Systematics and biogeography of amphibians of the African Eastern Arc Mountains

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For my mother, my father and Jane

Abstract

The Eastern Arc Mountains (EAM) of Tanzania and Kenya are a biodiversity hotspot with remarkable patterns of endemism. Many taxa are restricted to single blocks of the chain, a pattern thought to reflect a history of persistent but fluctuating forest cover so that the mountains are analogous to an island chain. EAM biodiversity has been studied for some time but is still poorly understood on many levels. Improved understanding of the evolution of EAM amphibians and of the biogeography of the EAM requires accurate phylogenies. Using amphibians, a group considered especially sensitive to environmental change, this study investigated the (1) Phylogenetic relationships of seven amphibian lineages (*Boulengerula, Scolecomorphus, Callulina, Probreviceps, Hoplophryne, Spelaeophryne and Arthroleptides*) to understand relationships among species and populations. Then (2) using molecular phylogenies from amphibians and the literature, temporal and spatial patterns were assessed using cladistic biogeography methods and molecular divergence dates to assess biogeography of the EAM (3) Lastly, the distribution of amphibians in the EAM were analysed using descriptive biogeographic approaches to investigate spatial patterns. Results from phylogenetic analysis of partial mitochondrial genes 12S, 16S and *cytb,* indicate that many populations occurring throughout the EAM are likely to represent distinct, previously unrecognized species. Apart from the genera *Spelaeophryne and Arthroleptides,* up to twice as many species might be recognized in each genus. The level of species diversity in the EAM is expected to increase rapidly with taxonomic refinements of these and other amphibian groups restricted to the EAM. Analysis of the biogeography of the EAM indicates that general spatial relationships are non-significant (both cladistic and descriptive reconstructions). Temporal data demonstrates that lineages divergence events are not co-temporal, even for taxa with similar dispersal ability. Overall, the historical biogeography in the EAM is likely to be complex. Geographic history of the EAM has been marked by repeated periods of fragmentation, isolation, contraction and expansion of forest habitats, and it is therefore not surprising that both temporal and spatial data are non-significant. Limitations to this study are discussed, particularly the effect of incomplete sampling of taxa and populations. Temporal estimates consistently support the ancient divergences of amphibian lineages, which suggest the EAM have persisted for a relatively long time. Persistence might have been important for the maintenance of biodiversity in the EAM. The EAM represent an important reservoir of phylogenetic diversity and its future conservation is critical for maintaining African biodiversity.

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Chapter One

Introduction

The thesis reports a study of the phylogenetic diversity of some amphibians in the rainforests of the Eastern Arc Mountains (EAM) of Africa, and an investigation of the biogeographic history of the region. It was undertaken because the area is of exceptional biological interest. The forests of the EAM are believed to have had a prolonged, fragmented and persistent history, even during climatic fluctuations and it is thought that this promoted remarkable levels of species diversity and endemism. The biogeographic history of Eastern Arc is poorly understood (Fjeldså and Lovett, 1997; Burgess *et al.* 1998; Burgess *et al.* in press), so that understanding patterns of speciation in amphibians, a suitable biogeographic indicator group, may help to better elucidate the biogeographic history of the region. Comparisons may also permit an evaluation of speciation patterns in tropical forests. Among only three other areas in Africa, the EAM of Tanzania and Kenya are considered a biodiversity hotspot (Myers *et al.* 2000) and consequentially a region of global importance.

Part 1: Background: Biological and climatic history of the tropical rainforests of the Eastern Arc Mountains

1.1 Introduction

1.1.1 Overview of tropical rainforests

Estimates suggest that more than half of the currently described terrestrial species are found in rainforests, despite rainforest habitats covering just 8% of the planet's land area (Wilson, 1988; Newmark, 2002). Whether this reflects their true share of Earth's diversity is uncertain, because rainforests are poorly explored. Recent reports of a previously unrecognised diversity of even relatively conspicuous species that await description (e.g. Biju, 2001; Meegaskumbura et al. 2002), suggest these are underestimates. Irrespective of this, rainforests, referred as the crucible of evolutionary processes (Wilson, 1988), represent a significant reservoir of the world's

biodiversity (Myers *et al.* 2000). However, there is a lack of understanding of these richly diverse areas compared to temperate regions (Hewitt, 2004). Of the few studies that have looked at the patterns of diversification in tropical forests, it appears that species generally show prolonged diversification patterns, though these differ between taxa and continental regions (e.g. Fjeldså and Lovett, 1997; Pennington *et al* 2004; Moritz *et al.* 2000) and overall suggest much complexity. More recent diversification patterns have been consistently drawn from studies of temperate species (Hewitt, 2004), which has led people to speculate on correlations between time of persistence and biodiversity (see review: Hewitt, 2004).

Tropical rainforests are distributed along the equatorial region of Africa, Asia and the Americas, reaching 10° south and 10° north, and below 3,000 feet, and are characterised by high rainfall. The distribution of rainforests worldwide, and by implication a large proportion of the planet's biodiversity, has changed radically through time. The waxing and waning of rainforests in particular regions are largely the result of plate tectonic movements and variations in climate (Whitmore, 1990). Our understanding of these changes, the causal factors and their effect on rainforest species is patchy and is derived from the geological record and the relationships and distributions of extinct and extant organisms.

1.1.2 Tectonic and climatic influences on rainforests

The occurrence of forest has fluctuated dramatically through time, in association with a number of influential factors. Movement of continental plates undoubtedly had a fundamental influence in a number of different ways on the evolution of forest systems (Hamilton, 1988; Morley, 2000; Whitmore, 1990): (1) Position of the continental plates relative to the equator, with moist forest tracking the land bisecting the equator (Hamilton, 1988); (2) The movement of landmasses resulting in the opening and closure of oceans, which has a significant effect on global climate patterns (Lovett, 1993a; Morley, 2000; Cane and Molnar, 2001); (3) Interchange of species, as continents separated and collided (Bonnefille, 1984; Biju and Bossuyt, 2003). In addition to the movement and position of continents affecting climatic circulation, other localised and worldwide climate patterns have had a major influence on the composition of rainforests and their occurrence (Jansson and Dynesius, 2002; Whitmore, 1990). Periodic oscillations in the climate, such as those due to Milankovitch cycles (the eccentricity of the Earth's orbit), have been shown to

correlate with changes in forest cover (e.g. Bonnefille, 1984; Lovett, 1993a). Climatic fluctuations during the Pleistocene have also been shown to influence changes in rainforest distribution (Van der Hammen, 1974; Hamilton, 1988).

1.1.3 Biogeography of tropical rainforests

Despite the complex nature of interactions between species and their environments, it is possible to reconstruct many of the changes that were critical in producing the distributions of rainforests seen today (Whitmore, 1990). Some of these patterns have been relatively straightforward to understand, based on tectonic plate theory and the distribution of organisms (Raven and Axelrod, 1974; Axelrod and Raven, 1978; Sneath, 1967; Cracraft, 1974; Hedges *et al.* 1996; Bossuyt *et al.* 2004; San Mauro *et al.* 2005). There are though other, more subtle patterns, which are difficult to detect (e.g. Roelants *et al.* 2005), and which are crucial to our understanding of the diversification of rainforests. A critical component to understanding the origins and determinants of forest systems are the signatures, sometimes cryptic, left in the phylogenetic relationships between extinct and extant species distributed both in and outside rainforests. These organisms can be important indicators of changes in habitat (Hamilton, 1988; Avise, 2000; Hewitt, 2004), and tectonic movement (Raven and Axelrod, 1974; Avise, 2000), provided that they are affected by these events in predictable ways.

1.1.4 Tropical rainforests: Broader issues

Understanding evolutionary diversification in rainforests is important to elucidate much broader questions fundamental to evolutionary biology, such as the mechanisms of speciation, adaptive radiations, definition of species, and conservation biology. Examples relevant to this study are given below.

1.1.4.1 Speciation in rainforests

The processes responsible for generating high rainforest diversity have been of great interest to biologists for over a century (Wallace, 1876; Darwin, 1872; Whitmore, 1990). Numerous theories have been advanced but few have been rigorously tested using modern approaches (Moritz *et al.* 2000). This debate has centred on two main competing hypotheses, though not necessarily mutually exclusive, the refuge model (dominant in temperate modes of species diversification) and the persistence model. The persistence hypotheses grew from speculation that the relatively stable conditions afforded by rainforest habitats resulted in low extinction rates and the

gradual accumulation of species over time (Fisher, 1960). This persistence hypothesis places significance on diversifying selection in distinct habitats, irrespective of geographic separation, but it has been challenged by evidence that suggests that climates have fluctuated dramatically, particularly recently in the Pleistocene, and this led to the formulation of the refuge hypothesis (Haffer, 1969). The refuge hypothesis proposes that changes in climate caused periodic contractions and expansions of forests, which would have isolated populations, thereby promoting allopatric speciation. This model of speciation involves the accumulation of genetic differences in isolated populations with the incidental emergence of reproductive isolation. The refuge theory has recently been challenged by a number of studies describing speciation patterns that do not correlate with global climatic fluctuations, particularly during the Pleistocene (Knapp and Mallet, 2003; Klicka and Zink, 1997, 1999; Zink and Slowinski, 1995; Colinvaux *et al.* 1996). Explanations however are still required to account for the increased endemism exhibited in Pleistocene refuges, which initially alerted biologists to the refuge hypothesis. At present time there is no consensus on which speciation model best describes, or best accounts for the main mechanisms of evolutionary diversification in tropical rainforests. This confusion is demonstrated by the differing conclusions derived from both Neotropical and Australian case studies (Hewitt, 2004). Patterns underlining the great diversity and historical complexity of these areas include examples of recent speciation processes (Pennington *et al.* 2004) and much deeper historical events, often, from the Pliocene (Hewitt, 2004; Moritz *et al.* 2000; James and Moritz, 2000). This issue is almost completely unexamined for Asia and Africa (Hewitt, 2004), and current theories in Africa are based largely on distribution data (Fjeldså and Lovett, 1997; Bruhl, 1997), evidence that is considered to have limited ability to inform on such subjects. Clearly these matters are significant, both for determining the patterns of diversification, but also for understanding fundamental evolutionary processes. The precise mechanisms of the speciation process are still poorly understood (Mallet, 2001; Barton, 2001; Moritz *et al.* 2000), however an increasing amount of phylogenetic data should provide an opportunity to evaluate competing hypotheses.

1.1.1.2 Conservation Biology of rainforest biodiversity

Humans are having a profound effect upon the natural world (Wright, 2005). Forest cover has been estimated to have reduced 20% globally, as a direct consequence of human induced changes, and numbers of threatened species are ever increasing. In

light of this biodiversity crisis (Wilson, 1988), it has become critical that we prioritise efforts to conserve the most biodiverse regions (Gaston and Williams, 1993). Deciding whether an area is more worthy for conservation is a tricky decision and of critical importance in a world of limited resources. If a complete inventory of the composition of each rainforest was known then this would be possible, but this is not realistic or even an economically efficient goal (Myers *et al.* 2000; Vane-Wright *et al.* 1991). Alternatively, an economically viable approach is to use surrogate measures of total biodiversity, including indicator species (e.g. Moritz et al, 2001; Moritz, 2002), habitat (as summarised by Brooks *et al.* 2004) and phylogenetic diversity (Ehrlich and Wilson, 1991; Sechrest *et al.* 2002). An interesting recent example suggested that conservation of all currently established hotspots (1.4% of the Earth's land surface area) would save nearly 70% of all carnivore and primate genetic diversity (Sechrest, *et al.* 2002). In conservation biology it is generally considered that added value or worth should be placed on assemblages that are distinctive phylogenetically (Vane-Wright *et al.* 1991). If we are to develop strategies to safeguard future evolutionary potential (as in Sechrest, *et al.* 2002) as well as conserve extant species, understanding the origins of diversity, particularly in rainforests, is an urgent priority.

1.2 Eastern Arc Mountains

1.2.1 Introduction

The EAM are a chain of isolated mountain blocks stretching over 700km from the Taita Hills in Kenya, to Mahenge Mountain in southern Tanzania (refer to Fig.1.1). They are comprised of the following mountains: Taita Hills, North Pares, South Pares, West Usambaras, East Usambaras, Nguu, Nguru, Ukaguru, Rubeho, Uluguru, Malundwe, Udzungwa, and Mahenge. These mountains, composed of ancient crystalline Precambrian basement rocks, contain the main proportion of East Africa's rain forests, and are notable for a high degree of plant and animal diversity (Clarke, 1988; Howell, 1993; Lovett, 1990; 1993b; 1998a; 1998b; Burgess *et al.* 1998a; Newmark, 2002; Burgess *et al.* in press), despite comprising just 0.1% of global rainforest. The Eastern Arc however is not the most speciose of global hotspots, but the number of species per km^2 is high, with remarkable levels of endemism in animals and plants (Lovett, 1993b; Hoffman, 1993). Large patches of undisturbed montane forests are still present, but the lowland and sub-montane layers of the

forest have suffered extensive losses and fragmentation caused by human disturbance (Newmark, 2002). Estimates suggest only 6.7% of the original primary vegetation remains (Myers *et al.* 2000). Preservation of these forests in light of increasing deforestation (Newmark 2002; Doggart *et al.* in press) has been singled out as a conservation priority (CEPF, 2003).

Despite the fact that the biodiversity and evolutionary history of the EAM has been studied for a comparatively long period (Barbour and Loveridge, 1928; Loveridge, 1937; Moreau, 1966; Rodgers and Homewood, 1982), they remain very poorly understood (Grimshaw, 2001). The exact historical origin of the EA forests is unknown, but is probably associated with a number of geological and climatic factors (see section 1.2.2). Essentially, once the continent of Africa reached its current equatorial position (~40mya), favourable climatic conditions would have promoted the growth of forest habitats (Lovett, 1993a). These habitats may have persisted over only small time periods, because of severe climatic fluctuations. However, once the EAM were uplifted, as a result of significant regional geological changes (25-10mya), the mountains would have then attracted substantial rainfall, condensing the moist air coming in from the Indian Ocean (orographic rainfall). From this point onwards it is believed that the Eastern Arc forests would have persisted; even during extreme dry phases forest would have contracted but not completely disappeared (Lovett, 1993a). The main source of evidence for the persistence of rainforest is based on the phylogenetic relationships between disjunctly distributed sister taxa (Burgess *et al.* 1998a). Loveridge (1937) noted the striking resemblance between the faunas of the montane forests of East and West Africa. Similarly, more recently authors have shown species linking to forest areas in Madagascar (Lovett, 1993b; Emberton *et al.* 1997; Huber, 2003), and southeast Asia (as summarised in Burgess *et al.* 1998a). The persistence of rainforest then allowed the diversification of numerous groups of animal and plant species (Burgess *et al.* 1998a; in press, see section 1.2.3.3).

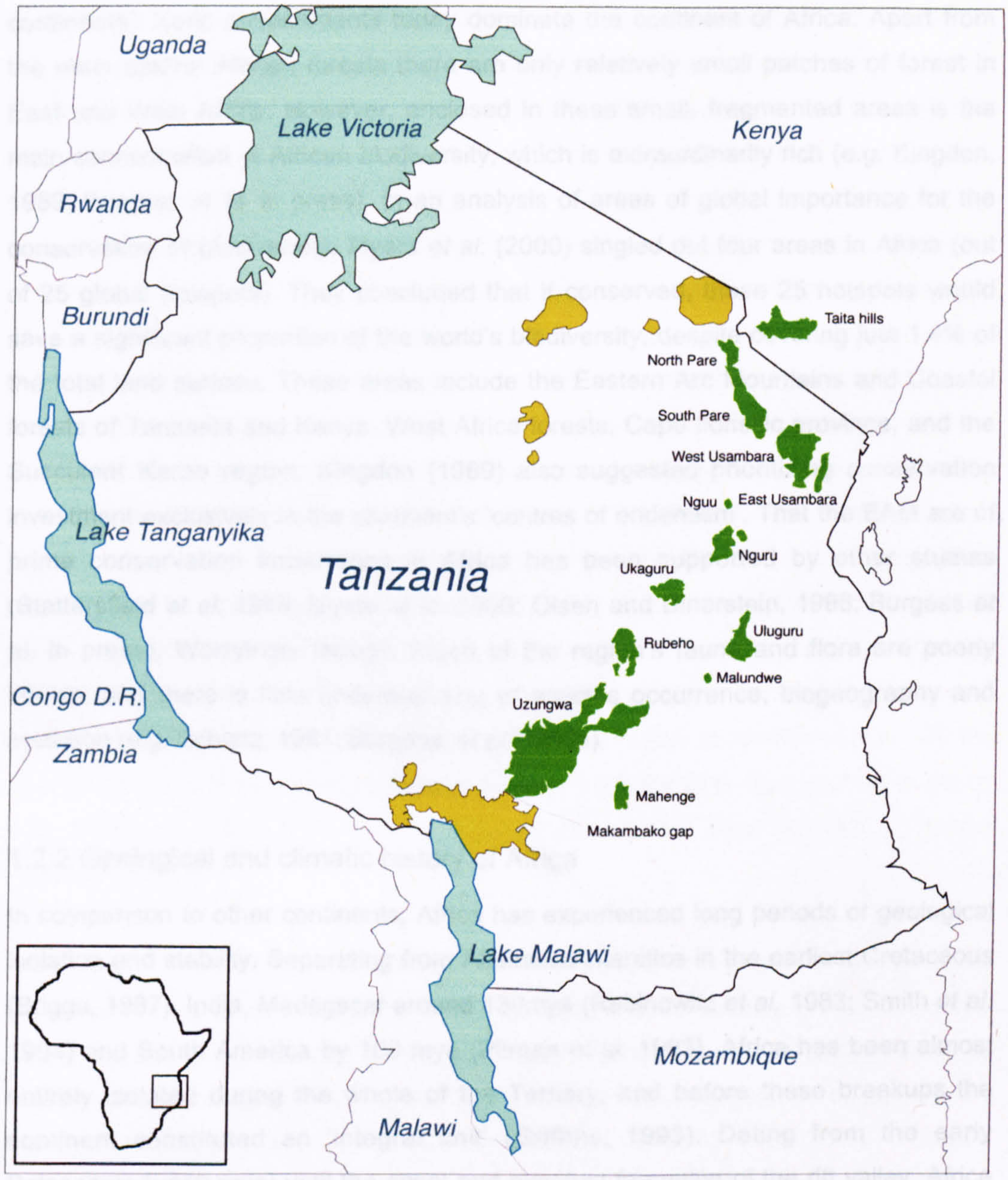

Figure 1.1.

Map of the Eastern Arc Mountains, modified from Menegon *et al.* 2004. Areas in green are Eastern Arc Mountains, areas in brown are of more recent volcanic origin.

Africa has been described as the 'odd man out' by Richards (1973), on account of the much lower species richness and relatively poor representation of phylogenetic lineages compared to elsewhere on the globe (Morley, 2000). Duellman (1993) noted this disparity when comparing the diversity of anurans in Africa and South America. Duellman (1993) among others (e.g. Morley, 2000) explained the difference in species richness by the disparity of 'xeric and humid environments on the two

continents'. Xeric environments today dominate the continent of Africa. Apart from the main central African forests there are only relatively small patches of forest in East and West Africa. However, enclosed in these small, fragmented areas is the main concentration of African biodiversity, which is extraordinarily rich (e.g. Kingdon, 1989; Burgess *et al.* in press). In an analysis of areas of global importance for the conservation of biodiversity, Myers *et al.* (2000) singled out four areas in Africa (out of 25 global hotspots). They concluded that if conserved, these 25 hotspots would save a significant proportion of the world's biodiversity, despite covering just 1.4% of the total land surface. These areas include the Eastern Arc Mountains and Coastal forests of Tanzania and Kenya, West Africa forests, Cape florisitic province, and the Succulent Karoo region. Kingdon (1989) also suggested prioritising conservation investment exclusively in the continent's 'centres of endemism'. That the EAM are of prime conservation importance in Africa has been supported by other studies (Stattersfield *et al.* 1998; Myers *et al.* 2000; Olsen and Dinerstein, 1998; Burgess *et al.* in press). Worryingly though, much of the region's fauna and flora are poorly known, and there is little understanding of species occurrence, biogeography and evolution (e.g. Schiøtz, 1981; Burgess, *et al.* 1998a).

1.2.2 Geological and climatic history of Africa

In comparison to other continents, Africa has experienced long periods of geological isolation and stability. Separating from Australia/Antarctica in the earliest Cretaceous (Briggs, 1987), India, Madagscar around 130mya (Rabinowitz *et al.* 1983; Smith *et al.* 1994) and South America by 100 mya (Pitman *et al.* 1993). Africa has been almost entirely isolated during the whole of the Tertiary, and before these breakups the continent constituted an 'integral unit' (Griffths, 1993). Dating from the early Palaeozoic (~440 mya) until the onset and eventual formation of the rift valley, Africa has also been little affected by major geological events (King, 1978; Hamilton, 1978; Livingstone, 1993; Lovett, 1993a). Drifting of the African plate has also been less severe than other continents (Briggs, 1987), with Africa moving slightly north east of its position during the Cretaceous (Hamilton, 1988; Smith *et al.* 1994). Geologically, the African landscape has been subject to slow long term erosional processes, punctuated by severe rifting and uplifting as a result of crustal melting during the Neogene (present time to 25 mya) (Griffiths, 1993).

In contrast to its relative geological stability, Africa has been marked by severe climatic fluctuations, most significantly during the Tertiary when the continent experienced rapid wet and dry cycles (Lovett, 1993a). This geological and climatic history of Africa is summarised in Fig. 1.2.

Under favourable climatic conditions, following the catastrophic extinctions that occurred at the end of the Cretaceous, the African continent showed 'continued diversification' (Morley, 2000) of indigenous taxa during the Palaeocene and Eocene (Axelrod and Raven, 1978). During this period, a thermal maximum was reached, with forest covering extensive areas of the continent, most probably a lowland equatorial forestbelt (Morley, 2000). This wet and humid episode is believed to have been the result of global climate patterns where moist air would have been directed from the Tethys Sea and newly emerging Atlantic and Indian Oceans over Africa, inducing high rainfall (Lovett, 1993a). At the onset of the Eocene (~55mya) numerous trans oceanic dispersal events are thought to have occurred between Africa and South America, Eurasia and India, as evidenced by the appearance of certain taxa of non-African origin (Morley, 2000), increasing the diversity of rainforest taxa. By the Late Eocene (~40mya), assemblages of a modern affinity were also starting to occur, with the appearance of taxa that 'today characteristise West African rainforest' (Morley, 2000).

The generally warm and moist conditions experienced over a prolonged period in the Palaeocene and Eocene came to an abrupt end in the Oligocene (~35mya), with a global cooling event (Lovett, 1993a). Although apparently not affecting Africa as extensively as other regions (Morley, 2000), it induced an expansion of grasslands, the contraction of rainforest areas and the extinction of certain taxa. There is though, very limited evidence for this specific time period (Morley, 2000), so any generalities concerning vegetational changes should be interpreted cautiously. The Middle and Late Oligocene showed a gradual diversification of forest species, as climatic conditions appeared to recover. During this time, uplift of the central African plateau was initiated (Burke and Wilson, 1972) and as a consequence East and West African rainforest began its slow separation, with the splitting of drainage patterns 'accentuating a divide' between East and West Africa (Lovett, 1993a). At this point the progenitors of the Eastern Arc may have resembled mountains, and if so, the massifs would have received increased rainfall suitable for development of rainforest. It is unclear how much vertical movement each mountain block had undergone

(Lovett, 1993a), but such topographic barriers would have increased rainfall, which would also have encouraged the development of rainforests.

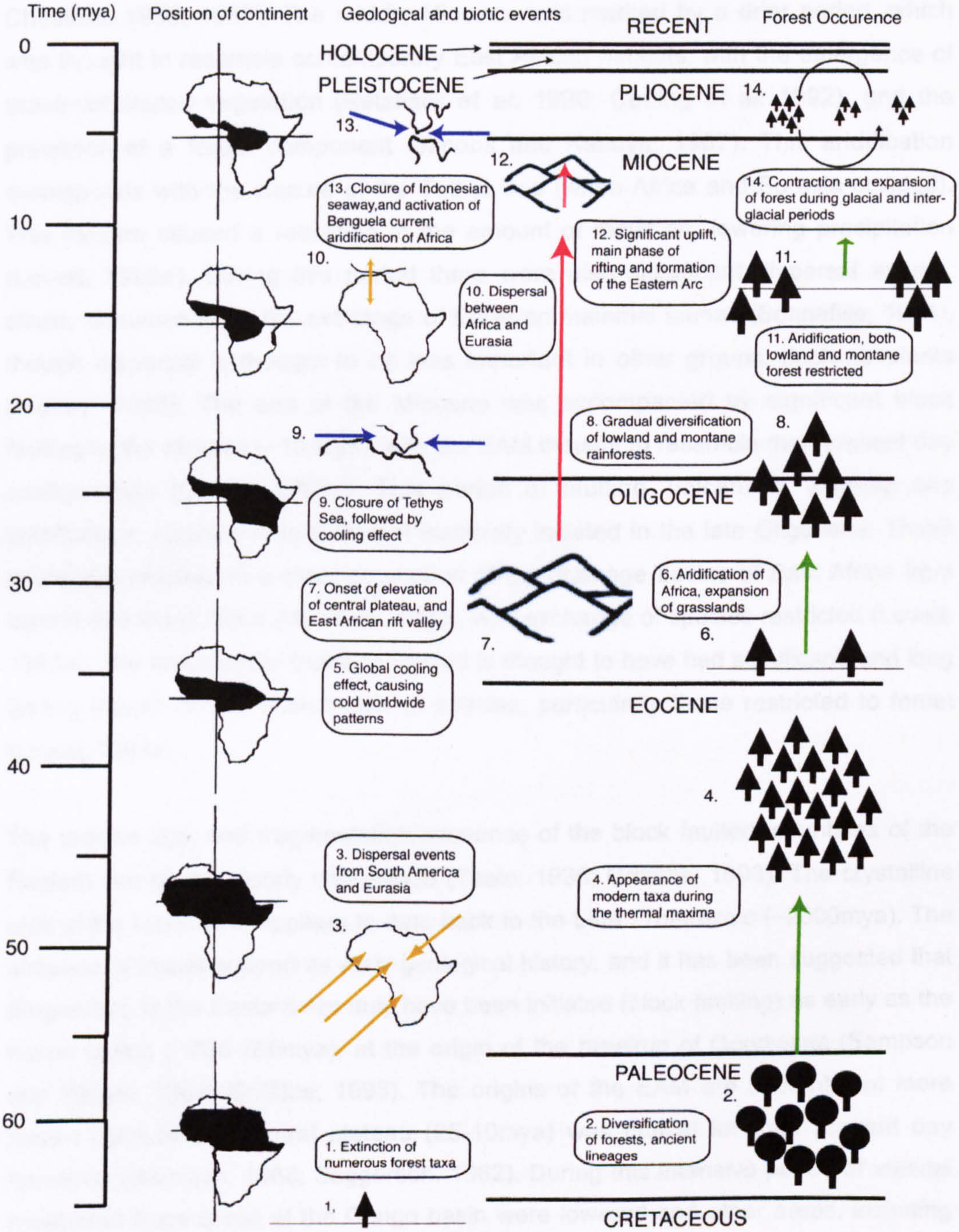

Figure 1.2.

Summary of the geological and climatic history of East Africa during the Tertiary (reconstructed from various sources; e.g. Morley, 2000; Hamilton, 1988). Black area on continent of Africa refers to approximate coverage of forest, based on reconstructions outlined in Hamilton, 1988.

The gradual increase in forest diversity carried on into the early Miocene, with rainforest extending across equatorial Africa (Andrews and Van Couvering, 1975; Chesters, 1955, 1957). The middle Miocene was marked by a drier period, which was thought to resemble contemporary East African habitats, with the emergence of grass-dominated vegetation (Retallack *et al.* 1990; Cerling *et al.* 1992), and the presence of a forest component (Jacobs and Kabuye, 1987). This aridification corresponds with the closure of the Tethys sea (when Africa and Eurasia collided). This closure caused a reduction in the amount of moist air, lowering precipitation (Lovett, 1993a). During this period there were also significant dispersal events, clearly documented in the exchange of Eurasian mammal faunas (Bonnefille, 1984), though dispersal is thought to be less important in other groups, such as plants (Morley, 2000). The end of the Miocene was accompanied by significant block faulting in the rift area (~10 Mya), with the EAM thought to resemble their present day configuration (Lovett, 1993a). This period of faulting, volcanism, warping and aridification, ended a long period of instability initiated in the late Oligocene. These processes resulted in a clear separation of the drainage basins of East Africa from central and West Africa (Hamilton, 1982), with exchange of species restricted (Lovett, 1993a). The arid corridor that was formed is thought to have had significant, and long lasting impact on the interchange of species, particularly those restricted to forest (Lovett, 1993a).

The precise age, and fragmentation sequence of the block faulted mountains of the Eastern Arc is very poorly understood (Teale, 1936; Griffiths, 1993). The crystalline rock of the mountains appears to date back to the Late Proterozoic (~2000mya). The absence of fossils support its early geological history, and it has been suggested that progenitors of the Eastern Arc may have been initiated (block faulting) as early as the Karoo period (~290-180mya), at the origin of the breakup of Gondwana (Sampson and Wright, 1964; Griffiths, 1993). The origins of the EAM are ancient, but more recent uplift of the central plateau (25-10mya) was critical for their present day formation (Hamilton, 1988; Saggerson, 1962). During this intensive period of vertical movement huge areas of the Congo basin were lowered and other areas, including the EAM, were pushed upwards. As a result of these changes, the presumably once continuous area of the EAM is believed to have became fragmented and separated (Quennel *et al.* 1956), and would have developed a highly localised climate that could have supported rainforest until the present day (Lovett, 1993a). A topographical formation like the EAM can confer climatic stability even during large

climatic fluctuations, limiting the magnitude of dry periods (Partridge *et al.* 1995; Fjeldså, 1994; Fjeldså and Lovett, 1997). The southern highlands, not considered to be an integral part of the EAM (Lovett, 1988) are also considered to have a long geological history, which is reflected in their rich biodiversity (Griffiths, 1993; Davenport, pers. comm.). More recent formations, such as the Late Tertiary volcanos of Kilimanjaro, Hanang and Meru have a relatively depauperate fauna and flora (Axmacher *et al.* 2004) compared to the EAM.

Late Tertiary climatic and geological history in Africa is relatively well understood, especially in East Africa, as a consequence of the interest in hominid evolution (Hamilton, 1988). The expansion of savanna habitats in East Africa occurred intermittently (deMenocal, 1995; Partridge, *et al.* 1995), causing extinction in many groups of animals and plants. There are a number of possible causes; closure of the Indonesian seaway (Cane and Molnar, 2001), southward movement of Antarctica initiating the cold Benguela current around Africa (Shackleton and Kennett, 1975; Morley, 2000), and the Mediterrranean salinity crisis (Hamilton, 1988). This was also matched by periods of warmer (Williamson, 1985), moist weather, which allowed the dispersal of some montane taxa such as *Podacarpus* (Hamilton, 1988; Morley, 2000). These fluctuations continued in the Quaternary, when again rainforests contracted and expanded (Hamilton, 1976; Hamilton, 1988; Morley, 2000) corresponding with glaciations at the poles (Jansson and Dynesius, 2002). Evidence has suggested climates fluctuating towards savanna from forest habitats on at least three occasions (e.g. 2.8, 1.7, 1.0Myrs) (deMenocal, 1995). It has been argued that temperature depression associated with the Ice Ages would have caused major extinctions of African forest organisms (Hamilton, 1988). The response of forest to the glacial and interglacial periods during the Pleistocene and Holocene has been investigated (Van Zinderen Bakker and Coetzee, 1972; Van der Hammen, 1974; Hamilton, 1976; Hamilton, 1988; Ambrose and Sikes, 1991; deMenocal, 1995; Marchant *et al.* 1998; Dupont, 2001) using the pollen record and lake level changes. These studies demonstrate that expansion and contraction of forest has occurred on numerous occasions. These studies suggest that altitudinal migration of montane forest occurs in response to warmer and colder periods (as shown in Fig. 1.3), with the lowering of vegetation zones by an altitude of about 1000m (Hamilton, 1976, 1988; Van Zinderen Bakker and Coetzee, 1972). There is no direct evidence for the persistence of Eastern Arc forest during the most arid phases in Africa's history. However, it has been suggested that during Pleistocene glacial maxima, coastal

regions (i.e. areas close to the EAM) were little influenced by these fluctuations, maintaining conditions suitable for forests (Prell *et al.* 1980).

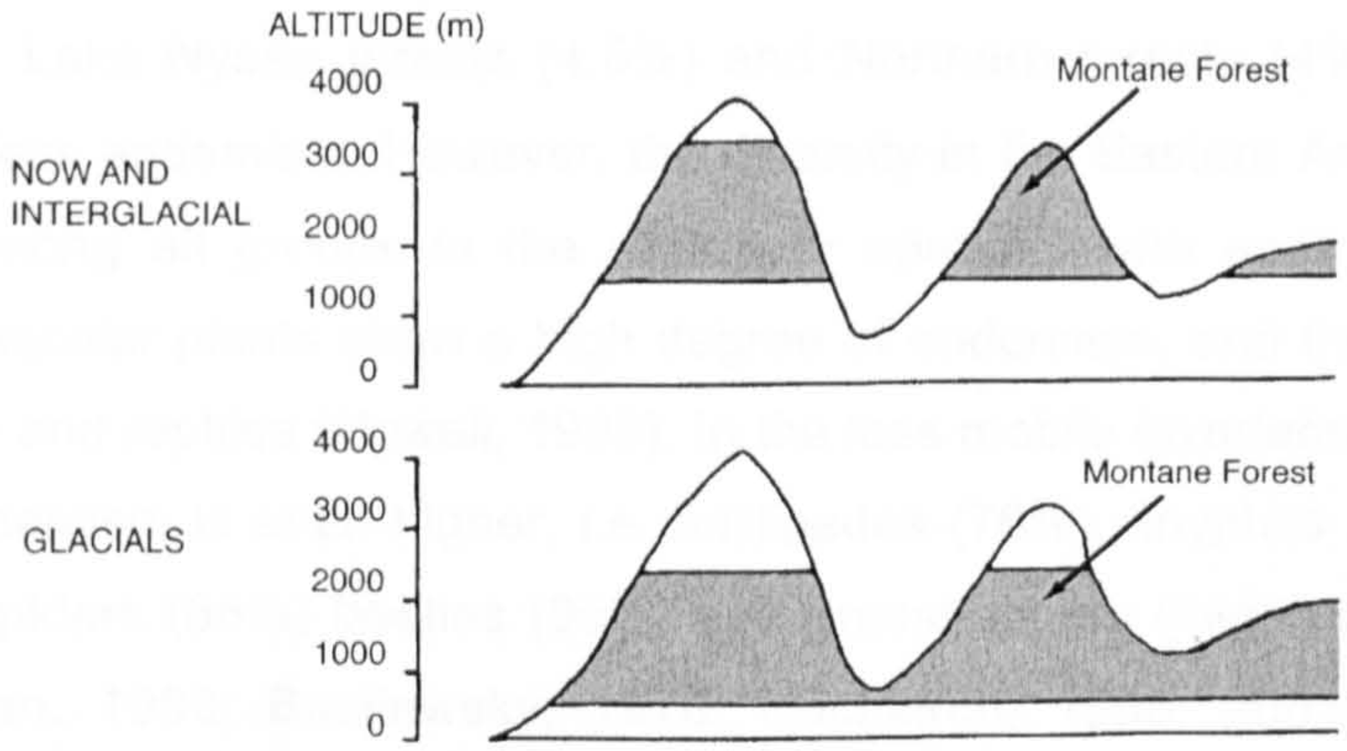

Figure 1.3

Altitudinal distribution of montane forest during glacial and interglacial periods, altered from Hamilton, 1988.

In total, this large body of evidence suggests that rainforests in Africa were fragmented during various periods of climatic instability, and over a considerable time period. This climatic instability appears to have had substantial consequences, limiting rainforests to small regional refugia, with the extinction of many species (Hamilton, 1976; 1988). This is consistent with the belief that Africa is faunistically and floristically depauperate compared to Asia and the Neotropics (Richards, 1973; Morley, 2000; Whitmore, 1990). Rifting of the East African block-faulted mountains ~25-10 mya also produced highly localised and stable forest habitats (Fjeldså, 1994; Fjeldså and Lovett, 1997; Partridge *et al.* 1995), though the precise nature of the fragmentation sequence and the timing is very uncertain.

1.2.3 Patterns of species diversity in the Eastern Arc Mountains

1.2.3.1 Taxonomic diversity patterns

Diversity patterns in the Eastern Arc Mountains are thought to correlate with the climatic and geological history of the area (Rodgers and Homewood, 1988; Lovett, 1998a,b), which appears to be supported by the patterns of endemism. When the

EAM are compared to the rest of Africa, both animal (Burgess *et al.* 1998b; Burgess *et al.* in press) and plant (Lovett, 1998a) groups show remarkable levels of endemism. Lovett (1998a) evaluated the diversity of vascular plants in the East African region, showing that 58% of vascular plants are endemic compared to coastal forest (14%), Lake Nyasa forests (4.5%) and Northern forests (4%), all showing substantially less endemism. However, the diversity in the Eastern Arc is not evenly distributed among all groups in the region or spatially with each mountain. As mentioned, vascular plants show a high degree of endemism, and the same occurs in amphibians and reptiles (Howell, 1993). In the less mobile invertebrate groups, the degree of endemism is even higher, i.e. milllipedes (76%), linyphiid spiders (82%), harvestmen spiders (88%) beetles (95%) and grasshoppers ('high') (Scharff, 1992, 1993; Hoffman, 1993; Basilewsky, 1976; Hochkirch, 1998; 2001). In contrast, mammals and birds show significantly less endemism (Stuart *et al.* 1993; Kingdon, and Howell, 1993). These contrasting levels of endemism show, unsurprisingly how the degree of endemism is highly correlated with the ability to disperse.

1.2.3.2. Spatial diversity patterns in the EAM

As a region, the EAM represent a considerable wealth of diversity. However a disproportionate number of species are found in three main areas (Usambara, Udzungwa, and Uluguru).

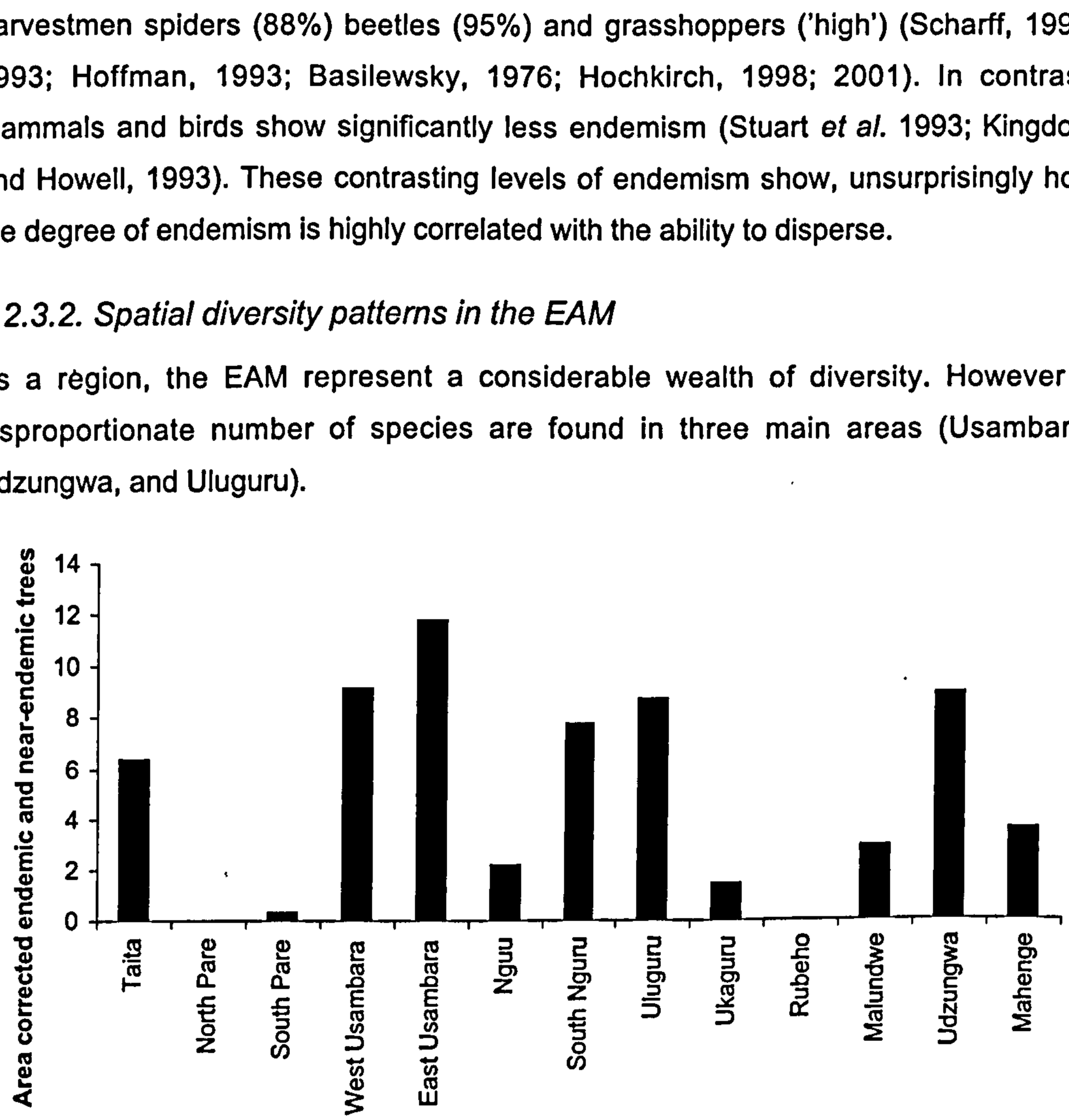

Figure 1.4.
Ranked importance for endemic and near-endemic tree species of the 13 mountain blocks within the Eastern Arc Mountain range (Burgess *et al.* in press).

In studies investigating the number of endemics in each EAM block, Burgess *et al.* (1998a; in press) illustrated that the Taita Hills, East Usambara, Uluguru, and Udzungwa 'ranked of greatest importance' on account of having the most number of endemics, even when factoring in the area of forest (see Fig. 1.4). There are a number of possible explanations for these differences, which were discussed by Burgess *et al.* (1998a; in press). Firstly, given that the numbers are representative of the actual differences and not collecting intensity (see below), the ranking may be indicative of the variations in geographical history, area, spatial distribution, climate and topographical complexities. Although collectively grouped together as the ancient crystalline mountains, each massif appears to have a distinct geological history (Griffiths, 1993; Lovett, 1993a). The Uluguru Mountains have been postulated to have more ancient faulting origins than other regions (Griffiths, 1993). Thus it seems reasonable to assume that certain blocks may have been activated earlier or later, and connected to other mountains or areas and this may have impacted on species diversity (Iverson, 1991; Lovett, 1993a). It would also be predicted based on island biogeography theory (MacArthur and Wilson, 1967), that the largest forest area would retain the greatest species diversity, which appears to be broadly correct (Udzungwa, East Usambara, Uluguru) (Newmark, 1998; Burgess *et al.* in press.).

The proximity of each EAM to the coast and relative position to the equator affects rainfall patterns, as shown by the different precipitation regimes among Eastern Arc blocks (Hamilton, 1988). The highest rainfall pattern correlates with the areas of highest endemism, for example East Usambara and Uluguru have the highest annual precipitation and endemism levels. Fjeldså and Lovett (1997) showed a positive correlation between areas in the EAM with stable climatic regimes and numbers of endemic species. Precipitation levels are altered dramatically by the position of mountains, for example the Rubeho Mountains (which have a relatively low species diversity) are in the rain shadow of the Uluguru Mountains. In addition to spatial position, topographical complexities may account for differences in the numbers or abundances of species. Some mountain blocks have a great proportion of eastern facing escarpments (e.g. Udzungwa and East Usambara), and large altitudinal ranges (e.g. Uluguru and Udzungwa) (Iverson, 1991; Burgess *et al.* 1998a). Preliminary quantitative data seem to support these differences, Burgess et al. (1998a; in press) showed high levels of vertebrate endemism occur on eastern facing parts of mountains in the Udzungwa and East Usambara.

The evidence that heterogeneous patterns of diversity in the EAM are the result of geographical and historic differences seems compelling, but there are likely to be sampling inequalities that will have biased or inflated differences. The three areas of highest diversity (East Usambara, Uluguru, and Udzungwa) are also those that have historically received the greatest attention (see above). Many of the other regions (Mahenge, Rubeho, North Pare, South Pare, Malundwe and Ukaguru) which show relatively depauperate levels of endemism are poorly known (as discussed by Stuart, 1991), and have only been recently explored for a few specific groups (e.g. Evans and Anderson, 1993; Akker and Highstead, 1992; Seddon *et al.* 1995; Doggart *et al.* in press; Loader *et al.* 2004a). Results from these surveys are somewhat contradictory. Burgess *et al.* (1998a; p. 49) believe that recent studies 'have not resulted in many new findings', indicating that the Eastern Arc probably does show significant differences in species composition between mountains. Recent herpetological surveys of Ukaguru, Rubeho and Mahenge however have resulted in new species descriptions and findings (Poynton, 2003b; Channing and Stanley, 2002; Channing *et al.* 2002; Loader *et al.* 2004a; Menegon *et al.* 2003b, 2004; Menegon and Doggart, in prep.), and new species continue to be described from Usambara, Uluguru and Udzungwa regions (de Sa *et al.* 2004; Menegon *et al.* 2003b; 2004; Burgess *et al.* 2002). It seems, at least herpetologically, that we are far from understanding the true diversity of the EAM, bearing in mind also our poor taxonomic understanding of several groups (Howell, 1993; Poynton, pers. comm.). It seems unlikely that Rubeho, Ukaguru, Pares, Mahenge, and Malundwe will show comparable levels of endemism to the Udzungwa, Uluguru and Usambara, given their drier forest habitats and smaller areas of forest. Even so, current inventories are likely to underestimate the biodiversity of these areas (Loader et al. 2004a). Until further fieldwork is carried out, a confident interpretation of endemism patterns in the Eastern Arc is hindered by uneven survey effort.

1.2.3.3 Regional relationships of the fauna and flora of the EAM

Hypothesised relationships of EAM species and to those in other regions in Africa have underpinned our understanding of the biogeographic history of the EAM, and provided the best indirect evidence for the archaic age of the forests (Burgess, 1998a). Spatially, the Eastern Arc lies adjacent to the coastal Zanzibar-Inhambane regional mosaic lowland forests. However, as noted earlier, there are significant faunal and floral differences between the coastal forests and the EAM (Clarke, 1998; Poynton, 1990; 2000b; 2003a), which suggests only a limited shared biogeographical

history. Species in the Eastern Arc show closer relationships with geographically more distant forests in central, and West African forest regions (Guineo-Congolian). Kirk-Spriggs (2003, p.152) mentions these affiliations as being a 'highly distinctive and well known distribution pattern of species restricted to forest', which is thought to be indicative of a former connection between these areas. This pattern of distribution has been shown in a number of animal groups, including Coleoptera (Wagner, 2001), Diptera (de Meyer, 2001), Odonata (Clausnitzer, 2001), and amphibians (Poynton, 1999). The significance of the close relationship between East and West African forest species was placed into further context when it was speculated that connections between these two regions were probably severely restricted from the Miocene onwards (see Geology section 1.2.2), and therefore many species would have been isolated and would have to have persisted from this time onwards. For some vagile organisms it is possible to reconcile disjunct distributions with possible dispersal events and these are likely to have produced patterns of recent relationships, e.g. between species. Furthermore, some species thought to be restricted to forest habitats may be able to tolerate more arid conditions, and would have been able to disperse through gallery woodland forest or lowland habitats which may have extended at various times between these areas (Hamilton, 1988). However, the biology of many species (the cold adapted afromontane element, *sensu* Poynton, 2000a) suggests that there are unlikely to be any plausible mechanisms for undertaking such a trans continental migration. In such cases, the most likely explanation is that the most recent forest connection in the Miocene was the last possible period of exchange between montane species (Lovett, 1993a), and that since then these taxa have persisted as 'biogeographically relictual species' (Fjeldså and Lovett, 1997). This hypothesis has been applied to explain some of the 'ancient' patterns of relationship between East and West African forest species (Kingdon, 1989; Lovett, 1993a; Burgess *et al.* 1998a).

1.2.3.4 Temporal patterns of diversity

The temporal origin of the endemic faunas of the EAM has only been preliminarily investigated, and this is one of the main foci of the thesis: The general consensus suggests a mixture of both recently evolved endemics (neo-endemics) and ancient lineages (palaeo-endemics) (Fjeldså, 1994; Fjeldså and Lovett, 1997). The occurrence of both ancient and newly evolved species indicates the EAM is both a centre of recent evolution and an ancient refuge (e.g. Burgess *et al.* 1998a). This

contrasts with the nearby lowland coastal forests, where recent speciation is less evident and most endemic species seem to be ancient (Burgess *et al.* 1998b; Matthee *et al.* 2004). Examples of genetically ancient lineages in the EAM include birds (e.g. Roy *et al.* 1997; Roy, 1997) with monophyletic forest groups thought to stretch back some 30 million years, dwarf pigmy chamaeleons (Mathee *et al.* 2004), angiosperms (Davis *et al.* 2002; Möller and Cronk, 1997; Lindqvist and Albert, 2001), grasshoppers (Hochkirch, 1998; 2001), molluscs (Emberton *et al.* 1997), caecilians and snakes (Wilkinson *et al.* 2003; Gravlund, 2002), elephant shrews (Douady *et al.* 2003b), and bryophytes (Pocs, 1998). Despite there being patchy evidence for the temporal diversity of lineages in the EAM, little work has been carried out to synthesise all this information to develop a coherent understanding of temporal patterns in the EAM.

1.2.3.5 Summary

The EAM show variable patterns of species richness and evenness. We currently have only a limited ability to interpret these diversity patterns, because sampling inequalities are notable (Howell, 1993; Burgess *et al.* 1998a; Poynton, *et al.* submitted) and only limited data are available. A preliminary extrapolation of distribution data and isolated phylogenetic case studies suggest that the isolation and persistence of mountain forests had a substantial influence on the diversity of species (e.g. Fjeldså and Lovett, 1997; Roy *et al.* 1997). It is unclear how biogeographically the different mountain blocks relate to one another, and how recent connections have been broken between each mountain and forest region, and consequently the impact this has had on the isolation of populations. A broad cladistic biogeographical investigation of the EAM is lacking. This study may allow a better interpretation of the history of the area.

Part 2: Investigations into the biogeography of Amphibians of the Eastern Arc Mountains

1.3 Amphibians and biogeography

1.3.1 Introduction

Amphibians are a model group for biogeographical studies, as shown by their often, restrictive habitats and breeding requirements (e.g. Poynton, 1962; Avise, 2000). Recent broad scale studies of amphibian biogeography have shown patterns consistent with our understanding of plate tectonics (e.g. Wilkinson *et al.* 2002; Gower *et al.* 2002; Biju and Bossuyt, 2003; Roelants *et al.* 2004), despite some striking examples of widely dispersing amphibians (Vences *et al.* 2003a; Vences *et al.* 2004). Numerous regional phylogeographic studies have also been carried out using amphibians (predominantly frogs) as indicators. These studies are generally congruent with the patterns of geographic changes, thereby confirming the idea that they are good indicators of geographical events (Avise, 2000).

1.2.2 Amphibian biogeography in Africa

The taxonomy of mainland African amphibians is very poorly understood (Mittermeier, *et al.* 1992; Lawson, 1993; Lawson and Klemmens, 2001; Rödel, 2000; as summarised by Poynton, 1999), particularly the fossorial herpetofauna (Gower and Wilkinson, 2005) and as would be expected there are only a few biogeographical studies (e.g. Wieczorek *et al.* 2000, Vences *et al.* 2004; see also section 2.6.2.2). Broad overviews of the historical events shaping the evolution of African amphibians are generally lacking, and we have only a very rough understanding of the possible biogeographical influences on their diversification (e.g. Poynton, 1962; Poynton and Broadley, 1991; Savage, 1973; Laurent, 1979; Duellman, 1993) compared to other groups (e.g. Mammals, Kingdon and Howell, 1993). There are various reasons for this, including; lack of phylogenies for cladistic biogeographical reconstructions (Kirk-Spriggs, 2003; see section 2.6.2.2), lack of fossil data (Howell, 1993), but probably most significant is the lack of sampling in vast regions of Africa (Lawson, 1993; Lawson and Klemens, 2001), and detailed taxonomic work (Poynton, 1999).

Herpetologists have long speculated on the origin and determinants of tropical diversity in Africa, and attention has focused on two distinct elements: the widespread lowland tropical fauna and the isolated montane fauna (e.g. Loveridge, 1937; Poynton, 2003a). The evolutionary histories of these elements are thought to differ significantly, with an archaic origin suggested for the montane fauna and a more recent origin for the lowland fauna. These differences are thought to be indicative of their respective biogeographic histories: the montane fauna is characterised by prolonged persistence, whereas the lowland fauna has been subject to the constant fluctuations of the turbulent African climate and have therefore experienced recent isolation, expansion, dispersal and extinction. Current understanding of these patterns and processes is poor and there is a dearth of quantitative and qualitative data. One would expect that isolation and persistence in montane habitats would generate specific phylogenetic, ecological and physiological patterns, and similarly, specific patterns would be anticipated for the lowland faunas. These predictions can be used to generate testable hypotheses to evaluate patterns of speciation of tropical amphibian fauna of East Africa.

Loveridge (1937) was the first to note the difference between lowland and montane amphibian fauna in his distributional survey of East Africa. He noted that 83% of the anurans occurring below 300m (lowland) were widely distributed, while only 48% in the highest zone (>1500m; montane). This montane fauna extends from South African to isolated highlands areas along the highland "spine" of Africa to Ethiopia and West African highlands (Poynton, 1962; Largen and Drewes, 1989; Largen, 1991; Poynton, 1998; Poynton, 1999). The Eastern Arc Mountains, a component of the montane fauna, has been singled out as a good example of a highly diversified fauna (e.g. Microhylids and Bufonids), which Poynton (1998; p.vi-vii) suggested was the result of 'prolonged isolation'. These EAM assemblages have been shown to be distinctly different from the proximally close lowland assemblages (Poynton, 2003a; Loader *et al.* 2004a).

