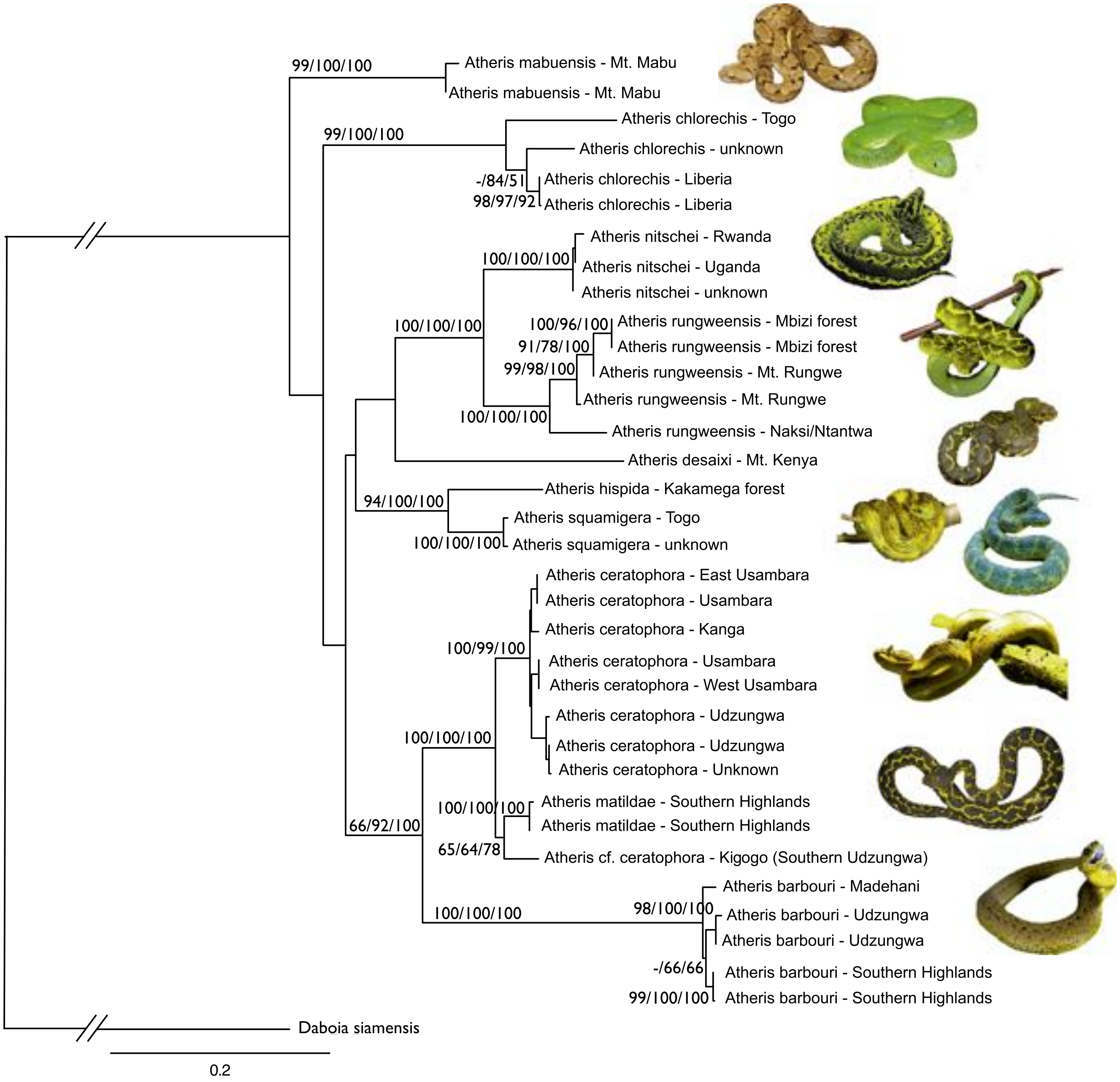

Fig. 1. Maximum likelihood tree with ML, MP bootstrap supports and Bayesian posterior probabilities (only when >50%).