Montane fauna of the EAM are confined to regions generally found above 400m, although the precise altitude is dependent upon the latitude and geography of an area. For example, for montane faunas distributed in mountains in a rain shadow, or at certain slope aspects, species have been found at generally higher altitudes (Emmett, 2004; Menegon, pers. comm). Below 400m, a generally widespread tropical lowland fauna is present. Between these two amphibian faunas there is a

complex transition zone where both assemblages overlap (Poynton, 2000a; Poynton, 2003a). Poynton (2003a; p.124) envisaged over geological time that subtraction zones between these two faunas would be periodically 'spreading and withdrawing...following cyclic changes in climate' which would have clear biogeographic implications on speciation patterns. Many authors have attributed high levels of endemism in the EAM amphibians (e.g. Loveridge, 1937) to this biogeographical history. These biogeographical patterns are not solely found in amphibians, but are congruent with patterns found in other species (Grimshaw, 2001), particularly poor dispersers, such as many invertebrates (Hoffman, 1993; Scharff, 1993). It is likely that the same, or similar biogeographic processes have influenced speciation patterns in the fauna and flora of the EAM, this however has yet to be tested.

Comparisons of the amphibian fauna with other continents have considered Africa to be 'depauperate' (Laurent, 1979; Duellman, 1993) however vast areas of forest are completely unknown, so until baseline herpetological research in tropical Africa is carried out, any such conclusions may be premature. However, it is likely that a continent dominated by arid habitats will have proportionally less amphibian species than in more forested continents. On mainland Africa, the areas of highest amphibian diversity are unsurprisingly located in regions covered by forest (see Table 1.1). In particular, montane elements are singled out as being important reservoirs for amphibian diversity, as shown by numbers of species in Cameroon, Tanzania, and South Africa (all in the top four mainland countries for amphibian diversity).

Table 1.1.

The number of Amphibian species in the top ten African countries, with land area.

Country	Land area (sqkm^2)	Number of species
Madagascar	581,540	219
Congo, The Democratic Republic of the	2,267,600	207
Cameroon	469,440	180
Tanzania	886,037	142
South Africa	1,219,912	114
Nigeria	910,768	98
Angola	1,246,700	90
Kenya	569,250	90
Ivory Coast	318,000	85
Zambia	740,724	84

Worryingly the areas richest for amphibian diversity; forest habitats, are also threatened by habitat destruction. Preliminary results from the recently compiled global amphibian assessment have shown that some 26% of African amphibians are threatened and 20% were evaluated as being data deficient (Stuart, pers. comm.). So not only are African amphibians at risk of becoming extinct, they are also relatively poorly understood compared to other regions of the world. Based on these findings, conservation projects in the hotspots of amphibian diversity are being established (as shown by CEPF global hotspots initiative, 2002; 2003). It is hoped that these projects will increase understanding of amphibian diversity in Africa, particular in the richly diverse rainforest areas, such as those found in the forests of Tanzania and Kenya (Howell, 1993).

1.4 Study taxa and molecular marker selection

1.4.1 Introduction

In a volume on the biogeography of East African forests, a number of authors (Hoffman, 1993; Scharff, 1993; de Jong and Congdon, 1993) suggested that it would be necessary to examine the evolutionary history of a monophyletic group endemic to the Eastern Arc to gain an insight into the biogeographic history of the area. Preferable would be species with a small range and low vagility (eg. Scharff, 1993). In the same volume, Howell (1993) suggested the favourable characteristics of Eastern Arc amphibians for use as biogeographical indicators (suggested previously Loveridge, 1937; Schiøtz, 1981). Amphibians, he suggested, would be suitable because they depend upon habitat connections rather than long distance dispersals for migration. Howell, (1993; p.195) also alluded to the methods that should be applied in such investigations, stating 'the study of mitochondrial and ribosomal DNA sequences would allow the refined insight into the ages and relationships of each herpetofaunal assemblage relative to the others within the EA system'. In addition to the appropriateness of amphibians as indicators in biogeographical analyses, the amphibian fauna of this region is very poorly known. As Howell (1993; p.173) introduced in his chapter on the herpetofauna of the eastern African forests 'of all the vertebrates, the amphibians and reptiles of the forests are the poorest known and receive the least attention from layman and biologists alike'.

1.4.2 Taxon selection

1.4.2.1 Introduction

In this study, I focus upon a series of taxonomically unrelated amphibian groups whose species show different distribution patterns. Although the lineages were not entirely chosen at random (species were selected partly on the basis of availability of tissues), the taxa chosen were not selected because of congruence in distribution, which may be seen to bias a biogeographical study. Species chosen for this study were selected mainly on the basis of their dependence on forest habitats. Species restricted entirely to forest habitats (part of the afro-montane element, *sensu* Poynton, 2003a) were favoured because these species were more likely to show patterns congruent with habitat changes. Habitat requirements are unknown for many species, and assumptions were made based on the localities of material previously collected and personal experience. Zonation of the amphibian fauna in Eastern Tanzania has been well documented (e.g. Poynton, 2003a; Loader *et al.* 2004a), and there has been shown to be distinct lowland and upland faunas.

Table 1.2.

Groups selected for study

Genus, or subfamily	Family	Amphibian order	Reproductive mode	Ecological niche
Scolecomorphus§	Scolecomorphidae	Gymnophiona	Viviparous	Fossorial & forest floor
Boulengerula§	Caeciliidae	Gymnophiona	Direct developing	Fossorial
*Arthroleptides**	Ranidae	Anura	Aquatic larval development	Forest floor & stream
Brevicipitines- *Callulina, Probreviceps, & Spelaeophryne*§	Microhylidae	Anura	Direct developing in the genus *Breviceps*	Arboreal, forest floor & fossorial
Hoplophryne†	Microhylidae	Anura	Aquatic larval development	Forest floor

§Contains species that are vulnerable, according to the IUCN redlist.

*Contains species that are threatened, according to the IUCN redlist.

†Contains species that are critically endangered, according to the IUCN redlist

Dispersal ability influences biogeographic patterns (Vermeij, 1991) and this needs to be accounted for in studies on biogeography. Taxa sampled in this study are

considered to be associated predominantly with the upland faunas with limited capacity for dispersal. However the taxa selected had different biologies, which potentially influence biogeographic patterns. Amphibians show an array of dispersal capabilities, reproductive modes, and habitat niches. For example, it may be that by virtue of their presumed relatively low dispersal capabilities, that fossorial amphibians retain more information than non-fossorial taxa on 'original' biogeographic patterns. Therefore, amphibians selected in this study show a range of dispersal abilities that would permit common biogeographical patterns to be more strongly evaluated. The following groups were selected (see Table 1.2):

1.4.2.2 Caecilians

Gymnophionan amphibians are thought to be ideal subjects for biogeographic studies because of their presumed limited powers of dispersal, and dependence upon forest habitats (Taylor, 1968; Nussbaum, 1985; Nussbaum and Hinkel, 1994). Knowledge of the caecilians of the Eastern Arc is extremely limited, and this is true for caecilians in general (Nussbaum and Wilkinson, 1989). Caecilians occur on all the mountains of the EA, where they can be found in moist soils; mainly in forest but also in agricultural land. Most EA mountain blocks harbour species of two genera found throughout the region, the caeciliid *Boulengerula* and the scolecomorphid *Scolecomorphus*. The systematics of these genera were relatively recently reviewed (Nussbaum 1985, Nussbaum and Hinkel, 1994), but there remain several outstanding questions. These caecilians are very different animals - *Boulengerula* is slender, has short tentacles and the eye is very reduced and not visible externally, while individuals of *Scolecomorphus* are usually more robust and reach a greater size, and they have eyes that are visible when they protrude, with closely associated, long tentacles (O'Reilly *et al.* 1996; Gower *et al.* 2004). *Boulengerula* lay eggs that develop directly (Nussbaum, 1994), while *Scolecomorphus* are viviparous (e.g. Nussbaum, 1985; Loader *et al.* 2003a,b). A recent ecological study of the species *Boulengerula boulengeri* and *Scolecomorphus vittatus* in the East Usambara Mountains (Gower *et al.* 2004) indicated differences between the proportions of captures above and below ground, and these results were taken to indicate different ecologies. *B. boulengeri* was interpreted as predominantly a burrower in soil, and *S. vittatus* as an animal spending a greater proportion of time above ground than *B. boulengeri*. Whether these ecological differences are reflected in all species of the genera *Boulengerula* and *Scolecomorphus* is uncertain. However, based on the morphological similarity among species in each genus, the ecological differences

shown between *S. vittatus* and *B. boulengeri* probably hold true for all members of each genus.

1.4.2.3 Frogs

Morphological studies on East African microhylid and petropedetid frogs (Channing *et al.* 2002; Poynton, unpublished) indicate the existence of distinctive species in each geographical area of the Eastern Arc (though not necessarily each mountain). Microhylid frogs are a fascinating group, showing enormous morphological diversity (Parker, 1934) from 'squat and small toad-like animals to arboreal frogs with expanded tips of the digits' (Duellman and Trueb, 1994; p.549). The EAM harbour six lineages, belonging to two distinct subfamilies (Brevicipitinae and Melanobatrachinae). Furthermore, within the Brevicipitinae there are a number of distinct morphological lineages that have interesting ecologies, including fossorial (*Probreviceps, Breviceps*), arboreal (*Callulina*) and open woodland/forest edge (*Spelaeophryne*). Breeding biology of brevicipitines is unknown for the endemic East African genera, although the presence of large pigmented ova in females suggests a direct developing mode of reproduction. The widespread African genera *Breviceps* has been shown to be a direct developer (Parker, 1934), which suggests brevicipitines may be exclusively direct developers. The small cryptic *Hoplophryne* and *Parhoplophryne* microhylids are forest dependent, aquatic developers (Parker, 1934; Barbour and Loveridge, 1928; Harper and Vonesh, 2002). Tadpoles of *Hoplophryne* are thought to possess a unique structure on the abdomen that has been postulated to help manoeuvring in small water-filled tree holes or bamboo cups that they occupy (Harper and Vonesh, 2002). The torrent frogs *Arthroleptides* are relatively large ranids, and are found near rocky streams in forests. They are characterised by their expanded heart-shaped digital discs, which allow them to cling to rocks in the stream. The tadpoles of *Arthroleptides* have an interesting morphology; the mouthparts are highly modified, wide, with densely keratinised jaw sheaths (Drewes *et al.* 1989), which allow them to adhere and graze on the surface of moss-covered rocks. In summary, all the taxa selected displayed features which were appropriate for biogeographical reconstructions, while displaying unique characteristics which may have interesting evolutionary implications both biogeographically and functionally.

1.4.3 Molecular markers

Molecular markers are used to discover patterns of gene flow between taxa, and the variations shown are expected to mirror processes such as fragmentation, long distance colonization, and population division (Avise, 2000). For an alternative perspective see Irwin (2002), Funk and Omland, (2003) and the discussion in section 2.5.1. Markers display a number of different properties and accordingly these characteristics make them suitable for addressing particular hypotheses. Most importantly, the level of variability a marker displays might be appropriate for investigating deep phylogenetic divergence patterns, but not population dynamics, so the choice of marker should be dependent upon the question that the study is attempting to address (Graybeal, 1993; Simon *et al.* 1994). Partial fragments of mitochondrial DNA (mtDNA) are widely used in phylogenetic studies, and this is because of their rapid rate of evolution at the nucleotide level, maternal inheritance, ease of amplification due to multiple copies and lack of recombination. Rapid rates of nucleotides substitutions between individuals make them suitable for reconstructing species relationships (Avise, 1994; Avise, 2000). Because mtDNA typically doesn't recombine, and are transmitted through maternal lines in most species, the sequences therefore represent organismal 'pedigrees' (Wilson, 1985), and therefore can be interpreted as a lineage sharing a pattern of common descent in phylogenetic analyses (Avise, 1994). These features have instilled the molecule as a marker of major importance in phylogenetics, and the results yield demographically relevant conclusions about historical population structure (Avise, 2000).

Mitochondrial markers that have been extensively utilised are 12S (small ribosomal subunit) and 16S (large ribosomal subunit). These markers are particularly favoured because they have regions that evolve at different rates, which mean they contain information from both old splitting events (in conserved domains) and recent speciation events (from fast evolving sites in more variable regions) (Avise, 2000). At a practical level also, mtDNA can be reliably amplified using "universal" primers, in particular 12S and 16S, which work for almost any species. Furthermore, sequences that can be easily accessed and downloaded from genetic databases (Benson *et al.* 1998) are mtDNA sequences. The ease at which mtDNA can be amplified and accessed, and its suitable phylogenetic properties have made mtDNA a popular genetic marker; 70% of all phylogenetic studies conducted involve analyses of mtDNA (Avise, 2000; see also section 2.5.1 for a further discussion).

In addition to 12S and 16S markers, there are a number of other genes that are frequently used in phylogenetic studies. Among a dozen or so protein coding genes in the mitochondrial genome, Cytochrome b (*cytb*) is widely used (Irwin *et al.* 1991; Hillis *et al.* 1996), particularly for species level questions, in frogs (e.g. Graybeal, 1993) and caecilians (e.g. Gower *et al.* 2002; 2005), and for which reliable universal primers are available (Goebel, 1999). Protein coding genes may provide valuable data for phylogenetic studies. Unlike 12S and 16S sequences for which homology is sometimes uncertain and alignment difficult, *cytb* sequences code for proteins, which restrict sequence ambiguities such as insertions and deletions. Furthermore, because *cytb* is a protein-coding gene with 1st, 2nd and 3rd codon positions, there are variable and predictable relative rates of substitution that increase its utility in phylogenetic studies (e.g. Graybeal, 1993).

One potential pitfall of using mtDNA is that, because of its matrilinear, non-recombining mode of inheritance, it will always exhibit a phylogenetic pattern of transmission. Within populations this branching pattern will represent specific ancestor-descendant lineages rather than phylogenetic relationships of a species (Puorto *et al.* 2001). Therefore, multiple haplotype clades do not necessarily imply multiple organismal lineages. Introgressive hybridisation can produce the same effect (Echelle and Echelle, 1994). There is also the possibility that, because mtDNA is maternally inherited, a strong geographical pattern will reflect only female gene flow and dispersal, which may differ from those patterns in males, thereby giving an inaccurate picture of phylogenetic relationships (Avise, 1994). Given these problems, nuclear markers are slowly beginning to be favoured, but have received far less attention in studies of population differentiation, as they show slow rates of molecular evolution and also currently lack reliable universal primers (Hillis *et al.* 1996). Previous studies of 12S, 16S and *cytb* on both caecilians (Gower *et al.* 2002; 2005; Presswell, 2002) and frogs (Graybeal, 1993; Vences *et al.* 2003a,b) have indicated their usefulness at reconstructing phylogenies of species and populations for biogeographic investigations. In addition, primers are available which worked reliably for target taxa (Presswell, 2002; Goebel *et al.* 1999). As a result, the three genes 12S, 16S and *cytb* were used in this study to investigate systematic and biogeographic patterns.

1.5 Aims of the project

The central aim of this project is to improve understanding of the historical biogeography of the EAM. This is tackled by reconstructing molecular phylogenies for several independent lineages of amphibians. Combined with species distribution data, these are used to explore area relationships through cladistic biogeography, molecular clock estimations and descriptive biogeographical methods.

Main hypotheses to be addressed:

(1) There is a significant area relationship(s) in the Eastern Arc Mountains.

(2) That area relationship(s) are temporally congruent.

(3) That congruent area relationships are consistent with nestedness patterns recovered in parsimony analysis of endemicity and similarity indices.

(3) Discordance in temporal and spatially relationships is correlated with dispersal ability (for example, fossorial vs. non-fossorial).

(4) That there is a strong correlation both temporally and spatially between area relationships and significant geographic events, e.g. uplift of the mountains in the late Miocene.

(5) Phylogenetic lineages are generally deeply divergent between monophyletic taxa distributed in the Eastern Arc, in association with a long period of geological and climatic stability.

In addition, analyses of multiple unrelated taxa can reveal common biogeographic patterns, which may contribute to understanding of the dynamics of speciation and rates of DNA evolution (Avise, 2000). Results from molecular phylogenies also provide an essential framework for studies on taxonomy, comparative morphology, ecology, and conservation.

Chapter Two

Materials and methods

2.1 Specimen collection

2.1.1 Introduction

In order to address the aims of this project, a wide geographical sampling of components of the Eastern Arc amphibian fauna was necessary. It was also crucial that material was available which would allow the extraction of DNA for generating molecular sequences. The following sources were utilised to achieve this goal: Material deposited at the Natural History Museum London (NHM), loans of material from international museum institutions, and new fieldwork in Tanzania and Kenya.

2.1.2 Sources of specimens

2.1.2.1 Natural History Museum, London.

The EAM has had a long history of collecting and study dating from the early missionary days and the German/British occupation of Tanzania and Kenya (Howell, 2000), and this is reflected in the collections of many national institutions. Specimens deposited in the herpetological section of the NHM London represent one of the largest worldwide collections of EAM amphibians, primarily through the collections made by Arthur Loveridge, Alice Grandison, and Kim Howell (Howell, 2000). These collections have been worked on by a succession of staff in the herpetology section, all of which have had an active interest in EAM amphibians (e.g. Boulenger, 1882; 1883; 1894; 1895; 1898; Parker, 1934; Grandison, 1983), which continues today (e.g. Clarke, 1988; Poynton, 2003b; Wilkinson, *et al.* 2004). Most of these collections were made many years ago, and were preserved in formalin, which has rendered them broadly unsuitable for extracting DNA.

One of the most significant recent contributions to the NHM collection has been the deposit of nearly ~4,000 specimens by Professor Kim Howell of the University of Dar es Salaam. Professor Howell has carried out biotic surveys and conservation work in conjunction with various individuals and non-government organisations over the past

thirty years in Tanzania. Collaborating with Kim Howell, two groups have made significant collections that are of relevance to the work outlined here, Frontier-Tanzania and the Tanzanian Forest Conservation Group (TFCG). These collaborations have focused, though not entirely, on baseline biodiversity surveys of EAM, which has included collections of amphibians (e.g. Frontier, 1999-2002; 2001; Johansson *et al.* 1998; Doggart *et al.* 2004; in press). A large proportion of the amphibians collected during these expeditions are sent to the NHM for taxonomic identifications that are then used in compiling species lists for biological inventories (e.g. Frontier, 2001). Professor John Poynton has identified amphibians sent by Frontier-Tanzania and TFCG over the past ten years. These collections have resulted in a number of significant discoveries (e.g. Poynton *et al.* 1998b; Poynton, 2003b; Menegon *et al.* 2004). The amphibian material collected by Frontier-Tanzania and TFCG is preserved in ethanol (70%), and is therefore suitable for extracting DNA[1]. The author worked for Frontier-Tanzania as a volunteer (1997) and an assistant research co-ordinator (2000) in biodiversity inventory surveys of the East Usambaras.

2.1.2.2 Other institutions

Other than the collection held at the NHM, many East African amphibian specimens are held at the Museum of Comparative Zoology in Harvard (MCZ), especially among the collections made by Arthur Loveridge in the 1920-30s. Unfortunately, Loveridge's collections were all preserved in formalin, creating difficulties in extracting long strands of DNA. Standard measurements of the type specimens held in MCZ were made for comparisons with other material used in this study. Similarly, material held at the Zoological Museum Berlin includes valuable historical collections. This material was principally described in the works of Tornier (1897) and Nieden (1910; 1912; 1913). Extraction of DNA was not attempted for any of this material; the age of the specimens, and the probable use of formalin for preservation meant that molecular work may have been inefficient. Measurements of the Berlin material were made for comparison with voucher specimens used for generating molecular data (Wilkinson *et al.* 2004). Material was borrowed from California Academy of Sciences (CAS), Zoological Museum of the University of Copenhagen (ZMUC), Museo Trentino di Scienze Naturali, Trento, Italy (MTSN), John Measey (JM), Transvaal Museum (TM), Museum d'Histoire Naturelle Geneva (MNHG), Univeristy of Texas, Arlington (UTA)

[1] Collections of amphibians by Frontier-Tanzania deposited at the NHM between 1989-1999 were preserved in industrial methylated spirits.

and Field Museum Chicago (FMNH). Material borrowed from these institutions and individuals provided specimens or tissues that were used for obtaining molecular sequence data (see Appendix 2).

2.1.2.3 Fieldwork

Based on the tissues assembled from various sources it was clear that there were still large gaps in the sampling of amphibians of the EAM. Sampling gaps were obvious for locations where specimens were present, but for which tissues were not available. However, with localities where no survey data existed it was less clear if species were likely to occur there. Disparity in the number of species found in each Eastern Arc mountain have often been attributed to inadequate sampling (e.g. Schiotz, 1981; Burgess *et al.* 1998a). I was optimistic that surveys of poorly known areas would result in range extensions and new species. Fieldwork carried out in November 2001 and March 2002 was planned to sample the less well known areas. It was however apparent that sampling inequalities between mountains could not be completely overcome. In addition, fieldwork carried out in 2001/2 was over a short period, during which I was unlikely to sample all amphibian species or areas. Despite these problems, sampling of all the Eastern Arc mountains was carried out (either through fieldwork or museum specimens) to a greater or lesser extent, apart from one locality, Malundwe Hill. This was difficult to sample logistically, being located within Mikumi National Park, and requiring official authorisation that I was unable to acquire. No single species was sampled in this study from this area, despite one known record of a caecilian *Scolecomorphus* sp. (Howell, pers. comm.). Malundwe Hill is the smallest patch of forest in the EAM (Burgess et al. 1998a), and possibly of least biological importance (see Lovett and Norton, 1989 and Lovett and Pócs, 1993) of all the EA massifs, and was therefore determined as being of lowest priority. In general, the EAM experience two main rainy seasons (though there are differences north to south between the mountain blocks), the short rains in November and December and the long rains from April to June. Experience shows that fieldwork is best carried out during the rainy seasons, when amphibians are most abundant and active and so fieldwork was timed to coincide with the onset of the rains. The schedule of fieldwork is shown in Table 2.1, and the specimens collected during these field trips are shown in Appendix 1.

Prior authorisation to carry out research in Tanzania was obtained in May 2001 through the Tanzania Commission for Science and Technology (COSTECH) and TAWIRI. The research permit RCA 2001-272 allowed me access to carry out

fieldwork in the EAM in Tanzania and to export the preserved specimens back to the UK. Before visiting any forest reserve, regional and local authorisation was necessary to carry out any work. Permission from regional offices in Tanga, Arusha, and Morogorro was necessary before embarking on fieldwork in specified areas.

Table 2.1.
Fieldwork carried out in 2001-2002.

Dates	Locality	Forest reserve	Notes
06/10/2001- 07/10/2001	Coastal Forest	Ras Kutani	Survey- 1 day
09/10/2001- 12/10/2001	Mahenge Mountains	Sali Forest	Survey- 3 days
13/10/2001- 15/10/2001	Dar es Salaam	-	Processing Nguu material from JB
16/10/2001-19/10/2001	Rubeho Mountains	Mafwemiro Forest	Survey- 2 days
20/10/2001- 22/10/2001	Nguru Mountains	Nguru South Forest	Survey- 2 days
26/10/2001- 29/10/2001	West Usambara Mountains	Mazumbi Forest	Survey- 3 days
28/4/2002- 29/4/2002	East Usambara Mountains	Magambo Forest	Survey- 2 days
04/05/2002- 05/05/2002	Ukaguru Mountains	Ikwamba Forest and Mamiwa-Kisara Forest	Survey- 2 days
09/05/2002- 12/05/2002	North Pare Mountains	Kindoroko Forest	Survey- 2 days
12/05/2002	Arusha	-	Processing Nguu and West Usambara material from JB
13/05/2002- 14/05/2002	South Pare Mountains	Chome Forest	Survey- 2 days
15/05/2002- 16/05/2002	West Usambara Mountains	Ambangula Forest	Survey- 1 day
18/05/2002	Bagamoyo	Ruvu ferry	Survey- 1 day
21/05/2002- 22/05/2002	Uluguru Mountains	Uluguru North Forest	Survey- 1 day

The first field season was carried out in conjunction with Dr Jean Mariaux of the Museum d'Histoire Naturelle Geneva, and the second with Dr Jean Mariaux, Dr David Gower and Dr Mark Wilkinson. Specimens that were deposited in MNHG are not listed. During all fieldwork night and day collections were carried out usually over two to three-day intensive survey periods. Drift fences were constructed in the forest to collect leaf-litter amphibians when there was enough assistance from residents of the area. Digging and searching through leaf litter and forest was carried out to locate burrowing caecilians and microhylid frogs. All specimens were killed by anaesthesia using MS222, and preserved in buffered formalin. Almost all specimens had tissue samples (liver) for DNA analysis taken and these were stored in 95%

ethanol. Live specimens were also obtained from Joe Beruducci (JB) in Arusha who captively breeds reptiles and amphibians.

A Garmin e-map GPS was used to obtain the coordinates of specimens collected during fieldwork. In addition, co-ordinates were obtained from maps and a gazetteer (www.gazetteer.com) for all other specimens. These co-ordinates were used for calculating distances between each locality using ArcView, assisted by Neil Cox of Conservation International. The distance is based on projected units of the data, so the data needed to be projected from latitude and longitude into a map coordinate system in meters. For these calculations, an Equidistant Azimuthal projection was chosen with the azimuth near the centre of the data's extent, minimizing the distortion that may be caused by the choice of projection. The tool calculates the distance from each feature in one dataset to the features of another layer. In this case, the points were brought into ArcView as an XY event theme and used as both the 'from' and 'to' dataset, this gives the distance between each point and all other points in the same layer. The data were then automatically added to the table of the input layer, creating the matrix of distances. This table was then exported to an Excel spreadsheet.

2.2 DNA extraction

A total of 243 tissue samples were processed (see Appendix 2). These samples included specimens that had come into contact with formalin, industrial methylated spirits (IMS), and ethanol. Extraction of DNA from these tissues required various methods to complement the method of preservation. Generally, tissues that were not preserved in ethanol were unlikely to produce fragments of DNA from which successful amplification could be carried out. However, there were exceptions to this, and a number of recently deposited tissues preserved in IMS yielded amplifiable DNA.. Storage in IMS appears to degrade DNA, because samples more than two years old did not produce adequate quantities for efficient amplification. DNA fragments were not obtained from any formalin preserved material (see Appendix 2).

Standard protocols for DNA extraction from ethanol fixed tissues were followed, as described in Sambrook *et al.* (1989). A small piece of tissue (1-2mm^3) was chopped with a sterile scalpel blade then suspended in 800µl digestion buffer[1] in a 1.5ml

[1] (Digestion buffer: 1MTris 1000µl, 5MNaCl 200µl, 0.5MEDTA 200µl, SDS 500µl, Prot. K 400µl (20 mg/ml), then add autoclaved water to make up to 10ml solution).

eppendorf. Samples were kept at 55°C for three hours in a shaking incubator, or overnight at 37°C. After the incubation period the sample was centrifuged (at 13,000rpm) for two minutes, and the supernatant was pipetted off, leaving behind any undigested tissue fragments. An equal volume (800μl) phenol/chloroform (1:1), was added to the digest sample which was agitated and centrifuged (at 13,000rpm) for 3 minutes. This process was repeated once usually, or twice with dirty samples. The supernatant was then pipetted off and added to 700μl chloroform, and vortexed and centrifuged for a further two minutes. The cleaned sample was then cleaned with an ultrafiltration MICROCON (100) column following the manufacturer's instructions. The resulting solution is then used directly as a template for PCR amplification. The approximate concentration of the DNA was checked by gel electrophoresis. 2μl of the template was run on an agrose gel (1-2%) with an ethidium bromide (1μl added) stain and was run for 60 minutes at 50mA. The gel was viewed on a short-wave UV transilluminator, and a digital photograph processed using the program Labworks 4.0 (UVP Inc. Upland, UK).

Extraction procedures for obtaining sequences from formalin and IMS preserved material were investigated for a number of tissues for species where fresh material was not available. DNeasy Kits (Qiagen®, UK) provide protocols for extraction of DNA from formalin preserved material. These extractions provided negligible levels of template for PCR reactions. Extraction of DNA from material preserved in IMS was more successful, standard methods with a prolonged digestion period sometimes resulted in DNA suitable for PCR amplification.

2.3 Polymerase chain reaction and PCR sequencing

2.3.1 PCR and gel purification

The methods adopted follow standard PCR protocols (as described by Palumbi, 1996). Fragments of the 12S and 16S rRNA genes and the cytochrome b (*cytb*) gene were amplified by the polymerase chain reaction (PCR) on a Hybaid Omnigene E, Mastercycler, and Perkin Elmer thermocycler, using specific primers (custom primers by Bioline, UK®) as listed in Table 2.2. The volumes used in the reactions are given: 18μl Water[2], 2.5μl Buffer (Bioline, UK®), 2.0μl $MgCl_2$ (25mM, Bioline,

[2] Alternative volume of water was used with the addition of a PCR additive buffer (Bioline, UK®), 13μl Water with 5μl of PCR high spec. additive.

UK®),1.0μl of each forward and reverse primer (10μM), 0.2μl dNTP (mixed dATP, dCTP, dGTP, dTTP), 0.15 μl Taq polymerase (Bioline, UK®), 2μl genomic DNA.

All reagents were prepared as a PCR master mix to which DNA was added before samples were placed in the PCR machine. Mineral oil was overlaid on reactions carried out using the Hybaid Omnigene E machine, which lacked a heated lid. The following cycles were specified on all machines:

1) 80°C for 2 minutes (pause to put tubes into PCR machine)
2) 1x (96°C 4min; 94°C 4min; 45°C 45 secs.; 72°C 1min)
3) 35x (94°C 4min; 45°C 45 secs.; 72°C 1min)
4) 1x (72°C 10min; 4°C hold)

Table 2.2
Primers used to amplify 12S, 16S and *cytb* in this study.

Name	Primer Location	Sequence 5'- 3'	Amplify Frog (F) or Caecilian (C)	Source
12Sa	12S	AAA AAG CTT CAA ACT GGG ATT AGA TAC CCC ACT AT	F and C	Kocher, *et al.* 1989.
12Sb	12S	TGA CTG CAG AGG GTG ACG GGC GGT GTG T	F and C	Kocher, *et al.* 1989.
12Sb (chicken)	12S	GAG GGT GAC GGG CGG TAT GT	F and C	After (Kocher, *et al.* 1989.)
16Sa	16S	GGC CTA AAA GCA GCC ACC TGT AAA GAC AGC G	F and C	After (Hedges, *et al.* 1993)
16Sb	16S	GAG GAT TTT TTA TTC TCC GTG GTC GCC CCA	F and C	After (Hedges, *et al.* 1993)
L14724	*cytb*	CCG AGC TTG ATA TGA AAA ACC ATCG TTG	F and C	Meyer and Wilson, 1990.
MVZ 15-L	*cytb*	GAA CTA ATG GCC CAC ACW WTA CGN AA	F	Moritz, *et al.* 1992.
CB1F	*cytb*	CCA TCC AAC ATC TCA GCA TGA TGA AA	F	Palumbi, *et al.* 1996.
CB1F (frog)	*cytb*	CCA TCA AAC ATT TCA TCA TTA TGA AA	F and C	Palumbi, *et al.* 1996.
CB2F	*cytb*	TGA GGA CAA ATA TCA TTC TGA GGG	F	Palumbi, *et al.* 1996.
CB2F (frog)	*cytb*	TGA GGA CAA ATA TCT TTT TGA GGG	F and C	Palumbi, *et al.* 1996.
CB2R	*cytb*	CCC TCA GAA TGA TAT TTG TCC TCA	F	Palumbi, *et al.* 1996.
CB2R (frog)	*cytb*	CCC TCA AAA AGA TAT TTG TCC TCA	F and C	Palumbi, *et al.* 1996.
CB3R	*cytb*	GGC AAA TAG GAA RTA TCA TTC	F	Palumbi, *et al.* 1996.
CB3R (frog)	*cytb*	GGC GAA TAG GAA RTA TCA TTC	F and C	Palumbi, *et al.* 1996.

Products from PCR reactions were electrophoresed in a 1% agarose gel (Bioline, UK®) stained with ethidium bromide for 60 minutes at 50mA, and were then viewed on a short-wave UV transilluminator, and photographed using a computerised Labworks 4.0 System (UVP Inc. Upland, UK). The PCR fragments of expected size were cut from the gel. It was then necessary to extract and purify the PCR products contained in the gel slices to use as a template for sequencing. In general, PCR products were purified using a silica method as described in Boyle and Lew (1995). In addition, PCR products were recovered using QIAquick™ Gel Extraction Kit (Qiagen, Crawley, UK) as described by Presswell (2002).

2.3.2 Sequencing reactions

Sequencing reactions were carried out at the Natural History Museum sequencing facility and University of Glasgow Molecular Biology Support Unit. Both facilities

analysed the reactions in an ABI 377 apparatus (Applied Biosystems, Perkin Elmer, UK). Sequencing in this study used a fluorescent dideoxy chain terminator method (as described by Hillis *et al.* 1996). Amplification of the template was carried out on a Mastercycler, and Perkin Elmer thermocycler, using the same primers as used in amplification. The volumes used in the reactions are: 3.5µl Water, 2.0µl buffer (BigDyes, PE Biosystems), 2.0µl Big Dye (BigDyes, PE Biosystems), 1.0µl of primer (2.5µM), 3.5µl sequencing template.

All reagents were prepared as a primer specific master mix to which sequencing template was added before samples were placed in the PCR machine. The following cycle for the sequencing reaction was used:

1) 80°C for 2 minutes (pause to put tubes into PCR machine)
2) 1x (96°C 5min; 95°C 20 secs.; 50°C 10 secs.; 60°C 4min)
3) 24x (95°C 20 secs.; 50°C 10 secs.; 60°C 4min)
4) 4°C hold

2.4 Sequence alignment

Forward and reverse electropherograms and their base calls were imported into the program Sequencher 3.1.1™ (Gene Codes Corporation, USA). The program automatically aligns forward and reverse strands, producing a consensus sequence, which is then checked by hand to resolve any ambiguities. Consensus sequences with primer sequences removed were then imported into BioEdit v. 5.0.9 (Hall, 1999) a manual sequence alignment program. In addition to these newly gathered sequence data, partial 12S, 16S and *cytb* sequences for various species were imported, either from unpublished data of the NHM herpetology group, or from published data held in Genbank (refer to Appendix 3 for all GenBank sequences utilised). Specific alignment procedures and outgroup selection will be discussed in each appropriate chapter section. In general, sequences were aligned manually, length differences were resolved by inserting alignment gaps, and positions that could not be aligned unambiguously were excluded. Hypervariable regions therefore were excluded from analyses. To include the maximum amount of information, analyses were carried out on two separate alignments for each group: (1) an alignment of all available sequences and outgroups to investigate the phylogenetic position of groups, and divergence times using appropriate taxa with calibration dates. Typically these alignments excluded more variable regions, because more

distantly related taxa were included in the alignment, and (2) an alignment of specific groups confined to East Africa (e.g. only *Scolecomorphus*, *Boulengerula*, *Arthroleptides*, or African microhylids).

2.5 Molecular phylogenetic analysis

2.5.1 Introduction

In this section I will outline the different approaches to analyse evolutionary relationships using molecular data. Only methods of relevance to this study will be covered, and therefore this will not be an exhaustive review of all methods. The principal aim of any phylogenetic analyses is to infer evolutionary relationships. Typically this involves finding evolutionary hypotheses that are most consistent with the available molecular or morphological data, and what we are willing to assume about how the data evolved (Page and Holmes, 1998). There are numerous methods that can be employed to infer phylogenetic relationships, and certain factors need to be considered when selecting the best methods to use, which will be discussed individually in the section on tree estimation (see section 2.5.3). Prior to any analysis, it is important to establish how reliable are the collected data (Hillis *et al.* 1996). There are various statistical methods that assess the overall structure of the data, the congruence among different gene partitions, and the informativeness of a data set (see section 2.5.2). Depending upon the type of analysis carried out, a single tree or multiple sets of trees may result from a phylogenetic analysis. In cases where there are multiple trees, consensus techniques can summarise the shared relationships (see section 2.5.4). *A posteriori* assessment of the relationships that phylogenetic trees describe can be made by various statistical measures and procedures (see section 2.5.5). It may often be the case that relationships differ from those expected prior to analysis, and in cases where there are alternative hypotheses which need to be explored it is possible to compare how significantly different a given suboptimal tree (a hypothesis) is to the one inferred (see section 2.5.6).

Once a phylogenetic tree is obtained, how confident can we be that the molecular data can reconstruct evolutionary relationships among species? Phylogenetic trees based on molecular data show relationships among the genes sampled; therefore, the phylogeny is in fact a gene tree. Whether these gene trees can be interpreted as representing the relationships among species (i.e. species trees) depends on the

genes being orthologous (Avise, 1986) and the genetic differences, which we assume show speciation don't reflect other patterns. Avise (1986; 2000) explained how there might be cases where gene trees and species trees are incongruent, and therefore phylogenetic hypotheses might be false. Avise demonstrated theoretically how mitochondrial haplotypes from a polymorphic ancestor subject to random lineage sorting could show a gene tree which conflicts with a species tree (Avise, 2000). Avise (1994) added that only after sufficient complementary haplotype extinction would species be recovered as monophyletic in gene trees. This process is called lineage sorting and causes incongruence between gene and species trees.

How common is lineage sorting in mitochondrial datasets? Quantitative data compiled by Funk and Omland (2003) on mitochondrial lineages and simulation studies by Irwin (2002) have independently suggested that correspondence between gene trees and evolutionary relationships (and their biogeographical interpretations) should not be presumed. In their survey, Funk and Omland (2003) suggest that species level paraphyly or polyphyly is abundant in the mitochondrial studies sampled (23%). Irwin (2002) also demonstrated in simulation studies how 'phylogeographic breaks' (divergence between two lineages) could result without a barrier to gene flow. This may explain why divergence within some lineages may not 'coincide with changes in other traits' (Irwin, 2002; p.2383). Irwin (2002) did offer a more optimistic view though for studies of certain organisms, suggesting the likelihood of observing discordant phylogeographic patterns (ancestral polymorphism) is decreased in species that show poor dispersal ability and have small populations. Presumably some amphibians would be expected to be less likely to demonstrate such patterns based on these characteristics. To detect species level paraphyly or polymorphism Funk and Omland (2003) suggest increased attention to sampling and better interpretation of results by both systematists and population geneticists. In addition to sampling from all the available populations, this study will make only preliminary interpretations of divergence patterns, and that any final conclusions will most probably await sampling of more populations and nuclear genes.

2.5.2 Data quality and homogeneity

Once data have been collected and an alignment constructed, manually or automatically, they can be tested to assess their phylogenetic utility and to determine

whether data partitions can be combined (Hillis and Huelsenbeck, 1992). There are a number of methods for making such assessments, including the permutation tail probability test (PTP) (Faith and Cranston, 1991), the incongruence length test (ILD) (Farris *et al.* 1994) and saturation plots. Some of these tests do not necessarily accept or reject the ability of a data set to reconstruct a phylogeny but provide useful indications on the quality of the data and appropriate approaches that need to be undertaken to estimate a robust phylogeny.

A permutation tail probability test is used to test for the absence of taxonomic structure in a data set (Faith and Cranston, 1991). It is a randomisation procedure in which the proportions of character states are maintained but are randomly reallocated among species and then trees are estimated, and this is repeated a number of times (Felsenstein, 2004). If the tree score for the randomly permuted data do not differ significantly enough from the most parsimonious solution then this indicates that the data contain no more hierarchical structure than random data (Bryant, 1992). Passing the test however does not demonstrate that the data are phylogenetically structured, and is therefore a minimum requirement (Wilkinson *et al.* 2002b). In addition to PTP tests there is a measure that uses the distribution of tree scores. The ILD test assesses the congruence in data partitions and is used as a guide to whether or not to combine them in a single phylogenetic analysis (Huelsenbeck *et al.* 1996). Felsenstein (2004) suggests caution in concluding that two data sets imply different trees, because the test appears to be affected by inequalities in rates of evolution (Dolphin *et al.* 2000).

A major problem in molecular phylogenetics is saturation (Page and Holmes, 1998), which occurs when rates of molecular evolution are rapid. Increased rates of molecular evolution cause problems in our ability to distinguish between homologous or homoplastic molecular sequences. For example, three lineages may share an A in common, but for one of the lineages this nucleotide may have changed from A to a C and then back to an A, resulting in an underestimation of the level of difference between lineages. Molecular sequences evolve at different rates, as changes between all nucleotides are not equally common (transitions and transversions), and there are inequalities in substitution rates at codon positions of protein coding genes. As a result of these inequalities, single sites may show increased rates of molecular evolution and therefore possibly multiple substitutions. Multiple substitution (back substitutions, parallel substitutions, convergent substitutions) rates at single sites

may potentially confuse phylogenetic reconstruction. Recognising the potential effect this can have on data is necessary before any phylogenetic analysis (Page and Holmes, 1998).

Assessing whether a data set suffers from saturation or not is often done by plotting the estimated numbers of transitions and transversions against the corrected pairwise differences. The degree of overlap between plots of transitions and transversions give an indication of the relative rates of these types of substitutions. It would be expected in an unsaturated data set that transitions would be proportionally greater than transversions. There are also other methods, such as plotting pairwise distances against "corrected" distances. In unsaturated data the observed and corrected distance should show a linear relationship whereas in saturated data a non-linear relationship would be expected. If saturation appears to be a problem in a data set then there are particular measures that can be made when inferring phylogenies. Since transitions are likely to become saturated first, and are therefore more likely to confuse phylogenetic relationships, these characters can be excluded or down-weighted and these analyses can be compared to data with transitions included. Evaluation of the difference in resolution of the phylogeny with these potentially confusing characters removed can be made. Third codon positions that show increased substitution rates can also be treated in a similar way.

2.5.3 Tree estimation methods

Depending upon the data set, there are a number of possible methods for inferring phylogenies. In the case of molecular sequence data the main approaches for determining evolutionary relationships are parsimony, likelihood, distance and Bayesian methods. Of these methods, a fundamental distinction between two types can be drawn (as summarised by Page and Holmes, 1998): (1) Methods that convert molecular sequence data into pairwise distances, so that trees are constructed based on overall similarity, e.g. distance methods (2) Discrete methods that analyse every single character separately, e.g. parsimony, likelihood, and Bayesian methods. These methods will be briefly discussed in the following sections.

2.5.3.1 Distance methods

Distance methods use the overall similarity of sequences as a basis for calculating evolutionary relationships. For each sequence pair in an alignment, the distance is a

value based on the proportion of positions that differ, known as the p-distance. However this p-distance can be an underestimation of the true difference. This is because some of the aligned nucleotides might be the result of multiple events (see section 2.5.2). In distance methods therefore, estimations of the number of substitutions (and overall similarity) that have actually occurred are attempted by applying specific evolutionary models. There are a number of substitution models that are derived from how we think molecular evolution might proceeds. For example, in simple substitution models such as Kimura's two-parameter model, distance calculations account for unequal transition and transversion rates (Kimura, 1980). More complex parameters can be included such as those accounting for base frequency differences, and site rate heterogeneity. Branch lengths as a result of these parameters will vary depending on the model used to calculate the differences. Once pairwise differences are calculated, a phylogeny can then be constructed using various tree-building methods. Summaries of all these methods are provided in Felsenstein (2004) and Page and Holmes (1998) and will not be discussed here. Essentially though, there are two main classes, algorithmic and optimisation methods, some of which are considered to be more appropriate than others (Page and Holmes, 1998).

Distance methods have been criticised for a number of reasons; the principal objections being that pairwise distances lose information of higher order combinations of character states, history of character evolution cannot be inferred, and negative branch lengths predicted by some distance methods may not be evolutionarily interpretable (as summarised by Page and Holmes, 1998; p.185-6). Despite the vigour of these criticisms, distance methods are still used to analyse sequence data because they are computationally efficient and therefore particularly useful for large datasets. In addition, the LogDet distance method is particularly useful for inferring relationships in the face of base compositional biases.

For distance analyses, as discussed, there is an array of possible models. Models with few parameters are not likely to be realistic and tend to give inaccurate estimates of evolution. Adding extra parameters is thought to produce more realistic models but increases sampling errors and the uncertainty of the resultant estimates because every parameter added reduces the information content (Swofford *et al.* 1996; Page and Holmes, 1998). Because of this conflict, contrasting models were used in this study: (1) the simpler Kimura two parameter model which takes into

account the differences between the number of transitions and transversions, and (2) the more complex GTR model, which in most cases was selected from several available models using hierarchical likelihood ratio tests and (3) LogDet method which is used to resolve sequences where there is variable base composition (Page and Holmes, 1998).

2.5.3.2 Parsimony methods

Parsimony is one of the most commonly used methods in analyses reconstructing evolutionary relationships (Swofford *et al.* 1996), and has been favoured by some phylogeneticists for its supposed philosophical foundation (Farris, 1978). Parsimony methods are easily understood; the preferred tree is the one that involves the 'minimum net amount of evolution' (Felsenstein, 2004). The goal in molecular phylogenetic analyses using parsimony methods is to search for the tree that assumes the least number of total nucleotide changes. Calculating the shortest tree can be straightforward based on a single cost for each substitution (Fitch, 1971). There are however a number of more complex models, all with the same goals of minimizing the number of steps. Swofford *et al.* (1996) summarise these methods, and they describe models that are believed to more accurately mirror substitutions in molecular data, in that not all sites are equally phylogenetically useful (eg. transitions vs. transversions). In addition to generating models that more accurately reflect evolution in molecular data, weighting schemes can be applied that place greater emphasis on more phylogenetically informative characters and thereby give a better estimation of the true tree.

Along with other methods, parsimony is known to be susceptible to long branch attraction (LBA) artefacts (Felsenstein, 1978), in which lineage specific rate heterogeneity confounds phylogenetic inference by grouping lineages with similar rates together. The problems of LBA have been shown to be particularly acute in sequences that show considerable rate variation, or when sequences are from quite divergent taxa (Anderson and Swofford, 2004). However, the impact of LBA is thought to be less important for phylogenies in which a large number of taxa are included (Page and Holmes, 1998).

2.5.3.3 Maximum likelihood

Maximum likelihood methods are widely used to estimate statistical parameters. The likelihood of a hypothesis is a function of the probability of the data given the model. Likelihood is taken to provide a natural preference order for selecting among competing hypotheses (Felsenstein, 2004). Calculating likelihoods requires an explicit model of how, in the case of phylogenies, the data evolved (Swofford *et al.* 1996). As implied then, likelihood methods are dependent upon how good the model is (Page and Holmes, 1998). The models constructed in likelihood analyses are based on the following three main parameters: the tendency of base to change to another, the composition (e.g. base frequencies), and the among site variation. The first model to be developed, and the simplest, is the Jukes-Cantor model (Jukes and Cantor, 1969), which assumes equal substitution and frequency between all the nucleotide bases. Further models extend this to include different rates of base changes, and frequencies (Page and Holmes, 1998) such as would be expected based on our understanding of transition and transversion changes in nucleotide sequences. The most complex variant of these models is the General Time Reversible model (GTR) (Lanave *et al.* 1984), which has six substitution types.

Further parameter rich models were developed to account for among site variation, which includes parameters that describe both the proportion of invariable sites in a data set and in more complex models the probability that any one position may belong to a specific rate class (e.g. quickly evolving site). Data sets not accounting for such variation, where it has been shown that the rates are highly variable among sites, can have serious consequences on phylogenetic inference, and result in misleading likelihood trees (Swofford *et al.* 1996; Foster, 2004). With all the possible permutations of these parameters, it is clear there are a number of different models that could be utilised in an analysis, which leads inevitably to the question 'which model should I choose?' Fortunately, a goodness of fit test has been developed which can quantitatively assess which model best fits the data, and this can be calculated automatically in the program ModelTest 3.06 (Posada and Crandall, 1998). Using hierarchical likelihood ratios or the Akaike information criterion, model test identifies a model that beyond which the addition of more parameters does not produce a significant improvement in the likelihood. As with every approach, likelihood has been subject to criticism. Opponents have questioned the model selection procedure. Most studies select (as assessed in the program ModelTest) the most complex model GTR + I + G or GTR + SS, which suggests that given the

addition of more parameters one could significantly improve the model. A recent paper has also suggested that parsimony outperforms likelihood analyses when there are heterogenous rates (sequences change non-identically over time) of molecular evolution (Kolaczkowski and Thornton, 2004).

Based on our understanding of stochastic changes in molecular sequences, models used in likelihood may be more or less accurate but most importantly sufficiently accurate to allow good phylogenetic inferences. It is likely that more complex models will continue to be developed which better depict how sequences evolve. Likelihood provides a framework for determining if improvement in likelihood is accompanied by including more parameters. The continuing emphasis on molecular data to understand evolutionary relationships means likelihood will continue to be a popular approach for determining evolutionary relationships.

2.5.3.4 Bayesian inference

Phylogenetic analyses using Bayesian methods have only recently been formalised (mid 1990s) and only within the last five years have programs been developed which can execute the analysis (Huelsenbeck and Ronquist, 2001). Bayesian analysis has made a striking impact on the systematic community, as a consequence of the ability to analyse large phylogenetic trees using complex evolutionary models, and the detection of the footprints of natural selection in DNA sequences (Huelsenbeck, *et al.* 2001). Bayesian inference is closely related to likelihood analyses in that it uses ratios of likelihoods to determine the probability of accepting proposals in Markov chain Monte Carlo (MCMC) chains. Summaries of Bayesian methods are given in Huelsenbeck and Ronquist (2001) and Lewis (2001) and the principles are outlined there and will not be covered here. Software implementations of Bayesian approaches already include a wide variety of stochastic models for nucleotide, protein, restriction site and morphological data. In addition, these methods allow modelling of heterogeneous substitution rates across the data (as in likelihood and distance methods), and subsets. As with all phylogenetic methods there are problems with Bayesian analysis, including overly high posterior probabilities (see section on measures for trees), sensitivity to taxon sampling, and uncertainty over convergence of MCMC chains on trees of high likelihood. Advocates of these approaches have suggested certain procedures that can alleviate these problems (as summarised in Lewis, 2001). As previously mentioned in likelihood methods, parametric methods (such as Bayesian) are thought to perform poorly when

molecular evolution is even moderately heterogenous (Kolaczkowski and Thornton, 2004).

2.5.4 Consensus methods

Systematists are often faced with the problem of interpreting multiple trees. There are a number of consensus methods for drawing inferences from, or for providing summaries of agreement and conflict among multiple trees, the utility of which is dependent upon the context (Wilkinson and Benton, 1996). In essence then, no single consensus method is universally applicable to all consensus problems. Consensus methods utilised in this study, include; strict and majority rule consensus methods in phylogenetic reconstructions, and the Nelson consensus in biogeographic analyses.

The simplest consensus tree is a strict consensus, which constructs a summary tree that contains all and only those clades that are found in all of the trees. However, strict consensus approaches are considered too stringent for the purposes of many studies. For example, where there are many most parsimonious trees, the strict consensus is often poorly-resolved and may be just a 'bush' that provides no information other than that there are no clades in common. As a result, there has been a drive to develop methods that provide better resolution through not being so strict. Majority-rule methods construct a tree that includes those clades that are present in a 'majority' of the trees whose consensus is being sought. For instance, majority rule consensus with 100% setting would only include groups that are found in 100% of all the trees, which would be equivalent to a strict consensus. Majority rule consensus methods however are usually set at a lower bound, such as 50%, and it is this flexibility that allows the user to obtain a summary of the percentage of relationships found among all parsimonious solutions. In biogeographic studies, a Nelson's consensus tree, as executed in the program Component 2.0 (Page, 1993), finds the largest clique of groups that are all compatible with each other, i.e. areas that are found grouped together in all trees.

2.5.5 Support for clades

There are a number of procedures for evaluating the robustness of a phylogeny and not surprisingly there is also much debate about which are the most appropriate measures and how they should be interpreted (Felsenstein, 2004). Measures that are automatically calculated in Bayesian analyses, are posterior probabilities, and these

provide 'credibility' values for clades (Huelsenbeck and Ronquist, 2001). There have been some highly critical appraisals of these measures, and certain authors have questioned the seemingly inflated probabilities on Bayesian trees (Erixon *et al.* 2003; Douady *et al.* 2003a), and comparisons with other measures (bootstrap) have shown inconsistencies. As a result, it has been recommended that clade credibility values should be interpreted with caution, and values of less than 0.95 are considered weakly supported (Erixon *et al.* 2003). A more traditional approach for evaluating support is the 'bootstrap', which is a standard statistical method for estimating sample variance by resampling from the original data. In phylogenetics, characters are randomly resampled with replacement to give many bootstrap replicate data sets of the same size as the original. The frequency with which clades are found in the analyses of the resampled data is the clade's bootstrap proportion and is taken as a measure of support. It is uncertain what exactly constitutes a significant result. Felsenstein (1985) suggested proportions of 95% or greater should be considered well supported. Bootstrap analyses can be applied to a wide range of analytical methods, including likelihood, distance and parsimony.

Bremer support or the Decay index is a measure that compares the length of a parsimony tree with a particular clade and the length of the best suboptimal tree without that clade (Felsenstein, 2004). The difference between these two values is given as the support value, and this is equivalent to the number of steps it takes to collapse that clade. There are difficulties in interpreting what these values equate to, as there is 'no immediate statistical interpretation' based on these values (Felsenstein, 2004; p331). Extensions to the decay analyses have been suggested (Wilkinson *et al.* 2000; Gatesy, 2000). In this study the methods discussed above are employed. Consistency between measures is interpreted as a more probable hypothesis, and conflicting relationships or measures of support are treated with caution.

2.5.6 Comparison of alternative hypotheses

We often want to test the phylogeny recovered in our analyses against alternative competing hypotheses. For example, we may ask whether the observed paraphyly of a particular grouping is strongly supported or if the tree we get from an analysis is significantly different from the suboptimal traditional phylogeny. There are several ways of doing this, depending on the criterion being used to estimate the phylogeny.

For parsimony, the Templeton test evaluates whether the number of steps between optimal and suboptimal trees is more significant than would be expected from random sampling error. For likelihood analyses Goldman (2000) recommends the use of the Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999) to compare the likelihood values. Whereas previously the Kishino-Hasegawa test (Kishino and Hasegawa, 1989) was used, which has been shown to be inappropriate unless all the trees have been specified *a priori*. It is possible to assess alternative hypotheses in Bayesian trees. This is achieved by filtering all the Bayesian trees recovered for the grouping of interest, and the number of trees recovered then provides a proportion from the total number of filtered trees. For example, if five trees with the suboptimal hypothesis are recovered from 1000 total trees filtered, the hypothesis shows 0.5% of trees include that hypothesis. It is however not entirely clear what proportion constitutes a rejection of the alternative hypothesis and this is not a statistical test of significance.

2.6 Biogeographical methods

2.6.1 Introduction

It is clear that species found on the planet are not randomly distributed, and this point is best exhibited in groups of species restricted to continents or islands. For example, marsupials have a restricted distribution and are not found randomly dotted around the globe. Found on the continent of Australia, New Guinea, and South America (one of the American opposums lives in North America), the current distribution of marsupials reflect recent migrations and the breakup of the continental landmasses. Therefore, distributions of species are determined by a range of causal factors; abiotic (e.g. plate tectonics) and biotic (e.g. dispersal ability). Biogeography is the branch of evolutionary biology that is concerned with understanding how non-random distributions are best explained. In addition to this, biogeographers are interested in the general patterns and the processes that have contributed to the present and past distribution of all organisms, which leads inevitably to fundamental questions regarding evolution; such as the relationship between biogeography and diversification. Biogeography therefore forms an integral part of evolutionary biology.