subsequent lineages appear to have originated in the mountains of East Africa, with two major radiations from a common ancestor that inhabited the Albertine Rift around 11 mya. About 7.5 mya, the common ancestor of *A. barbouri*, *A. matildae* and *A. ceratophora* complex occurred in the Southern Highlands of Tanzania. Both clades have their ancestral area in the Southern Highlands and they gave origin to two highly morphologically diverging snake species. The ancestral area of the *A. ceratophora*/*matildae* complex, including the still unassigned lineage sister to *A. matildae*, is the Southern Highlands of Tanzania, where *A. matildae* occurs. The unassigned lineage, herein called *A. cf. ceratophora*, occurs in the forest fragments of the southern plateau of the Udzungwa Mountains. Interestingly, the MRCA within *A. ceratophora* sensu stricto seems to have originated in the Usambara Mountains, suggesting a pattern of multiple colonisations/extinctions of the Eastern Arc Mountains.

4. Discussion

4.1. Molecular phylogeny and taxonomic implications

In previous molecular phylogenetic analyses of Viperidae the number of nominate *Atheris* species sampled was particularly limited with only a single representative per species (Lenk, 2001; Wüster et al., 2008). Our phylogenetic reconstruction includes nine out of the ten known *Atheris* species occurring in East Africa, (for the single known specimen of *A. acuminata* DNA samples was not available). In many cases, several specimens from different localities for each species were sequenced, allowing a more thorough estimation of phylogenetic and geographic structure within these taxa. Our phylogenetic results extract five well supported main clades of *Atheris* species, consistent with geographic distribution (Fig. 1): (1) *Atheris mabuensis*, (2) a clade representing the Albertine

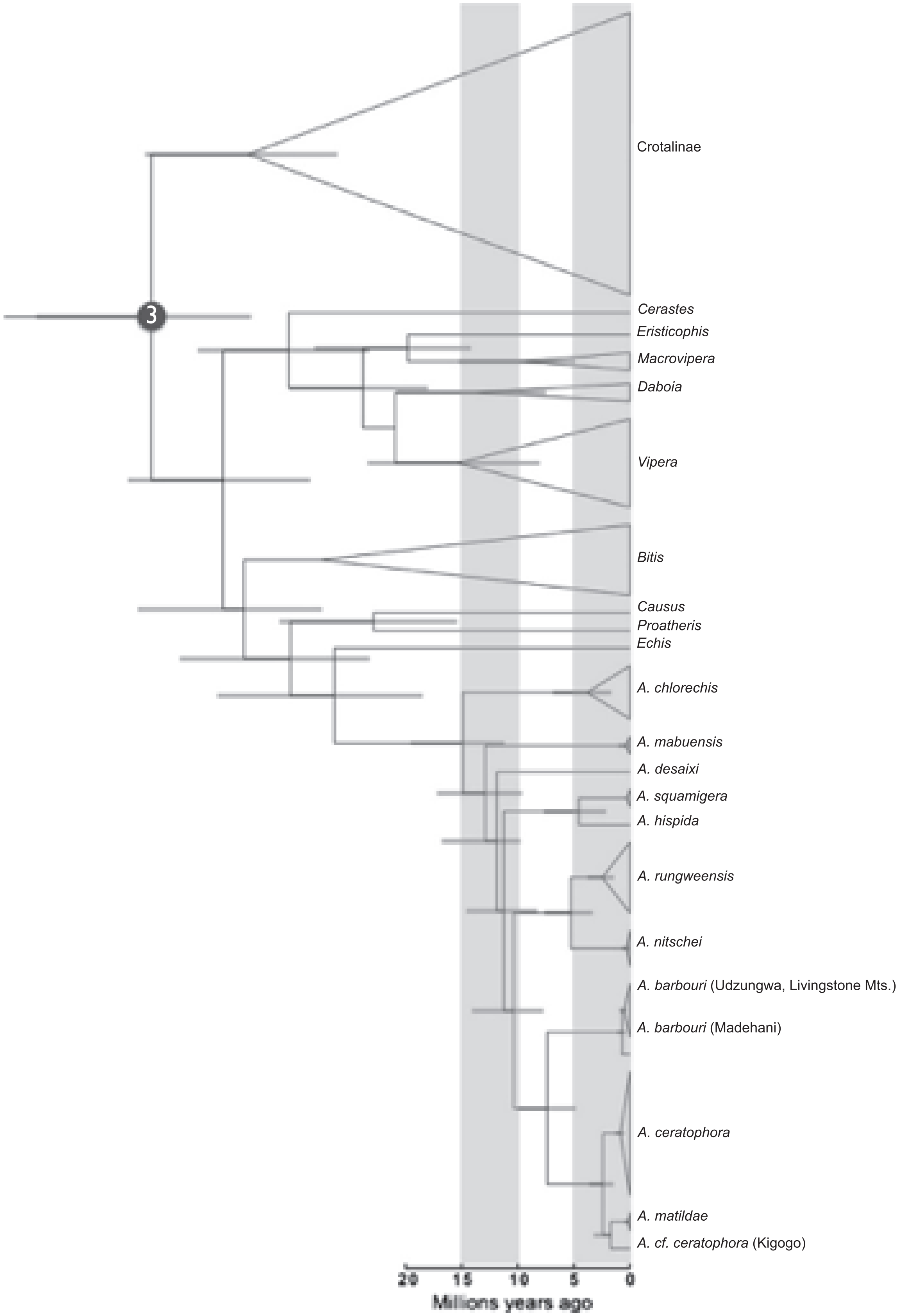

Fig. 2. BEAST (Drummond and Rambaut, 2003) maximum credibility ultrametric tree showing the timing of the evolution of the *Atheris* species and other Viperidae. Horizontal gray bars indicate 95% confidence intervals. Number 3 refers to the 3rd calibration point in the text. The other used calibration points fall within the collapsed clades of Crotalinae and *Vipera* and are not displayed in the figure.

Table 2
Estimated date and 95% confidence interval (CI) of main nodes (MRCA = Most recent common ancestor) with associated inferred ancestral area from the Bayesian binary MCMC (BBM) analysis. The node labels correspond to those in Fig. 3.

Node	Event	Estimated age in mya (95% CI)	Inferred ancestral area (ML value)
A	MRCA within the genus	15.1 (11.2–19.6)	West Africa (0.3) Mount Kenya (0.2)
B	Split between *A. desaixi* and the rest of the genus (without *A. chlorechis* and *A. mabuensis*)	12.6 (9.1–16.8)	Mount Kenya (0.53)
C	Split between *A. squamigera* + *A. hispida* group and the rest of East Africa species	11.4 (8.3–14.6)	Albertine Rift (0.73)
D	Split between the Albertine Rift species and the Eastern Arc species	10.8 (7.7–14.1)	Albertine Rift (0.65)
E	Split between *A. rungweensis* and *A. nitschei*	5.4 (3.3–7.6)	Albertine Rift (0.63)
F	Split between *A. squamigera* and *A. hispida*	4.8 (2.1–7.7)	Albertine Rift (0.89)
G	Split between *A. barbouri* and *A. ceratophora*/*A. matildae* group	7.4 (4.8–10.1)	Southern Highlands (0.94)
H	Split between *A. matildae* and *A. ceratophora*	2.5 (1.5–3.6)	Southern Highlands (0.9)
I	MRCA within *A. ceratophora*	0.9 (0.5–1.3)	East Usambara (0.45) Southern Highlands (0.35)
J	MRCA within *A. barbouri*	0.7 (0.4–1.1)	
K	MRCA within *A. rungweensis*	2.5 (1.4–3.8)	
L	MRCA within *A. chlorechis*	4.1 (1.7–6.9)	
M	MRCA within *A. matildae*	0.2 (0.0–0.5)	
N	MRCA within *A. nitschei*	0.3 (0.0–0.7)	

Table 3
Estimates of *p*-distance between major clades of *Atheris* species. The analysis involved 22 haplotypes of 1737 bp (combining parts of both cytochrome *b* and ND4 genes). Evolutionary analyses were conducted in MEGA v5.1 (Tamura et al., 2011).