There are a number of analytical methods that have been developed to investigate the patterns and the processes that have led to the distribution of extant and extinct

taxa (Morrone and Crisci, 1995; Cecca, 2002). In this study I consider two main schools of biogeography, and these will be covered in the following sections on descriptive and cladistic methods. These sections will not include an exhaustive discussion of all methods, but more importantly those relevant to this study. At the end of these sections is a discussion of molecular clock methods, which provide another important source of data for understanding biogeographical events (Donoghue and Moore, 2003). Although there is considerable debate and continuing uncertainty concerning the best biogeographic methods (Platnick and Nelson, 1978, 1981; Lieberman, 2000; Upchurch and Hunn, 2002; Donoghue and Moore, 2003; Humphries and Parenti, 1999; McDowall, 2004), for the purpose of this study an attempt is made to apply all potentially useful methods and search for commonalities in the results. Using different methodological approaches to investigate the biogeography of an area has, as claimed by some authors (e.g. Cracraft, 1994; Cecca, 2002), been important in verifying biogeographical conclusions made using one method. The approaches are therefore not considered as 'mutually exclusive alternatives' (Morrone and Crisci, 1995). It is with this rationale that this study was conducted. Combining the results from each analysis into a single-meta analysis was not attempted. Rather as suggested by Racheli (2004) comparisons were made between each method.

2.6.2 Descriptive biogeographical methods

2.6.2.1 Introduction

In endeavouring to elucidate the relationships between areas or communities, it is common to compare species occurrence and abundance (Krebs, 1999). Based on distributional data, biologists have been able to make either quantitative or qualitative (depending on the data, abundance or presence/absence respectively) measures of similarity between organismal assemblages. Descriptive methods derive their primary source of information from the presence and absence of taxa in an area. They have been considered analogous to phenetic methods because of the estimation of pairwise overall similarities of species distributions of areas. Using this approach, relationships among areas are inferred using the presence or absence of taxa. Relationships between areas are estimated by assuming that the relative recency of the biogeographic association of two areas is directly proportional to the number of taxa that are shared between them. It has been argued that the varied ages, ecology

and dispersal capabilities of species making up these biotas make it difficult to infer historical processes from phenetic assessments of the relationships among assemblages (Morrone, 1994). Similarly, Farris (1981) suggested phenetic biogeographical methods do not accurately reflect historical relationships, but appear to be sensitive to local extinction or differentiation and not the common biogeographic history of the area. In summary, the species lists and similarity indices, the basic data of descriptive biogeography, may be useful descriptors of assemblage differences and similarities but not the most appropriate tools for elucidating the biogeographic history of an area.

Investigations of the biogeography of the African fauna and flora have mostly used descriptive methods (e.g. Keay, 1954; Stuart, 1991; Tattersfield *et al.* 1998) and current understanding, certainly of East African amphibians, is based on the application of such approaches (Poynton, 1998; 2000; 2003a ; Poynton and Boycott, 1996; Loader *et al.* 2004a). There are a number of possible explanations for this (e.g. authors' methodological preferences), but the main reason would appear to be the lack of phylogenetic studies of African taxa that are suitable for using cladistic biogeographical techniques (Kirk-Spriggs, 2003).

2.6.2.2 Similarity coefficients of assemblages

All similarity co-efficients take the number of species shared between areas as the foundation of the measure. There are subtle differences between each coefficient, and it has been argued that particular measures are more useful than others (Bloom, 1981; Krebs, 1999; Cecca, 2002). Which measures employed depends upon the type of data; presence absence data (Jaccard and Sorenson indices) or abundance of species (Bray-Curtis index). For the purpose of this study, both Jaccard and Sorenson indices are utilised and compared. These two measures are most commonly used (e.g. Inger and Voris, 2001; Ramanamanjato *et al.* 2002). The formulas for Jaccard (Cj) and Sorenson (Cs) are given below:

1. $Cj = a/(a + b + c)$

2. $Cs = 2a/(2a + b + c)$

Where, a = number of species common to both sites, b = number of species in site B, but not in A, c = number of species in site A, but not in B

Once similarity indices are calculated for each pairwise comparison, a hierarchical cluster analysis is carried out on these symmetrical matrices to investigate patterns of nestedness among the assemblages (Cecca, 2002). Hierarchical cluster analysis is performed using a distance algorithm (Krebs, 1999) for which there are a number of different criteria. Based on the multivariate analysis a dendrogram can then be constructed, which provides a summary of the relationships, and a framework for interpreting results.

There have been a number of criticisms concerning the use of these similarity measures. Krebs (1999) cited ambiguities concerning the selection of measures and their evaluation. He argued that similarity measures are descriptive coefficients, and are not 'estimators of some statistical parameter' (Krebs, 1999). Therefore evaluating the reliability of the data is not possible unless by some type of Monte Carlo procedure (Krebs, 1999). For the purpose of this study, the PTP randomisation test (e.g. PTP; Faith and Cranston, 1991) is used as in phylogenetic studies to assess if the data contain no more structure than randomly permuted data. In addition the data matrix will be bootstrapped to assess how robust groupings are. Choosing the appropriate measure and the methods to analyse their relationships can also be tricky. There are a number of different similarity coefficients ('some two dozen', Krebs, 1999) that are widely used in the literature (e.g. Cecca, 2002). There are also as many methods for assessing their hierarchical relationships (Krebs, 1999) using multivariate analyses. Thus, there is no standard methodology that a biogeographer may use to assess the similarity of assemblages and their hierarchical relationships.

2.6.3 Parsimony Analysis of Endemicity

Parsimony analysis of endemicity (PAE) is used to investigate the relationships among areas using the occurrence of species in an area (same data as used in section 2.6.2), and analysing the matrix using cladistic algorithms (Rosen, 1988; Rosen and Smith, 1988). The method is analogous to cladistic methods in classifying areas (taxa in phylogenetic cladistics) by their shared taxa (characters in cladistics) using parsimony (Rosen, 1988; Cox and Moore, 2000). There is however a fundamental difference between PAE and phylogenetic cladistic methods in that the former is not using historical signatures of relationships among taxa (see section 2.6.4; Wiley, 1988). The resulting PAE cladograms consist of a nested set of endemic biotas as represented in a branching diagram (Cecca, 2002). As with

interpretations of similarity indices and their resulting dendrograms, historical comparisons in PAE rely on the assumption that similarities are the result of shared history. Interpretation of these branching diagrams can be difficult, because similarity between localities may result either from 'greater ecological similarity or from more recent biotic links' (Cox and Moore 2000; p.170). and therefore their biogeographic meaning can be difficult to understand (Rosen, and Smith, 1988; Rosen, 1992; Brooks and van Veller, 2003).

A number of authors (Rosen, 1988; da Silva, and Oren, 1996; Ron, 2000; Racheli and Racheli, 2003) have used PAE 'cladograms' to make inferences about historical relationships. Da Silva and Oren (1996) and Racheli and Racheli, (2003) have also found that PAE area 'cladograms' were congruent with area cladograms obtained using cladistic biogeographical methods. Despite these examples, PAE has been used relatively infrequently (Cecca, 2002), and this is fundamentally associated with the problems many critics have with the interpretation of the results (as summarised by Brooks and van Veller, 2003; Upchurch, 2004). Morrone (1994) has suggested that PAE area cladograms may be used as a tool to delimit areas of endemism on an 'intracontinental scale', when several species are included in the analysis, which may be an important prior step before defining areas for cladistic biogeographical analyses.

2.6.4 Cladistic biogeographical analysis

2.6.4.1 Introduction

Biogeography has been revolutionised by the finding that the earth's surface has undergone considerable geological change; continental plates have combined, broken away and migrated around the globe (Wegener, 1966). Based on this radically new understanding, biologists re-interpreted species distributions, and this provided solutions to previously intangible problems (Sneath, 1967). Furthermore, relationships among some groups were shown to be congruent with the fragmentation sequence of continental plates, as first shown by Brundin's (1966) study of chironomid midges (Cox and Moore, 2000). As evidence accumulated, the splitting of areas and the species occurring there (vicariance) was shown to have a considerable influence upon relationships in many animals and plants. It had long been appreciated that species phylogeny often accurately reflects biogeographical

events (Sneath, 1967), but analytical biogeographical methods were not formalised until Nelson and Platnick (1981). Nelson and Platnick (1981) provided a cladistic framework for understanding biogeographical distributions, as well as providing evidence for reconstructing palaeogeographies (Lieberman, 2000). The following sections will outline the general theory behind cladistic biogeography and its practical application. It concludes with remarks concerning current debates surrounding cladistic biogeography that are of relevance to this study.

2.6.4.2 Cladistic biogeography

In cladistic biogeograpy the singular aim is to evaluate the congruence in the branching patterns of species relationships that occur in the same areas. Species phylogenies should reflect biogeographical changes that have occurred (as discussed 2.6.4.1) and it is this correspondence between taxonomic and area relationships that underpin these methods. There are two basic steps to be made in any cladistic biogeographical analysis. The first is to replace all the terminal taxa on a species phylogeny with the areas that these species occur in (Fig. 2.1a, b). These branching diagrams are called taxon-area cladograms (TACs) (Sanmartin and Ronquist, 2002). In the second step, a common biogeographic signal may be revealed by comparison of TACs from different taxa occurring in the same areas (see Fig.2.1a). A 'consensus' of all the relationships in the source TACs is summarised in a single tree called a general area cladogram (GAC). It is possible that there are cases where there remains no biogeographical signal, as shown in Fig 2.1b.

Examples 1a and 1b are simplistic cases, and it is often the case in biogeographical analyses that the TAC that are compared to formulate a GAC are much more problematic. For example, there are times when there are incomplete or conflicting area relationships, as shown in Figure 2.1c, d, e. These examples (2.1c, d, e) are the three main sources of difficulty in cladistic biogeographic approaches; widespread taxa, redundant distributions, and missing areas (Morrone and Crisci, 1995). Each of these can result in conflicting area statements that may obscure a common biogeographical pattern. There are several different methods that have been developed to deal with these problems (Brooks and van Veller, 2003). In summary, *a priori* methods modify the TACs that are analysed, based on a set of assumptions that account for conflicting relationships (as in 2.1c, d, e) and then attempt to maximise the fit of TACs to a GAC (eg. Component 2.0; Page, 1993; see

the next section for overview). *A posteriori* methods do not alter the TACs, instead TACs are optimised to a common distribution (GAC) and any conflicting distributions (e.g. widespread species and sympatric taxa) are explained as 'post-speciation dispersal, or speciation by colonization' using a parsimony analysis (Brooks and van Veller, 2003; p.820). For the purpose of this study, *a priori* methods are investigated in order to assess the topological congruence between TACs generated in Chapters 3-6, as well as published phylogenies. Future work could investigate *a posteriori* methods but these are currently not deemed critical for addressing the aims of the project.

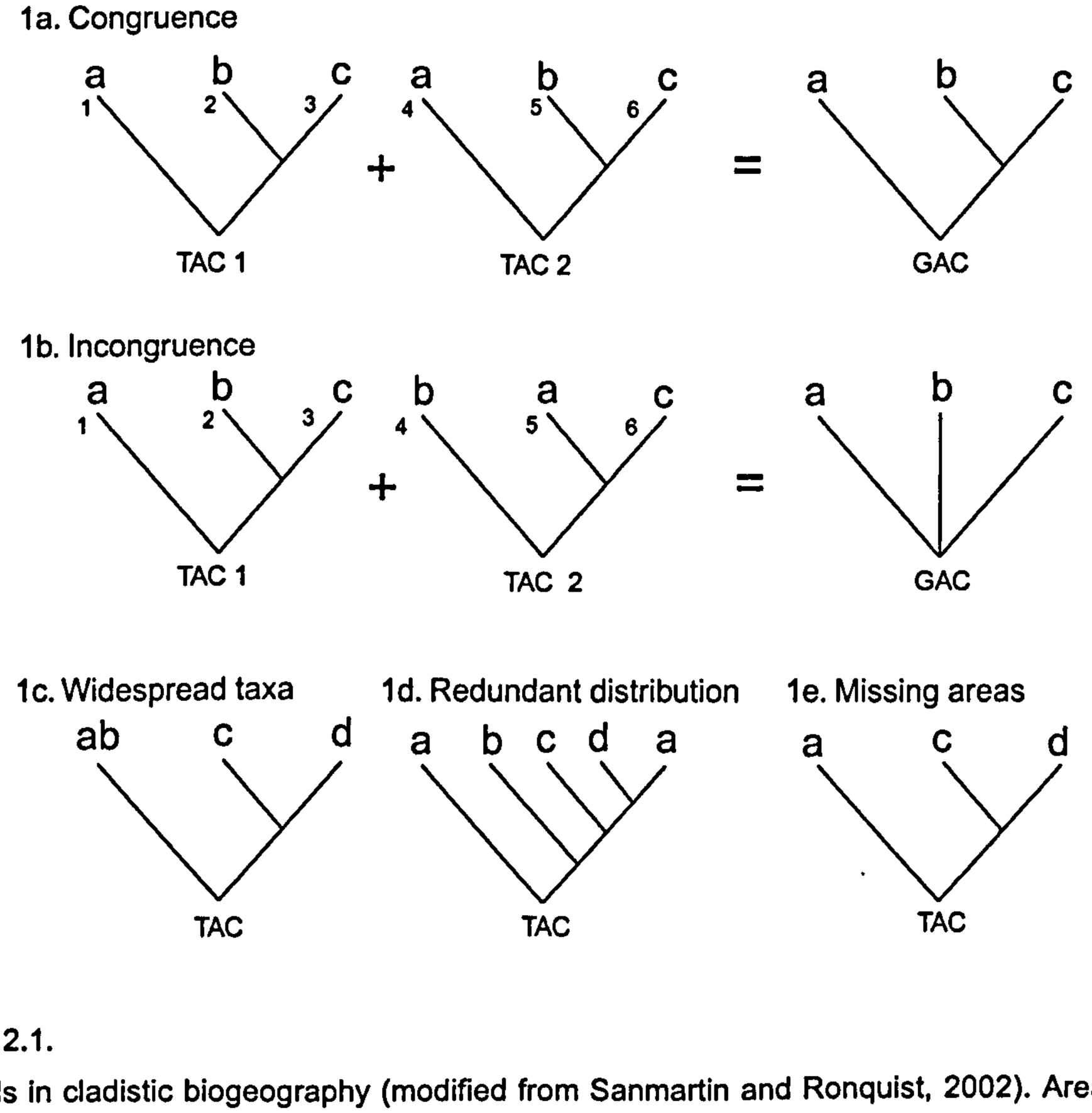

Figure. 2.1.
Methods in cladistic biogeography (modified from Sanmartin and Ronquist, 2002). Areas are given as letters at the end of branches, and species are numbers. 1a. Congruence: comparison of two TAC's with species 1-6 results in congruent GAC. 1b. Incongruence: comparison of two TAC's with species 1-6 results in incongruent GAC. Conflicting TAC's (see text for explanation): 1c. Widespread taxa. 1d. Redundant distribution. 1e. Missing areas.

To investigate the common history of the area using *a priori* methods, it is first necessary to convert the conflicting TACs into resolved area cladograms (RAC). This

means that for each phylogeny used each area is represented by 'one terminal [branch]' (Sanmartin and Ronquist, 2002; p.77) so the RAC can then be assessed along with other resolved TACs or previously conflicting RACs. The conversion of a TAC into a RAC is by no means a straightforward process, and it is a topic of debate (eg. Van Veller *et al.* 1999; Sanmartin and Ronquist, 2002). Marco van Veller *et al.* (1999; 2003), Morrone and Crisci (1995) and Sanmartin and Ronquist, (2002) all provide good summaries on the procedures for resolving TACs, when applying different assumptions. Essentially, the different assumptions allow for patterns such as extinction and dispersal (Van Veller *et al.* 1999), processes common in biogeography, to be resolved so as to maximise common patterns. These assumptions have been connected to different biogeographic processes, Assumption 0= vicariance, Assumption 1= vicariance + extinction, Assumption 2= vicariance + extinction + dispersal (Van Veller *et al.* 2003). The application of these different assumptions has not been without its critics, not least because examples from the same dataset applying different assumptions have produced significantly different GAC (Morrone and Crisci, 1995). There also appears to be no basis for choosing one assumption over another. Assumption 0 and 1, have been criticized as being 'too restrictive and unrealistic', and assumption 2, 'indecisive and uninformative' (summarised by Sanmartin and Ronquist, 2002; p.77). There is still no real consensus of opinion on the best approach to be taken. Most advocates suggest an experimental ethos, and that multiple trials of different approaches should be taken to evaluate all possible hypotheses and compare this to other quantitative approaches (Morrone and Crisci, 1995).

Once it is established whether or not there is a common biogeographical signal (e.g. resolved GAC), it is necessary to interpret the result. If there is no common biogeographic signal, then it could be indicative of (1) complex biogeographical history which may be intractable, or that the (2) actual lineages or (3) the number of lineages used are inadequate for resolving the biogeography of the area. For explanation (1), certain areas may have extreme fluctuations in climate and geology that affect organisms in significantly different ways that cannot be reflected in general biogeographic patterns accessible by any method. For (2) it might be clear that some of the lineages used in the analysis may not be suitable, such as vagile organisms which are less likely to share a common biogeographic history with a species unable to disperse long distances. Lastly, (3), analogous to increasing phylogenetic accuracy in phylogenetic analysis (Hillis, 1996), the addition of more

phylogenies to an analysis may improve resolution. Pennington *et al.* (2004; p 531) however makes the point that 'there is no precedence in the literature for having faith that addition of taxon cladograms will aid resolution' of biogeographical patterns.

If an analysis from a cladistic biogeographic study results in a fully resolved GAC, the interpretation of a common biogeographic pattern is again not straightforward, and cannot be 'confidently attributed to any particular cause' (Donoghue and Moore, 2003; p.261). Cladistic biogeography assumes that once a well-supported GAC is produced from the RACs, the patterns produced are that of vicariance, as these are the only patterns that can be consistently repeated. However, can it be safely assumed that repeated spatial patterns are caused by vicariance? Take the simple example in Fig. 2.2.a, which shows two TAC with two congruent area relationships, i.e. x (y,z). Given these patterns, a cladistic biogeographer would interpret the pattern as a result of fragmentation of the areas, with y,z sharing a more recent history than x. Now look at Fig. 2.2b, which shows 'pseudocongruence', in this example species share a common pattern, but the causal events that have resulted in their distribution differ.

It is also possible to imagine other examples where patterns may suggest a common history, implying fragmentation, and synchronous speciation in TACs which actually do not correspond to the historical reality or fragmentation sequence at all (Donoghue and Moore, 2003; p.262, fig.1 provide a good summary). 'Pseudocongruence' was also discussed by Hunn and Upchurch (2001). They demonstrated that the assumption of vicariance as the sole factor in imposing repeated spatial patterns was incorrect. Vicariance-mimicking events may confound cladistic interpretations such as 'the appearance of a dispersal route that allows members of several different clades to simultaneously populate a new area' (Hunn and Upchurch, 2001; p.395). Hunn and Upchurch (2001) urge an interpretation of GAC as relationships being indicative of 'recency of biotic interaction' instead of classic cladistic interpretation implying a fragmentation sequence. Distinguishing between vicariance and geodispersal is difficult; additional information such as the timing of geological events is necessary to assess the likely probability of these processes (Donoghue and Moore, 2003).

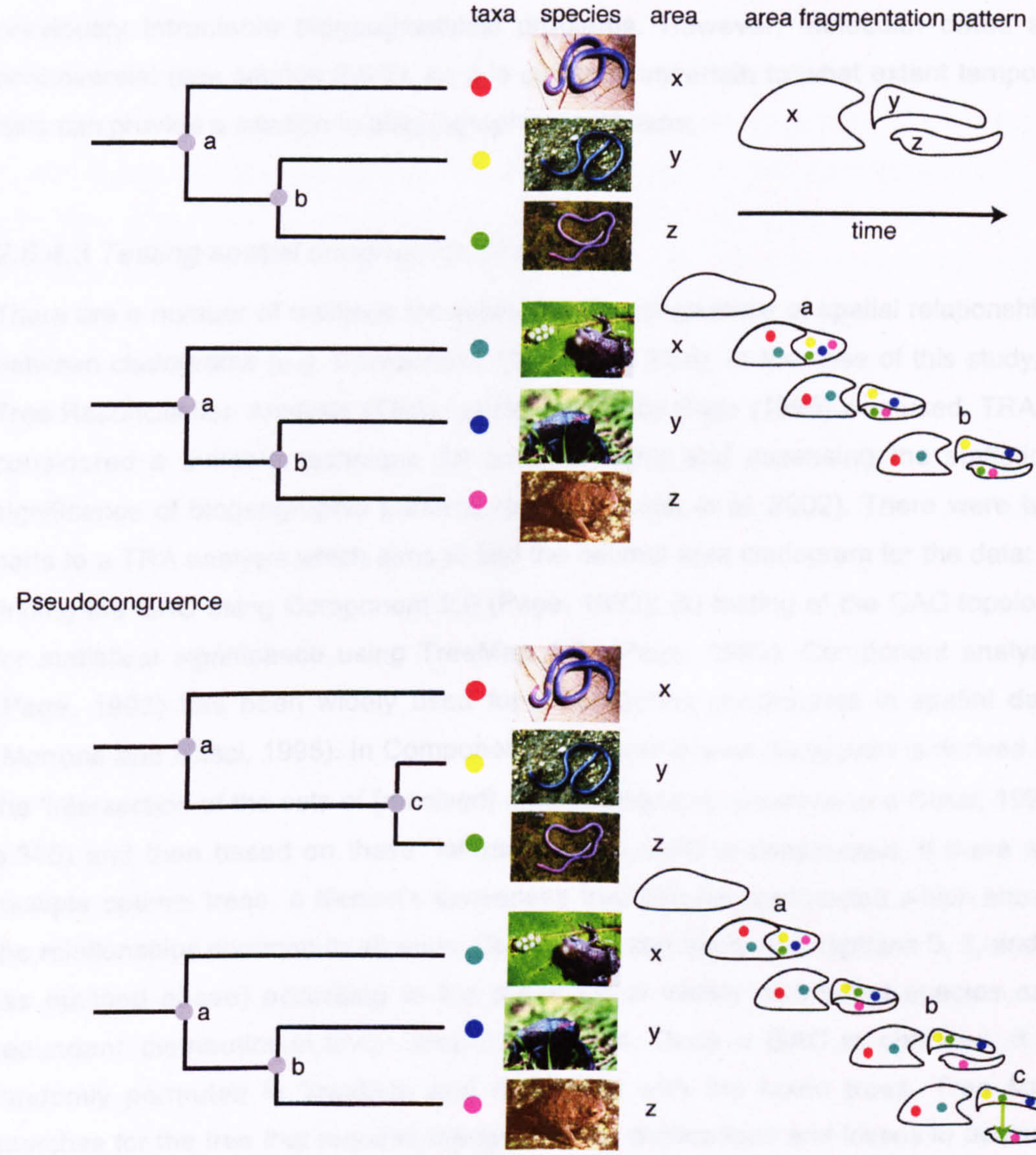

Figure 2.2.

Hypothetical biogeographic scenarios; above- temporal and spatial congruence below- pseudocongruence (see text for explanation).

Based on these criticisms and others made by Hunn and Upchurch (2001) it is clear that cladistic biogeographic methods, which focus exclusively on spatial congruence, are imperfect (Donoghue and Moore, 2003). One solution to the interpretation of cladistic reconstructions is absolute dating of the diversification of lineages, so that pseudocongruence can be detected (Cunningham and Collins, 1994). For example in Fig. 2.2.b, molecular dating on the timing of speciation at node b,c would provide important data for resolving pseudocongruence. Donoghue and Moore (2003)

suggested that absolute dating could provide vital information for teasing apart previously intractable biogeographical problems. However, molecular dates are controversial (see section 2.6.5), so it is currently uncertain to what extent temporal data can provide a solution to biogeographical problems.

2.6.4.3 Testing spatial congruence of areas

There are a number of methods for assessing the congruence of spatial relationships between cladograms (e.g. Component, BPA, and TASS). In the case of this study, a Tree Reconciliation Analysis (TRA), as formulated by Page (1995) was used. TRA is considered a suitable technique for reconstructing and assessing the statistical significance of biogeographic patterns (e.g. Upchurch *et al.* 2002). There were two parts to a TRA analysis which aims to find the optimal area cladogram for the data: (i) finding the GAC using Component 2.0 (Page, 1993); (ii) testing of the GAC topology for statistical significance using TreeMap 1.0. (Page, 1995). Component analysis (Page, 1993) has been widely used for investigating congruence in spatial data (Morrone and Crisci, 1995). In Component, the general area cladogram is derived by the 'intersection of the sets of [resolved] area cladograms' (Morrone and Crisci, 1994; p.386) and then based on these 'intersections' a GAC is constructed. If there are multiple optimal trees, a Nelson's consensus tree can be constructed which shows the relationships common to all trees. Component can apply assumptions 0, 1, and 2 (as outlined above) according to the presence of widely distributed species and redundant distribution in taxon area cladograms. Once a GAC is obtained, it is randomly permuted in TreeMap and reconciled with the taxon trees. Tree Map searches for the tree that requires the fewest total duplications and losses to account for a set of TACs or RACs. The significance of the GAC can then be assessed by comparing the number of duplication events to that of the randomly permuted data. The presence of congruent or incongruent area relationships can then be accepted or rejected.

2.6.5 Molecular clocks and dating

2.6.5.1 Introduction

One of the most promising but controversial uses of molecular sequence data in systematics is for the estimation of divergence times (Fitch, 1976; Hillis *et al.* 1996).

In the early studies of molecular evolution, inferences of stochastic changes in sequences were made (Zuckerkandl and Pauling, 1965). Based on this even, clock-like change in molecular sequences, dates for nodes on phylogenetic trees could be inferred (Wilson *et al.* 1985). On the whole, sequences appeared to evolve at a constant rate, and an approximately linear relationship between evolutionary distance and time of species divergence was generally found (Kumar and Hedges, 2003). However, as sequence data accumulated it became clear that there was not a globally constant rate of molecular evolution (Wu and Li, 1985; Martin and Palumbi, 1993). Significant differences in evolutionary rates among species and genes have been demonstrated (DeSalle and Templeton, 1988; and as summarised by Gillespie, 1991) and the fidelity of the universal clock has been questioned (Avise, 1994; Ayala, 1986). It is now accepted that the 'molecular clock' does not have a globally constant rate (Avise, 1994; Li and Grauer, 1991), and discrepancies in rates have been suggested to be largely the result of lineage specific rate variation (Martin and Palumbi, 1993) caused by differences in, for example, body size, metabolism, generation time and DNA repair. The realisation that sequence evolution is more complex has led to a more sophisticated approach to the application of molecular clocks. Identification and implementation of complex rate variation of sequence data into phylogenetic analyses and clock estimates is now possible (Posada and Crandall, 1998; Posada, 2003; Robinson *et al.* 1998; Sanderson, 1998; Kishino *et al.* 2001). Consensus of opinion suggests that the clock-like behaviour of a data set should be initially treated as a hypothesis for each gene and lineage, and accepted or rejected following analyses of rate variation (e.g. Robinson and Huchon, 2000; Posada and Crandall, 1998).

In the case of some of the data sets utilised in this study, it has already been demonstrated that there is lineage specific rate variation (Wilkinson *et al.* 2002a; Wilkinson *et al.* 2003; Loader *et al.* 2004). In this study divergence estimates are calculated using rate-smoothing methods (Sanderson, 1997; 1998; 2002a,b; 2003). There are methods in these programs that can estimate divergence times for datasets which do not show clock like rates of evolution, and thereby provide a better estimation of molecular divergence times, both relative and absolute (see section 2.6.3).

2.6.5.2 Molecular clock tests

Rate heterogeneity has been shown to have a negative impact on phylogenetic estimates, giving rise to spurious relationships (Swofford *et al.* 1996). An approach that is commonly applied to test for rate heterogeneity is the relative rates test (Sarich and Wilson, 1973), as implemented in programs such as rrTree (Robinson and Huchon, 2000). In this test, a branch length comparison is made between an ingroup and a reference taxon (usually the outgroup). If each ingroup taxon differs from the reference group by a similar amount (no significant branch length differences) then rate homogeneity cannot be rejected. Critics of the relative rates test (e.g. Ayala, 1986) have shown that it is biased towards finding equal rates, and has high Type II error rates. Despite these problems it is utilised to determine rate variation in various phylogenetic studies (e.g. Wilkinson et al., 2002a; 2003). The benefits of the relative rates tests are that problematic taxa with specific lineage rate variation can be identified. A molecular clock can also be tested using a hierarchical likelihood ratio test (Posada, 2003). A likelihood estimated tree with a molecular clock enforced is compared to a likelihood tree without a clock enforced (a stricter version of the NJ method, e.g. Posada, 2003). The likelihood score for the former (L0) is subtracted from the latter (L1) then multiplied by two (e.g. $\Delta = 2^*(L1 - L0)$). The sum should then be compared on a chi-squared table to determine the p-value; where the degrees of freedom equal the number of taxa minus two.

2.6.5.3 Rate smoothing methods

Molecular data for which the assumption of constant rates over time has not been violated are not necessarily required, for example when divergence times for DNA sequence data are estimated using the software r8s (Sanderson, 2003). This uses a likelihood estimation to assess variance in substitution rates, and takes account of any variances in the estimation of ages of lineages. The program also allows calibration using multiple constraints on node ages. Depending upon the rate variation in the dataset, different algorithms are utilized. For data where there is significant rate variation, penalized likelihood is the most suitable approach (Sanderson, 2002a). Penalized likelihood is used to identify an optimal rate smoothing parameter, which constrains the amount of change among ancestors and descendants. The optimal smoothing parameter is determined by a cross validation approach whereby the parameter chosen best predicts the overall terminal branch lengths in a saturated rate model (Sanderson, 2003). The Langley Fitch method is

better applied to data where there is no significant rate variation (e.g. clock like dataset) (Sanderson, 2002b).

2.6.5.4 Calibration points

Crucial to the estimation of 'absolute' time on a phylogenetic tree is at least one calibration point. Calibration points come in various guises. Most of those utilised in the literature are fixed by fossil or geological evidence. For example, Biju and Bossuyt (2003) utilised (among other calibrations points) the minimum age of Cryptobranchidae (164 Myr) to date their amphibian tree. This is marked by the recent discovery of basal members of Cryptobranchidae from the volcanic deposits of the Jiulongshan Formation, China. Evidence from geological data includes events such as Gondwana fragmentation, in which separation of areas where ancestors were assumed to be continuously distributed but then became separated (e.g. caeciliid fauna, Wilkinson *et al.* 2002), or colonization of an area which was volcanic, previously submerged and thus uninhabitable (e.g. Carranza *et al.* 2000; Vences *et al.* 2003a). Calibrations based on previous molecular dating estimates have also been used (Roelants *et al.* 2004). In a few cases, calibrations are fixed based on sequences of extinct lineages with known divergence times, as shown in bird divergence estimates calibrated using extinct moas (Cooper *et al.* 2001; Haddrath, and Baker, 2001).

Calibration points can be problematic. Geological data may not be appropriate for dating vicariance in taxa that may have the ability to disperse beyond potential barriers and the quality of plate tectonic data may also be uncertain, especially for island fragments whose origin and evolution is complex. Similarly, the incompleteness of the fossil record suggests any date is subject to imprecision. Fossils only provide a minimum estimate for the divergence of two lineages (Gingerich, 1983; Reisz and Müller, 2004). Further problems derive from using single calibration points, especially where these are fixed by taxa with lineage specific rate variation (Bromham, 2002). The use of multiple calibration points is clearly advantageous for a more thorough estimation of divergence times. Despite the problems mentioned above, confidence in temporal information is increasing, as a result of the development of methods that better integrate fossil and molecular data (Donoghue and Moore, 2003; Sanderson, 2003; Rambaut and Bromham, 1998; Thorne *et al.* 1998; Aris-Brosou and Yang, 2002; Hedges and Kumar, 2004).

The following calibration points are used in this study:

Frogs: The divergence between two members of the strictly freshwater aquatic family Pipidae is used as a calibration point. The African genus *Hymenochirus* and South American genus *Pipa* are sister groups (Canatella and Trueb, 1988) and must have diverged by the time that America and Africa separated at 101 mya ago (Pitman *et al.* 1993). The oldest known age of the Comoran Island Mayotte (8.7 Mya ago; as described by Vences, 2003 from Nougier *et al.* 1986) provides additional calibration points between the pairs of Comoroan *Boophis* sp. and *Mantidactylus* sp. and Madagscan *Boophis tephraeomystax* and *Mantidactylus wittei*.

Caecilians: The node joining India-Seychellean to Afro-American caeciliids (Wilkinson, *et al.* 2002) is calibrated by the minimum age of separation of Madagascar-India-Seychelles from Africa and America Gondwana, estimated to be 130 Mya (Smith *et al.* 1994). An additional calibration of 101 Mya is used for the node joining the African *Schistometopum* and South and central American *Dermophis*, recovered in recent analyses (Hedges *et al.* 1993; Wilkinson *et al.* 2002; and Wilkinson *et al.* 2003) based on the separation of Africa from South America from dated at 101 mya (Pitman *et al.* 1993). Any bias of the calibrations will be unidirectional, with calibrations representing probable underestimates of the divergence ages. All ages should therefore be considered as conservative.

2.6.5.5 Synchronous splitting events

One of the most fundamental questions this study is attempting to address is: Are divergences between commonly distributed, but independent amphibian lineages temporally congruent, and by implication plausibly driven by a common causal factor? Given that the absolute date estimations calculated from molecular data are correct, and the genes sampled between groups overlap (e.g. Zamudio and Greene, 1997), hypotheses concerning temporal congruence can be addressed as well as their possible association with changes in climate or geology. Confidence intervals provide the upper and lower limits for the overlap of each temporal estimate, and therefore determine the significance of temporal congruence. However, there might be concerns that the absolute dating estimates are incorrect, and if there are consistent errors made within and between lineages, then correspondence may be coincidental rather than informative. Molecular clock dates are controversial (see

section 2.6.5.1) and it is likely that there are substantial negative influences on absolute time estimates, e.g. under estimation of divergence dates, and saturation of data. In light of these concerns, estimation procedures that account for rate variation are investigated here, and quantitative assessment of the data sets is undertaken. Absolute dates are critical for assessing temporal congruence with specific biogeographic events (see section 2.5), but are not important for testing the hypotheses of synchronicity between cladogenetic events. Lineages divergences can be shown to be congruent using relative temporal estimates, even if the absolute timing may be uncertain.

2.7. Specifications

2.7.1 Phylogenetic Analysis

Using PAUP 4b10 (Swofford, 1998) all the molecular data sets were evaluated for differences between randomly permuted data (Faith and Cranston, 1991), incongruence in data sets (e.g. between 12S, 16S and *cytb*) and saturation. The level of saturation in each partition was investigated, from plots of uncorrected pairwise divergences against Tamura-Nei divergences, for transitions and transversion. The Tamura-Nei divergences take account of deviations from equal base composition and differences in substitution rates among bases (Tamura and Nei, 1993). Deviation from the isometric line on such a plot indicates increasing saturation of substitution for transitions or transversions. Furthermore, transitions were plotted against transversions and both a power curve and linear regression line plotted, and the r-squared value compared. If the power curve line is shown to have a higher r-squared value then there is some appreciable level of saturation in the data.

Protein coding sequences (*cytb*) were translated into amino acids using MacClade (Maddison and Maddison, 2002), which was used to verify the position of codons. Parsimony, maximum likelihood and distance analyses were all carried out in the program PAUP* 4.0b10 (Swofford, 1998). Kimura 2 parameter, LogDet and Maximum Likelihood distance analyses used the minimum evolution objective function on all runs. Likelihood analyses used models of evolution selected using Modeltest (Posada and Crandall, 1998). Alignment gaps were treated as missing data. Tree searches were heuristic with 100 (parsimony and distance analyses) or 10 (ML) random addition sequences and TBR branch swapping. Bayesian analyses

were performed using MrBayes 3.01 (Huelsenbeck, and Ronquist, 2001) using the selected ML model. The Metropolis coupled, Markov chain Monte Carlo analysis was run with 4 chains for 1,500,000 generations. Trees were sampled every 1000 generations, with the first 1000 generations discarded.

Support for clades was measured with bootstrap proportions (Felsenstein, 1985) (1000 pseudoreplicates) using both parsimony and distance options. Bayesian posterior probabilities are also calculated automatically in MrBayes analyses. Decay indices (Bremer, 1988) for clades were determined by enforcing converse topological constraints to find suboptimal trees; this was done manually in PAUP. The significance of length differences between most parsimonious and suboptimal trees found in constrained analyses were assessed using a non-parametric test (Templeton, 1983). Similarly, suboptimal ML trees, conforming to various *a priori* hypotheses, were found through searches enforcing user-defined topological constraints. Differences between optimal and suboptimal ML trees were assessed using the Shimodaira-Hasegawa test (Shimodaira & Hasegawa, 1999) using RELL with 1000 bootstrap replicates.

2.7.2 Molecular divergence estimates

To estimate molecular divergence dates between specific clades a number of taxa were included that provided calibration points (see section 2.6.5.4 for precise details). As suggested by Bromham *et al.* (2000), the likelihood ratio test was used to evaluate whether the molecular clock held for the analysed sequences (i.e. a single rate of molecular evolution). This was achieved by estimating a likelihood tree with a molecular clock enforced, which was compared to a likelihood tree without a clock enforced (see section 2.6.5.2). For the program r8s, a tree with branch lengths needs to be provided. ML trees obtained in analyses were used to estimate all the divergence times. Branch lengths for this tree topology and the best fitting substitution model selected by Modeltest were estimated using likelihood with PAUP 4b10 for all r8s analyses. Divergence times were calculated using both likelihood methods assuming a molecular clock (Langley fitch), and methods relaxing the molecular clock assumption (penalized likelihood), i.e. methods allowing for lineage specific rate variations (see Sanderson, 2002a, 2002b, 2003).

Confidence intervals on molecular divergence estimates are based on Langley-Fitch *s*-unit support limits (Cutler, 2000; see also Sanderson, 2002b). *S*-units support limits are the parameter estimates at which the log-likelihood drops by an amount of *s* units. As pointed out by Sanderson (2002b), interpreting *s*-units support limits of divergence time estimates is not straightforward. In fact, a drop of 2 units in the log-likelihood values equals the 95% confidence interval in the case of normally distributed parameters. However, molecular divergence times are unlikely to be normally distributed. Therefore, 2-units support limits cannot be assumed to encapsulate the 95% confidence intervals around the maximum likelihood estimate. Accordingly, Sanderson (2002b) suggested conservative *s* values (of at least 4) should be preferred when defining *s*-units support limits for divergence time estimates. In this study, 4 units were used to calculate the approximate confidence intervals for temporal estimates.

2.7.3 Biogeographical analyses

The definition of areas of endemism for use in biogeographic analyses is often difficult to define because there is not always a clear geographical separation of areas used in analyses (Morrone and Crisci, 1995). In many cases, such as continuous continental land areas, the designation of taxa to areas can be difficult which has an influence on biogeographic analyses (Morrone and Crisci, 1995). In the case of this study, it is fortunate that areas are clearly separated by the topography of the region. The Eastern Arc Mountains of Tanzania and Kenya are mountains uplifted, and are therefore clearly defined against the low lying savanna region. There was never any ambiguity in designating a species or population to an area, as the mountains are clearly marked and non-overlapping.

2.7.3.1 Descriptive Biogeography

Similarity Indices

A data matrix was constructed where "0" is coded for the absence of the taxon from the area and "1" for the presence in the area (see Appendix 4). Amphibian species lists were compiled from various sources, see details in Appendix. Jaccard and Sorenson indices were then calculated using the program Community Analysis Package (CAP) (1999). To elucidate the biogeographical relationships between areas, I conducted cluster analyses (Krebs, 1999) using both Jaccard and Sorenson indices. Dendrograms are automatically constructed from these calculations in the program. Using PAUP 4b10 (Swofford, 1998) the data matrix was also evaluated for

differences between randomly permuted data (Faith and Cranston, 1991) by carrying out a PTP test.

PAE

Exactly the same data matrix used for calculating similarity indices, was also used in PAE analyses (see Appendix 4). The matrix was constructed in MacClade and then exported into PAUP4b10 (Swofford, 1998). Exhaustive searches were carried out on the matrix, which calculates all possible trees. The cladogram was rooted using a hypothetical area coded with all zeros, as suggested by Rosen (1988). In addition support for clades was measured with bootstrap proportions (Felsenstein, 1985) (1000 pseudoreplicates) using parsimony options.

2.7.3.2 Cladistic Biogeography

Taxon cladograms were generated from analyses of 12S, 16S and *cytb* using the following tree building methods: Parsimony, Maximum Likelihood, Bayesian analyses for the following groups: *Arthroleptides, Boulengerula, Scolecomorphus, Brevicipitines, and Hoplophrynines*. Details of the phylogenetic relationships of these species are given in Chapters 3-6. Usually the best likelihood tree was used for biogeographic reconstructions, as these analyses require fully bifurcating trees. In addition to these phylogenies, all suitable trees were included from the literature (e.g. *Andropadus*, *Lobelias*, *Crotaphapeltis*, *Saintpaulia, Nectarina, and Rhampholeon*). All phylogenies utilised were derived from molecular data, and consequently terminal taxa in molecular phylogenies represent lineages and not necessarily species. In this study when a species or population is represented by a single accession or accessions which are monophyletic then it is safe to assume that the accession represents a single species/population and can be coded for the areas that it occurs in. Both the amphibian and a combined dataset were then subjected to a cladistic biogeographic analysis, referred to as 'Tree Reconciliation Analysis'. Firstly, resolved area cladograms were tested for the presence of repeated area relationships using Component 2.0 (Page, 1993). Following this, the statistical significance of GAC topology was tested using Tree Map version 1.0 (Page, 1995).

Chapter Three

Systematics and Biogeography of *Arthroleptides*

3.1 Aims

This chapter aims to investigate the systematic and biogeographical patterns in the frog genus *Arthroleptides*. *Arthroleptides* belong to a poorly known amphibian family with uncertain phylogenetic affinities. I will briefly examine the phylogenetic position of East African genus *Arthroleptides*. Using the wide sampling of *Arthroleptides* populations available I will also investigate the monophyly of the genus, species limits, and investigate population difference within the Eastern Arc. By investigating the differences between species and populations, I will also consider their biogeographical implications, including large-scale patterns of African biogeography and regional biogeography. In particular I will explore the possible effect the suggested fragmentation and prolonged isolation of mountains of the Eastern Arc have had on the genus.

3.2 Introduction

3.2.1 *Arthroleptides*

Arthroleptides are ranid frogs found restricted to the montane forest regions of East Africa, and are associated with riverine habitats, hence the name torrent frog. Of the three currently described species, two are endemic to the Eastern Arc (Channing *et al.* 2002; see Fig. 3.1) and are almost continuously distributed along the mountains. They are currently assigned to the family Petropedetidae. Nieden (1910) described the first species *Arthroleptides martiensseni* (Fig. 3.2a), based on material collected from Amani (or presumed from, see Channing *et al.* 2002), in the East Usambara Mountains. Nieden (1910) thought his specimens resembled members of the two ranid genera *Arthroleptis* and *Petropedetes,* sharing the absence of vomerine teeth and foot morphology of the former, and the strongly broadened finger and toe tips in the latter (Neiden 1910). However, specimens were differentiated enough from both

of these genera to warrant their own generic status. A second species, *A. dutoiti* (Fig 3.2b.), was described from the volcanic Mount Elgon, which lies on the Kenyan and Ugandan border (Loveridge, 1935). Since the description of *A. dutoiti* (Loveridge, 1935), no further specimens have been collected, despite recent surveys in the region (Lötters, pers. comm.). In light of the apparent absence of *A. dutoiti* in Mount Elgon, the species is considered critically endangered, and may even be extinct (G.A.A., 2004). *Arthroleptides dutoiti* can be easily distinguished from *A. martiensseni* by its smaller size and reduced webbing.

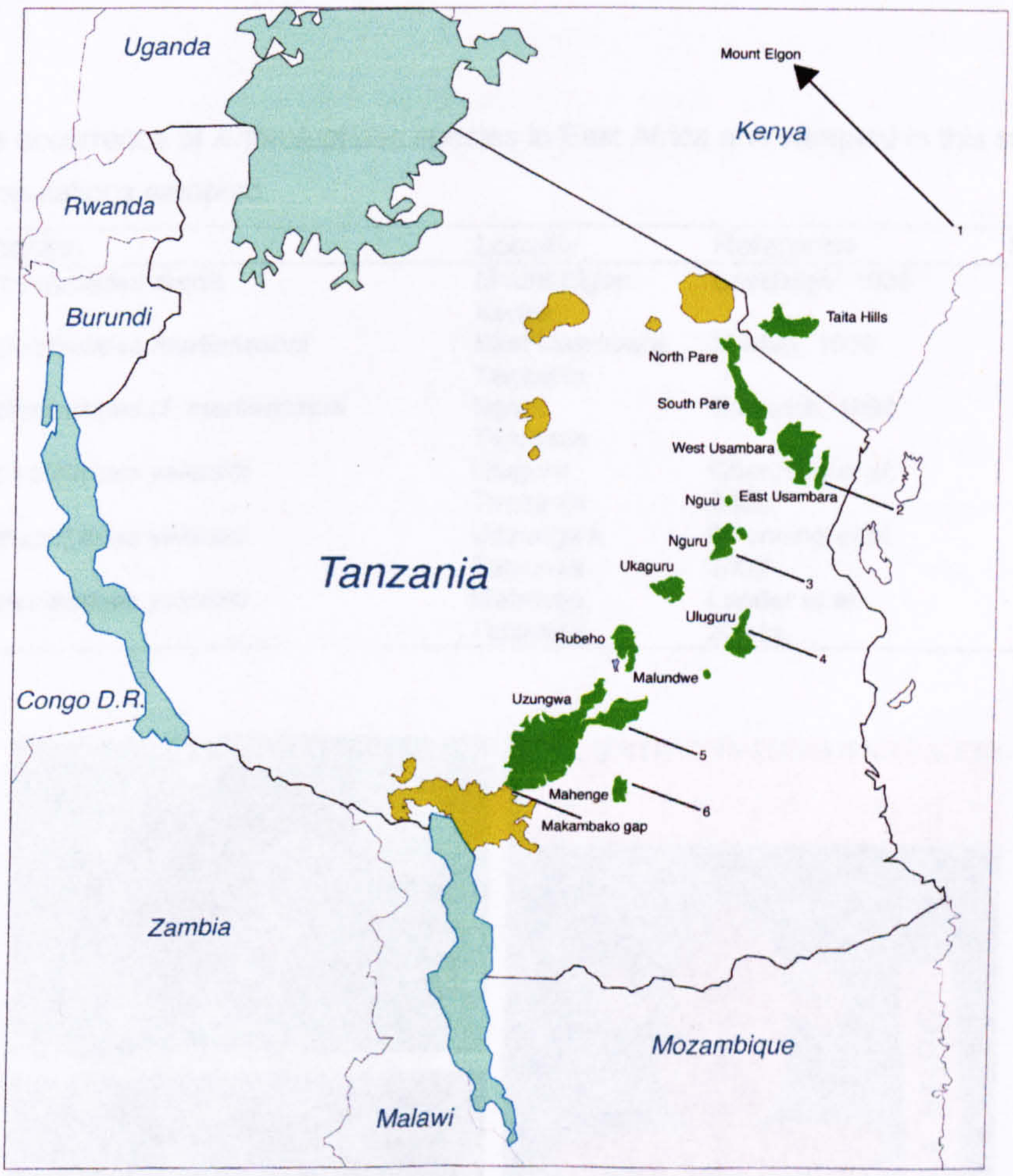

Figure 3.1
Distribution of the *Arthroleptides* in East Africa (see Table 3.1 for details). Areas in green are Eastern Arc Mountains, areas in brown are of more recent volcanic origin. See Table 3.1 for key to numbers.

The most recently described species from this genus was *Arthroleptides yakusini* (Fig 3.2c.), found in the southern montane region of Tanzania (Uluguru, Udzungwa, and Mahenge) (Channing *et al.* 2002; Loader *et al.* 2004a). This *Arthroleptides* species

has a large body size, distinguished by its extensive webbing, and reduced supratympanic ridge (Channing *et al.* 2002). Prior to the description of the southern species *A. yakusini* ('ya kusini' means from the south), *A. martiensseni* was thought to occur throughout the Eastern Arc (East Usambara, Ngurus, Uluguru, and Udzungwa) (Frost, 2002). The description by Channing *et al.* (2002) of *A. yakusini* split the distribution; with *A. martiensseni* restricted to the East Usambara. The specific status of material collected from the Ngurus (Emmrich, 1994), is however still in doubt because this material has not been examined (Channing *et al.* 2002).

Table 3.1

List of the occurrence of *Arthroleptides* species in East Africa and sampled in this study. With 66% of populations sampled.

	Species	Locality	Reference	Sampled
1	*Arthroleptides dutoiti*	Mount Elgon Kenya	Loveridge, 1935	X
2	*Arthroleptides martiensseni*	East Usambara, Tanzania	Nieden, 1910	√
3	*Arthroleptides* cf. *martiensseni*	Nguru, Tanzania	Emmrich, 1994	X
4	*Arthroleptides yakusini*	Uluguru, Tanzania	Channing *et al.* 2002.	√
5	*Arthroleptides yakusini*	Udzungwa, Tanzania	Channing *et al.* 2002.	√
6	*Arthroleptides yakusini*	Mahenge, Tanzania	Loader *et al.* 2004a.	√

Figure 3.2

Pictures of *Arthroleptides* (a) *A. martiensseni* (Amani, East Usambara) (b) *A. dutoiti* (Mount Elgon), scale in cm (c) *A. yakusini* (Udzungwa). Pictures kindly provided by Vonesh, Rosado and Menegon, respectively.

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3.2.2 Phylogenetic relationships among Petropedetidae frogs and outgroups

Noble (1931) erected the subfamily Petropedetinae for the two genera *Arthroleptides* and *Petropedetes*, which were similar in their 'size and palates' (p.520). A confused history then followed in which certain "ranid" genera were gradually placed in the subfamily, and taxonomic ranks were changed (Dubois, 1992). The changing of taxonomic ranks in ranids was argued as not being adequately justified (Inger, 1996) but is generally followed today (Frost, 2002). The group is now formally recognised in the family Petropedetidae (Frost, 2002), which consists of the following 12 genera: *Anhydrophryne, Arthroleptella, Arthroleptides, Cacosternum, Dimorphognathus, Ericabatrachus, Microbatrachella, Natalobatrachus, Notophryne, Petrodepetes, Phrynobatrachus, Phrynodon, Poyntonia* (Duellman and Trueb, 1994). There are approximately 102 species currently described from this family, and half of these species belong to the pan-African genus *Phrynobatrachus* (68 species). The petropedetids also comprise a number of monotypic genera (*Anhydrophryne, Dimorphognathus, Ericabatrachus, Microbatrachella, Natalobatrachus, Nothophryne, Phrynodon, Poynotonia*), which show highly derived features. Possibly, as a consequence of these highly derived forms, phylogenetic relationships among Petropedetidae are poorly understood, and even the monophyly of the group is far from well established (Blommers-Schlösser, 1993).

Despite the various controversies concerning the taxonomic status of ranoid frogs (Frost, 2002), petropedetids are considered part of the superfamily Ranoidea (*sensu* Ford and Canatella, 1993). Their 'ranid' affinities have been supported by various morphological studies (e.g. Noble, 1931; Griffiths, 1957, 1963). Griffiths suggested the grouping of petropedetids with dendrobatids in a ranid clade, but this has been disputed (Ford, 1993). Recent morphological studies have provided only a limited understanding of relationships among Petropedetides (Scott, 2002; Largen, 1991). Interestingly there appears to be support for the genera *Arthroleptides, Ericabatrachus, Petropedetes, and Phrynodon* forming a 'natural phylogenetic group' (Largen, 1991; p.150), which Scott (2002) partially agreed with, excluding *Phrynodon* from this clade. If correct, this finding is consistent with distribution patterns shown in other afromontane amphibian groups, with species showing closer relationships between montane East and West African and Ethiopian highlands than with species in close proximity, as also shown in bufonids, microhylids and caecilians (Largen, 1991; Poynton, 1999; Nussbaum, 1985).

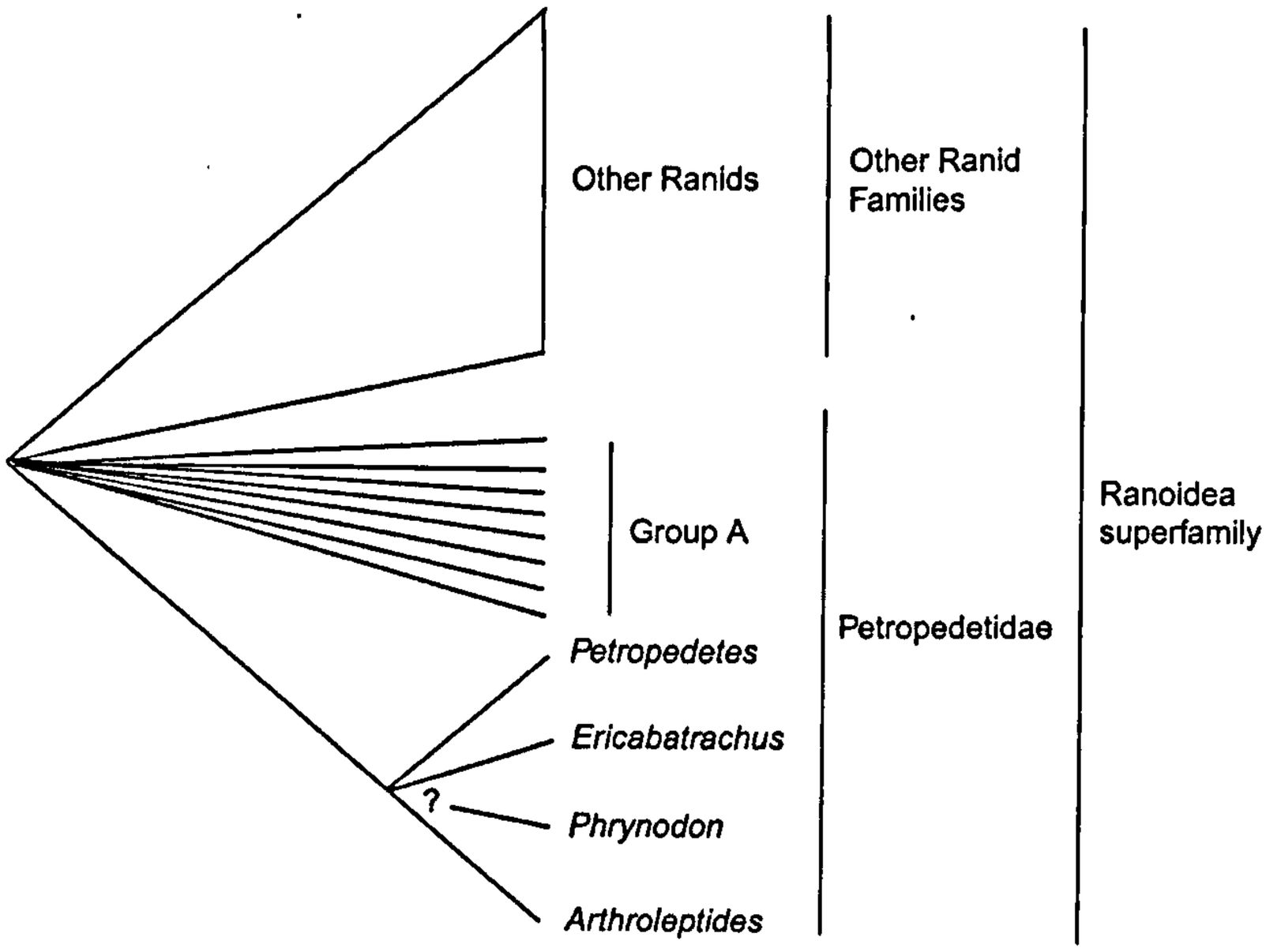

Figure 3.3.