	A. matildae	*A. squamigera*	*A. rungweensis*	*A. nitschei*	*A. chlorechis*	*A. mabuensis*	*A. barbouri*	*A. ceratophora*	*A. desaixi*
A. matildae									
A. squamigera	0.111								
A. rungweensis	0.094	0.110							
A. nitschei	0.109	0.119	0.056						
A. chlorechis	0.104	0.090	0.096	0.100					
A. mabuensis	0.116	0.108	0.098	0.109	0.089				
A. barbouri	0.105	0.133	0.127	0.128	0.124	0.137			
A. ceratophora	0.032	0.119	0.094	0.104	0.105	0.117	0.104		
A. desaixi	0.129	0.114	0.107	0.118	0.124	0.132	0.148	0.119	
A. hispida	0.122	0.054	0.109	0.121	0.094	0.107	0.145	0.121	0.116

Rift/Mt. Kenya radiation including *A. nitschei*, *A. rungweensis* (though *A. desaixi* position is not strongly supported) (3) The Albertine Rift/lowland forest clade including *A. hispida* and *A. squamigera* (4) *A. chlorechis* clade, and (5) a Tanzanian mountain radiation, including *A. barbouri*, *A. ceratophora* and *A. matildae*. The sister pairing of *A. squamigera* and *A. hispida* is consistent with previous phylogenetic reconstructions (Lenk, 2001; Wüster et al., 2008) and with morphological and immunological studies (Groombridge, 1987; Hermann and Joger, 1997). The recently discovered and geographically isolated *A. mabuensis* forms a clade separated from the rest of the East African species.

The analysis recovered two distinct clades among *Atheris rungweensis*, with each clade representing distinct geographic locations. The first clade includes samples from Southern montane forest localities, e.g. Nkuka forest on Mount Rungwe, and Mbizi forest on the Ufipa Plateau, and the second comprises a single sample from Central areas, Nkasi/Ntantwa, a more open area, at lower altitude with a different type of forest, close to Lake Rukwa, with scattered small forest fragments. The two clades have a p-distance 3.4% and deserve closer morphological analysis to see if they should be recognized taxonomically. The type locality for *Atheris rungweensis* corresponds to the areas sampled on Mount Rungwe, and therefore the Nkasi/Ntantwa would likely represent a new species if this population is shown to be distinct. Populations of *Atheris rungweensis* are also known both from Mahale and Gombe Stream National Parks, along the eastern shore of Lake Tanganyika (Menegon et al., 2011) but no sample from this region was successfully amplified.

Our phylogenetic analyses show that the Eastern Arc endemic *Atheris ceratophora*, displayed genetic differences across its range, with populations between Usambara, Kanga, and Udzungwa (Kigogo population excluded) showing variation in p-distance of 0.2–1.0%, the Udzungwa population being the most different. Within this clade, Emmrich (1997) found some differences in body scalation, for instance between Udzungwa and the other populations sampled. Thus, further analyses across this range are required to assess the taxonomic variability, but our genetic analyses suggest that the differences are likely not great enough to warrant taxonomic distinction. Interestingly, the morphologically distinct and newly described species *Atheris matildae* was regrouped with a population that, even if only based on a single specimen, seems morphologically similar to *Atheris ceratophora* (herein called *A. cf. ceratophora* from Kigogo on Figs. 1 and 2). Even if the genetic difference is lower between this single individual and *A. matildae* (p-distance: 2.4%) than with *A. ceratophora* (p-distance 2.9–3.1%), the support from our data is limited. We cannot be sure that this *A. cf. ceratophora* sample does not form the sister group to the Eastern Arc ceratophora clade – seemingly more in line with its general morphology. Further sampling, morphological and genetic analyses are required to resolve the grouping among these populations. However, regardless of its precise phylogenetic position, the Kigogo population appears to be distinct from all previously known taxa and consequently requires further morphological study.