Summary of the relationships among Petropedetidae frogs, group A represents all other eight Petropedetidae genera, summarised from various sources.

Molecular investigations on petropeditids have been sparse and not particularly informative, because only one or two relevant taxa have been sampled. For example, Vences *et al.* (2003a) found the genus *Petropedetes* to form a weakly supported clade with the African ranid *Ptychadena*, and Biju and Bossuyt (2003) found good support for the placement of *Petrodepetes* as sister to consecutive lineages of Asian ranids (*Meristogenys*), Indian rhacophorids (*Philautus*) and mantellids (*Boophis*). Only one African ranid was included in the former study, and no African ranids were included in the latter study, so interpretations are very limited. The most significant finding is that shown by Van der Meijden *et al.* (2004) who provided evidence for the paraphyly of petropedetids using a nuclear marker, RAG 1. Their study found that the south African genus *Cacosternum* forms a clade with *Ptychadena*, with *Petropedetes* basal to this group (no other relevant taxa were included), thus suggesting that petropedetines are paraphyletic, unless *Ptychadena* is considered a petropedetidae. Clearly, further research, both molecular and morphological is needed to establish the relationships among this family, as well as the wider relationships among ranids.

Certain outgroups were utilised in this study which provided suitable calibration points for molecular dating estimates, including the Pipid genera; *Pipa* and *Hymenochirus*, Hyperoliid *genera; Tachycnemis, Heterixalus, Afrixalus, Hyperolius,*

Mantellid genera; *Boophis* and *Mantidactylus*. The phylogenetic relationships among these groups are better understood, but will not be treated exhaustively here, for reviews see Cannatella and Trueb (1988) Drewes (1984) Emerson *et al.* (2000) Vences *et al.* (2003a,b) and Biju and Bossuyt (2003). A brief summary is provided below.

The Hyperoliidae, commonly known as Treefrogs (Schiotz, 1999), comprise over 200 species of African, Madagascan, and Seychellean species (Drewes, 1984; Ford and Cannatella 1993; Duellman and Trueb, 1994; Frost, 2002). Ford and Cannatella (1993) defined Hyperoliidae as the name for the descendants of the hyperoliid genera (*Acanthixalus, Afrixalus, Callixalus, Chrsobatrachus, Cryptohylax, Heterixalus, Hyperolius, Kassina, Kassinula, Leptopelis, Nesionixalus, Opisthothylax, Phlyctimantis, Tachycnemis, and Tornierella*). Drewes and Wilkinson (2004) recently returned to the synonomy of *Hyperolius* the Sáo Tomé Hyperoliid genus *Nesionixalus*. Hyperoliid phylogeny has been analysed based on morphology (Liem, 1970; Drewes, 1984) and this has provided evidence for the monophyly of hyperoliids. Recently the relationships among hyperoliids have been questioned using molecular data, showing a more complex picture. Vences *et al.* (2003a,b) and Van der Meijden *et al.* (2004) questioned the monophyly of Hyperoliidae, with *Leptopelis* forming a clade with the non-hyperoliid genus *Arthroleptis*. Broader sampling will be necessary to fully test the monophyly of Hyperolidae, which is not the subject of this study.

3.3 Materials and methods

3.3.1 Specimens

The specimens sampled are given in Table 3.2

3.3.2 Phylogenetic analyses

To investigate the phylogenetic relationships among *Arthroleptides* and their molecular divergence times, two datasets were compiled in BioEdit. The first dataset included all the taxa shown in Table 3.2, and this alignment was used to estimate divergence times. The second data set included only *Arthroleptides* and

Petropedetes taxa, thereby including a larger proportion of the original sequence data, because there were fewer hypervariable regions. Following analyses (among many others, Biju and Bossuyt, 2003; Vences *et al.* 2003a; Hertwig *et al.* 2004) that show the basal position of pipids relative to all neobatrachids, the two pipid species *Hymenochirus boettgeri* and *Pipa parva* were designated as the outgroup and used to root trees for analyses of the complete dataset. In the second alignment, the West African species *Peteropedetes parkeri* was used as the root of the tree, as this showed robust support as the sister group to *Arthroleptides* in all preliminary analyses.

3.3.3 Dating estimates

Specifications for the molecular dating estimates are given in Chapter 2.

Table 3.2.

Arthroleptides and outgroups analysed in this study, *= sequences obtained from Genbank.

Sequence number	Specimens	Species	Locality	Forest Reserve
T282	MW 1844	*Arthroleptides yakusini*	Mahenge	Sali FR
T285	MW 1852	*Arthroleptides yakusini*	Mahenge	Sali FR
T286	MW 1854	*Arthroleptides yakusini*	Mahenge	Sali FR
T414	KMH 22148	*Arthroleptides yakusini*	Udzungwa	West Kilombero Scarp FR
T415	KMH 21533	*Arthroleptides yakusini*	Uluguru	Kasanga FR
T416	KMH 21535	*Arthroleptides yakusini*	Uluguru	Kasanga FR
T417	KMH 21215	*Arthroleptides martiensseni*	East Usambara	Nilo FR
T419	KMH 21188	*Arthroleptides martiensseni*	East Usambara	Nilo FR
T458	RdS5862	*Arthroleptides yakusini*	Uluguru	Uluguru North FR
T459	RdS5946	*Arthroleptides martiensseni*	East Usambara	Amani NR
T464	MW 3044	*Hyperolius puncticulatus*	Ukaguru	Ikwamba FR
T465	MW 1837	*Afrixalus uluguruensis*	Mahenge	Sali FR
T466	MW 2338	*Arthroleptis tanneri*	West Usambara	Mazumbi FR
n/a	n/a	**Pipa parva*	South America	n/a
n/a	n/a	**Hymenochirus boettgeri*	West Africa	n/a
n/a	n/a	**Petropedetes parkeri*	West Africa	n/a
n/a	n/a	**Mantidactylus sp.*	Mayotte	n/a
n/a	n/a	**Mantidactylus wittei*	Madagascar	n/a
n/a	n/a	**Boophis sp.*	Mayotte	n/a
n/a	n/a	**Boophis tephraeomystax*	Madagscar	n/a
n/a	n/a	**Heterixalus tricolor*	Madagscar	n/a
n/a	n/a	**Tachycnemis seychellensis*	Seychelles	n/a

3.4 Results

3.4.1 Phylogeny

3.4.1.1 Data Quality and details

All PCR amplifications from DNA templates yielded products of the expected size, which when analysed in Sequencher 3.1.1™ contained minimal levels of site ambiguity. The PTP test rejected the null hypothesis of no more structure in the data than randomly permuted data (P> 0.001). There was no significant difference in base composition across all taxa (chi-squared tests for homogeneity, $P = 1$). Further comparisons were made between each partial gene fragment to investigate the extent to which the data sets individually result in different trees. Incongruence length difference test as applied in PAUP (partition homogeneity test) and showed no significant incongruence between each data set (P> 0.99), suggesting that combining the datasets would not be problematic (Cunningham, 1997). Each gene was subjected to combined and separate analyses, and no significant topological differences were noted for Petropedetidae groups. Branch lengths indicated different rates of molecular evolution between *cytb*, 12S and 16S (Fig. 3.4a-c), with *cytb* evolving more rapidly and basal splits being more strongly compressed. Furthermore, analysis of each *cytb* site showed 3rd positions to have a increased transition/transversion rates than 1st and 2nd positions, which is consistent with what is known about codon evolution e.g. Graybeal (1993) (see Fig. 3.4d-f). Third position analysis shows greater resolution at tree tips and increased number of changes relative to other positions (Fig. 3.4d-f). Saturation plots were calculated for each gene partition, and these indicate that the data appear not to be saturated (summarised in Fig. 3.4g), as shown by significant r^2 value, and separation of transition and transversion regression lines. In *cytb* for the third position, the most rapidly evolving sites, greater transition and transversion rates are shown than other partitions, which might suggest saturation in the data. Overall however, relationships inferred from each data partition, and reconstruction methods have almost entirely congruent tree topologies.

The first, larger alignment, including all taxa consisted of 22 taxa and 1537 characters: see Table 3.3 for details. The second, petropedetidae alignment, consisted of 11 taxa and 1710 characters: see Table 3.4 for details. Constant characters comprised 55% of the first alignment and 82% of the second.

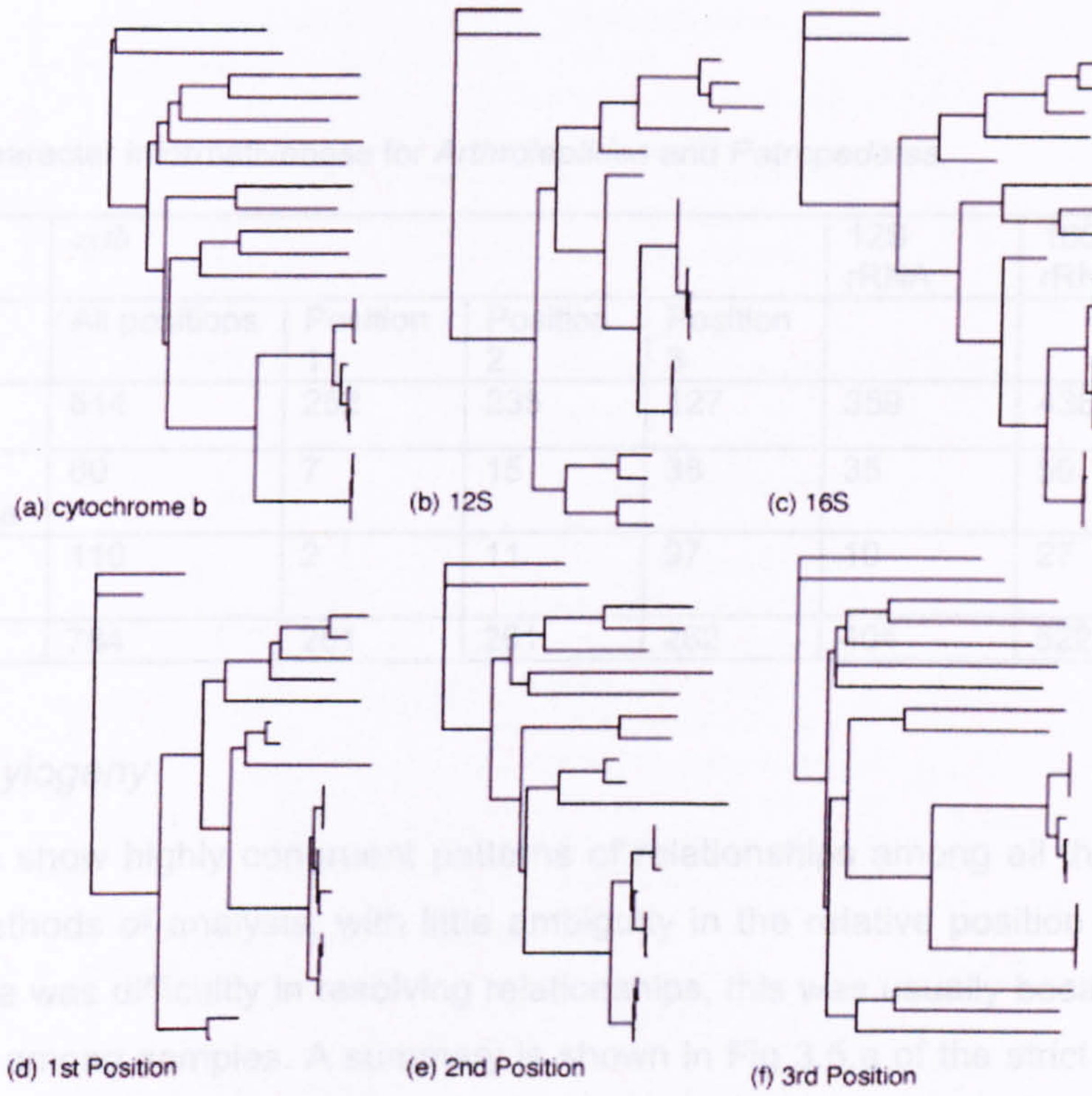

Figure 3.4

(a-f) Comparison of branch lengths for different data partitions (g) Plot of substitution of transversions and transitions, indicating levels of saturation, with transitions in purple, and transversions in blue, r^2 value for transitions ($r^{2=}0.9152$) and transversions ($r^{2=}0.9212$).

Table 3.3.

Details of character informativeness for the full alignment of 22 taxa.

	cytb				12S rRNA	16S rRNA	Total
	All positions	Position 1	Position 2	Position 3			
Constant	389	216	161	12	180	279	848
Variable-uninformative	62	19	27	16	33	42	137
Parsimony informative	333	26	73	234	100	119	552
Total	784	261	261	262	313	440	1537

Table 3.4.
Details of character informativeness for *Arthroleptides* and *Petropedetes*.

	cytb				12S rRNA	16S rRNA	Total
	All positions	Position 1	Position 2	Position 3			
Constant	614	252	235	127	359	436	1409
Variable-uninformative	60	7	15	38	35	59	154
Parsimony informative	110	2	11	97	10	27	147
Total	784	261	261	262	404	522	1710

3.4.1.2 Phylogeny

The results show highly congruent patterns of relationships among all the partitions and the methods of analysis, with little ambiguity in the relative position of all taxa. Where there was difficultly in resolving relationships, this was usually because of little divergence among samples. A summary is shown in Fig 3.5.a of the strict consensus of MPTs and in Fig.3.5.b the single maximum likelihood tree.

All data partitions and combined analysis agreed upon the relationships shown in Fig. 3.5 apart from in some data partitions where populations of *A. yakusini* and *A. martiensseni* were not as well resolved, the African treefrog sister group to the treefrog genera *Heterixalus* and *Tachycnemis*, and the position of *Arthroleptis* relative to treefrogs and mantellids. The position of the treefrogs, as shown in Fig. 3.5, was well supported in all combined analyses, and this resolution is a significantly better fit to the data than suboptimal groupings in both likelihood (P=<0.001) and parsimony topology tests (P=<0.001). Only in separate likelihood analyses of 12S and 16S was this topology shown to be poorly resolved in a polytomy. Likelihood analysis of 16S data also showed weak support for the grouping of *Arthroleptis* and mantellids, whereas all other analyses show there was moderate support for *Arthroleptis* as sister group to the Hyperoliids sampled in this study.

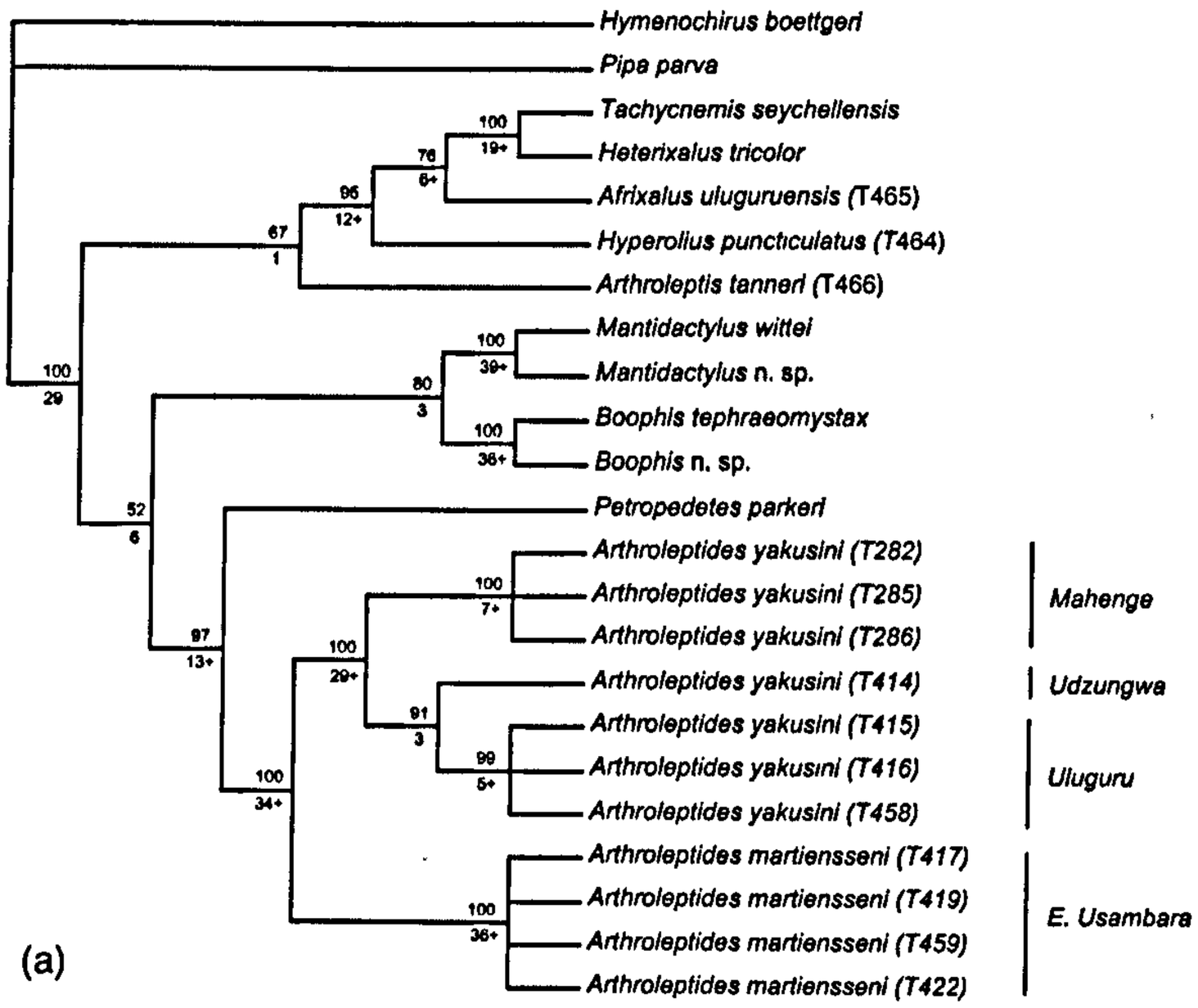

Figure 3.5

(a) Strict consensus of 11MPT, tree length 1910. Bootstrap proportions shown above branches, Decay Index values shown below, along with Templeton test result (+= <0.05 significant). (b) Maximum likelihood tree (LnL= 9722.46810), GTR+I+G model using Modeltest. Base frequencies estimated at 0.3349, 0.2644, 0.1291 and 0.2716 for A, C, G and T respectively, substitution rates =1.0000, 4.5963, 1.0000, 1.0000, 8.8352, and the proportion of invariant sites set at 0.3256 and a gamma distribution shape parameter of 0.4430. Values on branches show Bayesian posterior probabilities, bootstrap proportions calculated using distance criterion; kimura 2 parameter, LogDet, and maximum likelihood. Below branches shows SH test results (+= <0.05 significant).

Divergence within the populations of the species *A. yakusini* and *A. martiensseni* is very limited (see Appendix 4), and it is because of this that the phylogenetic relationships within these populations are not well resolved in any analyses. This is clearly shown in the second *Arthroleptides* alignment, which shows a majority of characters being constant (82%). Among the *Arthroleptides* populations, only within *A. martiensseni* and Mahenge *A. yakusini* are there any substantial phylogenetic structure between haplotypes, which is shown between Nilo FR and Amani NR, and Sali FR but this is only weakly supported (Bootstrap=68 and 70 respectively, see Fig. 3.5).

Overall, the phylogeny provides robust support for the recognition of the groups including hyperoliids, mantellids, Petropedetidaes and *Arthroleptides*. The monophyletic grouping of *Petropedetes* and *Arthroleptides* is well supported. Within *Arthroleptides*, the divergence of two main clades represented by the species *A. martiensseni* and *A. yakusini* is shown to be robustly supported and an unambiguous resolution. In addition there is significant support for the recognition of sub populations in *A. yakusini*, characterised between each mountain block of occurrence (Mahenge, Uluguru, and Udzungwa).

3.4.2 Molecular divergence estimates

3.4.2.1 Consistency of calibration estimates

The reliability of the calibration points were investigated in two ways; (1) by fixing one of the two calibration points, and using this fixed point to then estimate the divergence time for an unfixed calibration point. The divergence times for each unfixed calibration point could be estimated and then compared to each fixed time. For example, fixing the split between *Hymenochirus* and *Pipa* at 101 mya, to estimate the divergence of unfixed *Mantidactylus* species pair. (2) Comparison of divergence estimates for taxa common to both this study and that of Vences *et al.* (2003a). Vences *et al's* (2003a) divergence estimates are based on nuclear gene fragments, whereas the study here compares partial mitochondrial gene fragments from the same taxa. Particular taxa were included into the alignment to test this (eg. *Heterixalus*, *Tachycnemis, Afrixalus*) and are compared to divergences given by Vences *et al.* (2003a). The calibration points appear to provide reasonably robust

reciprocal estimates of each other (see Table 3.5a). The confidence intervals given allow the rejection of the hypothesis that single fixed calibration point estimates show substantial difference in divergence estimates from multiple fixed calibration points, and thereby validating their usage as calibration points. Comparison with the estimates given in Vences *et al.* (2003a) to the dates estimated using mitochondrial data here show good correspondence for the *Tachycnemis Heterixalus* clade. The overall congruence and consistency in the estimation of divergence is also not surprising given that the data provide a robust phylogenetic hypothesis of relationships, and appear to be little affected by saturation.

Table 3.5.

Consistency of dating estimates (a) Single calibration point estimation (b) Comparison of molecular divergence dates between Vences *et al.* 2003a and this study.

	Estimated time using single fixed calibration points	
(a)	*Hymenochirus* and *Pipa*	*Mantidactylus* species pair
Hymenochirus and *Pipa*	113.23 (97.34-126.23)	Constrained
Mantidactylus species pair	Constrained	10.62 (7.67-10.98)
	Calibration= 101 mya	Calibration= 8.7 mya

(b)	Vences et al., (2003a)	This study
Tachycnemis Heterixalus	11-21 Myr	14.97

3.4.2.2 Absolute time estimates for Arthroleptides

Estimation of divergence times was carried out using both Langley-Fitch and Penalized likelihood approaches. Rate heterogeneity in the data set was shown to be marginally significant, as shown by the likelihood ratio test (Δ=31.59294, P=>0.05, d.f.=20) demonstrating that with the molecular clock enforced there was a significant difference between likelihood scores. Two estimates were carried out on an alignment including all taxa, and one with the removal of *Petropedetes*, which contained only a partial segment of *cytb* sequenced for all other taxa. The two estimates are shown in Table 3.6. Alternative data partitions were also investigated (not shown), with *cytb* data removed (including the third positions). The results of these did not conflict with the results shown below.

Table 3.6.

Absolute divergence times in Myr. for clades within Petropedetidae.

Most recent common ancestor (MRCA)	All taxa		*Petropedetes* removed	
	Penalized Likelihood	Langley-Fitch	Penalized Likelihood	Langley-Fitch
Petropedetes Vs. Arthroleptides	50.35	50.12 (40.16-60.17)	n/a	n/a
A. martiensseni Vs. A. yakusini	18.94	19.88 (15.17-25.32)	19.88	22.01 (18.23-26.62)
A. yakusini Mahenge Vs. Udzungwa, Uluguru	4.56	3.65 (2.76-4.81)	2.34	2.53 (1.94-4.01)
A. yakusini Udzungwa Vs. Uluguru	2.03	2.55 (1.69-2.76)	1.71	1.79 (0.75-1.79)

3.5 Discussion

3.5.1 Phylogeny

3.5.1.1 Higher level relationships

Taxonomic sampling of African ranids is very limited, and therefore this analysis offers only a tentative understanding of their phylogenetic relationships. There have been a number of molecular phylogenies including ranids published recently, and these data have formed part of the sequences analysed here (Biju and Bossuyt, 2003; Vences *et al.* 2003a, 2003b). However, based on the newly collected samples added to these sequences, there are new insights that can be made (see Fig. 3.5). The optimal trees provide strong support for the monophyly of Hyperoliidae, with a basal *Hyperolius*, and *Afrixalus* sister to *Tachycnemis and Heterixalus.* This result is entirely congruent with the combined results from molecular phylogenies of Vences *et al.* (2003a,b) and van der Meijden *et al.* (2004), and in addition to combined morphology and molecular datasets (Emerson *et al.* 2000) and morphological analyses of Drewes (1984) where there is overlap in sampling.

The position of *Arthroleptis* as basal to the Hyperoliidae is also congruent with other molecular studies (Emerson *et al.* 2000; Biju and Bossuyt, 2003; Vences *et al.* 2003a,b). The close grouping of "Mantellidae" (sensu Vences *et al.* 2003b) with Petropedetidae is clearly resolved in this analysis, and supported in previous analyse

(Biju and Bossuyt, 2003; Vences *et al.* 2003a,b), which indicate a more recent common ancestor shared between these two clades than with arthroleptid or hyperoliids. The grouping of the petropedetides genera *Arthroleptides* and *Petropedetes* in this analysis is unsurprising based on their morphological similarity (Largen, 1991). Without sampling any further petropedetid lineages, or African ranids, this analysis provides only tentative insights into the relationships of petropedetids. The grouping of the petropedetid *Cacosternum* with *Ptychadena* (not a petropedetid), albeit weakly, in van der Meijden's (2004) study highlights the poor understanding we have of the relationships among petropedetidae and ranids in general. Ford (1993) suggested that the petropedetids might be paraphyletic with respect to other African ranids, which is supported by Scott (2002). Further analysis of other putative petropedetidae lineages and ranids will be necessary to resolve the relationships among this group of frogs which is beyond the scope of this study. Given the poor understanding we have of the content and meaning of 'Ranoidea' as it stands (Inger, 1996), a wide sampling strategy will be necessary to fully resolve these groups.

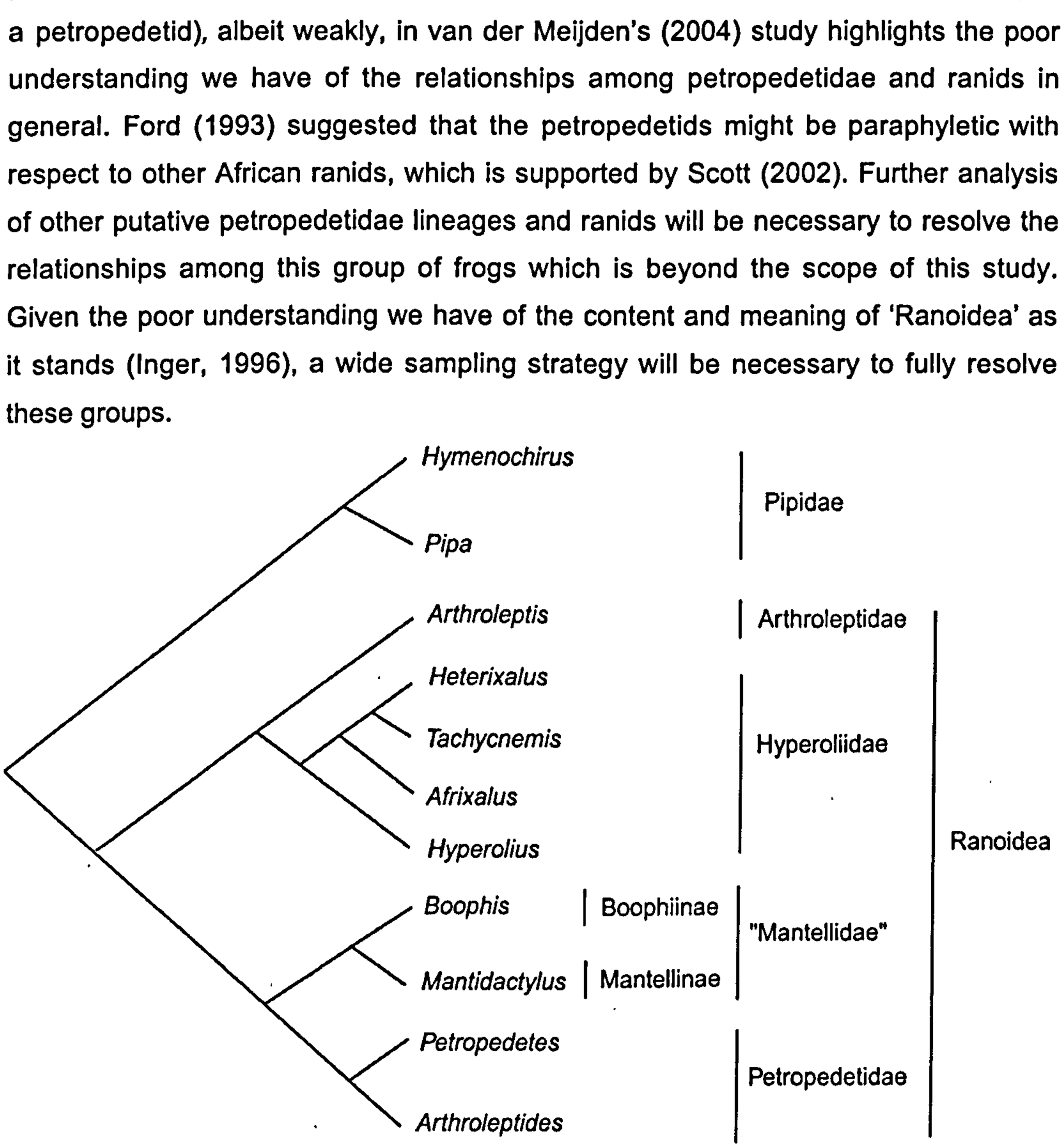

Figure 3.6.

Summary of the higher relationships inferred from 12S, 16S and *cytb* data presented in this study.

3.5.1.2 Arthroleptides: species and population differences

Species limits in Arthroleptides

Three species of *Arthroleptides* are currently recognised (Channing, *et al.* 2002) and molecular evidence in this study provides good support for two of these species. The monophyly of *Arthroleptides* can be further tested if *A. dutoiti* is included in future molecular analyses. The distinction of a southern Eastern Arc clade, as recently recognised morphologically by the species *Arthroleptides yakusini,* is found in all analyses. Pairwise comparisons (see Table 3.7) show considerable percentage sequence difference (7.8%) between the *A. yakusini* clade and *A. martiensseni* clade, a percentage difference that is substantially greater than that exhibited between other amphibian species using the same genes (e.g. Wieczorek *et al.* 1997). Based on the data presented here, and morphological studies (Channing *et al.* 2002), the status of *A. yakusini* as a distinct species is well corroborated. Within the species *A. yakusini* there is clear geographical structure, with distinct populations in Uluguru, Udzungwa, and Mahenge Mountains all strongly supported. These populations appear to show limited infraspecific haplotype diversity within each mountain block, but between mountains there are 1-2% differences, which suggest a period of substantial isolation. The Mahenge clade also appears to show the greatest divergence from the other mountain populations (in *A. yakusini)*, as demonstrated by pairwise differences (See Table 3.7).

Data provided in this study suggest that the Mahenge population of *Arthroleptides yakusini* might be a distinct lineage, and therefore a candidate for a previously unrecognised species. Further morphological and molecular studies will be necessary to see if there are any distinct phenotypic characters in the population that may provide evidence for the separation of this population from Uluguru and Udzungwa populations. No endemics have yet been detected in the Mahenge amphibian fauna, but evidence from other groups (Keilland, 1990; Lovett and Pocs, 1993; Mariaux, pers. comm.) suggests this area might be rich in endemics. The sampling of *Arthroleptides* in each mountain population is somewhat restricted and this limitation makes population interpretations tentative. Further understanding of the haplotype diversity and population level differences in these species will require a greater sampling of populations and genetic markers.

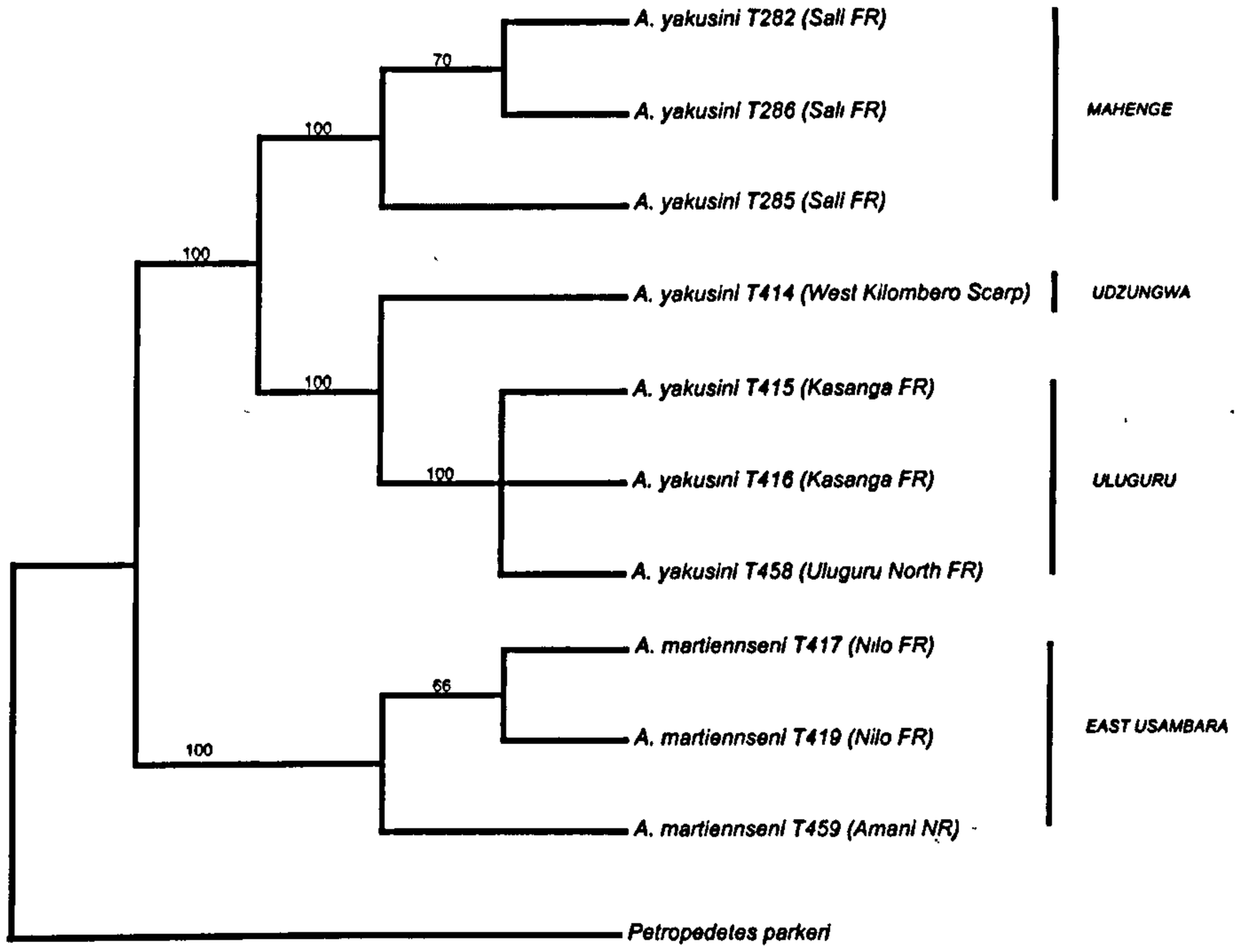

Figure 3.7

Phylogeny of *Arthroleptides* alignment, with Bootstrap proportions shown above branches.

Table. 3.7

(a) Percentage pairwise difference in *Arthroleptides*. (b) Geographical distance between mountain blocks in the Eastern Arc Mountains.

(a) % Sequence variation	Within population	Between population	To sister species
A. yakusini (Mahenge)	0.01-0.07%	1.3-1.56%	7.87-7.90%
A. yakusini (Uluguru)	0.13%	0.52-1.56%	7.92-7.97%
A. yakusini (Udzungwa)	n/a	0.50-1.30%	7.68-7.71%
A. martiensseni	0.01-0.07%	n/a	7.87-7.97%

(b) Geographical distance	Mahenge (Sali)	Uluguru (Kasanga)	Udzungwa (WKS)	E. Usambara (Amani)
Mahenge (Sali)	-	270km	155km	449km
Uluguru (Kasanga)	-	194km	217km	
Udzungwa (WKS)		-	405km	
East Usambara (Amani)			-	

3.5.2 Biogeography

3.5.2.1 East and West African rainforest biogeography

An ancient vicariant event separating the forests of East and West Africa has been hypothesised as being critical in shaping the evolution of rainforest taxa in Africa (see Chapter 1) (Kingdon, 1989; Lovett, 1993a; Burgess *et al.* 1998a). Data presented here suggest that the separation of two montane taxa, the West African *Petropedetes* and East African *Arthroleptides* predate a recent dispersal/vicariant event between these two regions, and even a Miocene event. The most conservative estimates show a date of 50 Mya, in the Eocene period; a phase substantially predating the initiation of the uplift of the central African plateau when East and West African forest started its slow separation (Lovett, 1993a). If the molecular clock estimates are correct then the separation is consistent with the hypothesis that dispersal of forest amphibians between both East and West Africa was restricted, however this is unlikely to be the causal factor for the split. Which causal factors might be important, given the poor understanding we have of this period in Africa (Morley, 2000), is uncertain. Future investigations may contradict the tentative conclusions made here, such as; finding of *Pedropedetes* in East Africa or *Arthroleptides* in West Africa, or opposite distribution to that seen today of post-Miocene fossils with paraphyly of this group and conflicting dating estimates. In addition, there might be concerns on the use of mitochondrial data for such ancient divergence events (Vences *et al.* 2003a). Future work on this should concentrate on estimates using nuclear data that may more accurately measure absolute time of splitting events. Sampling of other Petropedetid genera (e.g. *Ericabatrachus*) would also further elucidate biogeographic history of Africa, as well as examining other groups with similar distributions (e.g. *Nectophrynoides* and caecilians).

3.5.2.1 Eastern Arc biogeography

The biogeographic history of the EAM is long and complex (see section Chapter 1). The main process that is thought to have driven the diversification of organisms in this region is the gradual isolation of EAM from other mountain regions (e.g. Southern Highlands) and between each EAM block, these events correlated with the orogenic activities in the region over the past 25 Myr. Since these orogenic events, contacts and separations between forests within and between mountains in the EAM

may have been initiated by climatic changes in the region, which will have directly influenced the distribution of forests organisms.

Temporal correlations in Arthroleptides

The results presented here show a strong correlation between phylogeny and distribution, an overview of which is shown in Fig. 3.8. Located in the south of the Eastern Arc, clades C, and D (*Arthroleptides yakusini*) form a well supported clade, and are deeply divergent from clade A (*Arthroleptides martiensseni*) in the north. This pattern could be explained as the results of geographical distance between each area, with clade C, and D and terminal B closely associated geographically, and therefore phylogenetically, or perhaps the pattern is indicative of a more definitive barrier to gene flow, which perhaps separated these two regions. It would be expected that a linear relationship between genetic pairwise distance and geographical distance would be observed if the patterns were the result simply of geographical proximity. However, it appears this is not the case (refer to Table 3.7) as can be seen by proportionally large differences. For example, pairwise differences within the southern clade show divergences of 1-2% for distances up to 270km, whereas a substantially smaller distance between Uluguru and East Usambara (217km) shows a 7-8% difference. If these results are representative of all the populations in each region, it seems more likely that sequential isolation of each mountain block population has been a major factor in the differentiation of this group. That is, that separation of East Usambaras occurred at an earlier period than that between Mahenge, Uluguru and Udzungwa. Bowie *et al.* (2004) showed that recent exchange between northern and southern EAM was rare, which they speculated was the result of a significant biogeographic barrier to dispersal.

Based on molecular clock estimations, substantial temporal divergence is shown between *Arthroleptides martiensseni* (clade A) and *Arthroleptides yakusini* (clade C), and this split might be correlated with the significant orogenic activity in the area around the Miocene period (~25-10 Myr). The rift valley was beginning to be formed during this period, and the Eastern Arc Mountains were speculated as beginning to become uplifted to their present arrangement (Lovett, 1993a). It is difficult to evaluate how significant this event may have been on the diversification of *Arthroleptides* given the lack of any other form of data that may allow testing of this hypothesis. Reliable geological data, such as the exact timing of uplift and isolation of each Eastern Arc mountain, will be necessary to make a full assessment of these possible

correlations. More recent events appear to characterise the differences shown between the southern mountain blocks, i.e. Mahenge, Uluguru and Udzungwa (2-4 Myr). There are numerous climatic events that occurred in East Africa around this Pliocene/Pleistocene period that could account for the changes in forest distribution (Zachos *et al.* 2001; Morley, 2000; Matthee *et al.* 2004; Trauth *et al.* 2005) and therefore population isolation that may have restricted the exchange between each mountain block. Wetter periods are associated around 2-6 Myr (Lovett, 1993a), which may correspond to the results given here.

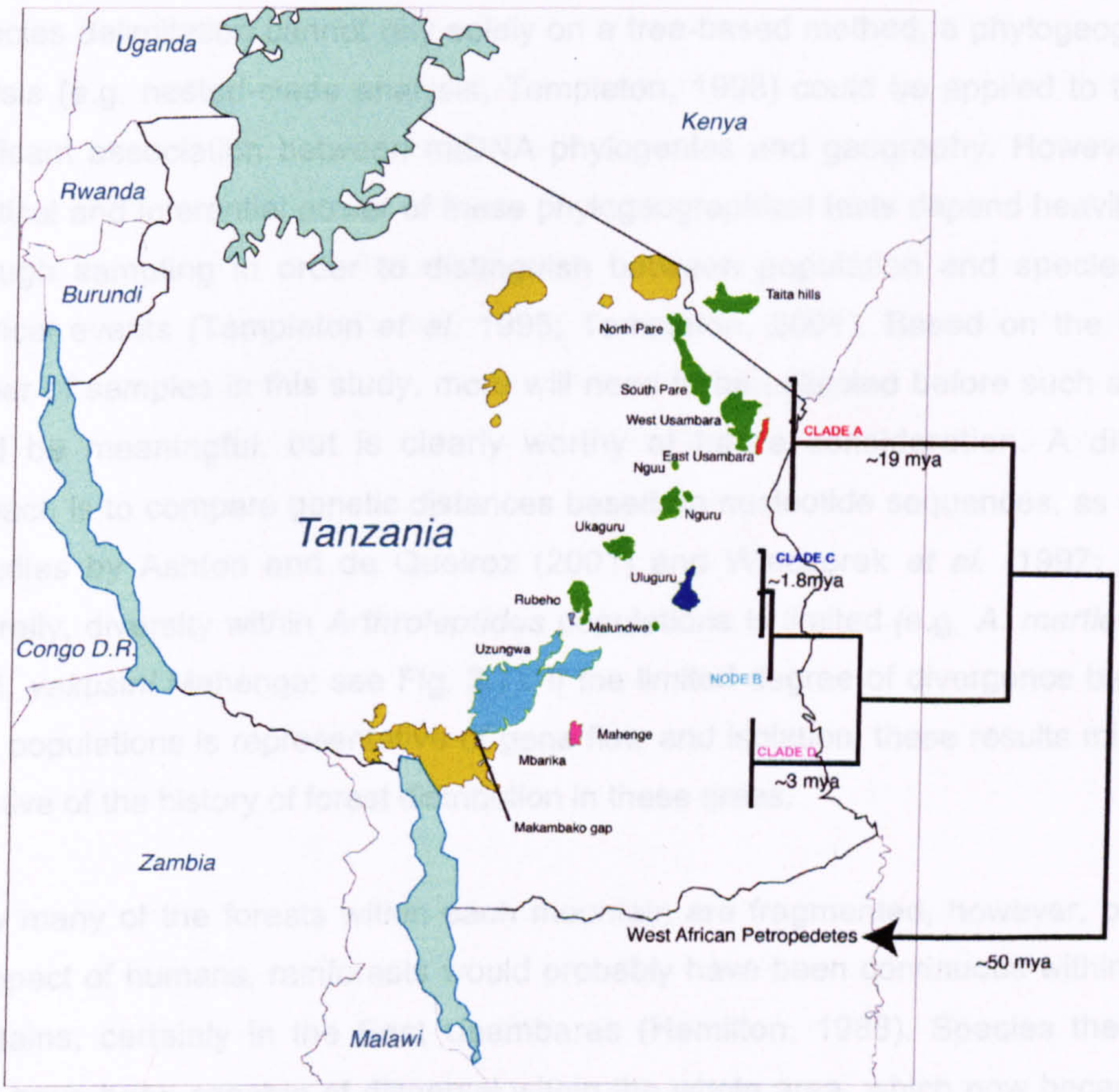

Figure 3.8
Summary of the relationships and divergence times in Arthroleptides (refer to text for explanation of clades and terminal).

Spatial Congruence in Arthroleptides

Based on the topology recovered in this analysis, which shows a distinct difference between north and south Eastern Arc Mountain blocks, there are consistent area patterns with other animal groups: snakes (Gravlund, 2002), birds (Roy, 1997), and

plants (Moller and Cronk, 1997; Lindqvist and Albert, 2001). However, there are examples from other groups that contradict this topology (Roy *et al.* 2001; Hochkirch, 2001). It is unclear whether the congruence in these groups is indicative of a common biogeographical signal or, as perhaps indicated in studies that contradict the area relationships, is simply reflective of common dispersal routes. Analysis of the biogeographical patterns shared among amphibians and other groups are discussed more fully in Chapter 7.

Population differentiation

If species delimitation cannot rely solely on a tree-based method, a phylogeographic analysis (e.g. nested-clade analysis, Templeton, 1998) could be applied to test for significant association between mtDNA phylogenies and geography. However, the statistical and inferential power of these phylogeographical tests depend heavily on a thorough sampling in order to distinguish between population and species-level historical events (Templeton *et al.* 1995; Templeton, 2001). Based on the limited number of samples in this study, more will need to be collected before such a study would be meaningful, but is clearly worthy of future consideration. A different approach is to compare genetic distances based on nucleotide sequences, as shown in studies by Ashton and de Queiroz (2001) and Wieczorek *et al.* (1997; 2000). Generally, diversity within *Arthroleptides* populations is limited (e.g. *A. martiensseni* and *A. yakusini* Mahenge: see Fig. 3.7). If the limited degree of divergence between these populations is representative of gene flow and isolation, these results might be indicative of the history of forest distribution in these areas.

Today many of the forests within each mountain are fragmented, however, prior to the impact of humans, rainforests would probably have been continuous within most mountains, certainly in the East Usambaras (Hamilton, 1988). Species therefore would have been capable of dispersal within the whole area, which now because of the patchiness of forest habitats is not possible. Thus the fragmented forest reserves today may have only recently restricted gene flow, and it would be anticipated that any phylogeographic divisions would be limited. For the East Usambara species *A. martiensseni*, small differences are noted between populations geographically separated, which might point to a recent interbreeding of these populations. Dense sampling of populations will be necessary to fully assess this hypothesis. However evidence from other biogeographic studies of amphibians within the Usambaras, although limited, show consistent phylogeographic patterns. De Sá *et al.* (2004),

showed limited genetic differences between Mazumbai FR and Ambangula FR populations in the species *Callulina kisiwamsitu* from the West Usambara. Furthermore, Loader *et al.* (2004) showed similar patterns of population homogeneity within the *Probreviceps macrodactylus macrodactylus* complex in the East Usambara from Nilo FR and Amani NR, though this was not explicitly discussed.

Samples of *Arthroleptides yakusini* from the Uluguru Mountains show greater haplotype diversity than other populations (as discussed above) despite similar geographical distance between population samples. The recent geographical history of the Uluguru Mountains is poorly understood, certainly compared to the Usambara. Perhaps the greater haplotype diversity is indicative of a more fragmented history or ecological diversification in these populations. Based on the available evidence it is difficult to assess these speculations, but perhaps warrants further study with an increased sampling of populations. A phylogeographic comparison between these mountain blocks maybe particularly enlightening for studies of speciation and the effects of ecological diversity, considering the differences taxonomically and ecologically between each mountain block (Fjeldså and Lovett, 1997; Menegon *et al.* 2004).

Chapter Four

Systematics and Biogeography of African Microhylids

4.1 Aims

This chapter assesses the phylogenetic relationships of African microhylid frogs based on mitochondrial sequence data. I examine the phylogenetic position of African Microhylids relative to each other and to some Asian Microhylids. This analysis includes representatives of all African subfamilies, six of the eight genera, and the enigmatic hemisotid *Hemisus*. In particular, sequence data are analysed and used to examine the taxonomic status of brevicipitine species and their relationships. Biogeographic hypotheses are examined in light of the findings gathered on the systematics of microhylids. A focus on Eastern Arc Biogeography is made, as this is one of the main areas where African microhylids are distributed.

4.1 Introduction

4.1.1 Microhylids

Microhylids are a diverse group of subterranean, terrestrial and arboreal frogs occurring in northern Australasia, South and Southeast Asia, sub-Saharan Africa, Madagascar, and North and South America. The approximately 350 nominate species are classified in 64 genera and 10 subfamilies. This is the largest number of genera in any amphibian family, comprising some 15% of all frog genera (Frost, 2002). The status, composition, inter- and intrarelationships of Microhylidae have not been studied in detail, and the family remains in general poorly understood. Indeed, even the monophyly of Microhylidae is far from established (see below). In association with their ecological diversity, microhylids display great morphological variation, particularly in their cranial and pectoral girdle structure (Parker, 1934; Carvalho, 1954; Blommers-Schlösser, 1993; Wu, 1994). The inadequate state of microhylid systematics partly stems from the lack of comparative morphological studies. Blair (1962) suggested the use of non-traditional character systems for

clarifying evolutionary relationships in frogs. More specifically, Largen and Drewes (1989) suggested molecular data would be useful for resolving relationships among African microhylids.

4.1.1 African Microhylids

The suprageneric taxonomy of Microhylidae has barely changed since the milestone monograph of Parker (1934) but, given the generally inadequate state of current knowledge, this is unlikely to prove stable. Currently, the eight African (excluding Madagascar) genera are divided into three subfamilies (Frost, 2002). The African Brevicipitinae consists of twenty species in five genera. Three of these genera (*Probreviceps, Callulina, Balebreviceps*) are found in evergreen forests of East Africa, whereas the remainder (*Breviceps, Spelaeophryne*) are also known to inhabit some drier habitats. Among the moist forest genera, *Probreviceps* is the most speciose (3 species) and apart from the Zimbabwean *P. rhodesianus* is found principally in the mountain forests of Tanzania (Howell, 1993). *P. macrodactylus* is subdivided into three subspecies (Parker, 1934), *P. m. macrodactylus* from the Usambara, *P. m. loveridgei* from the Uluguru and Udzungwa, and *P. m. rungwensis* from Rungwe and the Udzungwa. The latter two subspecies are sympatric in the Udzungwa Mountains, suggesting that they may be separate species. *Callulina* is also found throughout the Eastern Arc Mountains, and is known from *C. kreffti* and a species recently described from the West Usambaras, *C. kisiwamsitu*. De Sá et al. (2004) anticipated that 'other disjunct populations throughout the Eastern Arc Mountains may also prove to be distinct species'. *Balebreviceps* is monotypic, with *B. hillmani* known from the Bale Mountains, Ethiopia (Largen and Drewes, 1989). The only species of *Spelaeophryne, S. methneri,* is found in both low and highland areas of southeastern Tanzania, and *Breviceps* (15 species) is confined to eastern and southern Africa, being 'concentrated in South Africa' (Poynton, 1964; see also Channing, 2001; Minter, 2003). The Indo-African Melanobatrachinae comprises four species: *Melanobatrachus indicus* (Western Ghats, India), *Hoplophryne rogersi* (East Usambara, Tanzania), *Hoplophryne uluguruensis* (Uluguru and Udzungwa, Tanzania), and *Parhoplophryne usambaricus* (East Usambara, Tanzania). These species all appear to be strictly confined to forests. The subfamily Phrynomerinae comprises five species of *Phrynomantis* that have a wide distribution across savanna and woodland habitats in sub-Saharan Africa (see Fig. 4.2 and Table 4.1 for summary).