Atheris barbouri, formerly placed in the genus Adenorhinos, showed limited genetic variation across its range, with populations in Udzungwa and Southern Highlands (principally southern

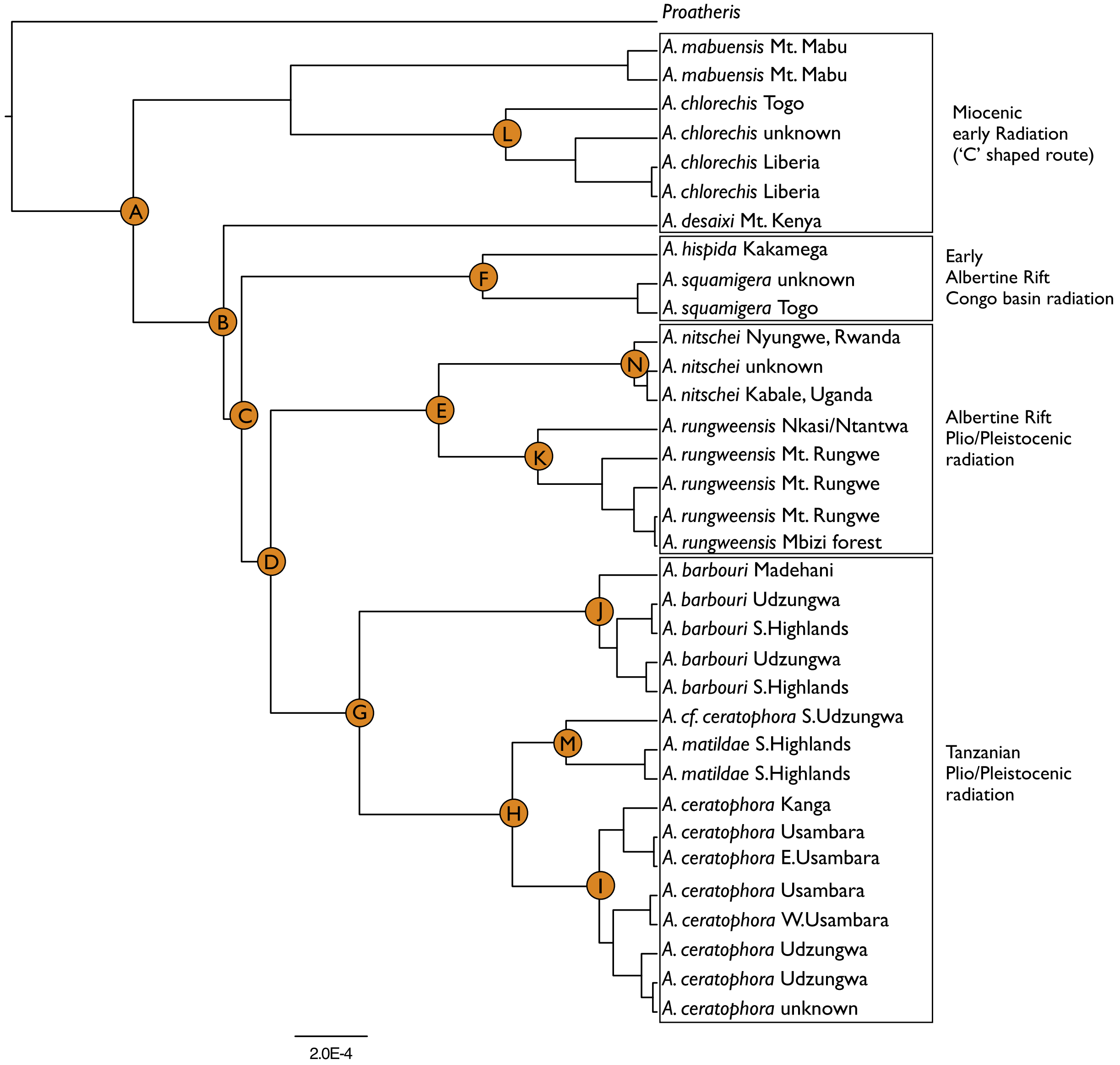

Fig. 3. Likelihood optimization probabilities for the ancestral areas represented by letters at each node in the *Atheris* ultrametric tree. The legend for the nodes is in Table 2. Terminal taxa are grouped according to the time and area of radiation.

Livingstone Mountains) forming a clade but with the exclusion of populations of Madehani in the northern Livingstone Mountains. Overall, the molecular analysis provided evidence for some cryptic genetic diversity, such as with the sample of *A. rungwensis* from Nkasi/Ntantwa, and an *Atheris* cf. ceratophora population from Kigogo. Both examples of cryptic diversity would require further molecular and morphological analyses to determine if new *Atheris* species can be supported.

4.2. Biogeography

Atheris has a wide distribution across Africa and the restriction of species to forest habitats makes this group an ideal candidate for exploring the historical fluctuations of forests Ancestral reconstruction of the genus *Atheris* shows an equivocal result – with similar likelihoods for both a western and an eastern African origin. The uncertainty may be due to several reasons, including extinction or under-sampling of species diversity, which may have provided a more conclusive result, for instance the inclusion of the other true West African species, A. hirsuta, could provide additional information on the overall biogeographic scenario of the genus. However, an alternative explanation would be that the pattern reflects a widespread distribution of the *Atheris* ancestor. It is notable that the apparent timing of the early diversification of the lineage (estimated between 15 and 18 mya) coincides with a period when a pan-African forest belt was present (Zachos, 2001; Böhme, 2003). This pan tropical forest would have harboured forest species over a much larger area than now. The basal part of the *Atheris* tree includes the taxa *A. mabuensis*, A. chlorechis and *A. desaixi*, which are species that cover almost the full geographic extremities of the genus' extent of occurrence across Africa. Both the timing of speciation events and the widespread distribution of ancestral *Atheris* species is consistent with more widespread forest habitats in the Miocene. The initial split (ca. 15 mya) within the *Atheris* genus was observed between the clade including *A. mabuensis* and *A. chlorechis*, and the rest of the genus, and shortly thereafter, the lineage containing *A. desaixi* also split from the rest of the genus. Subsequently (12.6–13.3 mya), there was a split between *A. chlorechis* and *A. mabuensis*, which today occurs in West Africa and Mozambique, respectively. These splits are timed within a period with pronounced aridification (Plana, 2004), resulting in the reduction and fragmentation of the tropical forest. This fragmentation could have contributed to the separation of biogeographic regions (such as Central, West and East African forests), resulting in the isolation of several *Atheris* lineages as observed in other reptiles, e.g. chameleons (Tolley et al., 2011) and amphibians (Loader et al., 2013).

The *Atheris* forest vipers appear to have been present in East Africa in the mid-Miocene (*A. desaixi* in Kenyan Highlands and *A. mabuensis* in Mozambique), but extant lineages in the Eastern Arc are much more recent. Ancestral reconstructions showed that the Albertine Rift is the likeliest origin for the recent East African radiation at various nodes (D–G) prior to the radiation in the Eastern Arc and Southern Highlands (*A. ceratophora*, *A. matildae*, *A. barbouri*) (Node H). The dated phylogeny suggests that *Atheris* was present in the Southern Highlands of Tanzania around 7 mya, and subsequently diversified across the Eastern Arc Mountains (*A. ceratophora*) with species penetrating the southern margins of the Eastern Arc (*A. cf. ceratophora/matildae*, *A. barbouri* in Udzungwa) from the Southern Highlands (Fig. 4). A pattern of old and new origins of the East African forest lineages has also been revealed in other taxa (Couvreur et al., 2008; Fjeldså and Bowie, 2008).