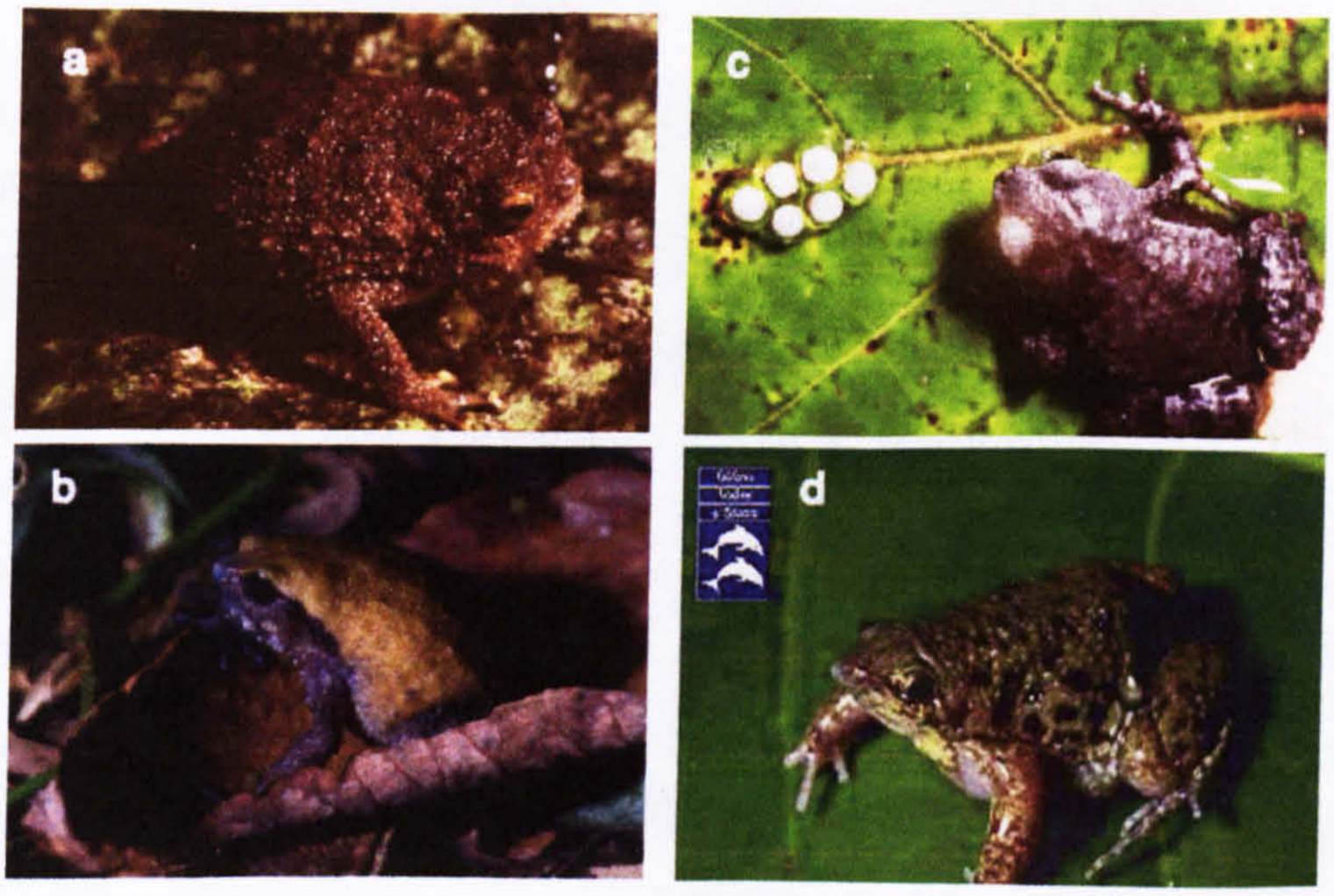

Figure 4.1

Photos of microhylids and *Hemisus* (a) *Callulina kreffti* (b) *Probreviceps* new sp. (Ukaguru) (c) *Hoplophryne rogersi* (d) *Hemisus marmoratum*. Photos provided kindly by Vonesh (a,c) Menegon, (b) and CAS (d). Scale unknown for all photos.

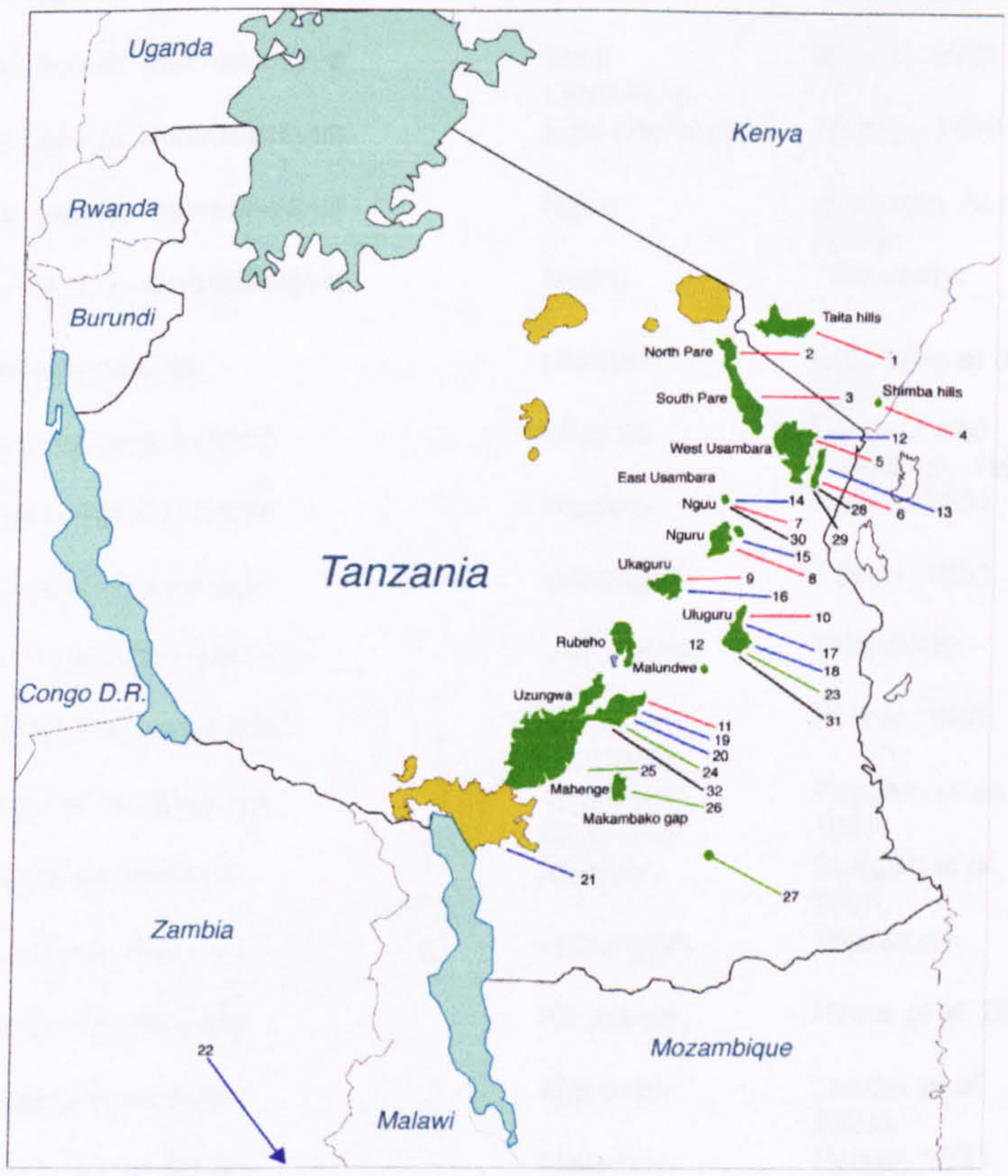

Figure 4.2

Distribution of East African Microhylids, distributed in Tanzania unless otherwise stated. Areas in green are Eastern Arc Mountains, areas in brown are of more recent volcanic origin. See Table 4.1 for key to numbers.

Table 4.1.

Occurrence of African Microhylids, species occurring in Tanzania unless stated otherwise.

With 66% of populations sampled.

	Species	Locality	Reference	Sampled
1	*Callulina sp.*	Taita hills, Kenya	Howell, 1993	√
2	*Callulina sp.*	North Pare,	This study	√
3	*Callulina sp.*	South Pare,	This study	√
4	*Callulina sp.*	Shimba hills, Kenya	AMNH collection	X
5	*Callulina sp.*	West Usambara,	de Sà, *et al.* 2004.	√
6	*Callulina sp.*	East Usambara,	Nieden, 1910.	√
7	*Callulina sp.*	Nguu,	Menegon, 2003.	√
8	*Callulina sp.*	Nguru,	Emmrich, 1994.	X
9	*Callulina sp.*	Ukaguru,	Akker and Highstead 1994.	√
10	*Callulina sp.*	Uluguru,	Barbour and Loveridge 1928.	√
11	*Callulina sp.*	Udzungwa,	Frontier, 2001	√
12	*Probreviceps m. macrodactlyus*	West Usambara,	Howell, 1993	X
13	*Probreviceps m. macrodactlyus*	East Usambara,	Nieden, 1910	√
14	*Probreviceps m. macrodactlyus*	Nguu,	Menegon, et al. 2003b	X
15	*Probreviceps m. macrodactlyus*	Nguru,	This study.	X
16	*Probreviceps new sp.*	Ukaguru,	Channing *et al.* 2002	√
17	*Probreviceps uluguruensis*	Uluguru,	Barbour and Loveridge, 1928.	√
18	*Probreviceps m. loveridgei*	Uluguru,	Parker, 1931.	√
19	*Probreviceps m. loveridgei*	Udzungwa,	Parker, 1931.	√
20	*Probreviceps m. rungwensis*	Udzungwa,	This study.	√
21	*Probreviceps m. rungwensis*	Southern Highlands,	Parker, 1931.	X
22	*Probreviceps rhodesianus*	Stapleford, Zimbabwe	Poynton *et al.* 1967	X
23	*Spelaeophryne methneri*	Uluguru,	Doggart *et al.* 2004.	√
24	*Spelaeophryne methneri*	Udzungwa,	This study.	X
25	*Spelaeophryne methneri*	Kilombero,	Hinde *et al.* 2002.	√
26	*Spelaeophryne methneri*	Mahenge	Loader *et al.* 2004a.	√
27	*Spelaeophryne methneri*	Matembo,	Parker, 1931.	X
28	*Parhoplophryne usambarica*	East Usambara,	Barbour and Loveridge, 1928	X
29	*Hoplophryne rogersi*	East Usambara,	Barbour and Loveridge, 1928	√
30	*Hoplophryne rogersi*	Nguu,	Menegon *et al.*	√

31	*Hoplophryne uluguruensis*	Uluguru,	2003b Barbour and Loveridge, 1928	X
32	*Hoplophryne uluguruensis*	Udzungwa,	Frontier, 2001	√

4.1.2 Phylogenetic Relationships of African Microhylids

Parker's (1934) monograph of the family Microhylidae stands as the most significant contribution to microhylid taxonomy, and the first attempt to understand relationships among Microhylids. The taxonomy of the family has changed very little since Parkers (1934) contribution (Duellman and Trueb, 1994). Parker recognised two subfamilies with African members; the African Brevicipitinae and the Indo-African Melanobatrachinae. The only alteration to Parker's (1934) classification was the inclusion of the genus *Phrynomantis* by Laurent (1941) as the third African microhylid subfamily (Phrynomerinae), which is recognised today (Frost, 2002). Parker (1934) recognised Brevicipitinae as a subfamily within the Microhylidae for *Breviceps* Merrem, 1820, *Callulina* Nieden, 1910, *Probreviceps* Parker, 1931 and *Spelaeophryne* Ahl, 1924. Although he did not comment specifically on the relationships between the genera within the subfamily he did provide some data from which a few basic inferences may be made. Parker (1934) wrote that breviciptines were exclusively African (actually confined to southern and eastern Africa; p. 10), and that the four genera were "closely allied" but were not apparently "particularly closely related to any of the other existing genera" within the Microhylidae. He also noted that the breviciptines were the only microhylid subfamily in which the complete shoulder girdle was retained in all genera. This may suggest breviciptines are a monophyletic and possibly basal clade with respect to other microhylids. The implication of monophyly is further supported by Parker's comment on the special nature of the vomer, reduced posteriorly but bearing a large anterior and medial expansion.

Parker (1934) made no specific comment on the relationships within the Melanobatrachinae, though by the affiliation of Hoplophryne and Parhoplophryne with *Melanobatrachus*, based primarily on the absence of auditory apparatus he suggested an affiliation. The arrangement of the sub family Melanbatrachinae has surprisingly not been questioned (Noble, 1931; Savage, 1973; Laurent, 1986). Parker (1934; p.11) did speculate briefly on the relationships of the Melanobatrachinae with other microhylids, suggesting that despite the group being 'incompletely known' they seemed to be a 'natural, probably archaic, assemblage...possibly a distinct family'

Two quantitative analyses have recently questioned Parker's groupings; 1) Blommers-Schlösser's (1993) analysis of 20 morphological characters and 2) Wu's PhD dissertation (1994), a cladistic analysis of the Microhylidae using 188 morphological characters. Both Blommers-Schlösser and Wu found that Brevicipitinae differ substantially from the remaining microhylids in a number of morphological features, and they share characters with the enigmatic genus *Hemisus*. These findings led Blommers-Schlösser to suggest removing the Brevicipitinae from Microhylidae and include it with *Hemisus* in a new family, Hemisotidae. In contrast Wu (1994) found that *Rhinophrynus* also grouped with *Hemisus* in a family he named 'Brevicipitidae'. Morescalchi (1973) provided supporting evidence for the divergence of breviciptids from all other microhylids with the similarities in 24 karyotype of *Breviceps, Ranids* and *Hemisus*, despite this, Bogart (1976, p.206) considering them to be 'best explained' as convergences.

Blommers-Schlösser's (1993) analysis was criticised by Channing (1995) who investigated the anatomy of *Hemisus* and Brevicipitinae, pointing out character differences between the genera, which has been further supported and extended in a recent analysis of osteology (van Dijk, 2001). Channing (1995) concluded that the association of *Hemisus* and Brevicipitinae was premature. The currently more orthodox view that brevicipitines are microhylids and only distantly related to *Hemisus* was summarised by Ford & Cannatella (1993). Recent studies of larval morphology (Haas, 2003) and DNA sequence data (Biju and Bossuyt, 2003; Vences *et al.* 2003b) have reinforced the view that *Hemisus* is only distantly related to a monophyletic Microhylidae, but none of these studies sampled any brevicipitine taxa. More specifically, Haas's (2003) study on the phylogeny of frogs using mainly larval characters robustly supported the monophyly of the Microhylidae, with *Scaphiophryne* placed as the most basal lineage. Included in his analysis of African taxa, he recovered *Phrynomantis* as the sister group to the enigmatic Madagascan microhylid *Paradoxophyla palmate*, and *Hemisus* grouped within the Hyperoliidae. Based on the latter finding, Haas suggested that *Hemisus* should be included in the Hyperoliidae. Haas however did not include any brevicipitids in his analysis, so the grouping of *Hemisus* with brevicipitines as suggested by Blommers-Schlösser (1993) and Wu (1994) could not be critically appraised.

Evidence has recently emerged from studies of both mitochondrial and nuclear datasets suggesting brevicipitines may not nest within the Microhylid family (Darst

and Canatella, 2004; Van Meide *et al.* 2004; Loader *et al.* 2004), agreeing with Blommers-Schlösser (1993) and Wu's (1994) morphological conclusions. All studies independently show that brevicipitines group with non-microhylids, though levels of support are relative weak, and only Loader *et al.* (2004b) show unambiguously that a tree topology recovering a monophyletic grouping of Microhylids is significantly worse than optimal trees. In this chapter, I will extend the preliminary analyses of Loader *et al.* (2004), including a denser sampling of African 'Microhylids' found in continental Africa. I focus especially on brevicipitines and hoplophrynines distributed in the Eastern Arc Mountains. *Hemisus* is also included, in order to explore the relationship of this genus with microhylids.

4.1.3 Biogeography of Microhylids

The current distribution of microhylids has been interpreted as reflecting the break up of Gondwana (Savage, 1973; Duellman and Trueb, 1994). Savage (1973) further speculated that the three extant African microhylid subfamilies (Brevicipitinae, Melanobatrachinae, Phrynomerinae) diversified prior to Gondwana fragmentation. In contrast, Duellman and Trueb (1994: p.489) argued that a brevicipitine-phrynomerine lineage diversified only after Gondwana fragmentation. No attempt has been made to quantitatively assess these hypotheses. At a finer scale, the high species diversity and strong patterns of endemism in amphibians (including microhylids) of the Eastern Arc is believed to be intimately associated to more recent geographic events (Fjeldså and Lovett, 1997; Howell, 1993). Microhylids have been mentioned as a group potentially affected by the geographic history of the EAM (Poynton, 1999a). In this study I make the first assessment of the likely effects of the breakup of Gondwana, and the potential impact of fragmentation and isolation in the EAM on the speciation and diversification of African microhylids.

4.2 Materials and methods

4.2.1 Specimens

A total of 55 terminal taxa were used in this study (Table 4.2). Sequences for 45 terminal taxa were generated from newly collected material from Tanzania, Kenya and Ivory Coast. These were supplemented by sequences for 10 species obtained from GenBank (Benson *et al.* 1998).

Table 4.2.

African Microhylids and outgroups analysed in this study, *= sequences obtained from Genbank, and P denotes members of the Pipidae.

Sequence number	Specimens	Species	Locality	Forest Reserve
T148	KMH 16360	*Probreviceps m. macrodactylus*	East Usambara	Amani-Sigi
T158	KMH 18974	*Probreviceps m. rungwensis*	Udzungwa	West Kilombero Scarp FR
T182	KMH 19152	*Probreviceps m. loveridgei*	Udzungwa	West Kilombero Scarp FR
T183	KMH 22702	*Probreviceps m. loveridgei*	Udzungwa	West Kilombero Scarp FR
T184	KMH 22067	*Probreviceps m. loveridgei*	Udzungwa	West Kilombero Scarp FR
T186	KMH 22060	*Probreviceps m. loveridgei*	Udzungwa	West Kilombero Scarp FR
T204	KMH 21570	*Probreviceps ulugurensis*	Uluguru	Uluguru South FR
T205	KMH 21577	*Probreviceps ulugurensis*	Uluguru	Uluguru South FR
T206	KMH 21575	*Probreviceps ulugurensis*	Uluguru	Uluguru South FR
T207	KMH 21461	*Probreviceps m. loveridgei*	Uluguru	Mkungwe FR
T208	KMH 21532	*Probreviceps m. loveridgei*	Uluguru	Kasanga FR
T209	KMH 21475	*Probreviceps m. loveridgei*	Uluguru	Mkungwe FR
T245	KMH 23136	*Probreviceps m. macrodactylus*	East Usambara	Nilo FR
T246	KMH 23137	*Probreviceps m. macrodactylus*	East Usambara	Nilo FR
T247	KMH 21399	*Probreviceps m. macrodactylus*	East Usambara	Nilo FR
T281	MW 1826	*Breviceps mossambicus*	Mahenge	Sali FR
T283	MW 1848	*Breviceps mossambicus*	Mahenge	Sali FR
T284	MW 1850	*Speleophryne methneri*	Mahenge	Sali FR
T303	MW 1968	*Callulina kisiwamsitu*	West Usambara	Mazumbai FR
T420	KMH 22723	*Hoplophryne ulugurensis*	Udzungwa	West Kilombero Scarp FR
T423	KMH 23534	*Callulina kreffti*	East Usambaras	Nilo FR
T424	KMH 23364	*Hoplophryne rogersi*	East Usambaras	Nilo FR
T425	KMH 21555	*Callulina kreffti*	Uluguru	Shikurufumi FR
T426	MW 3050	*Callulina kreffti*	Ukaguru	Ikwamba FR
T428	MW 3058	*Probreviceps new sp.*	Ukaguru	Ikwamba FR
T429	MW 3065	*Callulina sp.*	North Pare	Kindoroko FR
T432	MW 3101	*Callulina sp.*	North Pare	Kindoroko FR
T446	MW 3197	*Callulina sp.*	Taita Hills	Ngangao FR
T447	MW 3215	*Callulina kisiwamsitu*	West Usambara	Ambangula FR
T448	KMH 22478	*Callulina sp.*	Uzungwa	West Kilombero Scarp FR
T449	KMH 19141	*Probreviceps m. rungwensis*	Uzungwa	West Kilombero Scarp FR
T450	KMH 19158	*Probreviceps m. rungwensis*	Uzungwa	West Kilombero Scarp FR
T452	MS 23	*Callulina kreffti*	South Pare	Near Chome FR
T453	L	*Phrynomantis bifasciatus*	Mkomazi	Ubani Mbuga
T455	MW 1856	*Hemisus marmoratum*	Mahenge	Sali FR
T461	KMH 21451	*Speleophryne methneri*	Uluguru	Mkungwe FR
T467	MW 3830	*Callulina kreffti*	Nguu Mountians	Nguu FR
T468	MW 3831	*Hoplophryne rogersi*	Nguu Mountians	Nguu FR
T470	Red Label	*Phrynomantis microps*	Ivory Coast	Comoe NP
T471	Yellow Label	*Hemisus sudanensis*	Ivory Coast	Comoe NP
T473	MW 3852	*Spelaeophryne methneri*	Kilombero Valley	
T493	SHCP287	*Probreviceps m. rungwensis*	Southern Highlands	Rungwe FR
T495	MTSN1	*Probreviceps new sp.*	Ukaguru	Mamiwa-Kisara FR
T496	MTSN2	*Probreviceps new sp.*	Ukaguru	Mamiwa-Kisara FR

n/a	n/a	**Pipa parva* "P"	South America	n/a
n/a	n/a	**Hymenochirus boettgeri* "P"	West Africa	n/a
n/a	n/a	**Mantidactylus sp.*	Mayotte	n/a
n/a	n/a	**Mantidactylus wittei*	Madagascar	n/a
n/a	n/a	**Boophis sp.*	Mayotte	n/a
n/a	n/a	**Boophis tephraeomystax*	Madagscar	n/a
T465	MW 1837	*Afrixalus uluguruensis*	Mahenge	Sali FR
n/a	n/a	**Kaloula taprobanica*	India	n/a
n/a	n/a	**Scaphiophryne brevis*	Madagscar	n/a
n/a	n/a	**Scaphiophryne gottlebei*	Madagscar	n/a
n/a	n/a	**Microhyla sp. FB-2000*	India	n/a

Although microhylids are also distributed elsewhere in sub-Saharan Africa, collecting was concentrated in Tanzania because all but one genus (*Balebreviceps* from Bale Mountains, Ethiopia (Largen and Drewes, 1989)) of African microhylids occur there. The sampling of species known to occur in Tanzania is complete except for *Parhoplophryne usambaricus*, which is known from only a single specimen (Barbour and Loveridge, 1928). All other Tanzanian species are represented in this study by at least one specimen, and across all but a few areas of their distribution. Beyond Tanzania, this study lacks intensive sampling of *Breviceps*, with only one of 15 species included. The sub-Saharan *Phrynomantis* is represented by two of the five known species. The only species of *Probreviceps* not included in this study is the Zimbabwean *P. rhodesianus*. Unsuccessful attempts were made to amplify DNA from museum specimens of *Probreviceps rhodesianus,* and *Balebreviceps hillmani* (see Appendix 2).

Four non-African microhylids were included, including representatives of at least two major lineages within the family, the Madagascan Scaphiophryninae (*Scaphiophryne*) and more cosmopolitan Microhylinae (*Microhyla, Kaloula*). All microhylid taxa for which 12S, 16S and *cytb* data are currently deposited in GenBank were included, with the exception of the Madagascan dyscophine *Dyscophus guineti*, for which the available data do not match the regions sequenced here, and contain several ambiguities. In addition to microhylids, I included a number of taxa for calibrating the molecular clock estimations; *Hymenochirus boettgeri*, *Pipa parva, Boophis* new sp., *Boophis tephraeomystax, Mantidactylus* new sp. *and Mantidactylus wittei.* The East African *Afrixalus uluguruensis*, *Hemisus marmoratus* and West African *H. sudanensis* were also included to investigate phylogenetic relationships of *Hemisus* relative to brevicipitines.

4.2.2 Phylogenetic Analysis

Two alignments were constructed to investigate the phylogenetic relationships of African microhylids. The first larger alignment included all taxa, following analyses (among many others, Biju and Bossuyt, 2003; Vences *et al.* 2003a; Hertwig *et al.* 2004) that show the basal position of pipids relative to all neobatrachids, the two pipid species *Hymenochirus boettgeri* and *Pipa parva* were designated as the outgroup and used to root trees. The second alignment included only Brevicipitine taxa, with species *Hoplophryne rogersi* used as an outgroup, as recovered in previous analyses (Loader *et al.* 2004b; de Sá *et al.* 2004).

4.2.3 Molecular Divergence Estimates

Divergence dates between clades were estimated by adding a number of taxa that provided calibration points (see section 2.6.5).

4.3 Results

4.3.1 Data Quality

For the full alignment a total of 1131 aligned sites were analysed, of which 564 were constant, 67 variable but parsimony uninformative, and 500 parsimony informative (see Table 4.3). For the brevicipitine alignment, 1165 sites were analysed, of which 709 were constant, 59 variable but parsimony uninformative, and 397 parsimony informative (see Table 4.4). Both data sets have a PTP of 0.01, allowing rejection of the null hypothesis that they contain no more hierarchical structure than expected by chance alone. There is no significant base composition bias for any taxon for both alignments, whether or not uninformative sites are considered. Rate heterogeneity was investigated using hierarchical likelihood ratio test and rrTree. The results show that both alignments show significant rate heterogeneity (Full alignment Δ=257.63108, P= <0.01, d.f.=53 and Brevicipitine alignment Δ=71.91698, P= <0.01, d.f.=36). Relative rates tests indicated that *Spelaeophryne methneri*, *Hemisus marmoratus*, *Hemisus sudanensis*, *Hoplophryne rogersi* (T424) and *Breviceps mossambicus* evolved more rapidly than the other taxa (p= <0.05). Plots of transitions vs. transversions (see Fig. 4.3.d for a summary) show a linear relationship for both substitution rates for all data partitions (not shown), all partitions are marked by an increased rate in transitions. Branch lengths also indicated different rates of

molecular evolution between *cytb*, 12S and 16S (Fig. 4.3a-c), with *cytb* evolving more rapidly with strongly compressed basal splits. Power regression lines (not shown) for plots of transition vs transversions show a better fit than linear regression lines (full alignment: linear r^2=0.86, power r^2=0.93, brevicipitid alignment linear r^2=0.91, power r^2=0.95), for both data sets, though greater in the full alignment. The results suggest that saturation may be a problem with these data, particularly for the full alignment. Furthermore, significant rate heterogeneity and incongruence in basal topologies recovered from different data partitions suggest that there may be a problem.

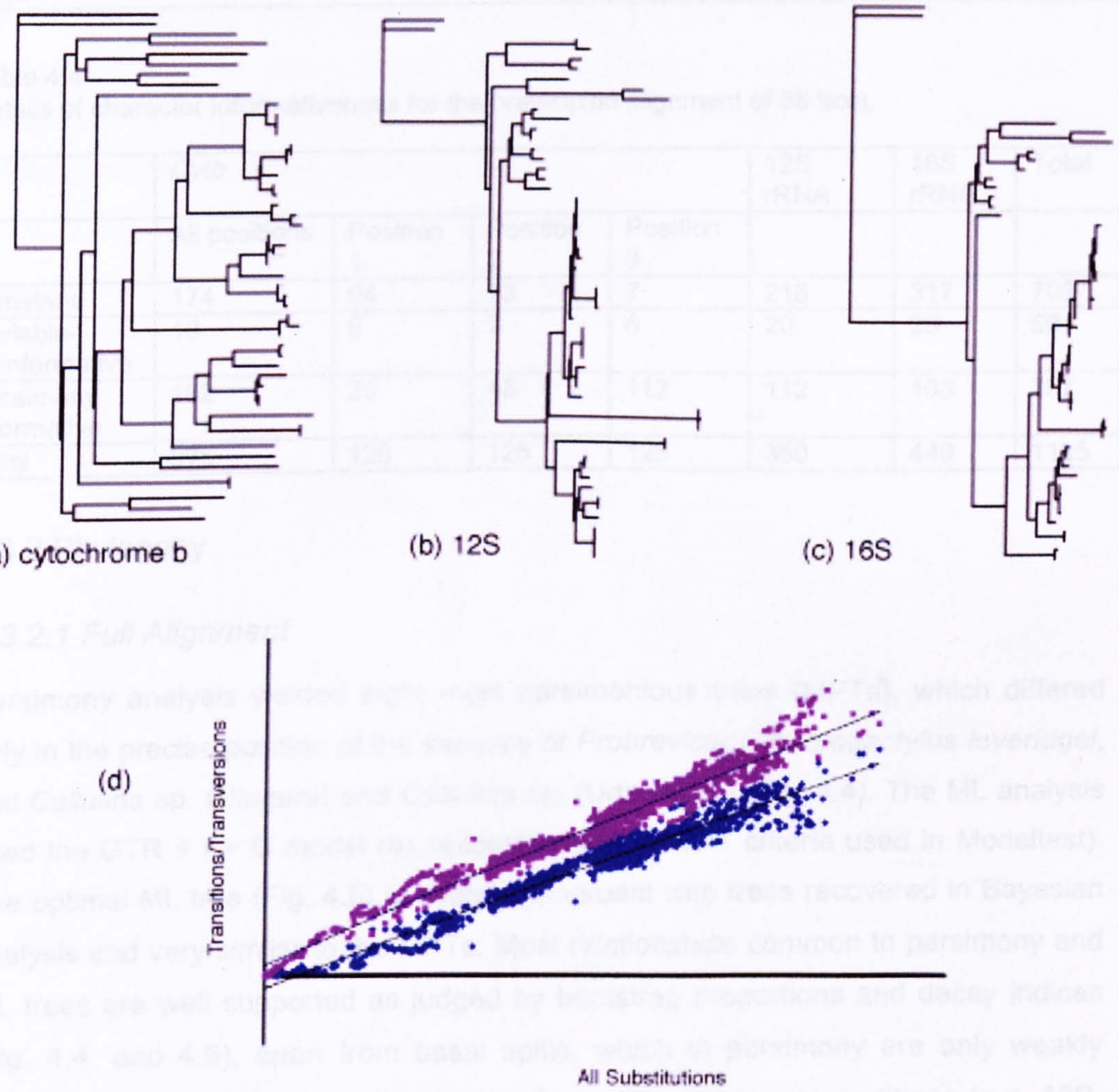

Figure 4.3

(a-c) Comparison of branch lengths for different data partitions (d) Plot of substitution of transversions and transitions, indicating levels of saturation, with transitions in purple, and transversions in blue, r^2 value for transitions ($r^{2=}$0.9666) and transversions ($r^{2=}$0.9572).

Table 4.3
Details of character informativeness for the full alignment of 55 taxa.

	Cytb				12S rRNA	16S rRNA	Total
	All positions	Position 1	Position 2	Position 3			
Constant	175	99	74	2	145	244	564
Variable-uninformative	17	6	7	4	19	31	67
Parsimony informative	222	33	57	132	141	137	500
Total	414	138	138	138	305	412	1131

Table 4.4
Details of character informativeness for the brevicipitid alignment of 38 taxa.

	Cytb				12S rRNA	16S rRNA	Total
	All positions	Position 1	Position 2	Position 3			
Constant	174	94	73	7	218	317	709
Variable-uninformative	19	6	7	6	20	20	59
Parsimony informative	182	25	45	112	112	103	397
Total	375	125	125	125	350	440	1165

4.3.2 Phylogeny

4.3.2.1 Full Alignment

Parsimony analysis yielded eight most parsimonious trees (MPTs), which differed only in the precise position of the samples of *Probreviceps macrodactylus loveridgei*, and *Callulina* sp. (Uluguru) and *Callulina* sp. (Udzungwa) (Fig. 4.4). The ML analysis used the GTR + I + G model (as recommended by both criteria used in Modeltest). The optimal ML tree (Fig. 4.5) is entirely congruent with trees recovered in Bayesian analysis and very similar to the MPTs. Most relationships common to parsimony and ML trees are well supported as judged by bootstrap proportions and decay indices (Fig. 4.4, and 4.5), apart from basal splits, which in parsimony are only weakly supported, as judged in bootstrap proportions. Alternative data partitions (e.g. 12S, 16S and *cytb* combined and separated) generally recovered the same topology, though showing weaker support for clades than combined results. Bayesian posterior probabilities are generally high (>0.90), perhaps unreasonably so, for all splits in the optimal ML tree (Fig. 4.5), including for relationships not found in the MPTs. The main differences observed between optimal parsimony and maximum likelihood trees are the sister group relationships to the *Probreviceps* clade and the relationships

recovered within the *Callulina* clade. For the latter, analyses of the brevicipitine alignment (see 4.3.2.2) show congruence between all methods, and this topology is recovered in parsimony analyses of the full alignment. (Fig. 4.5). Further discussions of these relationships are therefore given in section 4.3.2.2. The position of *Spelaeophryne* relative to *Probreviceps* and *Callulina* is unstable. Parsimony analysis show weak support for *Spelaeophryne* as sister group to *Callulina* and *Probreviceps*. However, the optimal likelihood and Bayesian tree show *Spelaeophryne* to be sister group to *Probreviceps*, which is also poorly supported (70). Neither hypothesis is strongly supported which means determining the best alternative scenario is not possible (however see 4.3.2.2). Basal relationships are poorly recovered in parsimony analyses, with weak support for the monophyly of non-Brevicipitine microhylids, brevicipitines, and *Hemisus*.

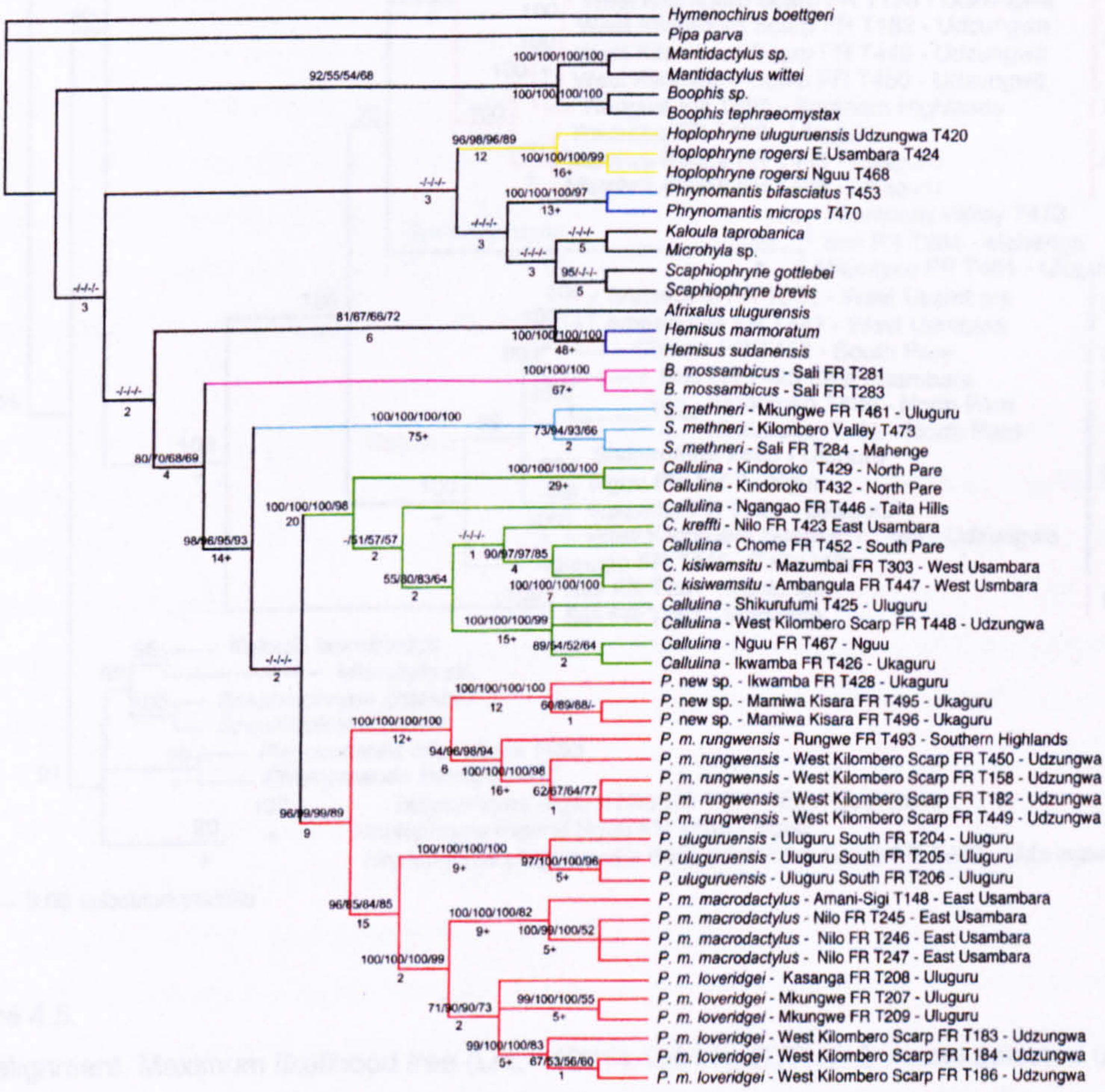

Figure 4.4

Strict consensus of 8MPT's for the full alignment, tree length 2505. Bootstrap proportions shown above branches (parsimony, kimura 2 parameter distance, maximum likelihood distance, log-det distance), Decay index values below along with templeton test result (+= significant at 0.05).

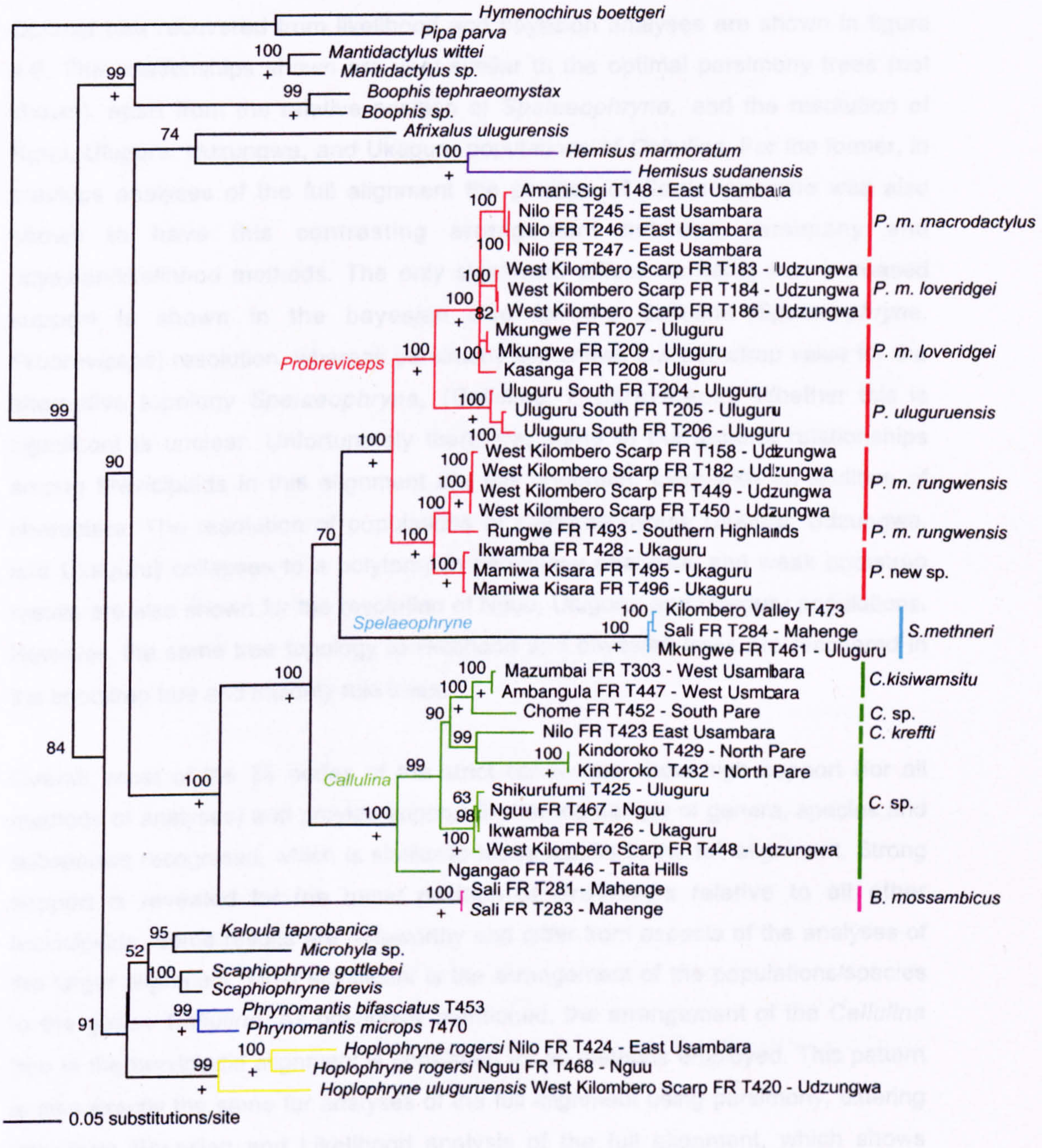

Figure 4.5.

Full alignment. Maximum likelihood tree (LnL= 12214.19611), GTR+I+G model selected using Modeltest. Base frequencies estimated at 0.31360, 0.27150, 0.16280 and 0.25210 for A, C, G and T respectively, substitution rates =2.4616, 7.3178, 3.8592, 0.9558, and 14.8339 and the proportion of invariant sites set at 0.3658 and a gamma distribution shape parameter of 0.6481. Values on branches show Bayesian posterior probabilities. Below branches shows SH test results (+= significant at 0.05).

4.3.2.2 Brevicipitine Alignment

Optimal tree recovered from likelihood and bayesian analyses are shown in figure 4.6. The relationships shown are very similar to the optimal parsimony trees (not shown), apart from the relative position of *Spelaeophryne,* and the resolution of Nguu, Uluguru, Udzungwa, and Ukaguru populations of *Callulina.* For the former, in previous analyses of the full alignment the position of *Spelaeophryne* was also shown to have this contrasting arrangement between parsimony and bayesian/likelihhod methods. The only significant difference being that increased support is shown in the bayesian tree for the *Callulina (Spelaeophryne, Probreviceps*) resolution, whereas parsimony still shows low bootstrap value for the alternative topology *Spelaeophryne,* (*Callulina, Probreviceps*). Whether this is significant is unclear. Unfortunately therefore, some of the generic relationships among brevicipitids in this alignment are still uncertain, even with an addition of characters. The resolution of populations of *Callulina* (Nguu, Uluguru, Udzungwa, and Ukaguru) collapses to a polytomy in parsimony analyses, and weak bootstrap results are also shown for the resolution of Nguu, Uluguru, and Ukaguru populations. However, the same tree topology to likelihood and bayesian trees are recovered in the bootstrap tree and majority rule trees.

Overall, most of the 35 nodes of the strict consensus show high support (for all methods of analyses) and provide support for the monophyly of genera, species and subspecies recognised, which is similar to those results in the full alignment. Strong support is revealed for the basal position of *Brevlceps* relative to all other brevicipitids. Some results are noteworthy and differ from aspects of the analyses of the larger alignment. Most significant is the arrangement of the populations/species in the genus *Callulina.* As previously mentioned, the arrangement of the *Callulina* tree in the brevicipitid alignment is consistent for all methods employed. This pattern is also exactly the same for analyses of the full alignment using parsimony, differing only from Bayesian and Likelihood analysis of the full alignment, which shows alternative positions for basal lineages (Taita Hills and North Pares). Considering the generally high support in the brevicipitid alignment for the position of *Callulina* populations in all analyses, and this arrangement shown in the full alignment in parsimony analyses then this is taken to represent the most robust hypothesis of relationships among populations of *Callulina* (see Fig. 4.6).

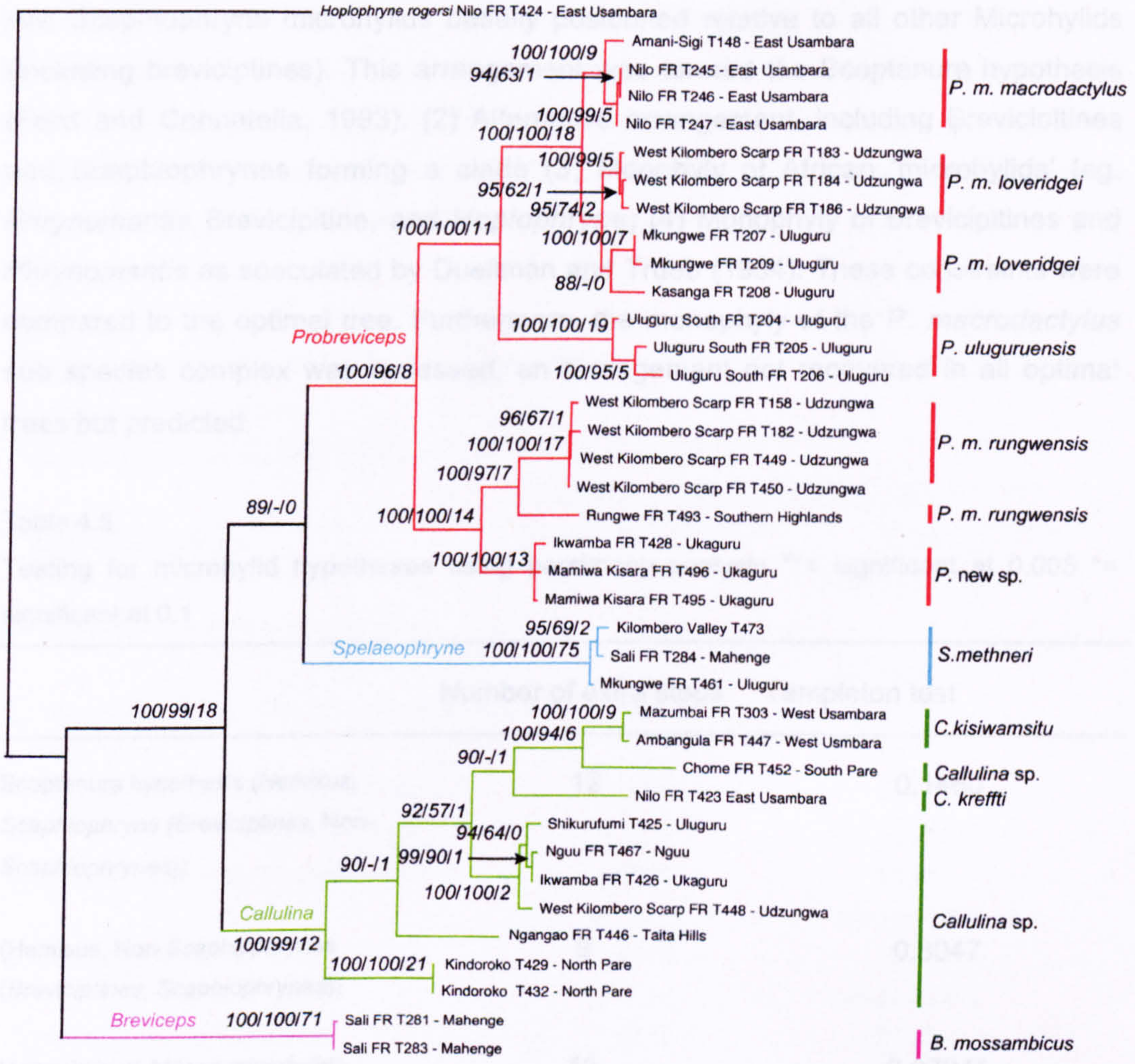

Figure 4.6

Brevicipitine alignment. Maximum likelihood tree (LnL= 7112.02925), GTR+I+G model selected by Modeltest. Base frequencies estimated at 0.30790, 0.27060, 0.16820 and 0.25330 for A, C, G and T respectively, substitution rates =2.9249, 10.6388, 5.4734, 1.4592, 19.0572, and the proportion of invariant sites set at 0.4493 and a gamma distribution shape parameter of 0.8015. Values on branches show Bayesian posterior probabilities, bootstrap proportions calculated using parsimony, and decay index.

4.3.2.3 Hypotheses

In addition to investigating the support of the recovered phylogenetic trees, alternative hypotheses were examined which were relevant to the interpretation of the systematics and evolution of African microhylids. Trees were constrained to investigate three main hypotheses concerning the relative position of the main Microhylid groups (e.g. Scaphiophrynes, Brevicipitines, and all other Microhylids): (1)

Are Scaphiophryne microhylids basally positioned relative to all other Microhylids (including breviciptines). This arrangement was termed the Scoptanura hypothesis (Ford and Cannatella, 1993). (2) Alternative arrangement, including Brevicipitines and Scaphiophrynes forming a clade (3) Monphyly of African 'microhylids' (eg. *Phrynomantis*, Brevicipitine, and *Hoplophryne*) (4) Monophyly of Brevicipitines and *Phrynomantis* as speculated by Duellman and Trueb (1994). These constraints were compared to the optimal tree. Furthermore, the monophyly of the *P. macrodactylus* sub species complex was assessed, an arrangement not recovered in all optimal trees but predicted.

Table 4.5
Testing for microhylid hypotheses using parsimony analysis **= significant at 0.005 *= significant at 0.1

	Number of extra steps	Templeton test
Scoptanura hypothesis (*Hemisus*, *Scaphiophryne* (*Breviciptines*, Non-*Scaphiophrynes*))	12	0.1460
(Hemisus, Non-*Scaphiophrynes* (*Breviciptines*, *Scaphiophrynes*))	9	0.3047
Monophyly of African microhylids	13	0.0704*
Monophyly of Brevicipitines, *Phrynomantis*	9	0.3047
Monophyly of *P. macrodactlylus* sub species complex	31	0.0001**

4.3.3 Molecular divergence estimates

Divergence estimates are given in Table 4.6. There are notable differences between the two dating methods, which given the rate heterogeneity exhibited in the data set is not unexpected. Sanderson's (2002a) penalized likelihood (PL) method has been used to estimate divergence times while allowing for lineage specific rate variation, and because of the preference for PL methods when data exhibits rate heterogenity these are here interpreted as providing more reliable estimates. The ML tree used to

estimate the divergence times was obtained from the full alignment, which when compared to the brevicipitine alignment, includes some suboptimal arrangements (e.g. relationships among *Callulina*). Based on the discrepancy among trees recovered using different methods, divergence times for these parts of the tree will be interpreted very conservatively.

Table 4.6

Absolute divergence times in Myr. for clades within African Microhylids. Refer to Fig.4.7 for precise position of nodes.

Most recent common ancestor (MRCA)	Estimation method	
	Penalized Likelihood	Langley-Fitch
1. MRCA Hoplophryne	91.04	71.78 (61.22-84.55)
2. Hoplophryne rogersi, H. uluguruensis	51.80	40.23 (25.86-48.59)
3. Hoplophryne rogersi (Nguu-East Usambara)	39.31	29.18 (22.00-37.31)
4. Phrynomantis bifasciatus, P. microps	24.75	17.67 (12.27-24.73)
5. Hemisus sudanensis, H. marmoratum	28.60	36.34 (29.74-44.88)
6. MRCA Phrynomantis	88.47	66.66 (59.24-78.27)
7. MRCA (Hemisus, Hyperolius)	78.77	91.25 (80.45-101.09)
8. MRCA (Hemisus, Hyperolius) Brevicipitids	107.66	120.39 (111.59-134.35)
9. MRCA (Hemisus, Hyperolius, Brevicipitids) Microhylids	110.95	123.18 (114.11-136.89)
10. MRCA Breviceps	88.91	103.50 (94.51-114.28)
11. MRCA Callulina	69.28	83.17 (74.77-91.89)
12. MRCA Spelaeophryne	61.69	74.81 (58.98-83.79)
13. MRCA Probreviceps	36.83	44.94 (37.62-53.07)
14. Spelaeophryne methneri (Mahenge, Kilombero) Uluguru	0.98	1.39 (1.13-3.08)
15. Spelaeophryne methneri (Mahenge, Kilombero)	0.32	0.47 (0.30-1.48)

16. Callulina (refer to node)	35.04	40.62 (33.34-48.61)
17. Callulina (refer to node)	23.33	27.31 (22.37-33.28)
18. Callulina (refer to node)	2.94	3.43 (2.33-4.37)
19. Callulina (refer to node)	20.64	24.27 (19.48-29.58)
20. Callulina (refer to node)	18.05	21.27 (16.84-26.66)
21. Callulina (refer to node)	7.80	11.50 (8.90-13.64)
22. Callulina (refer to node)	1.62	2.13 (0.57-4.74)
23. Probreviceps (refer to node)	20.67	22.58 (19.86-25.82)
24. Probreviceps (refer to node)	11.55	12.03 (9.46-15.21)
25. Probreviceps (refer to node)	4.77	5.55 (3.27-9.29)
26. Probreviceps (refer to node)	3.37	4.25 (3.02-7.60)
27. Probreviceps (refer to node)	18.52	22.92 (17.92-28.65)
28. Probreviceps (refer to node)	12.21	12.50 (9.25-15.89)
29. Probreviceps (refer to node)	0.59	0.57 (0.31-2.03)
30. Probreviceps (refer to node)	1.50	1.74 (0.36-3.09)
30. Probreviceps (refer to node)	2.37	2.58 (1.28-3.89)

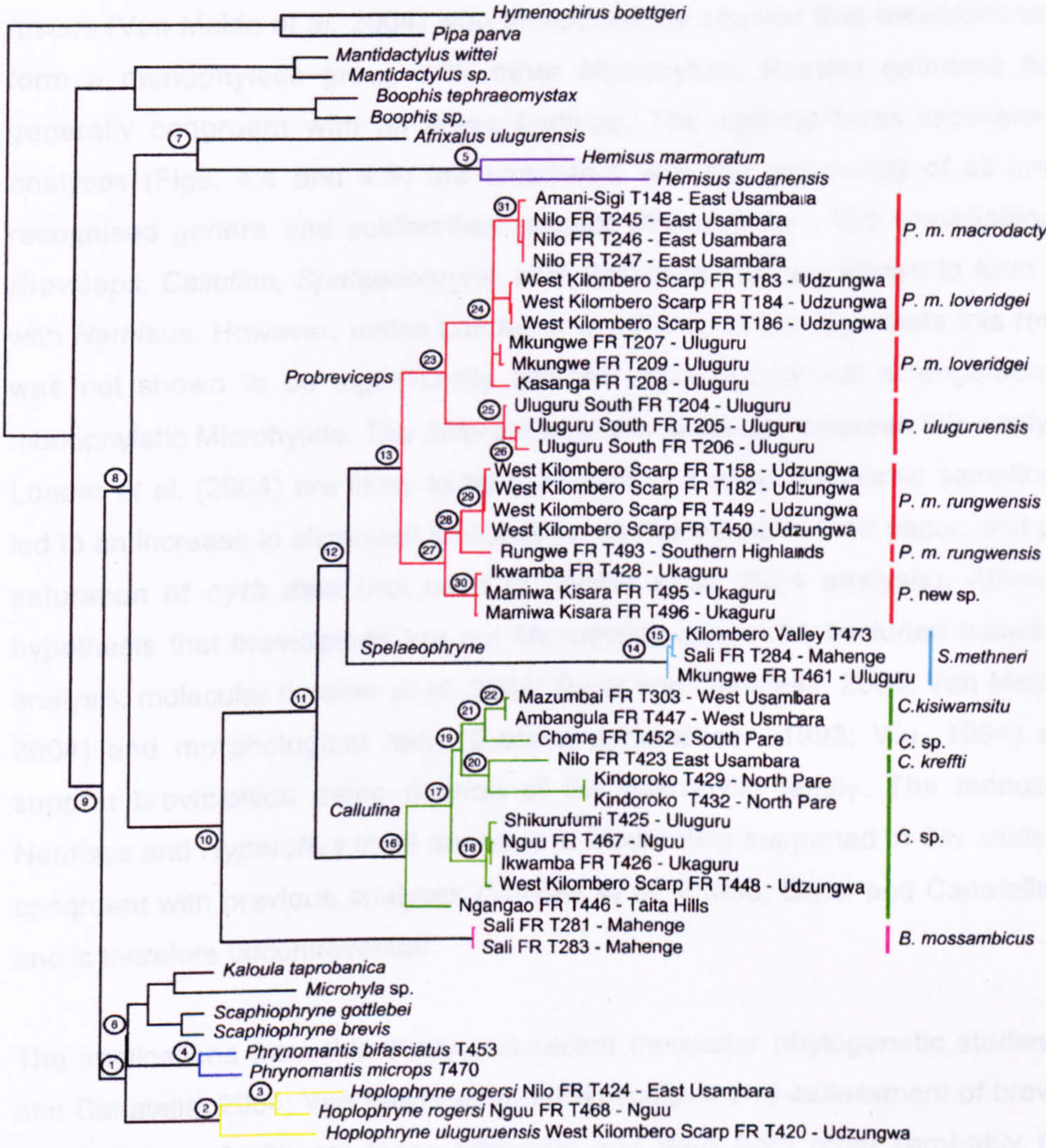

Figure 4.7

Phylogeny of African Microhylids with nodes calculated for molecular divergence times.