The origins of the East African *Atheris* lineages (ca. 15 and 7 my) coincide with known periods of aridification and geological activity in Africa that isolated the rainforests of Central/West African from East Africa. Our results point to a role for aridification in the structuring of biodiversity in African forests, and suggest that repeated reconnections between rain forest blocks allowed for biotic exchange at specific times, while the break-ups might have induced speciation via isolation and vicariance (Couvreur et al., 2008).

The presence of *Atheris* lineages (primarily *A. ceratophora*) only relatively recently in the Eastern Arc Mountains, indicates an important biogeographical pattern. The Eastern Arc Mountains are among the oldest and climatically most stable mountain ranges in Africa (Schlüter, 1997; Burgess et al., 2007) and harbour lineages of forest-associated species predating the Miocene climatic optimum (Fjeldså and Bowie, 2008; Tolley et al., 2011). Despite this, and the fact that *Atheris* species were already present (*A. desaixi* from Kenya and *A. mabuensis* from Mozambique) across East Africa, only a recent radiation within *Atheris* is present in the Eastern Arc, A possible cause of the absence of old lineages is extinction of once present taxa. Fossil data would be required to evaluate the likelihood of this hypothesis. An alternative explanation is that the genus was never distributed in the Eastern Arc prior to its recent dispersal (Fig. 4). From our ancestral reconstructions, a west to east colonization (nodes D to G) pattern is suggested. According to our time-tree, the timing of the speciation event between west/central areas and the Albertine Rift corresponds to large geological perturbations, including rifting and the formation of Lake Tanganyika. The rift-associated lakes and mountains are traditionally believed to be composed of two distinct segments: an older, volcanically active eastern branch and a younger, volcanic western branch (McConnell, 1972). However, recent studies have shown that initiation of rifting in the western branch began more than 20 mya, much earlier than previously thought, and contemporaneously with the eastern branch of the *Atheris* genus (Roberts et al., 2012). These geographical changes could have constituted a barrier to the movement of forest-dependent species. Consequently, these barriers might have contributed to a “C-shaped” (Fig. 4; areas 2 and 3) dispersal route into East Africa and the subsequent, more recent, radiation into the Tanzanian Mountains, from south of lake Tanganyika. A possible inferred scenario of *Atheris* dispersal in the Tanzanian mountains considers an early radiation from the south, across the Eastern Arc Mountains, north to the Usambaras. There followed a subsequent wave of extinctions and a re-colonisation of Mount Kanga, in the Nguru Mountains and Udzungwa Mountains (and possibly Uluguru Mountains) from relict populations that had survived in the Usambaras.

Our results are consistent with some reports that show that montane forest faunas are a mixture of old, paleo-endemic lineages, and recent *in situ* speciation (Fjeldså and Bowie, 2008; Demos et al., 2013). Such patterns have been show in avian and mammal assemblages, which are a combination of relict taxa (since the mid-Tertiary thermal optimum) and recently speciated clades (Fjeldså and Lovett, 1997; Fjeldså and Bowie, 2008; Demos et al., 2013). Contrasting this, are other forest groups, like