4.4 Discussion

4.4.1 Phylogeny

4.4.1.1 Higher relationships

Loader *et al.* (2004b) analysed a subset of the data and taxa presented here. They found that despite uncertainties over the position of the root, that the Brevicipitids are the sister group to *Hemisus* (i.e. a clade containing non-brevicipitine Microhylids). Support for the paraphyly of Microhylids was also provided from recent investigations of the brevicipitid species *Callulina kreffti* (Darst and Canatella, 2004) and *Breviceps*

fuscus (Van Meide *et al.* 2004) who independently showed that brevicipitines do not form a monophyletic group with other Microhylids. Results gathered here are generally congruent with all these findings. The optimal trees recovered in all analyses (Figs. 4.4 and 4.5) are consistent with the monophyly of all previously recognised genera and subfamilies (except Microhylinae). The brevicipitines (e.g. *Breviceps, Callulina, Spelaeophryne,* and *Probreviceps*) are shown to form a clade with *Hemisus*. However, unlike Loader *et al.* (2004), in topology tests this resolution was not shown to be significantly different from suboptimal arrangements, e.g. monophyletic Microhylids. The differences in the resolution between this analysis and Loader *et al.* (2004) are likely to be the result of greater taxonomic sampling which led to an increase in alignment ambiguities, as mentioned in their paper, and possibly saturation of *cytb* data (not used in Loader *et al.* 2004 analysis). Although the hypothesis that brevicipitids are not Microhylids cannot be excluded based on this analysis, molecular (Loader *et al.* 2004; Darst and Canatella, 2004; Van Meide *et al.* 2004) and morphological data (Blommers-Schlösser, 1993; Wu, 1994) strongly support brevicipitids being outside of the microhylid family. The monophyly of *Hemisus* and *Hyperolius* in all analyses is moderately supported in this study, and is congruent with previous analyses (Vences *et al.* 2003b; Darst and Canatella, 2004) and is therefore uncontroversial.

The implications from this study and recent molecular phylogenetic studies (Darst and Canatella, 2004; Van Meide *et al.* 2004) suggest a re-assessment of brevicipitids is necessary. Additional taxon sampling and data from other (probably nuclear) genes and/or from morphological systems will be needed to further resolve phylogenetic relationships before this can be undertaken with confidence. Also, of pressing concern are the implications these studies have for the classification of Brevicipitids. Darst and Canatella (2004) suggest the superfamily Brevicipitoidea could be erected to include all Arthroleptidae, Hyperoliidae, Hemisus and Brevicipitines as one alternative to revising the taxonomy of brevicipitids and ranids (see Fig. 4.8 for summary and Darst and Canatella, 2004). Although logically this may appear to be the best arrangement, based on the evidence at hand, stability of taxonomic classifications needs to be considered and any major changes in the arrangement should be tempered with the expectation that given our limited understanding of the content of the clade Ranoidea (as defined by Ford and Cannatella, 1993) more changes are likely to occur. Further areas of interest include the putative grouping of the African genera *Hoplophryne*, *Parhoplophryne* with the

Indian genus *Melanobatrachus* in Parker's (1934) subfamily Melanobatrachinae. The two monotypic genera *Parhoplophryne* and *Melanobatrachus* are currently unsampled. However, based on their large disjunct distribution (India and East Africa) and morphological inconsistencies (Savage, 1973) it is likely the content of this subfamily grouping may change in the future.

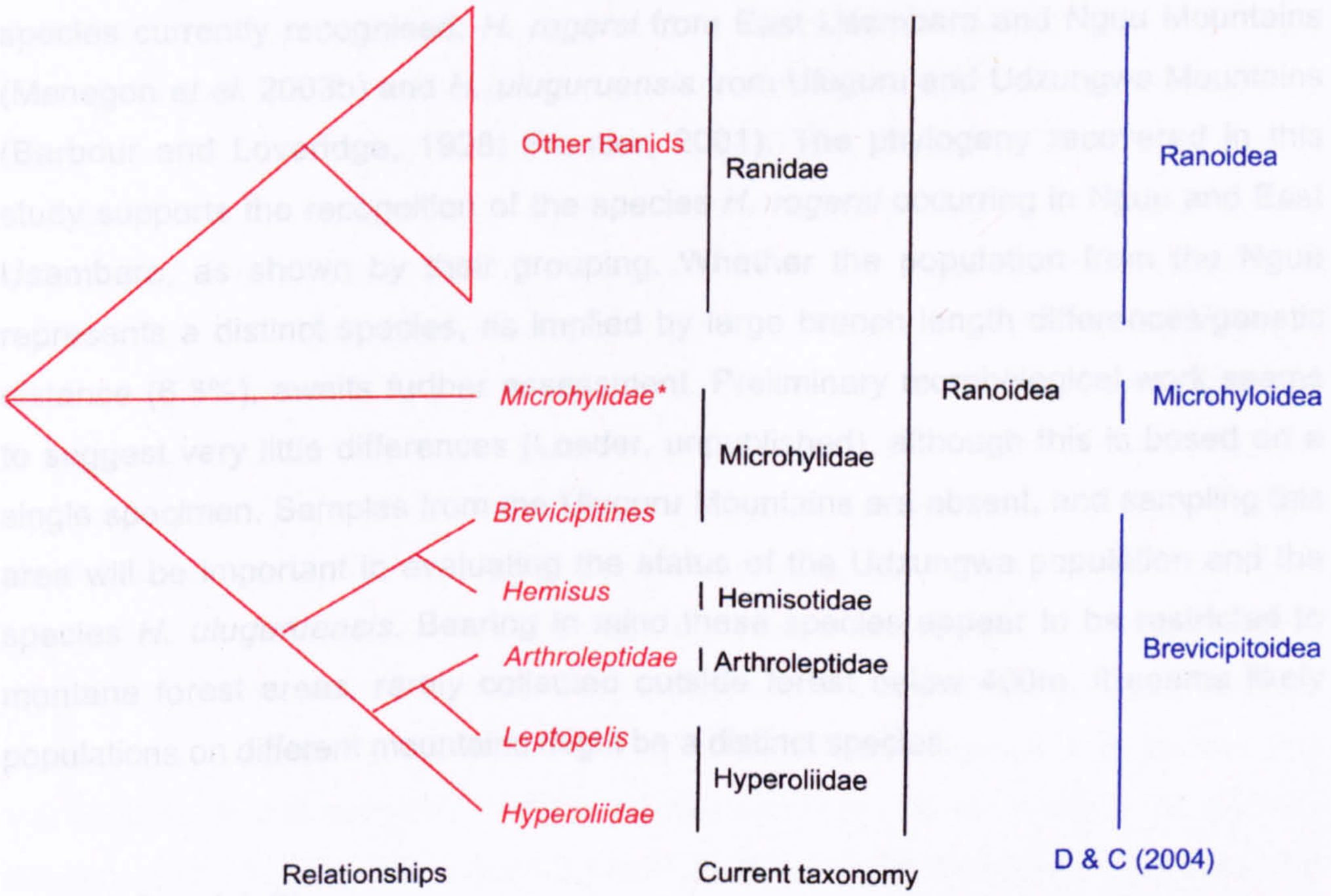

Figure 4.8

Summary of the higher relationships among Microhylids, Ranids, Hyperoliids and Brevicipitines.

4.4.1.2 Non-brevicipitine microhylids

For the non-brevicipitid microhylids all optimal trees show these taxa recovered in a single clade, although weakly supported in all analyses. Inter-relationships among the genera *Scaphiophryne, Hoplophryne, Phrynomantis, Microhyla,* and *Kaloula* are also poorly resolved, and little can be interpreted from these results. However, the position of *Hoplophryne* within a putative clade comprising a mixture of widely geographically distributed, non-brevicipitine microhylids is uncontroversial (Parker, 1934). The similar nesting of *Phrynomantis* is supported by detailed studies of morphology (Laurent, 1941; Haas, 2003). Noble (1931) placed *Phrynomantis* in its own subfamily, not closely allied to any other microhylids. Parker (1934) excluded *Phrynomantis* from Microhylidae based on the presence of intercalary cartilages, a

character now known to be present in other microhylids (Wu, 1994). Data from larval morphology strongly support the nesting of *Phrynomantis* within a clade of non-scaphiophrynine microhylids (Haas, 2003).

The rarely collected, cryptic microhylid *Hoplophryne* is endemic to the EAM, with two species currently recognised; *H. rogersi* from East Usambara and Nguu Mountains (Menegon *et al.* 2003b) and *H. uluguruensis* from Uluguru and Udzungwa Mountains (Barbour and Loveridge, 1928; Frontier, 2001). The phylogeny recovered in this study supports the recognition of the species *H. rogersi* occurring in Nguu and East Usambara, as shown by their grouping. Whether the population from the Nguu represents a distinct species, as implied by large branch length differences/genetic distance (6.3%), awaits further assessment. Preliminary morphological work seems to suggest very little differences (Loader, unpublished), although this is based on a single specimen. Samples from the Uluguru Mountains are absent, and sampling this area will be important in evaluating the status of the Udzungwa population and the species *H. uluguruensis*. Bearing in mind these species appear to be restricted to montane forest areas, rarely collected outside forest below 400m, it seems likely populations on different mountains might be a distinct species.

4.4.1.3 Brevicipitines

Monophyly of subfamily

Likelihood and bayesian analyses support the monophyly of Brevicipitinae. Posterior probability for this group is high, and Shimadairo-Hasegawa tests (p= 0.0402) do not suggest this resolution can be attributed to sampling error (Fig.4.5). Parsimony analyses for this node however provide less convincing support, with bootstrap of 80, decay index of 4 and Templeton test (p = 0.0891). Overall though the strong support in likelihood and bayesian analyses, other molecular data sets (Darst and Canatella, 2004; Loader *et al.* 2004; Van Meide *et al.* 2004) and corroboration from morphological studies (Parker, 1934; Blommers-Schlösser, 1993; Wu, 1994) means the monophyly of brevicipitine is the most likely resolution. Certain morphological characters have previously suggested brevicipitids non-microhylid features, Parker (1934) commented on the special nature of the brevicipitine vomer, reduced posteriorly (post-choanally) but bearing a large anterior and medial expansion. Parker noted other characters, I.e retention of a complete shoulder girdle, which

readily distinguished brevicipitines from all other Microhylids. Further work is required to determine derived and plesiomorphic conditions.

Generic Relationships

Phylogenetic relationships of Brevicipitinae genera have been explored by Poynton (1964; 1999), Poynton and Pritchard (1976), Largen and Drewes (1989) and Wu (1994). As their names suggest, *Probreviceps* and *Breviceps* have been thought as being closely related, and Poynton (1999: p.515) proposed that *Breviceps* 'can be derived from sylvicolous East African *Probreviceps'*. This was based on the observation of clinal variation in the lengths of limbs and digits along the continuous North to South distribution of the two genera (Poynton and Pritchard, 1976). *Probreviceps* from Tanzania have the longest limbs and toes, followed by *P. rhodesianus* (further South, in Zimbabwe), then *Breviceps* (which occurs further southwards) with the shortest. In contrast, Wu (1994) hypothesised that *Callulina* and *Probreviceps* comprise a clade, with successive sister groups formed by a paraphyletic *Breviceps*, and *Spelaeophryne*. Focussing on pectoral girdle morphology, Largen and Drewes (1989) questioned the monophyly of *Probreviceps* + *Breviceps* by suggesting that *Probreviceps* is more closely related to *Balebreviceps* (not included in this analysis). Analyses presented here and in Loader *et al.* (2004b) strongly exclude *Breviceps* from a clade comprising *Probreviceps*, *Callulina* and *Spelaeophryne*. Judged by the Templeton test ($p < 0.04$), it is unnecessary to attribute the difference (14 steps) between our MPTs and the best trees containing a *Probreviceps* + *Breviceps* clade to random sampling error. Despite this, the optimal trees recovered in the analyses (Figs. 4.5, 4.6, and 4.7) do not preclude the possibility that *Breviceps* evolved from a *Probreviceps*-like ancestor, in keeping with Poynton's hypothesis.

Bootstrap support for the *Spelaeophryne* + *Callulina* + *Probreviceps* clade, and for the monophyly of the constituent genera is high in all analyses, although the best trees in which *Probreviceps* is constrained to be non-monophyletic do not have a significantly worse fit to the data (Fig. 4.5). The relationships among these three genera are not clearly resolved, although no analyses recovered one of the three possible resolutions, i.e. the pairing of *Callulina* + *Spelaeophryne*. Currently, morphological data that might provide decisive support for one of the two competing hypotheses (in the optimal parsimony and ML trees) are lacking. The conflict and lack of resolution might be caused by heterogeneous rates of molecular evolution

(i.e. *Spelaeophryne* relative to other brevicipitines), inadequate taxon sampling (*Balebreviceps hillmani*; additional species of *Breviceps*), or simply too few sequence data. Clearly more data both molecular and morphological are needed, before an adequate appraisal of intergeneric relationships in the Breviciptinae can be presented.

Callulina

Prior to de Sá, *et al.'s* (2004) description of the species *Callulina kisiwamsitu* from the West Usambara, the genus *Callulina* contained one single species *Callulina kreffti,* considered to be widespread throughout the EAM (Barbour and Loveridge, 1928; Howell, 1993; Frontier (Udzungwa) 2001). The recognition of two species disjunctly distributed in the geographically adjacent mountain blocks of the Usambaras in de Sá, *et al.'s* (2004) paper have implications for the status of all other *Callulina* populations known to occur in the EAM. De Sá *et al.* (2004) anticipated that other 'distinct populations of *Callulina* throughout the Eastern Arc Mountains may also prove to be distinct species' (de Sá *et al.* 2004; p.223), although their status was not addressed. In light of the evidence that *Callulina* species are different between East and West Usambaras (de Sá *et al.* 2004), and the level of amphibian endemism in the EAM (e.g. Howell, 1993; Menegon *et al.* 2004) the other *Callulina* populations would appear to be likely candidates as new species.

The recognition of *Callulina kisiwamsitu* based on morphology and molecular data is again strongly supported here by our molecular analyses, as also demonstrated by de Sá *et al.* (2004) and Loader *et al.* (2004b). As outlined in their paper de Sá, *et al.* (2004) showed that the two samples of *C. kisiwamsitu* in the West Usambara Mountains (Ambangula FR T447 and Mazumbai FR T303) that form a clade, are geographically more distant from each other (31.13 km apart), than samples from Mazumbai are to *Callulina kreffti* from Nilo, East Usambara (19.73 km apart). This suggests that the phylogenetic relationships do not appear to be the result of clinal variation among now separated populations, but indicative of the populations of the East and West Usambara mountains being specifically distinct. This assessment is also consistent with the known differences in the amphibian assemblages of the East and West Usambara Mountains (Howell, 1993; Menegon *et al.* 2004; Poynton, pers. comm.). Sampling of *C. kreffti* populations from the type locality of Amani in East Usambara would be useful, and necessary to fully evaluate phylogeographic patterns in this species.

The relationships among almost all of the known populations of *Callulina* (apart from Nguru Mountains) are summarised in Fig. 4.9. As can be seen from this figure, this study provides evidence for the tentative recognition of many new species of *Callulina*, as supported by the large degree of divergence in molecular sequences, a conclusion supported also by morphology (Loader, unpublished). As a measure for the detecting whether mitochondrial lineages may represent new species, pairwise genetic comparisons can be made between currently designated *Callulina* species and other amphibian studies.

For example, between the species *C. kreffti* and *C. kisiwamsitu* genetic distances are approximately 7.4%, which can be regarded as particularly high for most amphibian studies of inter-specific genetic heterogeneity between species (e.g. Wieczorek and Channing, 1997; Gower *et al.* 2002). Taken that this high and somewhat conservative estimate approximates species differences in *Callulina*, then almost all the species identified in Fig.4 would be considered new, as shown by genetic distances of >7%. Only relatively smaller divergences are exhibited between South Pare and West Usambara (4.7%) populations, and West Usambara and Clade '*Callulina* sp.2' (>6%), and even these values exceed published estimates of genetic differences between amphibian species. Although such estimates may not be an appropriate measure for designating new species, the molecular data highlights need for further work.

It is noteworthy that although there are many new disjunct populations (see above), there is also a clade comprised of a number of populations from disjunct mountain blocks, with very limited genetic diversity (0.2-1%). This clade includes Udzungwa, Uluguru, Ukaguru and Nguu populations, and based on inferences from the phylogeny and genetic pairwise distances these probably form a single species. It is uncertain whether this clade also shows congruent morphological patterns, but geographically these populations are closely aligned, and therefore a recent contact among these areas can be imagined which would allow genetic interchange between these populations. It is likely that populations from the Nguru Mountains (the only mountain population missing from this study) nests in this clade, based on its geographical proximity. Sampling will be necessary to establish if this is correct.

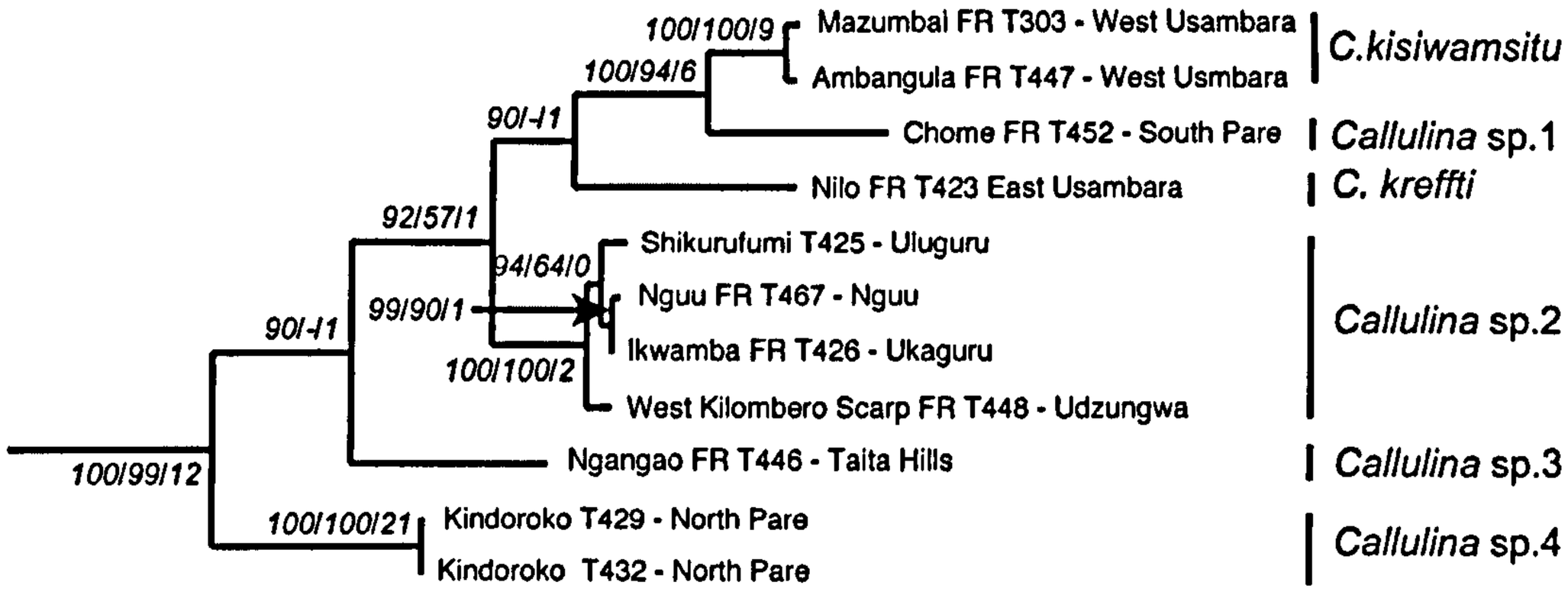

Figure 4.9

Relationships in *Callulina* based on Brevicipitine alignment. Values on branches show Bayesian posterior probabilities, bootstrap proportions calculated using parsimony, and decay index.

Table 4.7

Summary of the geographical distance (km) (above the diagonal) and % genetic distances between *Callulina* populations (below the diagonal).

	1	2	3	4	5	6	7	8	9	10	11	12
1. *Probreviceps*	-	-	-	-	-	-	-	-	-	-	-	-
2. *Callulina* T303 West Usambara- M	15.3%	-	19.7	283.4	131.1	241.6	153.1	153.1	162.0	31.1	411.2	86.4
3. *Callulina* T423 East Usambara	15.8%	7.5%	-	283.3	141.7	249.4	170.7	170.7	172.7	35.6	415.5	105.5
4. *Callulina* T425 Uluguru	15.6%	6.2%	7.2%	-	181.2	108.0	382.0	382.0	432.0	252.3	146.6	320.4
5. *Callulina* T467 Nguu	15.2%	6.4%	7.3%	0.8%	-	113.0	200.9	200.9	255.8	106.3	288.8	141.3
6. *Callulina* T426 Ukaguru	15.2%	6.2%	7.2%	0.6%	0.2%	-	301.7	301.7	365.5	213.9	178.1	250.4
7. *Callulina* T429 North Pare	15.3%	10.1%	9.2%	9.5%	9.5%	9.4%	-	0	86.1	171.9	479.1	70.6
8. *Callulina* T432 North Pare	15.3%	10.1%	9.2%	9.5%	9.5%	9.4%	0.0%	-	86.1	171.9	479.1	70.6
9. *Callulina* T446 Taita Hills	14.8%	8.2%	9.7%	7.8%	8.0%	7.8%	9.5%	9.5%	-	190.5	543.5	115.3
10. *Callulina* T447 West Usambara- A	15.2%	0.4%	7.4%	6.2%	6.4%	6.2%	9.9%	9.9%	8.0%	-	381.3	101.8
11. *Callulina* T448 Udzungwa	15.4%	6.3%	7.3%	1.1%	1.0%	0.9%	9.0%	9.0%	8.2%	6.3%	-	428.2
12. *Callulina* T452 South Pare	14.8%	4.7%	9.0%	7.1%	7.4%	7.2%	10.6%	10.6%	8.8%	4.7%	7.1%	-

There does not appear to be a significant correlation (r^2= 0.1513) between geographical distance and pairwise genetic, though with increasing distance populations are more likely to show increased pairwise genetic distance, as shown

by the positively inclined trendline (see Fig.4.10). This finding suggests that populations are not freely interbreeding, which would be anticipated given the isolation of populations on 'islands' of forest along the mountain chain.

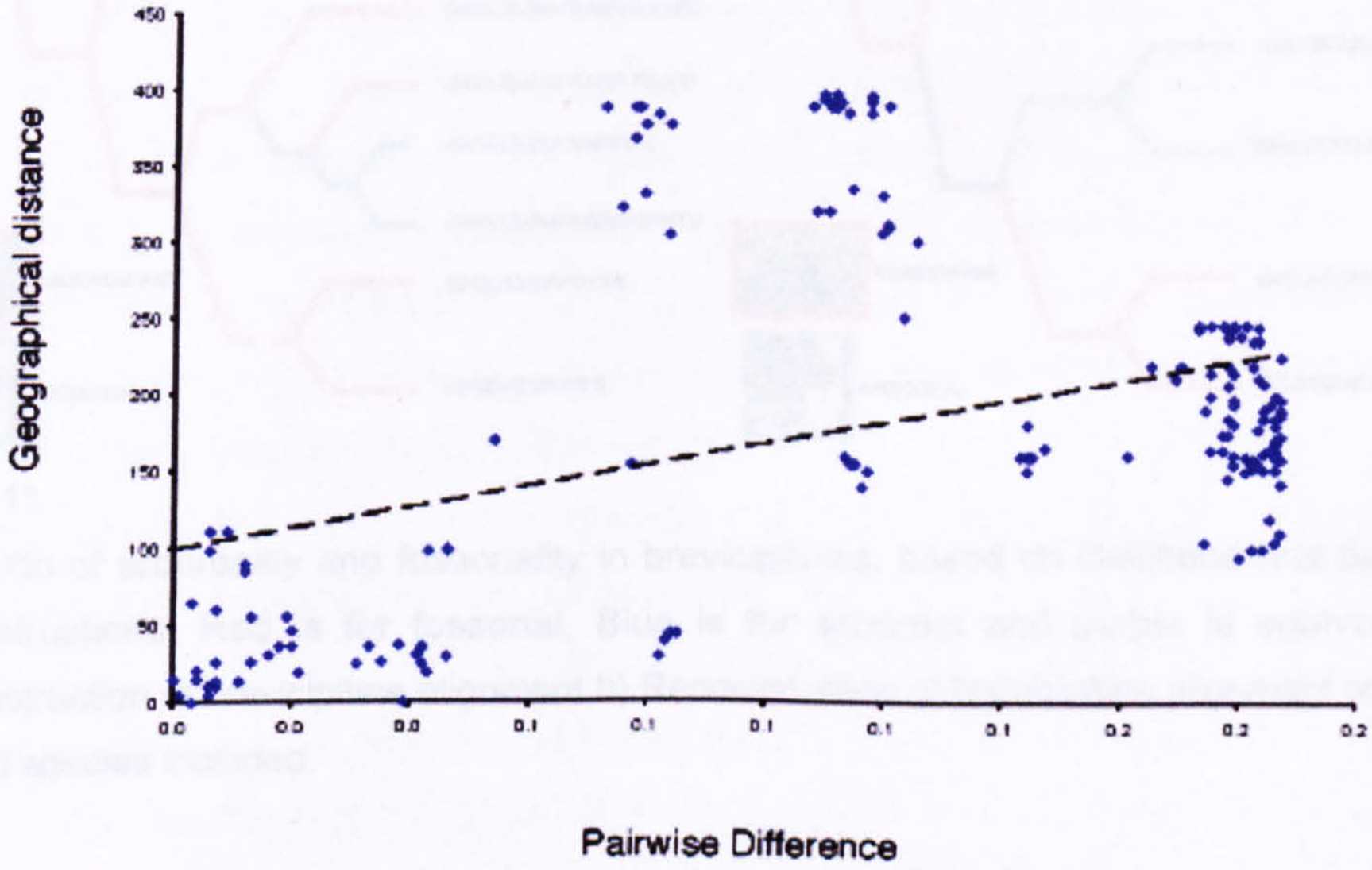

Figure 4.10.

Geographical distance against corrected pairwise differences, $r^2 = 0.1513$.

Phylogenetic relationships among genera of brevicipitines show strong support for the monophyly of the genus; topology tests and the degree of support are evidence for this. The topology of the optimal *Callulina* tree shows basal clades being geographically located in northern areas of the EAM (Taita Hills and North Pare), which are genetically highly divergent from all other clades (7.8-10.0%). These two clades are also morphologically most divergent, and show consistent morphological differences. Most significant are the differences in the 'mode of life' in these prospective species. Taita Hills and North Pare populations appear to be fossorial, which is in contrast to all other *Callulina* species and populations that appear to be arboreal. As a consequence of these significant differences in lifestyles, there are correlated morphological changes in fossorial species; e.g. lack of a tympanum, absence of expanded digital discs, stouter body, and shorter limbs (Loader, unpublished). Fossoriality appears to be the ancestral condition in the genus *Callulina*, which is also the dominant mode of life in brevicipitines (see Fig. 4.10a). Without the inclusion of Taita Hills and North Pares populations, the interpretation of the evolution of fossoriality and arboreality in brevicipitines would be less clear (Fig. 4.10b).

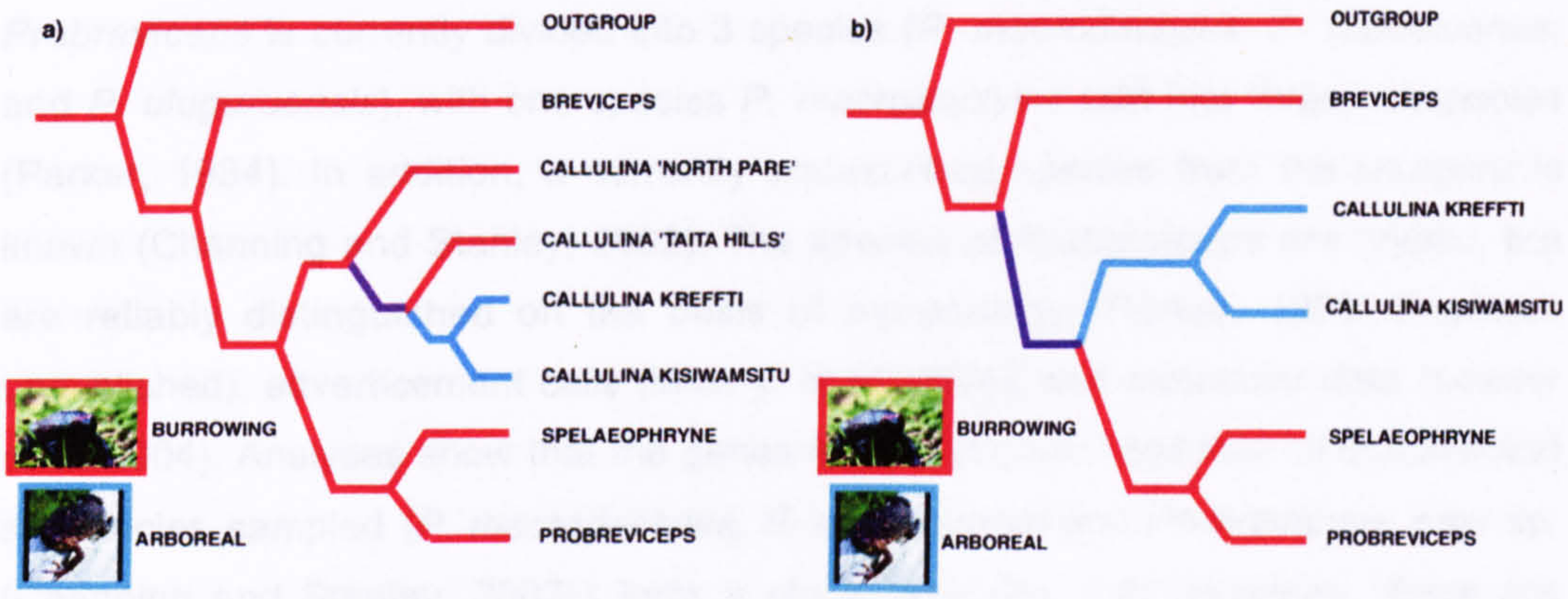

Fig. 4.11

Evolution of arboreality and fossoriality in brevicipitines, based on likelihood and bayesian reconstructions. Red is for fossorial, Blue is for arboreal and purple is equivocal. a) Reconstruction of brevicipitine alignment b) Reconstruction of brevicipitine alignment with only named species included.

Relationships among clades nested within *Callulina*, outside of Taita Hills and North Pare populations show a distinctive phylogeographic split between northern and southern clades, as mentioned in previous studies (Gravlund, 2002; Roy, 1997; Moller and Cronk, 1997; Lindqvist and Albert, 2001) with the grouping of northern species and populations (Usambara and South Pares), and southern areas (Udzungwa, Uluguru, Ukaguru, and Nguu). It is interesting to note the closer relationship shared between South Pare and West Usambara, than the latter to East Usambara where there are closer faunal similarities between amphibians (Howell, 1993). Whether the South Pare population represents a new species or *Callulina kisiwamsitu* is uncertain and awaits morphological data. However, a significant genetic pairwise distance of 4.7% suggests South Pare population might be distinct. Further collections will be necessary to ascertain the status of this population.

Before the description of the species *Callulina kisiwamsitu* (de Sá *et al.* 2004) the genus *Callulina* was considered a monotypic genus. Evidence presented in this study provides a valuable insight into a poorly understood group, in particular the diversity of species which currently is severely underestimated, both in terms of numbers (>5) but also morphological diversity (e.g. arboreal and fossorial forms).

Probreviceps

Probreviceps is currently divided into 3 species (*P. macrodactylus, P. rhodesianus,* and *P. uluguruensis*), with one species *P. macrodactylus* split into three subspecies (Parker, 1934). In addition, a currently undescribed species from the Ukaguru is known (Channing and Stanley, 2002). The species of *Probreviceps* are cryptic, but are reliably distinguished on the basis of morphology (Parker, 1934; Poynton, unpublished), advertisement calls (Mkonyi *et al.* 2004), and molecular data (Loader *et al.* 2004). Analyses show that the genus is monophyletic, and that all species and subspecies sampled (*P. macrodactylus, P. uluguruensis* and *Probreviceps* new sp. (Channing and Stanley, 2002)) form a clade (see Fig. 4.6). However, there are notable results that question the specific status of species, and subspecies, and therefore call for a re-assessment of the genus.

Prior to Loader *et al.'s* (2004) study, the status of the *Probreviceps macrodactylus* complex has not been investigated in a phylogenetic context. Limited morphological studies on *P. macrodactylus* species complex had been carried out (Parker, 1934; Poynton *et al.* in prep.). Results from these studies suggested only very little differences between the sub species *P. macrodactylus macrodactylus* from East Usambara and *P. m. loveridgei* from Uluguru and Udzungwa. The third sub species *Probreviceps macrodactylus rungwensis* however could be distinguished from other *Probreviceps,* by its large tympanum and notably pointed snout (Poynton, pers. comm.), which suggested it could represent a distinct species. Data from advertisement calls (Mkonyi *et al.* 2004) has recently suggested that there are distinct differences between the calls of *P. macrodactylus macrodactylus P. m. loveridgei,* and *P. uluguruensis* (calls of *P. m. rungwensis and P. rhodesianus* were not collected), which might be indicative of them being separate species.

Analyses presented here (and in Loader *et al.* 2004) suggest that there are few genetic differences between the subspecies *P. macrodactylus macrodactylus* and *P. m. loveridgei.* In addition, and contrasting to 12S and 16S data presented by Loader et al. (2004b), *P. m. macrodactylus* and *P. m. loveridgei* are resolved into separate clades, although only moderately supported. For the subspecies *P. m. macrodactylus* and *P. m. loveridgei* data recognise the division of populations, consistent with the subspecific designations of Parker (1934), the likelihood that they represent distinct species is uncertain. Based on the calls of *P. m. macrodactylus* and *P. m. loveridgei* which can be easily distinguished (Mkonyi *et al.* 2004) it could be suggested that the

populations might be distinct at species level. However, morphologically the subspecies are indistinguishable (Poynton, pers. comm.) and therefore their status as subspecies or species remains uncertain. For the third subspecies *P. m. rungwensis* sampling from both the type locality of Rungwe in the Southern Highlands and the Udzungwa were carried out, analyses show this subspecies to be paraphyletic. Suboptimal topologies, including a monophyletic *P. macrodactylus* sub species complex is significantly different from optimal paraphyletic solutions presented in Fig. 4.12. Based on the molecular results presented here, as in Loader *et al.* (2004b), and their morphological differences (Poynton *et al.* in prep), it seems clear that *P. m. rungwensis* should be considered a distinct species and not a subspecies of *Probreviceps macrodactylus*.

In Loader *et al.'s* (2004) study of African Microhylids sampling for *P. m. rungwensis* included populations from the Udzungwa, and not the type locality for the sub species in Rungwe (Parker, 1934). Loader *et al.* (2004) recommended that sampling Rungwe would be necessary to evaluate the status of this species 'particularly in light of the apparently significant biogeographical barrier between these populations (the 'Makambo Gap', e.g. Lovett, 1990; Keilland, 1990; Gravlund, 2002). The phylogeny presented here shows significant divergence between these two populations (~4%), and indicate that the Udzungwa population might be distinct. The Southern Highlands have been shown to be a centre of endemism with strong zoological affinities to the Eastern Arc (Davenport, pers. comm.). Analyses of the snake genus *Crotaphopletis* (Gravlund, 2002) and the bird genus *Andropadus* (Roy, 1997) indicate these regions show extensive levels of divergence between populations from Southern highlands and the Udzungwa Mountains. Based on these findings, populations of amphibian species (e.g. *Nectophrynoides viviparous*, *Arthroleptis reichei*, *Scolecomorphus kirkii*) distributed throughout the Udzungwa and the Southern Highlands may prove to be distinct with more refined taxonomic approaches.

Based on morphology, Channing and Stanley (2002) suggested the presence of a new species of *Probreviceps* from the Ukaguru Mountains. Molecular data supports the presence of a distinct species as demonstrated by the high support for this clade and genetic divergence (~5%). The recognition of the species as being distinct is further supported by call data, which is highly distinctive, being a slow series of clicks (Loader *et al.* in prep.). Interesting morphological similarities are also shown by its

close relationship to *P. m. rungwensis*, which both share a pointed snout, apparently keratinised in the new species from Ukaguru, and a large tympanum (which is strongly sexually dimorphic in both species).

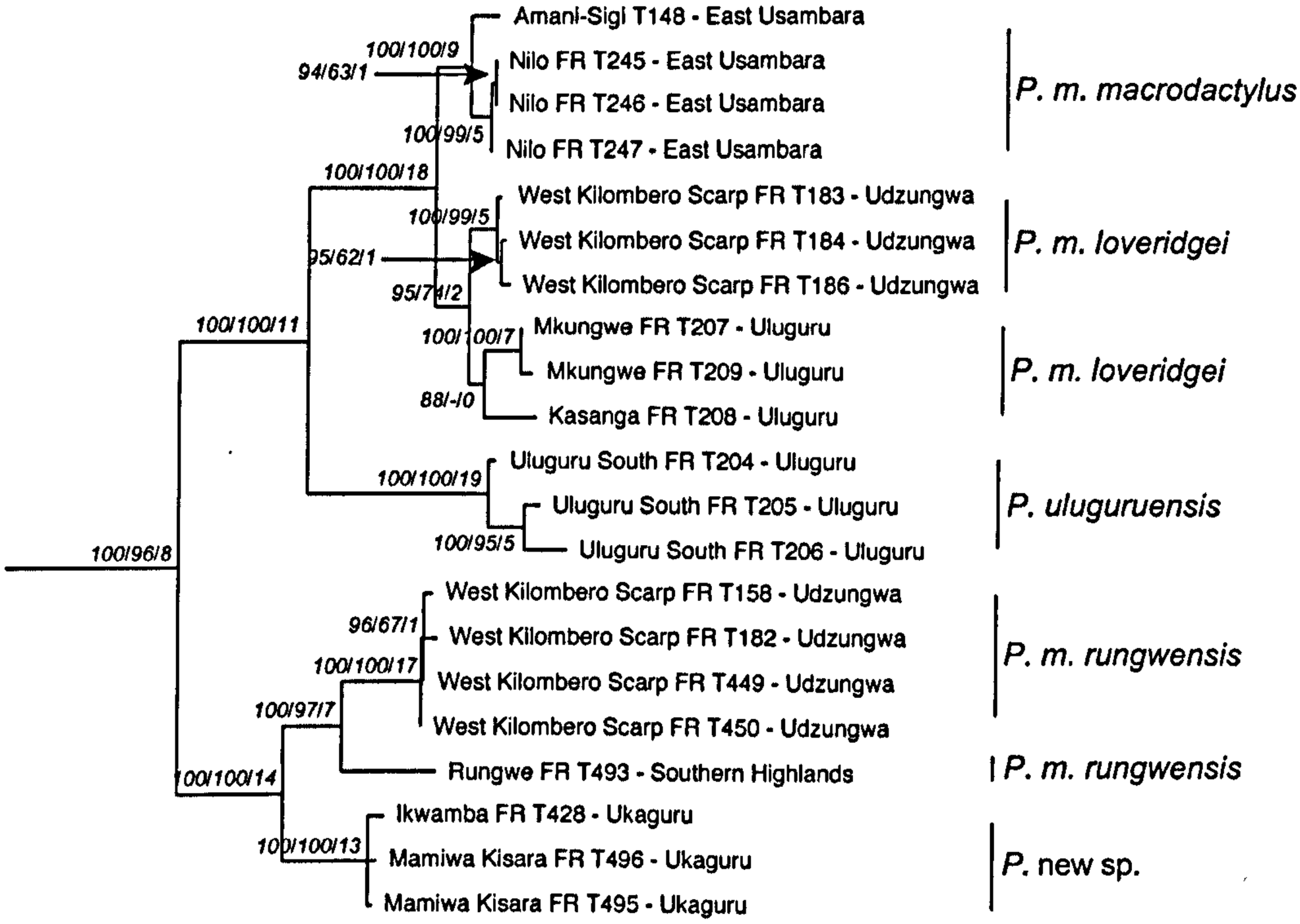

Figure 4.12

Relationships in *Probreviceps* based on Brevicipitine alignment. Values on branches show Bayesian posterior probabilities, bootstrap proportions calculated using parsimony, and decay index.

Spelaeophryne

The enigmatic species *Spelaeophryne methneri* has a scattered but wide distribution throughout SE coast of Tanzania and the Eastern Arc region (Parker, 1934; Howell, 1993). Despite its wide distribution throughout East Africa, covering some 500km, only a single species is known for this monotypic genus. This cryptic species is found in a range of habitats and altitudes, but is usually found in woodland or forest areas. The species is highly distinctive with a red band across the top of the head (though in one specimen this marking is absent), resembling the dorsal patterning of a honey badger. Morphologically all these populations appear to be homogenous. Results from sequences of three genes show limited genetic variation (0.5-0.6% between populations in Uluguru, Kilombero (=Udzungwa), and Mahenge), which when compared to the degree of difference seen in other brevicipitid genera from species distributed in both areas (*Probreviceps macrodactylus loveridgei* 0.8-1%; *Callulina*

1%) is moderately less. Although this study does not sample all the known populations of *Spelaeophryne*, based on the molecular data there do not appear to be any cryptic species. It is likely that the generalist habitat of *Spelaeophryne* compared to the evergreen forest restricted species of *Callulina* and *Probreviceps* may have influenced the lower genetic heterogeneity in this genus.

Conclusions

The use of molecular data for investigating phylogenetic relationships among African microhylids show large genetic heterogeneity between mountain populations, and it is likely that many of these populations will be recognised as being new upon subsequent study of morphology. With the exception of *Spelaeophryne*, nearly all of the groups sampled show twice as many species as previously anticipated (*Probreviceps*: 3 species- now estimated 5-6 species; *Callulina* 2 species- now estimated 6 species; *Hoplophryne*: 2 species- now estimated 3-4 species). Some of the previously unrecognised forms represent cryptic species, ones which are morphologically similar, but which display divergent mitochondrial lineages (e.g. *C. kreffti* from Uluguru; *Probreviceps rungwensis* from Rungwe and Udzungwa). However, many of the 'new' species represent newly collected populations, which are both morphologically and molecularly distinct (e.g. *Probreviceps* from Ukaguru; *Callulina* from North Pares and Taita Hills). The only anomaly in the pattern of high species diversity among EAM microhylids is shown in the brevicipitine taxa *Spelaeophryne*. As previously mentioned, the significant differences in habitat preference of *Spelaeophryne* to all other Microhylids may account for the different phylogenetic patterns. This result might be significant: of all the Microhylid taxa that occur in the EAM, the species *Spelaeophryne methneri* has the least restrictive habitat preference, occurring in woodland and lowland areas. All other microhylid genera (eg. *Hoplophryne* and *Callulina*) are restricted to the upper montane rainforests (Poynton, 2003) and therefore presumably show restrictive dispersal capabilities among mountain blocks. For forest restricted species there tends to be new species between each mountain block, which is not repeated in *Spelaeophryne*. Perhaps a fuller geographical sampling of *Spelaeophryne* throughout its distribution may uncover significant genetic variation. Overall, the phylogenetic patterns in microhylids are congruent with the idea that fragmentation and isolation may have been important in generating high species diversity in the EAM (Lovett, 1993a).

4.4.2 Biogeography

4.4.2.1 African Biogeography

Savage (1973) speculated that the three extant African microhylid subfamilies (Brevicipitinae, Melanobatrachinae, Phrynomerinae) diversified prior to Gondwana fragmentation. In contrast, Duellman and Trueb (1994: p.489) argued that a brevicipitine-phrynomerine lineage diversified only after Gondwana fragmentation. Based on the tree recovered in this analysis Duellman and Trueb's hypothesis can be rejected because there is no rooting of our optimal trees in which *Phrynomantis* and brevicipitines form a clade. However, suboptimal solutions are not statistically significant, which contrasts to the results presented by Loader *et al.* (2004b) who were 'not compelled to attribute the difference (9 steps) between our MPTs and the best trees in which *Phrynomantis* and brevicipitines are a potential clade to sampling error (Templeton test, $p < 0.02$)'. The inclusion of more outgroups, and *cytb* data in this analysis may have obscured the optimal tree and their relationships. Hertwig *et al.* (2004) has shown that the use of partial mitochondrial data for investigating early branching events in amphibians is unsuitable. The inclusion of appropriate molecular data (e.g. nuclear) will be needed to resolve these questions, particularly more slowly evolving gene fragments.

Estimates of molecular dates provided another source of data to evaluate biogeographic hypotheses. If dating estimates are correct, they indicate that the three major clades; non-brevicipitine Microhylids, brevicipitines, *Hemisus* all diverged from each other around the same time period. Firstly, as indicated by the optimal tree non-brevicipitine Microhylids diverged from *Hemisus* and Brevicipitines around 111 Myr (Langley Fitch 114-136 Myr), followed soon after by *Hemisus* and Brevicipitines splitting around 107 (Langley Fitch 111-134 Myr). If the Langley Fitch estimates confidence values are considered, then the estimates overlap with the final phase of Gondwana fragmentation, which cannot reject the hypothesis given by Savage (1973) that brevicipitines and hoplophrynines/phrynomantis diverged prior to the fragmentation. However, Savage also speculated that Hoplophrynines and *Phrynomantis* also diverged at this time, whereas estimates here suggest a much more recent divergence time (91 Myr; Langley Fitch 61-84 Myr). Overall the tree and dating estimates dispute Duellman and Trueb's hypothesis that the brevicipitines and phrynomerines diverged after Gondwana fragmentation but is less clear on hypotheses proposed by Savage (1973).

Phylogenetic results presented in this study do not support the classification of brevicipitines as Microhylids. Microhylids surveyed in this study include the genera *Scaphiophryne, Hoplophryne, Phrynomantis, Microhyla,* and *Kaloula.* Traditionally, the patterns of relationships among Microhylids have been interpreted as reflecting the breakup of Gondwana, with distinct elements from Africa, Madagascar, Asia, and America. There appears to have been little crossover in the Microhylid faunas from these areas based on classificatory systems, although there are exceptions (e.g. Melanobatrachine*)*. Based on the molecular dates, the divergence of hoplophrynines from all other microhylids occurred around 91 Myr (Langley Fitch 61-84 Myr). This date is much more recent than the breakup of Gondwana, post dating the separation of Madagascar-India-Seychelles complex from Africa, and if correct could imply: a transoceanic dispersal event, incorrect geological reconstructions of Gondwana, or stepping stone terrains that are now absent. More recent dates (not shown or calculated) would also be inferred for the splits between *Phrynomantis* and *Scaphiophryne*, *Microhyla*, and *Kaloula,* which again imply more recent links between East Africa, Madagascar and India. Long distance transoceanic dispersal events have been suggested in amphibian species, e.g. *Ptycadena mascarenensis* and Hyperoliid Tree Frogs (Vences *et al.* 2004a,b). However, these examples are likely to be exceptions to the rule. Forest-restricted microhylids would be unlikely to make transoceanic dispersals. That three splits in the base of the tree consistently show dates more recent than would be predicted could suggest molecular clock estimates might be proportionally underestimating, perhaps the result of saturated data. Until more data is collected, which is not saturated (e.g. nuclear genes), conclusions should be viewed cautiously. There is clear potential in the future for testing hypotheses concerning the diversification of microhylids and how this corresponds to Gondwana fragmentation (as investigated in chamaeleons by Raxworthy *et al.* 2002).

Forest restricted species have been predicted to show biogeographic patterns that mirror the significant geographic changes (geological and climatic) that have occurred to the forests during Africa's history. Most importantly, the timing of separations and the degree of isolation forests have undergone. East and West Africa contain the main proportion of Africa's montane rainforests, and many of the species are related, though only distantly because of the long period of separation (e.g. Loveridge, 1937). Brevicipitines and Hoplophrynines do not have any affinities with any known West African forest species, however the microhylid genera

Phrynomantis and the ranid *Hemisus* occur both in East and West Africa, though not necessarily in forest habitats. Sampling of these genera show deep divergences between East and West African species, despite the actual species themselves being relatively widely distributed. *Phrynomantis* species are widely distributed in moist savannas of sub-Saharan Africa, with *P. bifasciatus* distributed from Kenya to Angola and southwards to Namibia and north South Africa (Channing, 2001). *P. microps* is restricted to grasslands, and savanna in Guinea to the Sudan savanna (Rödel, 2000). For *Hemisus*, the species *Hemisus marmoratum* is found in both savannas and moist rainforest (contrary to Channing, 2001) from Senegal to Eritrea south to north South Africa. *Hemisus sudanensis*, formerly a subspecies of *marmoratum*, is found in similar habitats, with an overlapping range from Senegal to Eritrea (Rödel, 2000). For the species *Phrynomantis microps* and *Phrynomantis bifasciatus,* and *Hemisus sudanensis* and *Hemisus marmoratum* respective dating estimates are: 25 Mya (12.27-24.73), and 29 Mya (29.74-44.88). The divergence estimates appear to correspond with the pronounced biogeographic separation between East and West Africa around 25 Mya (Lovett, 1993a). The correlation however may simply be coincidental as such a biogeographic event would probably not be significant for savanna living amphibians. For example, savanna dwelling mammals show more recent patterns of divergence between East and West African regions (eg. Hamilton, 1988; Kingdon, 1989; Pitra *et al.* 2002). This study identifies groups that potentially could be useful for investigating the biogeography of non-forest habitats in Africa, and if the patterns of divergence correspond to forest species or other taxonomic groups living in similar habitats.

4.4.2.2 Eastern Arc Biogeography

The age and changes in the size and contiguity of rainforest habitats in the EAM have been predicted to have a significant influence on speciation patterns in forest dependent species. Tree topologies and estimates of divergence times allow an opportunity to investigate these temporal and spatial patterns in the Eastern Arc Mountains. Tanzanian Brevicipitines and Hoplophrynines are almost entirely confined to upland evergreen forest of the isolated constituent blocks of the Eastern Arc Mountains and Southern Highlands (e.g., Howell, 1993). Taken at face value, the optimal phylogeny recovered in the analyses and relative molecular dating estimates (Fig. 4.12) suggest that divergence of lineages (*Probreviceps, Callulina* and *Hoplophryne*) giving rise to extant species occurring in the Udzungwa, East

Usambara, Uluguru, Ukaguru and Nguu, has occurred at least twice. For example, splits between lineages occurring in the Ulugurus show different degrees of divergence (Fig. 4.13). Combined distributional and phylogenetic evidence does not fit with a simple, single vicariance/dispersal event, but is seemingly in accordance with the hypothesis that a combination of both vicariant isolation, possibly through fragmentation, and climatic fluctuations have repeatedly isolated and connected populations along the EAM.

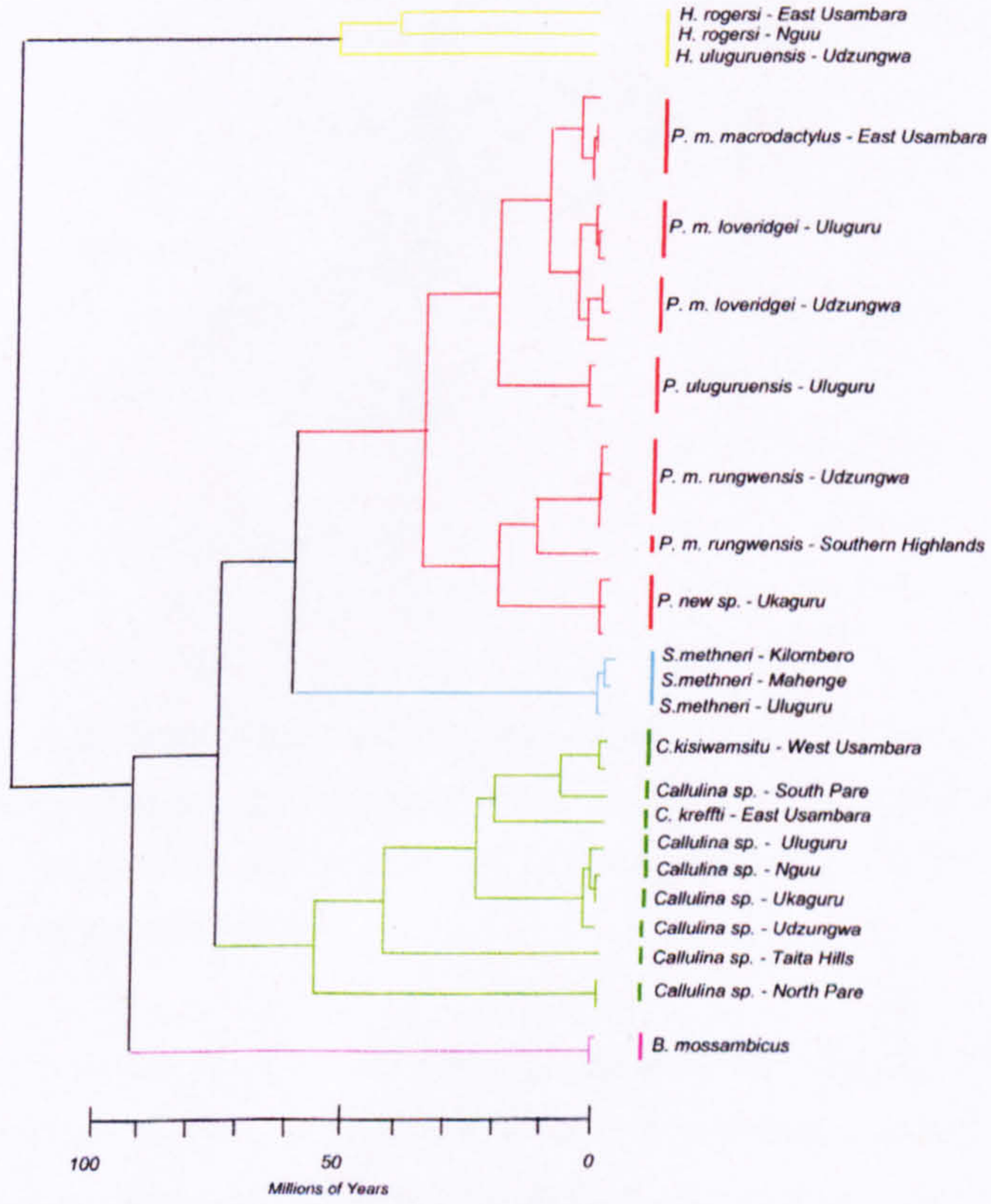

Figure 4.13

A chronogram of 'Microhylid' species found distributed in the Eastern Arc Mountains.

Divergence times suggest that each brevicipitine and hoplophrynine lineage has persisted for at least 40 Myr, and by implication the habitats where they occur. An archaic origin has long been speculated for the forests of the EAM (Lovett, 1993a), and is supported here using microhylids as indicators. These results are also congruent with divergence times presented in other groups that show a long period of persistence; e.g. snakes (Gravlund, 2002) and chamaeleons (Matthee, 2004).

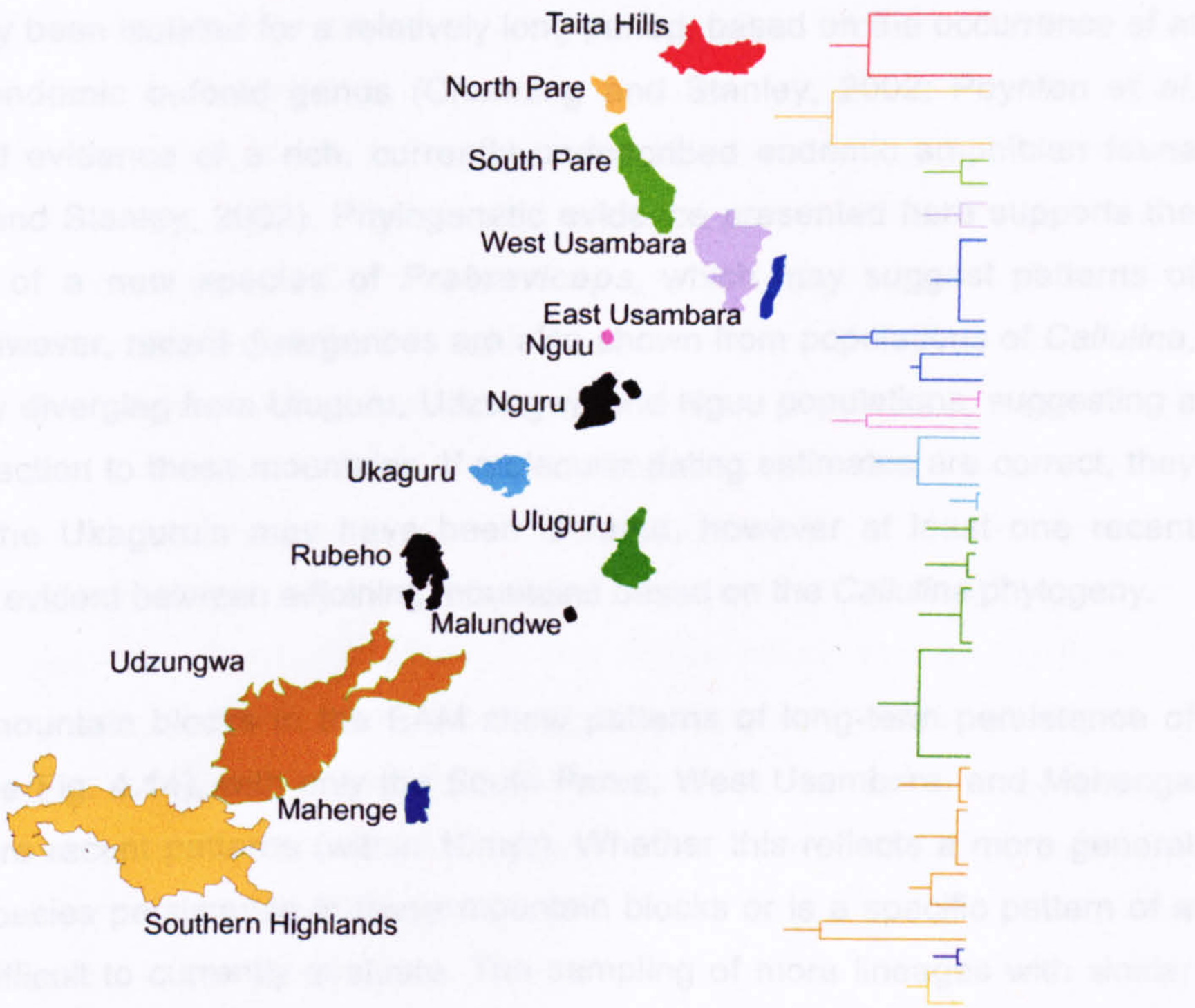

Figure 4.14

Speciation patterns of Microhylids in the Eastern Arc Mountains. Chronograms of Microhylid populations (Hoplophrynines and Brevicipitines) and their closest sister group, for each mountain block. Mountains are colour coded with each split, black areas have no chronograms of microhylids available.

The fragmentation and uplift, of presumably a once continuous and relatively shallow Eastern Arc Mountain chain, are believed to have influenced speciation in this region. Furthermore, the subsequent prolonged isolation of mountains is believed to have allowed a substantial period of time, which would have generated high levels of endemism. Phylogenetic evidence from Microhylids presented here show that some lineages may have been isolated for a long time in single mountain blocks. For example, *Callulina* populations in Taita Hills and North Pares, shown to be separated for more than 40Myr. The Taita Hills have been considered as an example of a mountain block separated for a considerable period of time, as shown by large molecular (e.g. Wilkinson *et al.* 2003; Lens *et al.* 2002) and morphological (e.g. Brooks *et al.* 1998; Perkin *et al.* 2003) differences between selected species. Brooks, *et al.* (1998; p.1) believed the Taita Hills were 'sufficiently differentiated...[that it]... should be merited consideration as a centre of endemism in its own right'. Another example includes the Ukaguru Mountains, which Menegon *et al.* (2004) suggested

has probably been isolated for a relatively long period, based on the occurrence of at least one endemic bufonid genus (Channing and Stanley, 2002; Poynton *et al.* 1998b), and evidence of a rich, currently undescribed endemic amphibian fauna (Channing and Stanley, 2002). Phylogenetic evidence presented here supports the recognition of a new species of *Probreviceps*, which may suggest patterns of isolation. However, recent divergences are also shown from populations of *Callulina*, only recently diverging from Uluguru, Udzungwa and Nguu populations, suggesting a recent connection to these mountains. If molecular dating estimates are correct, they imply that the Ukaguru's may have been isolated, however at least one recent exchange is evident between adjoining mountains based on the *Callulina* phylogeny.

Almost all mountain blocks in the EAM show patterns of long-term persistence of lineages (see Fig. 4.14), with only the South Pares, West Usambara, and Mahenge showing more recent patterns (within 10myr). Whether this reflects a more general pattern of species persistence in these mountain blocks or is a specific pattern of a lineage is difficult to currently evaluate. The sampling of more lineages with similar distributions would be an appropriate step to correctly estimate such patterns. Patterns of speciation in microhylid frogs indicate a complex temporal history, which show splits between species occurring at both ancient and recent periods. Whether these splits correspond to particular geographic events is unclear, particularly when bearing in mind the uncertainty of molecular divergence time estimates. Molecular dates may not be able to confidently correlate speciation events with geological events, however in this study they allow the ability to differentiate between single, or multiple speciation events occurring in an area.

Spatially, the phylogenetic patterns within brevicipitines and hoplophrynine species are incongruent. Based upon tree topology, the inferred ancestral area for *Callulina* is the northern part of the Eastern Arc, with the southernmost species being nested well within the genus. In contrast, the inferred ancestral areas for *Probreviceps* and *Hoplophryne* are the southern ranges of the Eastern Arc Mountains. The geographic history of the region appears to have influenced each brevicipitine and hoplophrynine lineage in different ways. This finding shows that, although there are strong indications that amphibian (Wilkinson *et al.* 2003; Loader *et al.* 2004b), and reptilian (Matthee *et al.* 2004; Gravlund, 2002) lineages have been influenced by the biogeographic history of the EAM, the history seems to be highly complex both spatially and temporally. The differences in phylogenetic patterns between lineages

might be accounted for by the differing ways that removing barriers affects species that share a common biogeographic history (Vermeij, 1991). For example, the variation in phylogenetic patterns in brevicipitines and hoplophrynines appears to be correlated seemingly with dispersal capability; forest dependent lineage *Hoplophryne, Probreviceps*, *Callulina* shows the strongest phylogenetic patterns of isolation, with *Spelaeophryne* able to tolerate many different habitats with shallow phylogenetic divergences among mountains. The degree to which speciation events show synchronous or delayed co- speciation depends on properties (population structure, reproductive mode, and habitat requirements) of the groups under study. Therefore, dispersal ability might be able to account for differing temporal patterns between lineages. However, in this example, it is highly unlikely that such differences between lineages could account for such divergent phylogenetic patterns, as seen in *Probreviceps* and *Callulina*. A better explanation for such discordant biogeographic patterns between lineages is a complex biogeographic history where many patterns are overlaid and there is no single biogeographic history for all lineages. This will be discussed more fully in Chapter 7.

Chapter Five

Systematics and Biogeography of Scolecomorphidae

5.1 Aims

In this chapter, I investigate the systematic and biogeographical patterns in the caecilian family Scolecomorphidae. The family is endemic to the mountains of East and West Africa. Monophyly of Scolecomorphidae has been supported on morphological grounds, however relationships among its species are poorly understood. The study reported here represents the largest molecular sampling of populations of the East African genus *Scolecomorphus*, including all the currently recognised species. Using molecular data I focus on species limits, and population differences, which allows the current taxonomy of *Scolecomorphus* to be tested. The data also allow an examination of biogeographical patterns, including both large-scale and local patterns in Africa. The wide geographical sampling throughout the Eastern Arc allows some assessment of the possible influence fragmentation and prolonged isolation of mountains of the Eastern Arc are purported to have had upon the family.

5.2 Introduction

5.2.1 Caecilians

Caecilians comprise the least understood of the three orders of amphibians (Nussbaum and Wilkinson, 1989). So scarce is the knowledge of these amphibians that they are probably the most poorly known order of tetrapods. This is true for all aspects of their biology, including their systematics. Caecilians are poorly understood for a number of reasons, but the main factors are probably their cryptic habits, tropical distribution, and lack of dedicated study (Nussbaum and Wilkinson, 1989). Caecilians are distributed throughout much of the wet tropics, occurring in South America, Africa, the Seychelles, the Indian subcontinent and parts of SE Asia. 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. Caecilian morphology appears well adapted to life underground. They have robust skulls that they push through the soil, and many have their eyes greatly reduced, sometimes to just a small group of cells concealed beneath bone and skin. In such forms, the eyes probably have a limited visual function, perhaps only detecting light and dark. One of the main sensory apparatus used by caecilians appears to be a pair of retractable, probably chemosensory tentacles on the snout that are unique to the group. Unlike other amphibians, caecilians are entirely limbless with elongate snake- or worm-like bodies. Their resemblance to worms is further enhanced by their moist, externally scaleless amphibian skin and its external subdivision into conspicuous rings or annuli.

Caecilians are often regarded as a small, conservative, and even 'primitive' vertebrate group. However, despite numbering only about 160 currently recognised species worldwide, caecilians are extremely diverse, and many novel morphological features and natural histories are evidence of this (e.g. Wilkinson and Nussbaum, 1997; Loader *et al.* 2003b). Amphibians in general show enormous morphological diversity, which is reflected in the habitat niches they occupy: aquatic, fossorial and arboreal to gliding forms. The presence of comparatively divergent morphological forms between groups has meant that the monophyly of the three orders (caecilians, salamanders and frogs) has generally been widely accepted (Duellman and Trueb, 1994). However, the relationships among the three living orders still remain somewhat uncertain (as summarised in Meyer and Zardoya, 2003). Most morphological (e.g. Duellman and Trueb, 1994), palaeontological (e.g. Milner, 1988) and recent molecular evidence (e.g. Zardoya and Meyer, 2001; San Mauro *et al.* 2004) seem to suggest that salamanders and frogs form a clade (Batrachia), with caecilians the sister group to this.

Caecilians are currently divided into six families, and relationships among these families appear to be reasonably well resolved at the base of the tree (see Fig.5.1). The South American family Rhinatrematidae is sister group to all other living caecilians, which is well supported by both morphological and molecular data (Duellman and Trueb, 1994; Wilkinson, 1997; Wilkinson *et al.* 2002a; San Mauro *et*

al. 2004). For the remaining caecilians, the Indian Uraeotyphlidae and Asian Ichthyophidae form a sister group to a clade coined the "advanced caecilians" (Nussbaum, 1991). The "advanced caecilians" comprise the three families Scolecomorphidae, Typhlonectidae, and Caeciliidae, with the family Scolecomorphidae thought to be basally positioned to the families Caeciliidae and Typhlonectidae.

Relationships among the "advanced caecilians" however are very poorly resolved (Hedges *et al.* 1993; Wilkinson *et al.* 2002a; Wilkinson *et al.* 2003), as shown by the polyphyletic distribution of caeciliids in the latest molecular analyses (see Fig.5.1).

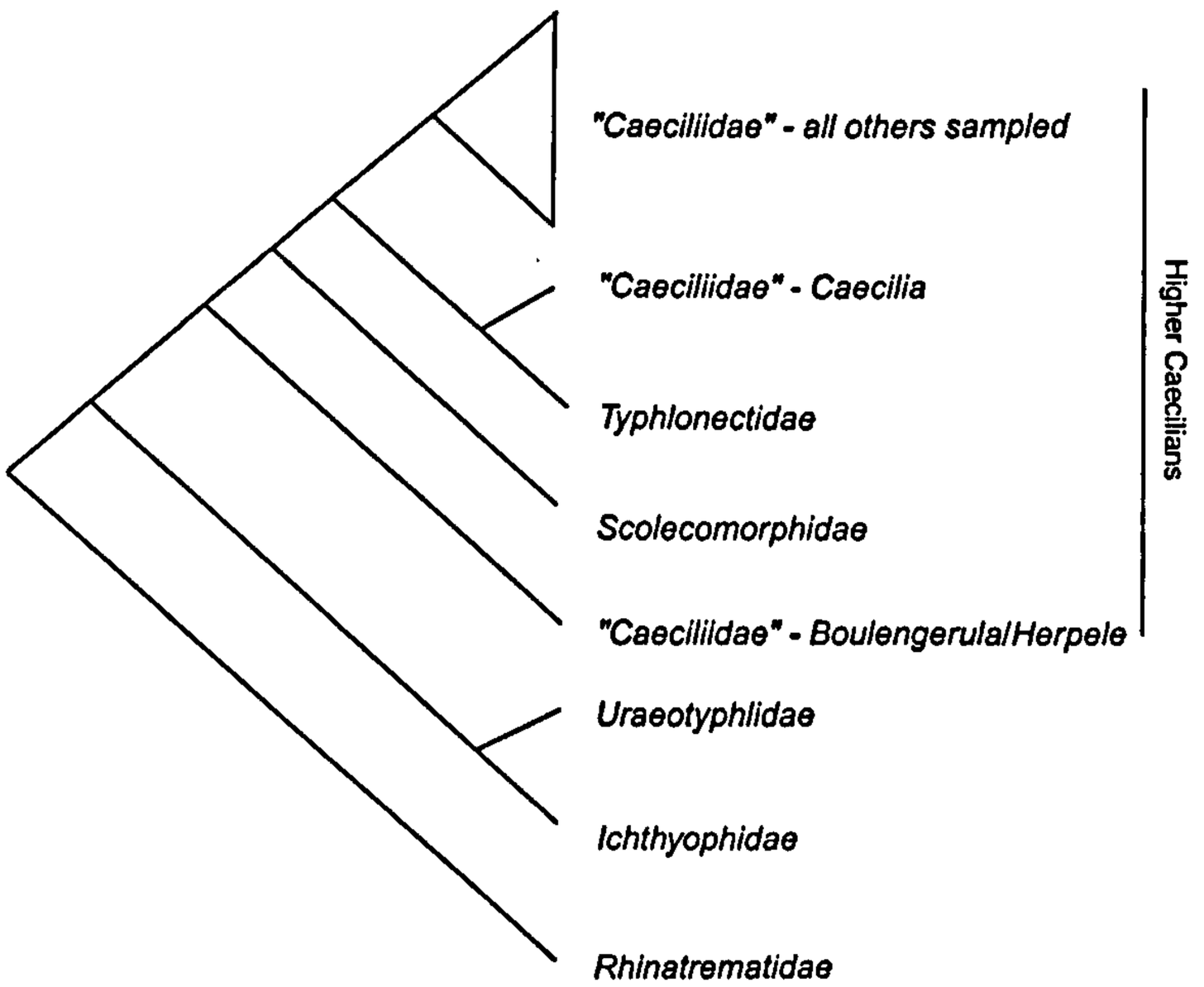

Figure 5.1
Summary of the most recent view of family relationships among caecilians (modified from Wilkinson *et al.*, 2003).

In molecular analyses of partial mitochondrial sequence data, *Caecilia* sp. has been shown as forming a clade with *Typhlonectes*. Furthermore, the African species *Herpele squalostoma, Boulengerula taitanus* and *Boulengerula boulengeri* form a sister group to all other "higher caecilians" (Hedges, 1993; Wilkinson *et al.* 2003), albeit weakly supported. Based on this evidence the phylogenetic relationships among "higher caecilians" are poorly understood, and await further taxon sampling (Wilkinson *et al.* 2003; San Mauro *et al.* 2004). Certain schemes have been proposed to resolve the paraphyletic status of the family Caeciliidae (e.g. Taylor, 1968;

Laurent, 1986), but were dismissed (e.g. Nussbaum and Wilkinson, 1989). Nussbaum and Wilkinson (1989) argued that these schemes were poorly conceived, inaccurate methods and characters, and therefore did not provide a robust alternative to the currently imperfect taxonomy.

Species limits are poorly understood in caecilian systematics, one of the main problems being the paucity of obvious external morphological features. Species accounts are dominated by counts of annuli, vertebrae and teeth (Taylor, 1968). In cases where these counts are inconclusive, workers have utilised alternative systems for investigating systematics. Certain systems have already been singled out as having good potential for such investigations; molecular data (e.g. Wilkinson *et al.* 2002a; Wilkinson *et al.* 2003; Gower *et al.* 2002), phallus morphology (Gower and Wilkinson, 2002), and life history features (Loader *et al.* 2003b). In this study I look at molecular data to resolve relationships among the poorly known caecilian family Scolecomorphidae, and investigate the implied biogeographical patterns.

5.2.2 African caecilians

African caecilians constitute a substantial proportion of the known diversity of the order. Approximately 13% of the recognised caecilian species are African, and two of the six caecilian families can be found in Africa, including the endemic family Scolecomorphidae. Recent molecular phylogenetic analyses (Wilkinson *et al.* 2003) have included eight taxa from five of the seven genera and therefore provided the best insight into the phylogenetic relationships of African caecilians. In addition to the paraphyletic position of the caeciliids *Boulengerula* and *Herpele* (with respect to the family Typhlonectidae) monophyly was supported in the genera *Scolecomorphus, Boulengerula,* and *Schistometopum*. The relationship of the West African caeciliid *Geotryptes seraphini* was poorly resolved in phylogenetic analyses. Wilkinson *et al.* (2003) noted significant base compositional biases and rate variation that may have hindered resolving the position of this species in the analysis. Wilkinson *et al.* (2003) rejected the hypothesis that the caecilian assemblages of Africa, and of East and of West Africa are monophyletic. The nestedness patterns of African caecilians and the deep divergences in Wilkinson *et al's* (2003; p.89) phylogeny suggested the 'current diversity of African caecilians predates the break-up of Gondwana' although no dating estimates were made. The caeciliid genera *Sylvacaecilia* and *Idiocranium* still await sampling in a molecular study. Nussbaum (1985) suggested that *Idiocranium*

represented a distinct lineage and does not appear to show close affiliations with other taxa. Africa is very poorly explored herpetologically, and this is particularly true for caecilians. Large areas of central Africa await sampling (Nussbaum and Pfrender, 1998) and it is likely we currently severely underestimate the diversity of caecilians found on this continent.

5.2.3 Scolecomorphid Caecilians

Species belonging to the family Scolecomorphidae are distributed in the montane forests of East and West Africa (Nussbaum, 1985). Scolecomorphids have a number of distinctive morphological synapomorphies, including the absence of stapes and in *Scolecomorphus* the presence of protrusible eyes that migrate from the orbit during development in association with the tentacle (Nussbaum, 1985; O'Reilly *et al.* 1996). Despite the fact that scolecomorphids have been known to have many derived features (e.g. Brand, 1956), it was not until Taylor (1969) that they were given a familial rank. The revision of the family by Nussbaum (1985) led to numerous changes in the definition and diagnosis of the family, genera, and species. Nussbaum (1985) re-examined Taylor's (1969) diagnosis of the family, which was based mainly on observations he made on East African species. The main problem with Taylor's diagnosis of the family was his understanding of cranial morphology, which conflicted with previous reports (de Villiers, 1938; Brand, 1956). The inconsistencies prompted Nussbaum to re-examine these cranial characters in addition to reviewing other features. Nussbaum (1985; p.3) redefined the family, using various skull, muscle, and hyobranchial characters. Some of the characters which Taylor (1969) placed an emphasis on diagnosing the family; strong diastema between the provomerine and palatine teeth, palatine teeth posterior to the maxillary teeth and squamosals and parietals separated by large diastema, were re-evaluated and redefined and used to differentiate between East and West African species, and this provided evidence for the subsequent recognition of West African Scolecomorphids in the new genus *Crotaphatrema* (Nussbaum, 1985).

The genus *Crotaphatrema* is known from a total of only eight specimens, with three species recognised (*C. bornmulleri, C. lamottei, and C. tchabalmbaboensis*) (Lawson, 2000). These species are separated on the number of annuli, colouration, and various head measurements. Little is known of their natural history, and extent of variation between and among each member of this genus. Species are found

distributed along the montane forests of Cameroon, and it has been speculated that more species exist in the poorly investigated tropical forests of this region of West Africa (Lawson, 2000). *Scolecomorphus* species are known from many more specimens, and currently three species are recognised (*S.kirkii, S.uluguruensis, S.vittatus*). There has however been much confusion regarding the status of certain *Scolecomorphus* species, as they have been poorly defined (Nussbaum, 1985). Confusion has arisen with the description of species and subspecies based, it seems, primarily on geographically separated populations and aberrant morphological characters (Barbour and Loveridge, 1928; Loveridge, 1953; 1957; Taylor, 1968).

Fig. 5.2.

Scolecomorphus vittatus from Amani Nature Reserve, East Usamabara. Scale unknown.

Boulenger (1883) described *Scolecomorphus kirkii* based on a single specimen, probably found 'from the vicinity of Lake Tanganyika'. Following this, further material became available to Boulenger (1895) from Malawi, which he also placed in the species *S. kirkii*. This species was then characterised by its distribution in Malawi, because this was the only definite locality. Correspondence between Dr H. W. Parker at the Natural History Museum, London and Arthur Loveridge (Loveridge, 1953) revealed that the locality of the holotype of *S. kirkii* was initially recorded as Mpwapwa, and was subsequently struck out and replaced with 'probably about Lake

Tanganyika' (Loveridge, 1953;p.332). The former locality might be considered a more probable location, because Mpwapwa is a village between the Ukaguru and Rubeho Mountains within the Eastern Arc Mountains, the centre of *Scolecomorphus* diversity, seemingly more probable than Lake Tanganyika where no other caecilian record exists. Fieldwork around the river basins of Lake Tanganyika provided no evidence for the occurrence of *Scolecomorphus* in the region (Loader, unpublished). However, the town of Mpwapwa is located on the train line connecting Western Tanzania to coastal Tanzania, and in former times an outpost village for travellers going from East to West (Howell, 2000), and therefore could conceivably account for the contradictory locality data of Mpwapwa and Lake Tanganyika.

Uncertainties over the provenance of the holotype of *S. kirkii* have caused difficulties for interpreting population and species differences in the genus. This problem is particularly acute when bearing in mind the level of amphibian endemism in the EAM (Howell, 1993), where many species are often restricted to single mountain blocks (e.g. Menegon *et al.* 2004). Nussbaum (1985) carried out a morphometric survey of *Scolecomorphus* in an attempt to address the question of morphological variation among populations of *Scolecomorphus* species, and thereby investigate species boundaries, and potentially, the affiliation of the holotype *S. kirkii,* among other questions. In this study, he demonstrated that the holotype of *S. kirkii* clustered with populations from Malawi, Southern Highlands (Ubena), Udzungwa, Uluguru and Rubeho and not with populations of *S. vittatus* or *S. uluguruensis*. Nussbaum (1985) felt he could confidently distinguish among all *Scolecomorphus* species,' based on multivariate statistical analyses, however no single morphometric or meristic trait could diagnose species. This difficulty in diagnosing species on a single morphological character was highlighted by Nussbaum's (1985;p.45) key for the genus, which is entirely based on colour. Taken at face value then, Nussbaum's results suggest the holotype probably originated from one of the *S. kirkii* localities, e.g. Malawi, Southern Highlands, Udzungwa, Uluguru and Rubeho, unless in the unlikely event specimens are eventually uncovered from Lake Tanganyika region (>2,000km away) that are morphologically similar to the holotype. Furthermore based on Nussbaum's analyses, the holotype cannot be excluded from occurring in Mpwapwa (Rubeho) a possible locality for the holotype. Defining the characteristics and distribution of *S. kirkii* is important for interpreting new populations (Nussbaum, 1985; Loader *et al.* 2003a; Gower *et al.* 2004).

Following Boulenger's 1883 description of *S. kirkii*, he described the new genus and species *Bdellophis vittatus* (1895) based on material from the Usambara, which he thought showed a distinct eye position, differing substantially from *S. kirkii*. However, the genus *Bdellophis* was later synonymised, and the species placed in the genus *Scolecomorphus* by Barbour and Loveridge (1928; p.179) who showed the eye differences accounted for by Boulenger (1895) was a 'sign of youth, ossification developing with age so that the eyes of adults are concealed'. Barbour and Loveridge recognised the species *S. vittatus* as occurring in the East and West Usambara Mountains and Uluguru Mountains, which was latter verified by Nussbaum (1985; p.42), despite a 'wide scatter' in multivariate analyses which he concluded may reflect geographical variation. More recently the species *S. vittatus* has been shown to occur in the Nguru, North Pare Mountains and possibly Mombassa in Kenya, the latter based on an old museum specimen held at the NHM (Nussbaum, 1985; Emmrich, 1994). A third species, *S. uluguruensis,* was described (Barbour and Loveridge, 1928), based on material collected by Loveridge from the Uluguru Mountains. Sympatric with both *S. kirkii* and *S. vittatus,* this species was shown to be different based primarily on a distinct colour pattern. Subsequent work has shown distinct differences in phallodeum ornamentation (Wake, 1998) and head proportions (Nussbaum, 1985).

In addition to *S. uluguruensis*, Barbour and Loveridge (1928;p.181) also described the species *S. attenuatus* from the same locality as *S. uluguruensis,* which it resembled but differed in body proportions and colouration, 'being jet-black' in colour. However, Nussbaum's (1985) reappraisal of this material showed that *S. attenuatus* is a junior synonym of *S. uluguruensis*. Nussbaum (1985) suggested the colour difference and slenderness of *S. attentuatus* was a result of the poor state of preservation.

Taylor (1968) made some modifications to the genus by dividing *Scolecomorphus kirkii* into two species, with *S. kirkii* occurring in Malawi and Uluguru, and the new species *S. convexus* from the south-central highlands (Iringa region- presumably Udzungwa and Southern Highlands), perplexingly placed geographically between *S. kirkii* populations of the South and North (see Fig. 5.3). However, Nussbaum (1985) also synonmised *S. convexus,* because these specimens were indistinguishable from *S. kirkii,* the differences Taylor used to differentiate the species were the result of desiccation, which resulted in an aberrant morphological feature (Nussbaum, 1985).

Currently, three species can be found distributed along all the Eastern Arc Mountains in Tanzania, and South to the highlands in southern Tanzania, Malawi and Mozambique, but are absent from the Taita Hills in Kenya (shown in Fig. 5.3 and an accompanying table).

The limited number of samples from 'scattered' localities available to Nussbaum (1985) precluded definite conclusions about colour variation (e.g. North Pare population of *S. vittatus* p.30). It seems likely that many cryptic species might be present, based on the level of speciation of other animal and plant groups (particularly amphibians and reptiles) in the area and limited data on variation in *Scolecomorphus* species (e.g. Nussbaum, 1985; Gower *et al.* 2004). The inadequate number of samples available to previous workers has meant a full appreciation of the scolecomorphids of the Eastern Arc has not been possible. Over the past 10 years, samples from Frontier-Tanzania and fieldwork carried out in this study have increased the number of specimens available from each mountain block, which has prompted a preliminary investigation of species boundaries and relationships in the EAM in this study.

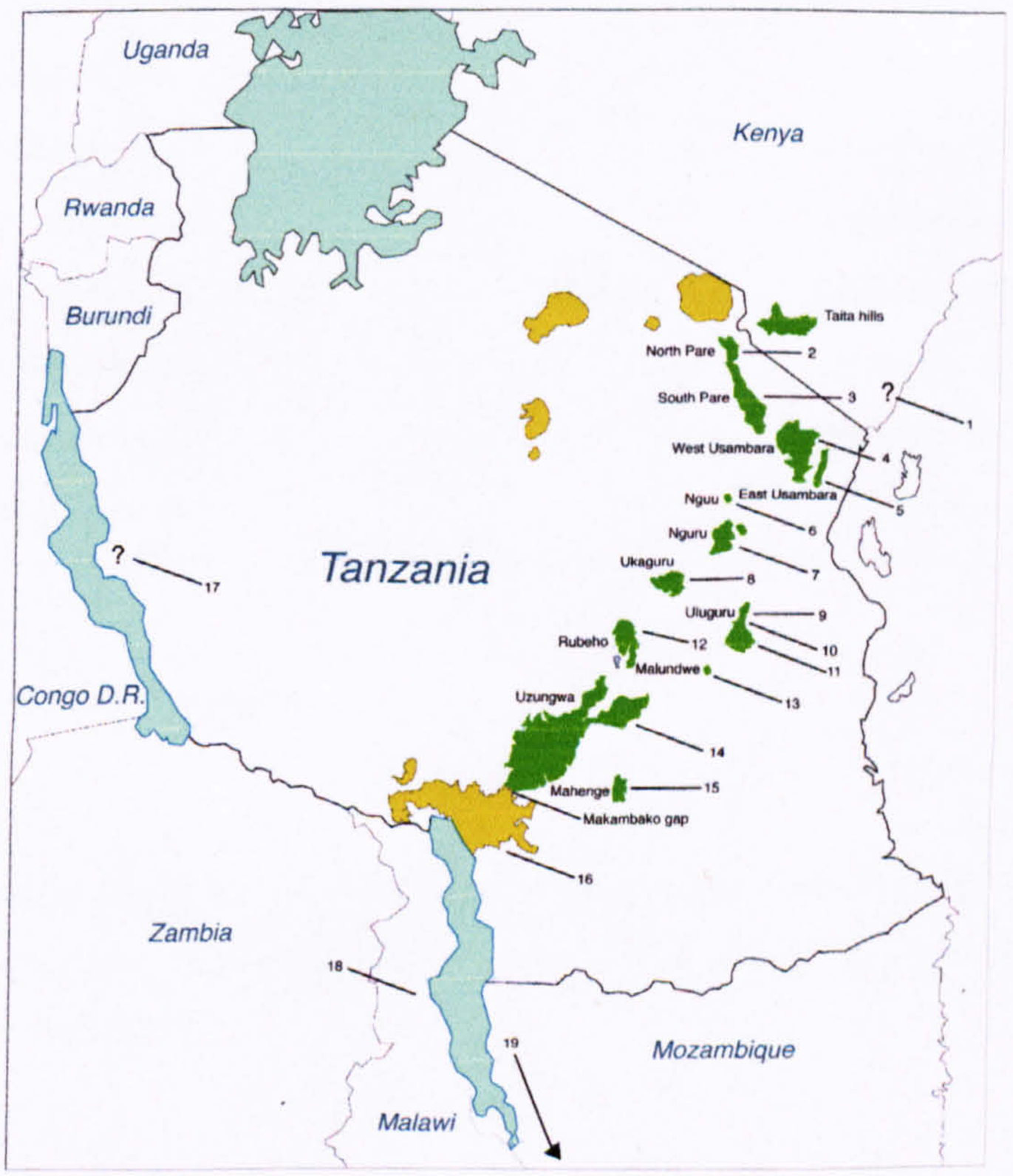

Fig. 5.3

Distribution of the caecilian *Scolecomorphus* in East Africa. See Table 5.1 for key to species.

Table

5.1. List of the occurrence of *Scoleomorphus* species in East Africa and sampled in this study. With 62% of populations sampled.

	Species	Locality	Reference	Sampled
1	*Scolecomorphus vittatus*	Mombassa, Kenya	Nussbaum, 1985	X
2	*Scolecomorphus vittatus*	North Pare, Tanzania	Nussbaum, 1985	√
3	*Scolecomorphus sp.*	South Pare, Tanzania	This study	√
4	*Scolecomorphus vittatus*	West Usambara, Tanzania	Barbour and Loveridge, 1928	√
5	*Scolecomorphus vittatus*	East Usambara, Tanzania	Boulenger, 1895	√
6	*Scolecomorphus sp.*	Nguu, Tanzania	Menegon, *et al.* 2003b	√
7	*Scolecomorphus kirkii*	Nguru, Tanzania	Emmrich, 1994	X
8	*Scolecomorphus sp.*	Ukaguru, Tanzania	This study	√
9	*Scolecomorphus vittatus*	Uluguru, Tanzania	Nussbaum, 1985	X
10	*Scolecomorphus kirkii*	Uluguru, Tanzania	Nussbaum, 1985	√
11	*Scolecomorphus uluguruensis*	Uluguru, Tanzania	Nussbaum, 1985	√
12	*Scolecomorphus kirkii*	Rubeho, Tanzania	Nussbaum, 1985	√
13	*Scolecomorphus sp.*	Malundwe, Tanzania	Howell, 1993	X
14	*Scolecomorphus kirkii*	Udzungwa, Tanzania	Loveridge, 1935	√
15	*Scolecomorphus kirkii*	Mahenge, Tanzania	Loader, *et al.* 2004	√
16	*Scolecomorphus kirkii*	Southern Highlands, Tanzania	Loveridge, 1935	X
17	*Scolecomorphus kirkii*	Lake Tanganyika? Mwapawpa?	Boulenger, 1883	X
18	*Scolecomorphus kirkii*	Cholo, Malawi	Loveridge, 1935	X
19	*Scolecomorphus sp.*	Nissa, Mozambique	Branch (pers. com.)	√

5.3 Materials and methods

5.3.1 Specimens

Specimens were collected from various sources (see Appendix 2). Once tissues were assembled, DNA extraction, amplification and sequencing were carried out (see section 2.3 for methods).

Table 5.2. *Scolecomorphus* and outgroups sequenced in this study.

Sequence number	Specimens	Species	Locality	Forest Reserve
T7	RAN 31529	*Scolecomorphus sp.*	Unknown	Unknown
T17	SG 5700	*Scolecomorphus vittatus*	West Usambara	Ambangula FR
T20	SG 5589	*Scolecomorphus vittatus*	East Usambara	nr Amani
T160	UFS 5662	*Scolecomorphus vittatus*	West Usambara	Mazumbai FR
T172	MW 901	*Scolecomorphus vittatus*	East Usambara	Amani-Kwamkoro
T174	KMH 22480	*Scolecomorphus kirkii*	Udzungwa	West Kiombero Scarp FR
T175	KMH 22703	*Scolecomorphus kirkii*	Udzungwa	West Kiombero Scarp FR
T176	KMH 22168	*Scolecomorphus kirkii*	Udzungwa	West Kiombero Scarp FR
T177	KMH 22716	*Scolecomorphus kirkii*	Udzungwa	West Kiombero Scarp FR
T178	KMH 22724	*Scolecomorphus kirkii*	Udzungwa	West Kiombero Scarp FR
T179	KMH 22041	*Scolecomorphus kirkii*	Udzungwa	West Kiombero Scarp FR
T188	CAS 168810	*Scolecomorphus vittatus*	West Usambara	Mazumbai FR
T197	KMH 25021	*Scolecomorphus sp.*	Uluguru	Uluguru South FR
T198	KMH 25024	*Scolecomorphus uluguruensis*	Uluguru	Uluguru North FR
T199	KMH 25000	*Scolecomorphus uluguruensis*	Uluguru	Uluguru North FR
T226	KMH 21262	*Scolecomorphus vittatus*	East Usambara	Nilo FR
T227	KMH 23333	*Scolecomorphus vittatus*	East Usambara	Nilo FR
T228	KMH 21263	*Scolecomorphus vittatus*	East Usambara	Nilo FR
T238	R 096037	*Scolecomorphus kirkii*	Udzungwa	Kilanzi Kihungulu FR
T244	KMH 23344	*Scolecomorphus vittatus*	East Usambara	Nilo FR
T271	MW 1842	*Scolecomorphus kirkii*	Mahenge	Sali FR
T272	MW 1846	*Scolecomorphus kirkii*	Mahenge	Sali FR
T276	MW 1897	*Scolecomorphus kirkii*	Rubeho	Mafwemiro FR
T427	MW 3054	*Scolecomorphus kirkii*	Ukaguru	Ikwamba FR
T430	MW 3070	*Scolecomorphus vittatus*	North Pare	Kindoroko FR
T431	MW 3072	*Scolecomorphus vittatus*	North Pare	Kindoroko FR
T433	MW 3114	*Scolecomorphus vittatus*	Nguu Mountians	Handeni side
T435	MW 3141	*Scolecomorphus vittatus*	South Pare	Chome FR
T440	MW 3278	*Scolecomorphus kirkii*	Uluguru	Uluguru North
T441	KMH 23346	*Scolecomorphus vittatus*	East Usambara	Mgambo FR
T442	MW 3048	*Scolecomorphus kirkii*	Ukaguru	Ikwamba FR
T443	MW 3115	*Scolecomorphus vittatus*	Nguu Mountians	Handeni side
T457	MW 3202	*Scolecomorphus vittatus*	South Pare	Chome FR
T469	MW 3832	*Scolecomorphus kirkii*	Udzungwa	Udzungwa Scarp Forest
T493	Ni197	*Scolecomorphus kirkii Crotaphatrema*	Mozambique	Nissa
N/a	UTA 51667	*tchabalmbaboensis*	Cameroon	Mount Tchabal Mbabo
N/a	UTA 38889	*Herpele squalostoma*	Cameroon	Mundemba
N/a	UTA 51487	*Dermophis mexicanus*	Guatemala	Izabal, Morales
T438	MW 3225	*Schistometopum gregorii*	Tanzania	Bagamoyo
N/a	MW 331	*Gegenophis ramaswami*	India	Thenmalai

5.2.2 Phylogenetic analyses

Phylogenetic analyses were carried out as detailed in section 2.7. Two alignments were used to investigate relationships among Scolecomorphids, (1) All scolecomorphids and outgroups used for calibrating molecular clock estimates (2) Only *Scolecomorphus* species, with the species *Scolecomorphus uluguruensis* used to root the tree. *S. uluguruensis* was shown to be unambiguously basally positioned in all preliminary analyses. This alignment resulted in the inclusion of data unalignable in the first alignment.

5.3.3 Molecular divergence estimates

Molecular divergence dates between specific clades were estimated by adding a number of taxa that provided calibration points (see section 2.7.2, and 3.2.3 for precise details and approaches for these calibrations).

5.4 Results

5.4.1. Phylogeny

5.4.1.1 Data Quality and Details

Partial 12S, 16S and *cytb* data were collected for all samples apart from T17 for which I was unable to amplify the first part of the *cytb*. The data are significantly different from randomly permuted data sets (P= 0.0001). No significant base composition differences were shown across all taxa analysed (chi-squared tests for homogeneity, P = 1). The full alignment showed significant rate heterogeneity, though only marginally (Δ=60.78668, P=<0.05). The alignment of only *Scolecomorphus* taxa was also shown to have significant rate heterogeneity (Δ=156.50658, P=<0.001). The incongruence length difference test showed no significant incongruence between any data partition (P<0 0.98).

Separate and combined analyses were carried out which resulted in a few minor differences, but overall the phylogeny was similar between all analyses whether different data partitions were combined or separately analysed which is congruent with the ILD test results. Branch lengths for *cytb*, 12S and 16S data partitions show similar rates of molecular evolution (Fig. 5.4a-c). An analysis of each *cytb* site showed 3rd positions to have a transition/transversion rate greater than other

positions (see Fig. 5.4d-f). Third position analysis shows greater resolution at tree tips and increased number of changes relative to other positions (Fig. 5.4d-f).

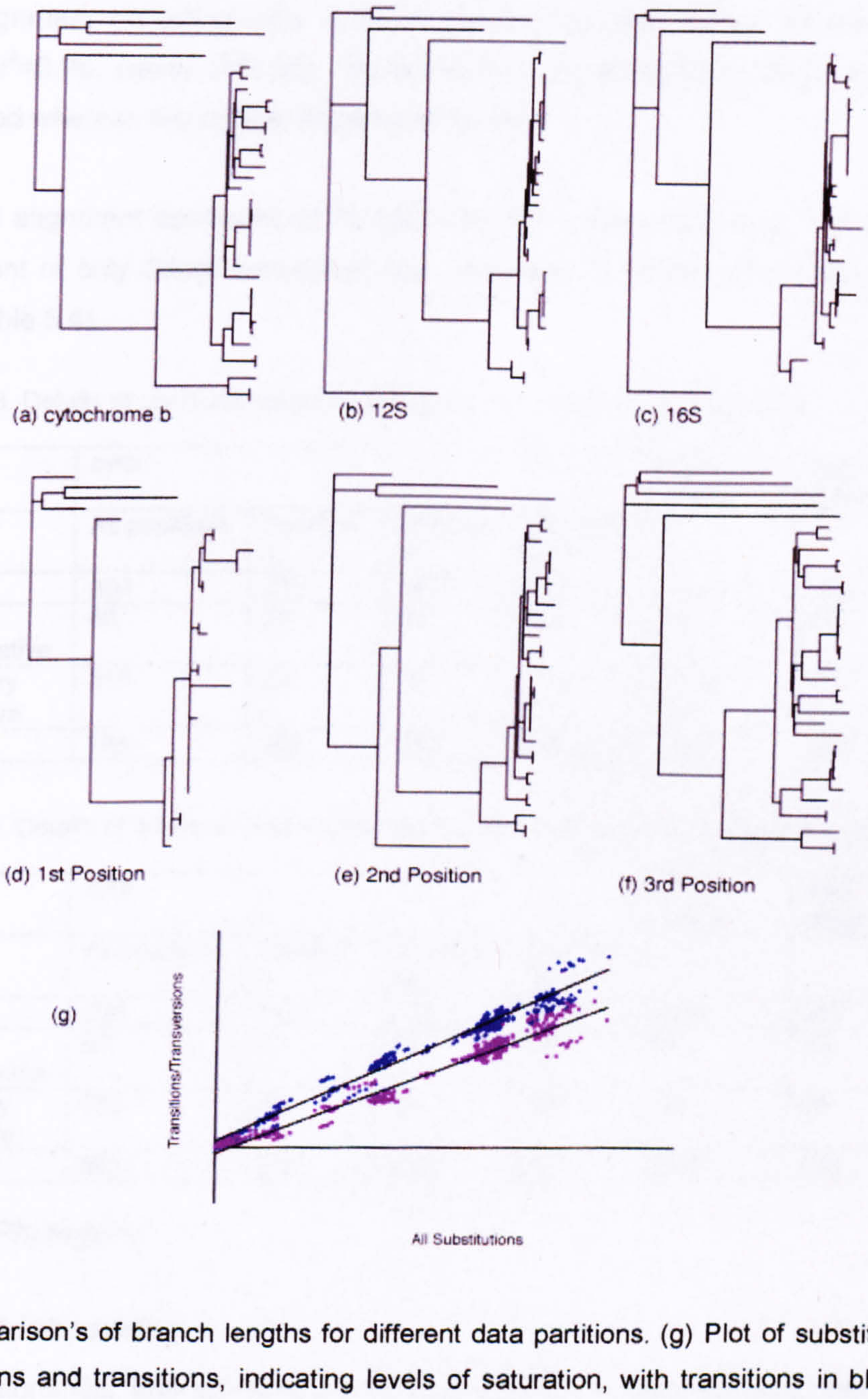

Figure 5.4.

(a-f) Comparison's of branch lengths for different data partitions. (g) Plot of substitution of transversions and transitions, indicating levels of saturation, with transitions in blue, and transversions in purple, r^2 value for transitions ($r^{2=}0.9726$) and transversions ($r^{2=}0.9356$).

Saturation plots were calculated for each gene partition and *cytb* codon position, these plots indicate that saturation is evident in the data (summarised in Fig. 5.4g) shown by substantial overlap in points of transitions and transversions. Saturation

levels were further investigated for each alignment by fitting a linear and power regression lines to a plot of transitions and transversions. Results (not shown) indicate that the full alignment is better fitted using power regression lines, whereas the alignment including only *Scolecomorphus* showed similar correlation levels (linear r^2=0.96, power r^2=0.95). These results indicate that the larger alignment is saturated whereas the smaller alignment less so.

The full alignment consisted of 40 taxa and 1575 characters (see Table 5.3). The alignment of only *Scolecomorphus* taxa consisted of 36 taxa and 1653 characters (see Table 5.4).

Table 5.3. Details of character informativeness for the full alignment of 40 taxa.

	cytb				12S rRNA	16S rRNA	Total
	All positions	Position 1	Position 2	Position 3			
Constant	384	215	157	12	178	274	836
Variable-uninformative	86	25	33	28	56	53	195
Parsimony informative	319	23	73	223	108	117	544
Total	789	263	263	263	342	444	1575

Table 5.4. Details of character informativeness for the *Scolecomorphus* alignment of 35 taxa.

	cytb				12S rRNA	16S rRNA	Total
	All positions	Position 1	Position 2	Position 3			
Constant	493	246	198	49	284	373	1150
Variable-uninformative	61	12	25	24	6	32	99
Parsimony informative	252	10	46	196	54	98	404
Total	806	268	269	269	344	503	1653

5.4.1.2 Phylogeny

5.4.1.2.1 Introduction

The relationships inferred from the mtDNA alignment from 40 taxa, including 36 Scolecomorphids are summarised in Fig.5.5. and 5.6. The alignment including only 36 *Scolecomorphus* samples, with an extra 51 characters, is summarised in Fig.5.7. The results of the analyses of the larger alignment using maximum likelihood and Bayesian methods produced almost identical topologies, but differed from parsimony. Analyses of fewer taxa (exclusively *Scolecomorphus* species) shows a similar

resolution, but showing a more robust support of relationships between leaves, as shown by the higher Bayesian posterior probabilities. This alignment also shows slightly greater correspondence between each tree building method, with fewer contradictory topologies. For this reason, the alignment including only *Scolecomorphus* species will be discussed more fully. The specific status of each species as described by Nussbaum is treated separately in section 5.4.1.2.4.

5.4.1.2.2 Full Alignment

In the full alignment all three methods clearly indicate the monophyly of scolecomorphids, the basal position of *S. uluguruensis* relative to all other *Scolecomorphus* species, and the monophyly of most (also see section 5.4.1.2.3) mountain populations (West Usambara, East Usambara, North Pare, South Pare, Ukaguru, Mahenge, and Udzungwa). The relative phylogenetic position of all the monophyletic populations, which may or may not represent the two described species *S. kirkii* and *S. vittatus*, is sensitive to the method of analyses, and is weakly supported at some nodes. Bayesian posterior probabilities suggest the following clades are well supported, though not necessarily how they relate to one another: (North and South Pares) (Ukaguru, East Usambara, Nguu) (Mahenge, Mozambique). Parsimony and distance analyses show only weak support for most relationships for populations of *S. kirkii* and *S. vittatus*, with the clades (North and South Pare) (Ukaguru, Nguu, and East Usambara) shown as being moderately supported. All analyses therefore are in agreement with the monophyly of almost all mountain haplotypes, and in addition the clades including Pare populations and an Ukaguru, Nguu, and East Usambara clades.

5.4.1.2.3 Scolecomorphus only alignment

With the exception of "*S. vittatus*" from the East Usambara Mountains, all analyses where more than one haplotype is sampled for each mountain population, the monophyly of these clades are robustly supported. The relationships among each mountain population show varying degrees of resolution, and depend upon the tree building method. Bayesian analyses and likelihood analyses show total agreement in relationships and the posterior probabilities also show high support for most nodes, with values of 95 or greater (see Fig. 5.7). This is in sharp contrast with parsimony bootstrap proportions (see Fig.5.6), which are substantially lower and may suggest Bayesian probabilities show inflated support values. Bayesian and likelihood

reconstructions show geographical proximity being significant in explaining the relationships among populations, with adjacently located areas usually associated. This is shown in the following clades: Southern EAM (Mahenge, Mozambique, Rubeho, Uluguru, and Udzungwa), and Northern EAM (Ukaguru, Nguu, East Usambara), (North and South Pare). The weakest part of the tree in all analyses appears to be the splits between southern EAM populations (e.g. Mahenge, Mozambique, Uluguru, Rubeho and Udzungwa- see Figure 5.7)

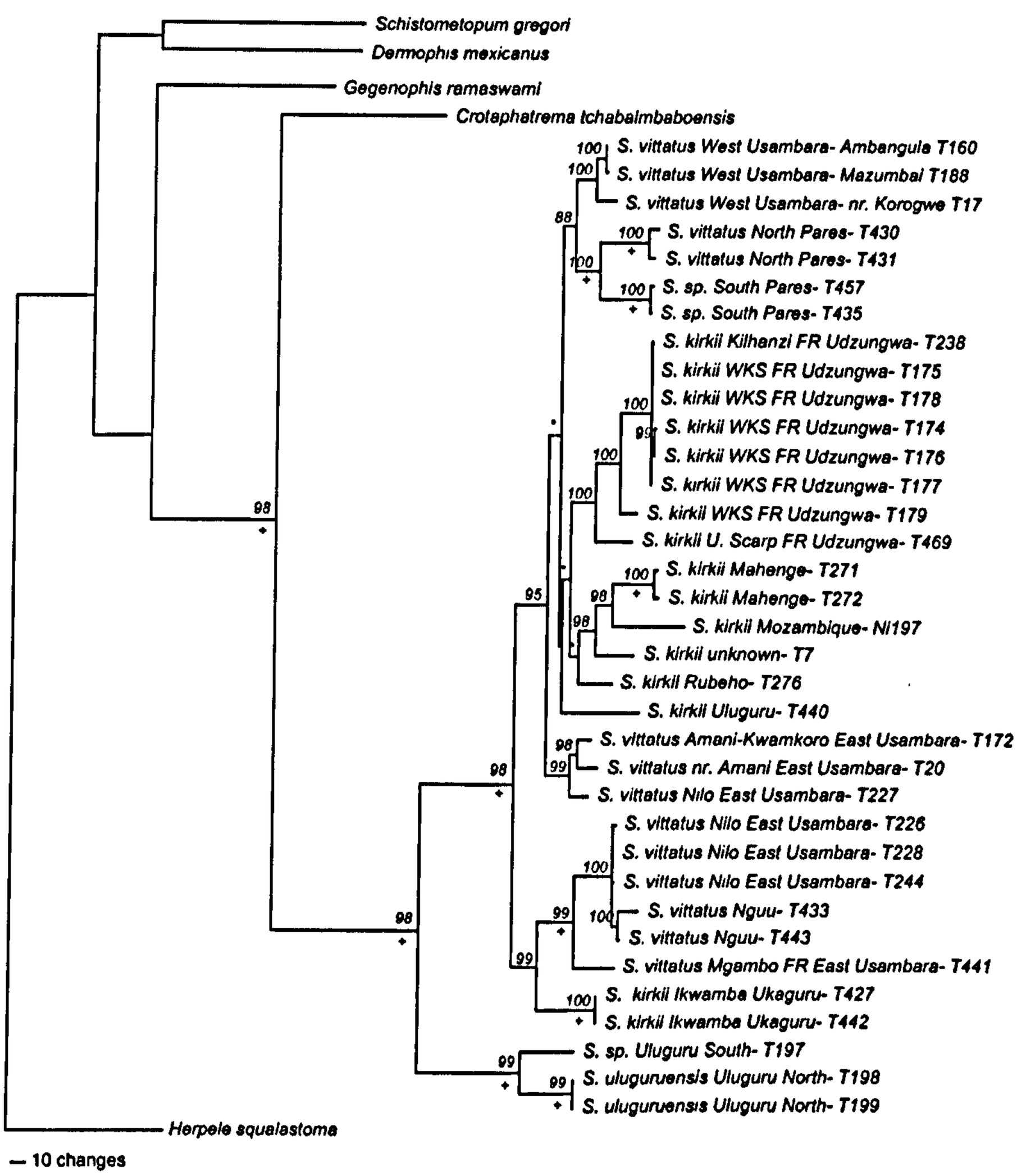

Figure 5.5

Maximum likelihood tree (LnL= 10990.51188), GTR+I+G model selected from Modeltest. Base frequencies estimated at 0.3700, 0.2375, 0.1129 and 0.2796 for A, C, G and T respectively, substitution rates =1.0000, 3.9850, 1.0000, 1.0000, 7.0603, and the proportion of invariant sites set at 0.3302 and a gamma distribution shape parameter of 0.5651. Values on branches show Bayesian posterior probabilities, *= less than 85. Below branches shows SH test results (+= significant at =<0.05).