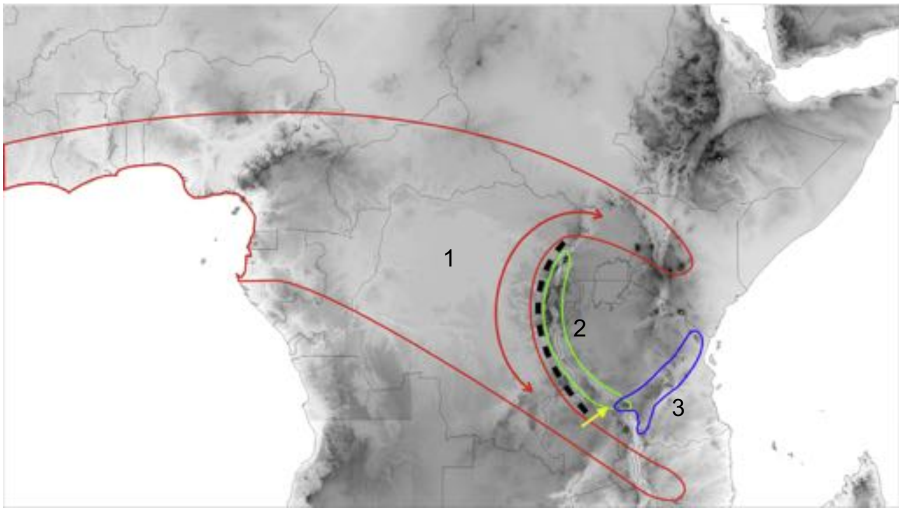

Fig. 4. The pattern of distribution of *Atheris* since the origin of the genus. (1) Possible minimum extent of range of the genus about 11 million years ago (mya), when the lineages of *A. chlorechis* in the west, *A. desaixi* in the northeast and *A. mabuensis* in the southeast were already split. (2) Albertine Rift radiation initiated 5.4 mya. (3) Southern Highlands of Tanzania colonization and subsequent radiation in the Eastern Arc Mountains initiated 7.4 mya. The yellow arrow represents the possible way of penetration of the genus into East Africa. The dashed line represents the barrier constituted by the early stages of the Albertine Rift depression 20 mya that may have forced the dispersing lineages to move north or south of the barrier (the ‘C-shaped’ route, represented by the red arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

chameleons in the genus *Kinyongia*, showed relictual distributions, with diversification substantially pre-dating the Miocene and Plio-Pleistocene climatic shifts associated with cladogenesis and little evidence of recent immigration (e.g. Tolley et al., 2011). The reasons for such contrasting phylogenetic patterns are not well understood but are likely to represent a combination of differences in the taxon's ability to disperse, historical events excluding or facilitating dispersal, and local extinctions (Dobrovolski et al., 2012). Further comparative studies are necessary to untangle the causal reasons for differences that might provide a more general explanation of the biodiversity patterns in this region.

5. Conclusions

We provide a molecular phylogenetic tree for the forest viper genus *Atheris*. The tree generally provides strong supports for the relationships amongst each species. Our study also reveals potentially new cryptic species. Our *Atheris* time-tree and area reconstruction showed that during the Miocene Climatic Optimum (18–16 mya) the genus was likely to be widespread within the rainforests of tropical Africa. Early differentiation in the genus corresponds to the Middle Miocene Disruption, around 15 mya, a period characterized by a wave of animal and plant extinctions, due to a global cooling (Böhme, 2003). The more recent cooling events appear to have restricted species to forest refugia (e.g. Central Africa, Kenyan Highlands, Albertine Rift).

Despite two *Atheris* lineages being present in East Africa soon after the formation of the genus (*A. mabuensis* and *A. desaixi*), the lack of occurrence of any old Eastern Arc lineage before late Miocene/early Pliocene suggests a recent dispersal into this region. A possible reason for this might have been a barrier to forest species, with both the formation of the western branch of the Great Rift Valley and associated local climate perturbations. This event would have split the previously contiguous tropical forests of East and Central Africa. This could have resulted in blocking the direct west–east dispersal routes of forest-associated organisms, promoting a 'C-shaped' dispersal route (Fig. 4), with a northern and a southern front. Our study underlines the potential role of the Rift Valley in shaping dispersal routes of forest-associated organisms. More broadly our study shows the biogeographical relationships among regions, and why we see such distinctive faunal assemblages in the Eastern Arc, Kenyan Highlands and Albertine Rift.

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