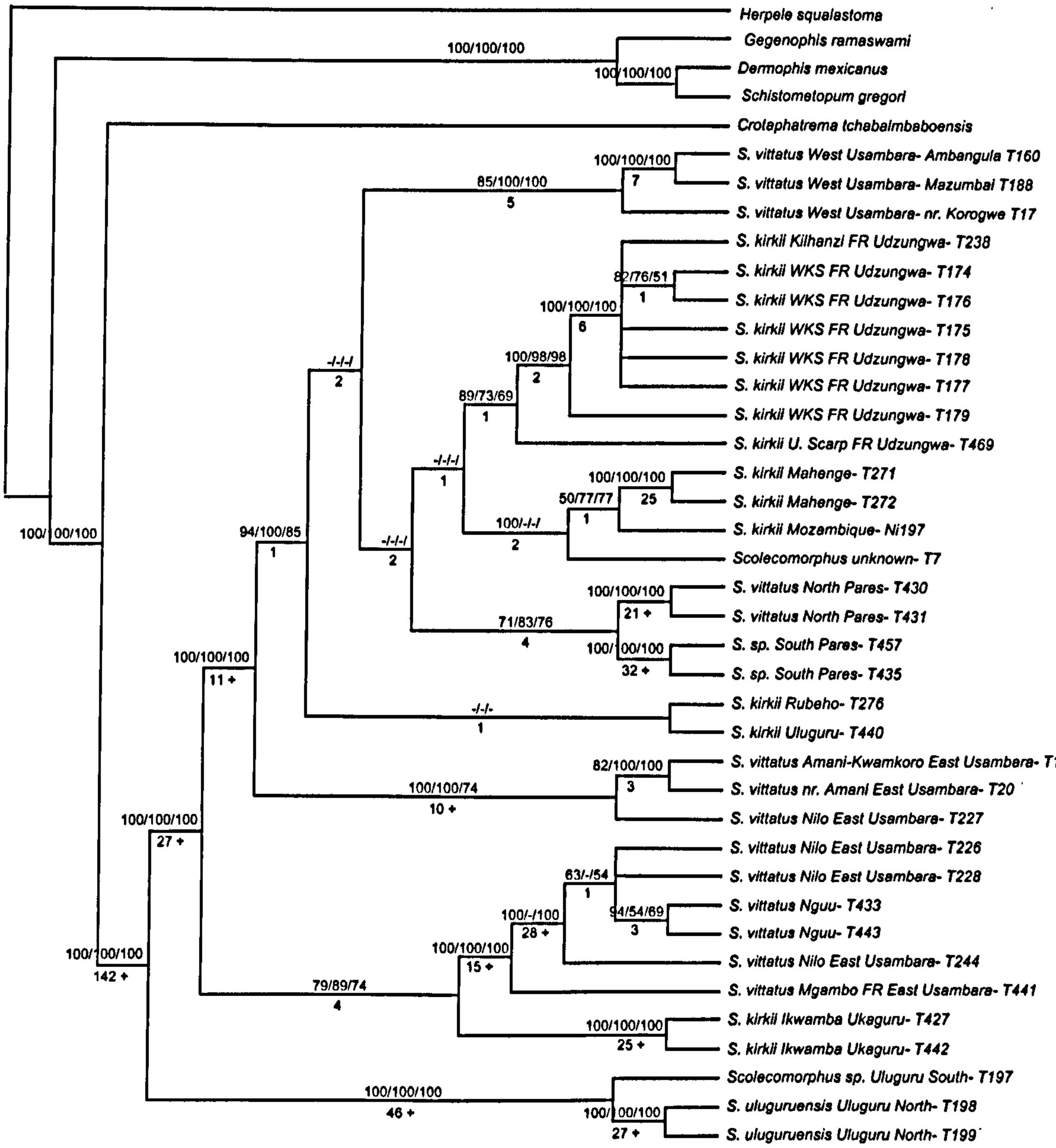

Figure 5.6.

Strict consensus of 14MPTs, tree length 2087. Bootstrap proportions shown above branches for parsimony, ML distance, and log-det distance. Decay Index values are shown below along with Templeton test result (+= significant <0.05).

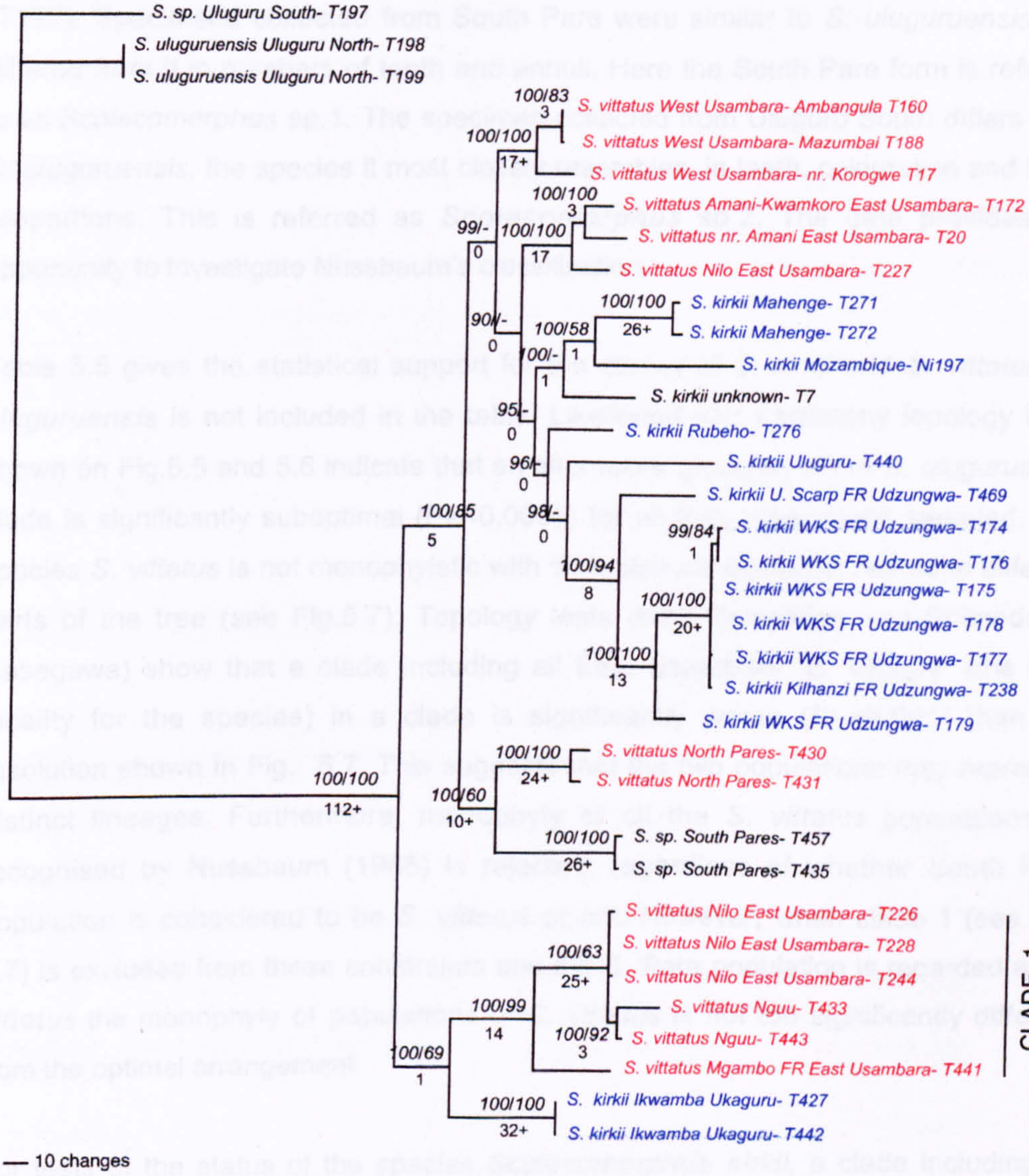

Figure 5.7.

Maximum likelihood tree (LnL= 7708.79510), GTR+I+G model using Modeltest. Base frequencies estimated at 0.33320, 0.21730, 0.15830 and 0.29120 for A, C, G and T respectively, substitution rates = 1.0000 3.9850 1.0000 1.0000 7.0603, and the proportion of invariant sites set at 0.5116 and a gamma distribution shape parameter of 0.7689. Values on branches show Bayesian posterior probabilities and bootstrap proportions for parsimony. Bremer support values are shown below, and SH test results (+= significant at <0.05). Red colour refers to specimens currently referable to *S. vittatus* and Blue colour refers to *S. kirkii*.

5.4.1.2.4 Species boundaries in Scolecomorphus

Each specimen sampled was identified based on the morphological key provided by Nussbaum (1985). All specimens could be assigned to a species apart from some specimens collected in this study from the South Pares and Uluguru South FR

(T197). Specimens collected from South Pare were similar to *S. uluguruensis*, but differed from it in numbers of teeth and annuli. Here the South Pare form is referred to as *Scolecomorphus* sp.1. The specimen collected from Uluguru South differs from *S. uluguruensis*, the species it most closely resembles, in teeth, colouration and head proportions. This is referred as *Scolecomorphus* sp.2. The data provides the opportunity to investigate Nussbaum's classification.

Table 5.5 gives the statistical support for the clades of *S. kirkii* and *S. vittatus*. *S. uluguruensis* is not included in the table. Likelihood and parsimony topology tests shown on Fig.5.5 and 5.6 indicate that an alternative grouping of the *S. uluguruensis* clade is significantly suboptimal (P=<0.0001) for all three specimens sampled. The species *S. vittatus* is not monophyletic with divergent clades found nested in different parts of the tree (see Fig.5.7). Topology tests (both Templeton and Shimadairo-Hasegawa) show that a clade including all East Usambara "*S. vittatus*" (the type locality for the species) in a clade is significantly worse (P=<0.001) than the resolution shown in Fig. 5.7. This suggests that the two populations may represent distinct lineages. Furthermore, monophyly of all the *S. vittatus* populations as recognised by Nussbaum (1985) is rejected, regardless of whether South Pare population is considered to be *S. vittatus* or not. However, when clade 1 (see Fig. 5.7) is excluded from these constraints and the S. Pare population is regarded as *S. vittatus* the monophyly of populations of *S. vittatus* is not too significantly different from the optimal arrangement.

For tests of the status of the species *Scolecomorphus kirkii,* a clade including all recognised populations is shown to be significantly suboptimal from the arrangement in Fig.5.6. However, if the Ukaguru population is excluded this is not shown to be a significantly different grouping.

5.4.2 Molecular divergence estimates

5.4.2.1 Consistency of calibration estimates

Calibration points were evaluated for their consistency, and thereby to quantify the potential confounding effects this may have on divergence estimates. Two calibration points were used to calculate the divergence times, and the reliability of each of these were scrutinised by fixing one calibration point and using this to estimate the

other. The confidence intervals shown in Table 5.6 allow the rejection of the hypothesis that multiple fixed calibration points based on geological evidence show substantial difference in divergence estimates to single fixed estimated calibration points.

Table 5.5. Statistical support for alternative hypotheses of phylogenetic relationships of *Scolecomorphus*, using parsimony methods for both alignments. * significant (P=<0.05).

Tree Includes	Number of extra steps	Templeton test
Monophyly of all recognised *Scolecomorphus vittatus* populations (without S.Pare).	19	0.0126*
	97	<0.0001*
Monophyly of all recognised *Scolecomorphus vittatus* populations (with S.Pare considered as *S. vittatus*).	26	0.0212*
	91	<0.0001*
Monophyly of all recognised *Scolecomorphus vittatus* populations (excluding clade 1)	12	0.0522
	15	0.0482*
Monophyly of all recognised *Scolecomorphus vittatus* populations (excluding clade 1 and S. Pare considered as *S. vittatus*)	4	0.6069
	12	0.08626
Monophyly of all recognised *Scolecomorphus kirkii* (all *kirkii*)	22	0.0042*
	85	<0.0001*
Monophyly of all recognised *Scolecomorphus kirkii* (all *kirkii* except Ukaguru population)	1	0.989
	1	0.976

Table 5.6. Consistency of dating estimates using single calibration point estimation.

	Dermophis- Schistometopum (constrained 101 mya)	*Gegenophis- (Africa clade)* (constrained 130 mya)
Divergence estimate as given from single fixed calibration point	PL- 111.15 LF- 111.28 (97.08-126.83)	PL- 118.56 LF- 117.98 (102.32-132.46)

5.4.2.2 Absolute time estimates for Scolecomorphus

Significant rate heterogeneity was identified in the data set, and thereby estimation of divergence times was carried out using methods that could account for rate differences, i.e. Penalized likelihood approach. Confidence intervals were obtained using the Langley-Fitch method. The two estimates are shown in Table 5.7. The different estimation methods show considerable overlap in divergence estimates, and there is little difference between dates, which might suggest rate variation is not sufficiently acute to render molecular dates unusable. PL methods generally show more recent divergence estimates. Analyses of data partitions with third positions of *cytb* data removed did not conflict significantly with any of the results shown below (not shown).

Table 5.7.

Absolute divergence times in Myr. for clades within *Scolecomorphus.* Refer to Fig.5.8 for precise position of nodes.

Most recent common ancestor (MRCA)	Estimation method	
	Penalized Likelihood	Langley-Fitch
1. Crotaphatrema, Scolecomorphus.	91.20	87.81 (82.56-93.98)
2. All *Scolecomorphus.*	43.31	43.45 (38.56-47.72)
3. Scolecomorphus uluguruensis clade.	11.79	11.90 (9.73-14.41)
4. Scolecomorphis uluguruensis-Uluguru North FR clade.	0.13	0.23 (0.10-0.56)
5. Scolecomorphus- Ukaguru, (Nguu, East Usambara).	14.21	15.23 (13.28-18.06)
6. Scolecomorphus- Mgambo, (Nguu, East Usambara).	8.00	8.60 (6.56-10.97)
7. Scolecomorphus- Nguu, East Usambara.	0.92	1.01 (0.81-1.28)
8. Scolecomorphus vittatus, S. kirkii clade-(refer to node).	19.43	20.19 (17.92-22.46)
9. Scolecomorphus- Rubeho, (Mozambique, Mahenge).	12.57	13.28 (11.78-16.27)
10. Scolecomorphus- (Mozambique, Mahenge).	10.44	11.01 (9.46-12.86)
11. Scolecomorphus-(refer to node)	13.18	13.78 (12.42-15.33)

12. Scolecomorphus- South Pare, North Pare.	11.07	11.95 (10.37-13.46)
13. Scolecomorphus- Mahenge.	0.75	0.81 (0.52-0.98)
14. Scolecomorphus- North Pare.	0.67	0.93 (0.73-1.16)
15. Scolecomorphus- South Pare.	0.34	0.50 (0.27-0.86)
16. Scolecomorphus- Udzungwa clade.	9.23	9.87 (8.20-11.53)
17. Scolecomorphus- Udzungwa clade.	4.63	4.93 (3.68-6.39)
18. Scolecomorphus- Udzungwa clade.	0.22	0.15 (0.11-0.55)
19. Scolecomorphus- East Usambara clade.	4.81	5.04 (3.48-6.88)
20. Scolecomorphus- West Usambara clade.	3.64	3.91 (2.57-4.79)
21. Scolecomorphus (refer to node)	12.91	12.94 (10.42-14.33)

It is notable that many recent speciation events appear to be clustered, and not continuous. According to molecular clock estimates, a large proportion of the lineage divergence events in *Scolecomorphus* seemed to correspond to 10-14Myr (nodes 3,5,6,9,10,11,12 and 21).

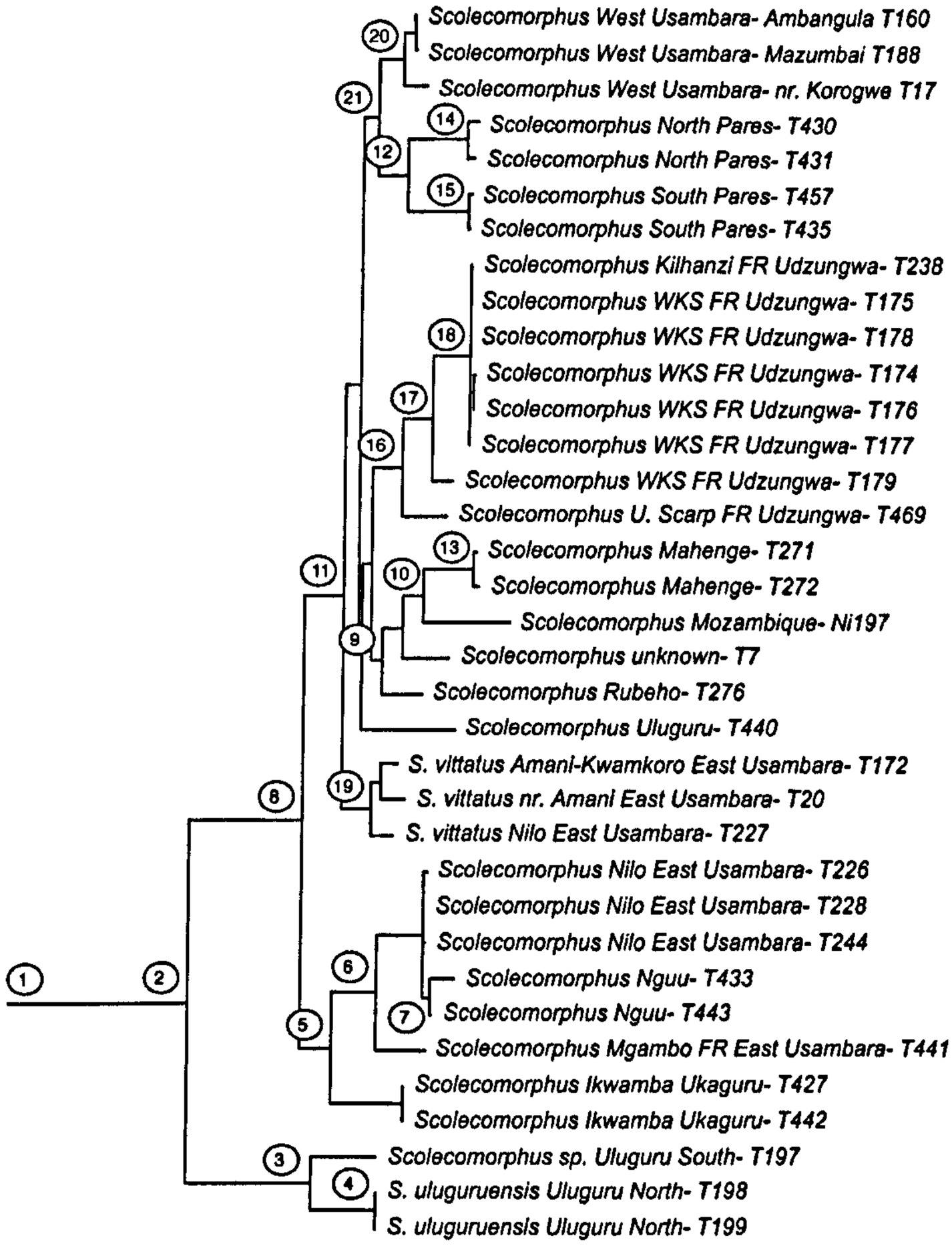

Figure 5.8.

Phylogeny of *Scolecomorphus* with nodes calculated for molecular divergence times using r8s.

5.5 Discussion

5.5.1 Phylogeny

5.5.1.1 Higher level relationships

Taking into consideration that this study presents only a very limited sampling of caecilian families, molecular data presented here support previous analyses of caecilian phylogeny (Wilkinson *et al.* 2003), whereby scolecomorphids are monophyletic. The Scolecomorphids sampled in this study were recovered as a putative clade in all analyses. This includes representatives of both genera; *Crotaphatrema* and *Scolecomorphus*, and a representative of almost all populations of *Scolecomorphus* known to occur. The clade comprising *Crotaphatrema* and

Scolecomorphus is robustly supported, which is consistent with the limited morphological data available (Nussbaum, 1985). A previous study by Wilkinson *et al.* (2003) unambiguously recovered a *Scolecomorphus* clade, and showed its relatively basal position (see section 5.2.3 for details). Furthermore, preliminary analyses of an alignment including the largest number of African caecilian species, and genera sampled (Loader *et al.* unpublished), including data gathered from this study, show congruence with the arrangement of scolecomorphids as shown by Wilkinson *et al.* (2003).

5.5.1.2 *Scolecomorphus*

Species level taxonomy

Scolecomorphus is distributed in an area of high endemism, and it would be predicted based on their presumed limited dispersal ability (Taylor, 1968) that as in other amphibian groups, many species would occur in this region. The most recent revision of scolecomorphids (Nussbaum, 1985) using morphology recognised three species with overlapping distributions in the EAM. The molecular phylogeny presented in this study allows the explicit testing of species limits and boundaries based on our current understanding of the genus *Scolecomorphus* (Nussbaum, 1985). Analyses presented in this study include a comprehensive sampling of all known species and almost all known and newly discovered representatives of *Scolecomorphus* populations.

Well-corroborated evidence from different mitochondrial genes and partitions suggest that the species *S. uluguruensis* is sister group to all other *Scolecomorphus* species, and is highly divergent from all other species. The distinctiveness of *S. uluguruensis* is also supported by limited morphological evidence, which suggests a similar resolution; males have spines on the phallodeum, and both sexes have a distinct colouration differing from *S.kirkii* and *S.vittatus* (Nussbaum, 1985; Wake 1998). Results suggest the existence of an undescribed species (T197), as shown by substantial molecular differences. This single specimen was captured from the Uluguru South FR, in the Uluguru Mountains (a new locality for *Scolecomorphus*) and is similar to *S. uluguruensis*, which is currently confined to Uluguru North (Nussbaum, 1985). Interestingly, the forest reserve Uluguru South is ecologically different from the submontane forests of Uluguru North FR; the reserve is covered by moist forest,

with upland grassland areas, swamps and forest patches of the Lukwangule plateau (Lovett, and Pocs, 1993; Menegon *et al.* 2004). The Ulugurus are an area of extraordinary rich amphibian diversity (Barbour and Loveridge, 1928), with many endemics. Menegon *et al.* (2004) showed that the Uluguru Mountains harbour many species of the bufonid *Nectophrynoides*, with some species restricted to either Uluguru North or Uluguru South. These results suggest the distinctness of these two areas. Further sampling of populations and diverse taxa will be necessary to test the suggestion that Uluguru South and Uluguru North have substantially different endemic faunas.

The statuses of *S. vittatus* and *S. kirkii* are much more complicated. For *S. vittatus* the data suggests that the current understanding of the distribution and morphological variability in this species as outlined by Nussbaum (1985) is inadequate. The first obvious problem is the recognition of two deeply divergent *S. vittatus* haplotypes in the East Usambaras, populations that are syntopic. A statistical assessment using topological constraints showed that grouping all specimens identified as *S. vittatus* (Nussbaum, 1985) from the East Usambara into a single clade was significantly suboptimal. The suggestion is that at least two species, one currently undescribed, can be recognised from the East Usambara. Morphological analysis of these populations will be necessary to investigate this more fully. However preliminary data collected during this study and by Gower *et al.* (2004) indicate both populations appear to show distinctive morphological variation. Alternatively, it should also be considered that the molecular differences could reflect a diverse polymorphism in the ancestral population, which as a result of random lineage sorting events have produced widely divergent mitochondrial haplotypes (Avise, 1994). Therefore, the results may not be indicative of non-interbreeding populations. This cannot be ruled out, but the consistent morphological and molecular differences would appear to favour the previous scenario at present.

Based on examination of the holotype of *S. vittatus* (BM1946.9.5.59) this species is characterised by a pointed snout, and dark thin mid-dorsal stripe which contrasts with a much more reddish ventral colouration. This phenotype agrees with that of the sequences for T172, T20 (voucher specimen not inspected), and T227. And based on this morphological similarity, these populations most closely represent *S. vittatus* as first described by Boulenger. In contrast the other haplotype of *S. vittatus* (Clade 1 in Fig.5.7) in the East Usambara (T226, T228, T244) has a broad rounded snout with

a larger mid dorsal stripe and lighter cream coloured ventral region. This larger headed population also attains a greater size (Gower *et al.* 2004) and appears to be more broadly distributed within the EAM; occurring in the Nguu Mountains (T433, T443), and the lowland woodlands (Mgambo FR- T441) and the submontane forests (Nilo FR- T226, T228, T244) in the East Usambara, whereas *S. vittatus* appears to be restricted to higher submontane regions of the East Usambara. The occurrence of the larger headed population at areas of both higher and lower altitude may account for the recent patterns of diversification between the Nguus and East Usambara. A careful assessment of these populations will be necessary, because curiously, populations of East Usambara (T226, T228, T244, T441 separated by only ~20km) are rendered paraphyletic by Nguu haplotypes (T433, T443 ~180km from East Usambara).

Confronted by small sample sizes and a broad scatter of morphological variation, Nussbaum, (1985) was unable to distinguish two species in the East Usambara. This may also have obscured his ability to distinguish distinct populations elsewhere. Populations outside of the East Usambara (e.g. West Usambara, Pares) may have shown overlapping characters with the combined populations in the East Usambara, but if these populations were treated as separate, other populations may have been distinguished. This problem it seems would have been further exacerbated by the fact that scolecomorphids show significant sexual dimorphism (Nussbaum, 1985) and that Nussbaum most importantly only had available a limited number of specimens and hence limited power in the statistical analysis. Considering the difficulties in distinguishing between caecilian species in general (Nussbaum and Wilkinson, 1989), even with large sample sizes (Gudynas *et al.* 1988; Nussbaum and Pfrender, 1998; Presswell, 2002) these findings are not surprising. Once each population from the East Usambara is correctly distinguished re-examination of the morphological variation of populations from Nguru, Pares, and Ulugurus may uncover additional cryptic species. These speculations seem to be borne out by the molecular trees recovered in all analyses that show a large genetic diversity exhibited between each mountain population, suggesting they are distinctive. Whether each population warrants recognition at the species level awaits detailed study of morphology. An indication that each population most probably represents a new species is suggested by the paraphyletic position of *S. vittatus* populations, albeit weakly supported in some analyses, and preliminary morphological work on populations from North and South Pare, which show that they are readily distinguishable. If both Pare

populations are considered as new species, then it would be logical to infer other populations (e.g. West Usambara) with similar levels of divergence are likely to represent new species. It is also noteworthy that recent detailed molecular and morphological studies of other amphibian groups from the EAM (presented in this thesis and published papers e.g. de Sà *et al.* 2004; Menegon *et al.* 2004) have suggested high levels of species diversity and endemism.

The specific status of *S. kirkii* cannot be explicitly tested in this molecular study, because sampling of the holotype was not possible (and of uncertain locality). A combined molecular and morphological study will be necessary to fully evaluate this species. However, based on the optimal trees it is possible to indicate the likely patterns in these populations that may focus morphological studies. The most significant result is the strongly supported placement of a species currently referable to *S. kirkii* from the Ukaguru, which lies outside of the main *S. kirkii* clade and more closely related to *S. vittatus*. Topology tests confirm that the placement of this group in a monophyletic *S. kirkii* clade is significantly suboptimal. The placement of this sample is robustly supported in all analyses and would therefore appear to represent a divergent lineage, probably an undescribed species (see Fig. 5.6). A clade including mainly southern located EA populations (Mahenge, Mozambique, Udzungwa, Uluguru and Rubeho) is relatively well supported in ML and Bayesian analyses. This resolution is not well supported in parsimony analysis, which only shows close affinities between Mahenge, Udzungwa and Mozambique haplotypes with the other clades unresolved in a polytomy. Within this clade the lineages are divergent, even within mountain blocks (e.g. Udzungwa) and potentially may represent distinct species. The recognition of mtDNA clades as putative species is precluded at present because of ambiguous support for clades and the uncertain morphological variation in these populations. Further data will need to be collected before species level taxonomy can be tackled satisfactorily.

Species Level Phylogeny

Poor hierarchic structure is found among samples in the large clades of "*S. vittatus*" *and "S. kirkii"*, with little resolution in the branching pattern between each mountain population. There are a number of possible explanations for the lack in resolution, with an emphasis placed either on the quality of the data, or interpreting the patterns as a phenomenon of the speciation process. Patterns may also be the result of both processes, so it is difficult to decipher between all the possible explanations. The

data presented could suggest the phylogeny is strongly compressed due to saturation of sites at deeper nodes, because there is significant rate variation and saturation in the dataset. However, in an alignment with only *Scolecomorphus* species saturation was shown to be less important and the branching pattern at basal nodes was still compressed and is similar to the results of the larger alignment. In addition, compression of these nodes is consistently shown in all data partitions, including 1st, 2nd, and 3rd position of *cytb* (see Fig.5.3).

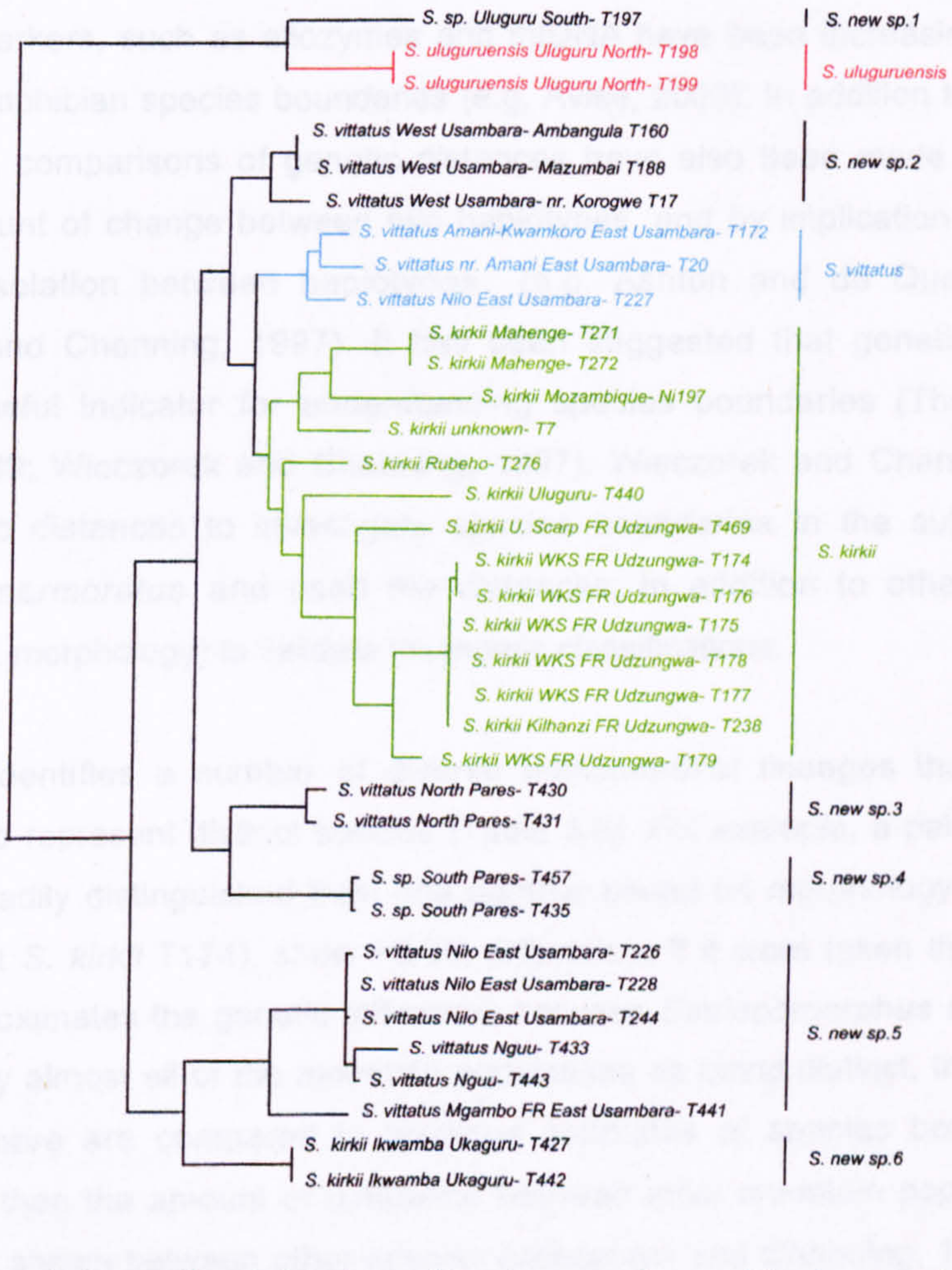

Figure 5.9.
Summary of the phylogenetic relationships of *Scolecomorphus* and the taxonomic findings.

If the poor hierarchic structure is not artifactual, it is possible that the patterns are due to rapid speciation over a short time, and or both that speciation may have occurred simultaneously due to a uniform extrinsic factor, suggesting a 'true polytomy'. Similar patterns have been shown in the clades of African greenbuls in the EAM (Roy, 2001), which suggests it might be indicative of speciation patterns in the area (see

Biogeography section for discussion). Determining which causal pattern is more likely is difficult bearing in mind the limited understanding we have of the region, caecilian biology, and the speciation process in general. Further work will be necessary to investigate this further, and collection of more sequence data may help to resolve these issues.

Genetic distance and species boundaries

a. Species

Molecular markers, such as allozymes and mtDNA have been increasingly used to determine amphibian species boundaries (e.g. Avise, 2000). In addition to evaluating phylogenies, comparisons of genetic distances have also been made to infer the relative amount of change between two haplotypes, and by implication the relative degree of isolation between haplotypes (e.g. Ashton and de Queiroz, 2001; Wieczorek and Channing, 1997). It has been suggested that genetic distances maybe a useful indicator for understanding species boundaries (Thorpe, 1982; Highton, 1989; Wieczorek and Channing, 1997). Wieczorek and Channing (1997) used genetic distances to investigate species boundaries in the subspecies of *Hyperolius marmoratus* and used the distances, in addition to other character systems (e.g. morphology) to validate taxonomic classifications.

This study identifies a number of diverse mitochondrial lineages that could be considered to represent distinct species (Table 5.8). For example, a pair of species which are readily distinguished from one another based on morphology (*S. vittatus* T172 against *S. kirkii* T174), show ~5.8% difference. If it were taken that this then roughly approximates the genetic difference between *Scolecomorphus* species this would identify almost all of the mountain populations as being distinct. In addition, if the results here are compared to previous estimates of species boundaries in amphibians, then the amount of difference between most mountain populations far exceeds that shown between other species (Wieczorek and Channing, 1997;>2% in 12S genes for frogs; Gower *et al.* 2002; >3% for Ichthyophid caecilians). Such conclusions would suggest that we currently severely underestimate the diversity of *Scolecomorphus* species. However, the uncertainties surrounding the factors that have caused the genetic heterogeneity (e.g. possible lineage sorting effects) mean that an attempt to define species boundaries based on these differences alone is not pursued or advised in general in this study.

Table 5.8. Summary of the geographical distance (km) (above the diagonal) and % genetic distances (for 12S, 16S and *cytb* genes) between *Scoleomorphus* populations (below the diagonal), using exemplars. Geographical distances provided by Neil Cox (Conservation International) apart from Mozambique, which was approximated.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. *S. uluguruensis* (T199) Uluguru		35	213	219	260	~630	144	35	189	353	288	244	110
2. S. sp. (T197) Uluguru	5.5		270	271	230	~600	129	35	158	376	313	273	110
3. *S. vittatus* (T188) W.Usambara	14.8	14.6		25	472	~815	275	213	381	172	102	36	214
4. *S. vittatus* (T172) E.Usambara	14.2	14.3	4.5		478	~815	292	219	393	189	120	26	230
5. *S. kirkii* (T271) Mahenge	14.8	14.4	5.1	5.1		~430	255	260	130	592	535	504	292
6. *S. kirkii* (Ni197) Mozambique	15.6	15.5	6.2	6.4	5.9		~600	~630	~500	~900	~860	~820	~600
7. *S. kirkii* (T276) Rubeho	14.6	14.4	5.2	5.3	4.5	5.9		144	130	352	306	311	62
8. *S. kirkii* (T440) Uluguru	15.7	14.8	6.2	5.9	6.6	7.7	5.8		189	353	288	244	110
9. *S. kirkii* (T174) Udzungwa	15.3	14.6	5.4	5.8	6.3	7.1	5.0	6.8		479	428	416	178
10. *S. vittatus* (T430) N.Pare	14.2	14.2	5.2	5.5	5.8	7.7	5.4	6.7	6.6		70	171	302
11. *S. vittatus* (T435) S.Pare	14.4	14.2	5.3	5.6	6.5	7.9	5.9	7.2	7.3	5.3		105	250
12. *S. vittatus* (T226) E.Usambara	14.6	14.5	7.7	7.5	8.5	9.5	7.6	9.3	8.4	8.9	9.1		249
13. *S. kirkii* (T427) Ukaguru	14.6	14.3	7.4	7.7	8.9	9.3	7.5	8.7	8.2	7.8	8.2	6.7	

b.Within Population Variation

Generally, each mountain population shows fairly limited levels of within lineage divergence (excluding the two highly divergent lineages of *S. vittatus*), and these patterns are consistent with a scenario of recent interbreeding within these mountain localities (e.g. Mahenge, Ukaguru, Pares). However, a number of populations show large genetic heterogeneity within potentially interbreeding populations (e.g. Usambaras, Udzungwa). Larger differences are exhibited between mountain populations where denser spatial sampling of haplotype lineages has been carried out, which perhaps would be expected. However, some divergences are still so large that the lineages may potentially be new species given the difference (e.g. Udzungwa *S. kirkii*) and these need to be examined carefully in future analyses. It needs to be ascertained based on a larger number of samples whether these divergent lineages

are simply a function of geographical distance, isolation, or perhaps the results are misleading as the gene tree is decoupled from the species trees (lineage sorting effects). The EAM show considerable geological, climatic, and ecological heterogeneity and this may have influenced diversification of lineages, even within mountain populations, resulting in the presence of more than one species for each mountain block.

Conclusions

Overall, the results suggest that there are a number of cryptic species of *Scolecomorphus*, but further analyses of morphology will be necessary to investigate this. Based on molecular data conservative estimates of species diversity in *Scolecomorphus* indicate the genera may contain twice as many species, though three times as many species is a more likely number. The current gross underestimation of species is a result of previously limited sampling of populations in the EAM and the difficulties encountered in diagnosing caecilian species. Future work should also investigate the association between geographic and genetic distances (see also below), which can be examined using Mantel tests or nested-clade analysis (NCA) (Templeton, 1998; Templeton, 2004). The molecular study presented here has provided a vital impetus and a starting point for re-evaluating the systematics of Scolecomorphidae.

5.5.2 Biogeography

5.5.2.1 East and West African Rainforest Biogeography

Certain geological and climatic events are thought to have influenced patterns of speciation in amphibians restricted to the montane forests of East and West Africa (Poynton, 1999). Phylogenies of appropriate amphibian species have not been available, which has prevented the testing of biogegraphic hypotheses (Kingdon, 1989; Lovett, 1993a; Burgess et al. 1998a). Molecular dating estimates presented here suggests that the separation of the West African *Crotaphatrema* and East African *Scolecomorphus* significantly predate a recent dispersal/vicariant event between the two forest regions, and remarkably date back some 90 Myr. Confidence intervals suggest times ranging from 82-93Myr, which significantly predate a period that has been hypothesised as restricting dispersal between regions (20-30Myr). It appears that perhaps once a barrier was established between the East and West

African forests in the Miocene, this may have excluded dispersal between montane areas and thereby restrict dispersal of *Scolecomorphus* or *Crotaphatrema*. Restriction of dispersal between the two montane forest regions has been indicated in similar studies of *Rhampholeon* (Matthee *et al.* 2004).

Estimates of the divide between East and West African scolecomorphids suggest that separation of forests regions in the Miocene did not influence divergence between the two genera. It is unclear what factors may have been critical in separating these two lineages, given the number of possible geological and climatic influences (Morley, 2000; Zachos *et al.* 2001; Trauth *et al.* 2005). The basal node of the *Scolecomorphus* clade is dated at 38-48 Myr, closer to the origin of East and West separation. The significance of this date is difficult to evaluate, but may be associated to the beginning of the restriction of Eastern Arc forests. Overall, the temporal data cannot reject the hypothesis that dispersal of Scolecomorphidae between both East and West African forest was restricted, which is consistent with the biogeographical patterns found in other groups (Grimshaw, 2001).

5.5.2.2 Eastern Arc biogeography

Indications that the isolation and persistence of EAM rainforests may have influenced speciation of amphibians, were first concluded from differences exhibited between the Ulugˈuru and Usambara Mountains assemblages (Barbour and Loveridge, 1928). Barbour and Loveridge (1928) noticed similarities between the fauna of Usambara and Uluguru, but more significantly they described many new species restricted to either mountain range (e.g. *Hoplophryne uluguruensis, Scolecomorphus uluguruensis,* and *Boulengerula uluguruensis*), which suggested a period of isolation that permitted the evolution of new species. The most recent studies (Howell, 1993; Channing *et al.* 2002a; Menegon *et al.* 2004; de Sá *et al.* 2004; Loader *et al.* 2004) have continued to emphasise that long periods of isolation and fragmentation have been important in determining the diversity of EAM forests. However quantitative temporal data is still lacking to support these speculations concerning the amphibian fauna.

a. Spatial Patterns in the EAM

Repeated patterns of area relationships among widely divergent lineages of organisms are thought to be indicative of a shared biogeographic history. A

comparison of *Scolecomorphus* phylogeny with other published phylogenies show interesting similarities. Spatially, Scolecomorphids show some interesting geographical relationships, with associations between northern located EAM populations (Pare, Usambara) and southern (Udzungwa, Uluguru, Rubeho, Mahenge), which is repeated in other studies (e.g. Roy, 1997; Moller and Cronk, 1997; Matthee *et al.* 2004). Furthermore the most southerly positioned mountain block (Mahenge) shows a close phylogenetic relationship with a scolecomorphid distributed south and outside of the EAM (Mozambique). These closer phylogenetic relationships within the northern and southern mountain blocks suggest a more recently shared biogeographical history in these areas, which is not a controversial finding.

There are some notable exceptions to the congruence in spatial relationships, whereby patterns between areas do not correspond with previous findings, expectations, and suggest a significant departure from common biogeographic patterns. This includes the placement of the Nguu clade in the phylogeny. Bowie *et al.* (2004) noted a distinct separation between Nguru and Usambara montane communities, which they suggested was the point at which northern and southern EAM mountains were probably biogeographically divided. Although this study does not sample populations that are known to occur in Ngurus (Emmrich, 1994), the geographically close Nguu (and floristically very similar, e.g. Lovett and Pocs, 1993) is deeply nested in an Usambara clade (see clade 1 Fig. 5.5). If populations from Nguu are similar to Nguru (as would be expected based on preliminary morphological evidence) then this significant biogeographical barrier between southern and northern EAM regions may not exist for scolecomorphid caecilians. Sampling of all the *Scolecomorphus* populations in this area is needed before these hypotheses can be fully tested. Preliminary evidence from other groups also suggests a close association between Nguru, Nguu and Usambara fauna (Menegon *et al.* 2003b; Howell, 1993) in addition to the Ulugurus (Doggart *et al.* 2004). Therefore, Bowie's (2004) findings may not be a general biogeographical pattern but possibly lineage specific. The equidistant position of the Ngurus and Nguu relative to the Uluguru and Usambaras would suggest a mixture from both assemblages is likely, in addition to its own endemic fauna, which is poorly understood (Emmrich, 1994; Menegon *et al.* 2003b).

Based on the geographical proximity of mountains and published phylogenetic studies, spatial patterns do not show congruent area topology in scolecomorphids. The position of the Ukaguru population in the optimal Scolecomorphid trees would be predicted to group more closely to those of southern mountains (e.g. Knox and Palmer, 1998). Instead, the clade is the sister group to the Nguu/Usambara clade. Recent evidence from amphibians (bufonids: Channing and Stanley, 2002; Poynton *et al.* 1998b; Menegon *et al.* 2004; and Microhylids; Channing *et al.* in prep) has shown the Ukagurus harbour a divergent amphibian assemblage in addition to the absence of a number of widespread Eastern Arc taxa (Menegon *et al.* 2004). Based on these findings the area is believed to have been isolated for a relatively long period (Channing and Stanley, 2002; Menegon *et al.* 2004; Channing *et al.* in prep; see also Chapter 4). The discovery of a divergent Ukaguru *Scolecomorphus* clade, possibly a new species restricted to this area, is then consistent with these findings. Whether these coherent amphibian phylogenetic patterns are reproduced in other groups (apart from Knox and Palmer, 1998), and by implication suggest a general biogeographic pattern, is currently uncertain, because our knowledge is still quite rudimentary.

Discordance in spatial patterns provides evidence for a complex biogeographic history in the region. Further evidence from optimal phylogenies (Figs. 5.5, 5.6) suggest that divergence of lineages giving rise to extant Uluguru, East Usambara populations has occurred at least twice. For these populations at least, the evidence suggests more than one single vicariance/dispersal event has occurred. Therefore, the relationships seem to be more in accordance with the hypothesis that more than one event has isolated and reconnected Eastern Arc montane forests (e.g. Roy, 1997; Loader *et al.* 2004b), possibly following an initial gradual fragmentation of EAM. Perhaps more recent dispersal events have obscured a common biogeographic pattern, such as the fragmentation of mountain blocks.

b. Temporal Patterns in the EAM

The most outstanding aspect of the temporal patterns in the genus *Scolecomorphus* is the tempo of speciation patterns. Based on the likelihood analyses and the resulting estimated dates, there is evidence for the long persistence of lineages, coupled by periods of rapid speciation in many lineages (see Fig. 5.10). *Scolecomorphus uluguruensis*, a basal *Scolecomorphus* lineage is estimated to have

persisted for ~40Myr, and if correct suggests the montane forest habitats (Barbour and Loveridge, 1928) that *Scolecomorphus* occur in have also persisted. Prolonged persistence of lineages is also evident in other forest-restricted taxa, supporting the patterns in the *Scolecomorphus* tree (Gravlund, 2002; Matthee *et al.* 2004).

The rapid speciation of many lineages during a relatively short time period may correspond to significant biogeographic events. Although the speciation events in all the lineages may not all be contemporaneous, the timing corresponds roughly to the end of a prolonged period of uplift in the EAM, when each of the EA mountain blocks became separated (Lovett, 1993), occurring between 25Myr- 10Myr (Partridge *et al.* 1995) (see Fig.5.10). Of the 14 lineages only one example shows a recent divergence times between mountains, e.g. East Usambara and Nguu (marked ß in Fig. 5.10). All other examples show substantial periods of isolation approximately ranging from 8-14Myr. The rapid isolation and timing among lineages seems to fit with a biogeographic history of fragmentation.

How plausible are such biogeographic scenarios? There are a few problems with evaluating biogeographic hypotheses. Firstly, ascertaining correlations between speciation and geographic events are generally very problematic, simply because the estimation of divergence times using molecular clock methods are not completely reliable (see section 2.6). Data that is perhaps more reliable than the mitochondrial data used in this study, such as nuclear gene fragments, may allow for more robust calculations, however even with these data sets there is a large degree of ambiguity in calculations (Ayala, 1986). Secondly, even when temporal data can be confidently utilised, many alternative biogeographic scenarios could be envisaged which could, based on the evidence at hand, explain the genetic diversity exhibited between lineages, e.g. vicariance mimicking processes (Hunn and Upchurch, 2001). Often, phylogenetic data are insufficient to allow discrimination between causal factors that can influence speciation patterns in similar ways. As a result, it is often difficult to tease apart the causal processes that have influenced speciation in any particular lineage.

However, even if molecular date estimates are incorrect, which, for example means questioning the precise length of time forests have persisted, such problems do not discount the ability to interpret the speciation patterns in *Scolecomorphus* being punctuated by at least two main periods of diversification. Such multiple speciation

events within a mountain block suggest a more complex biogeographic history than just simple fragmentation, unless significant inter-individual molecular rate difference are used to explain the differences, an unlikely and unprecedented explanation. Fragmentation of the EAM might be invoked as an explanation for influencing the biogeographic history of *Scolecomorphus*. It is unclear if fragmentation is the driving force behind the existing phylogenetic patterns in *Scolecomorphus* and not merely vicariance mimicking patterns. Further data of Scolecomorphids, other lineages, and geological data will be necessary to assess the biogeographic patterns in the region and their causes more effectively. Single studies are inherently speculative, but they do allow the generation of hypotheses that can be tested with other groups or more data.

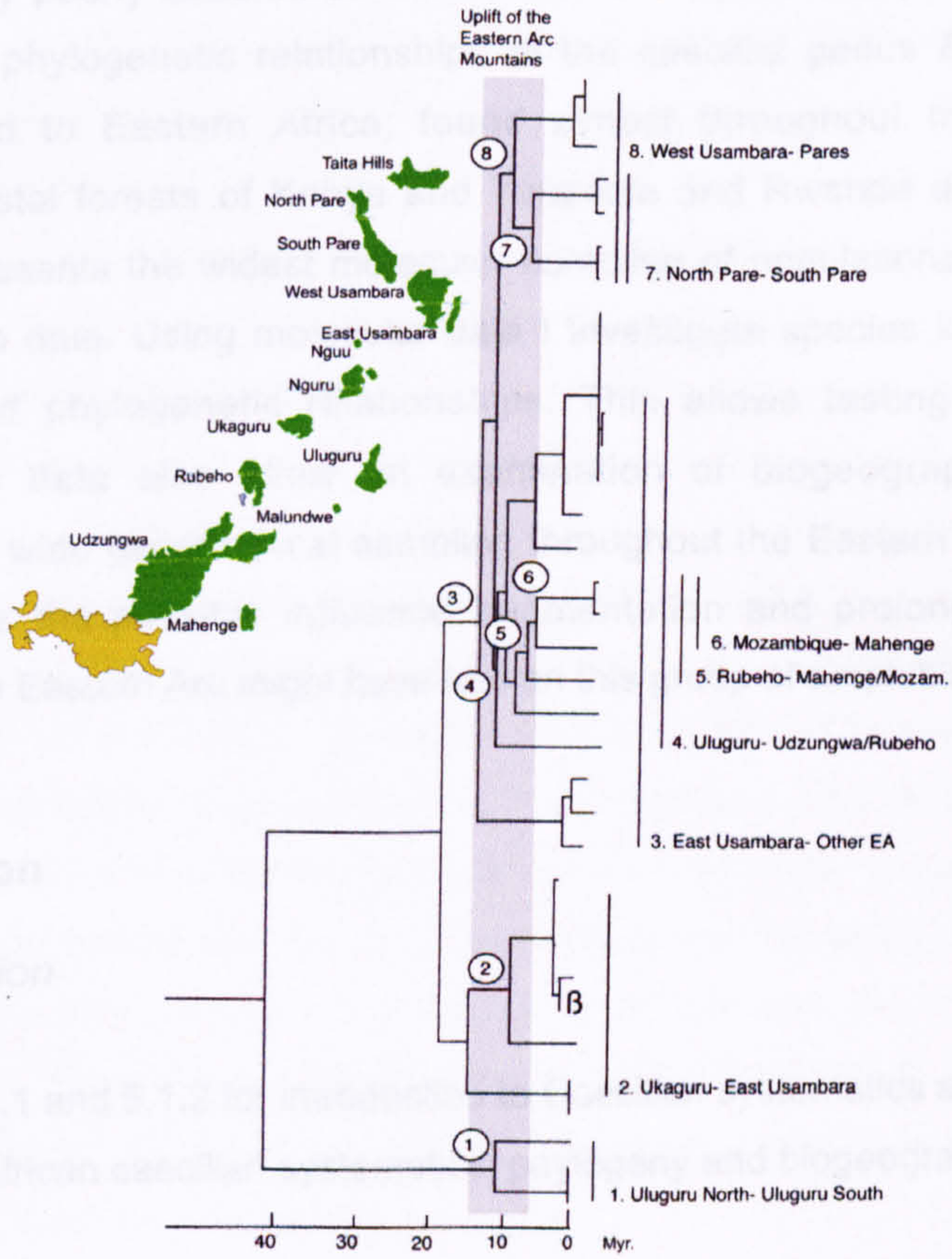

Figure 5.10

Summary of the biogeographical history of *Scolecomorphus*, showing the possible correspondence between speciation events and the fragmentation of the EAM.

Chapter Six

Systematics and Biogeography of *Boulengerula*

6.1 Aims

The caecilian family Caeciliidae has a cosmopolitan distribution, found in Africa, Asia and South and central America. The relationships among genera and many of the species are very poorly understood due to lack of detailed study. In this chapter I investigate the phylogenetic relationships of the caeciliid genus *Boulengerula*, a group restricted to Eastern Africa; found almost throughout the Eastern Arc Mountains, coastal forests of Kenya and Tanzania and Rwanda and Malawi. The study here represents the widest molecular sampling of populations and species of *Boulengerula* to date. Using molecular data I investigate species limits, population differences, and phylogenetic relationships. This allows testing of the current taxonomy. The data also allow an examination of biogeographical patterns. Considering the wide geographical sampling throughout the Eastern Arc and coastal forests I assess the possible influence fragmentation and prolonged isolation of mountains of the Eastern Arc might have had on this group of amphibians.

6.2 Introduction

6.2.1 *Introduction*

(See section 5.1.1 and 5.1.2 for introduction to Caecilian systematics and background to African caecilian systematics, phylogeny and biogeography).

The caeciliid fauna of Africa, excluding the Seychelles, includes six genera and 17 currently recognised species (Wilkinson *et al.* 2004). These species are primarily distributed close to the coast line of East and West Africa, with only 1 species confirmed as occurring in central Africa (*Boulengerula fisheri* from Rwanda; Nussbaum and Hinkel, 1994). Only the genus *Shistometopum* transects the continent of Africa, with one species *S. thomense* (Nussbaum and Pfrender, 1998), described

from San Thome in West Africa and another, *S. gregorii,* from the coastal plains of Tanzania and Kenya (Nussbaum and Pfrender, 1998). All other genera are confined either to the East (*Boulengerula,* and *Sylvacaecilia*) or the West (*Herpele, Idiocranium,* and *Geotryptes*) of equatorial Africa (Nussbaum and Hinkel, 1994). Of these genera, *Boulengerula* is the most speciose African genus, in addition to being one of the more widely distributed.

6.2.1 *Boulengerula*

Boulengerula are found throughout the mountain forests of the Eastern Arc and lowland coastal areas (see Fig.6.1). As currently conceived (Wilkinson *et al.* 2004) the genus comprises 6 species. Tornier (1897) described the first of these, *Boulengerula boulengeri,* from Amani in the East Usambara Mountains (see Fig. 6.1) based on five specimens collected by the German explorer Eisner. Fifteen years later *B. denhardti* was described from the region of the Tana River Delta of Kenya based on a single poorly preserved specimen (Nieden 1912). Although dehydrated, Nieden (1912) was able to easily identify this specimen as being distinct from the only other *Boulengerula* species, *B. boulengeri* by the greater number of annuli. Following Nieden and Tornier, Arthur Loveridge herpetologist at the Museum of Comparative Zoology, Harvard, became a significant force in East African herpetology. He pioneered many expeditions throughout the region, collecting in the many un-surveyed parts of East Africa (Howell, 2000). Loveridge was first in East Africa during the First World War, in the then named German East Africa (Tanzania). During his service, Loveridge made some collections of reptiles and amphibians, including a single caecilian thanks to a fellow colleague, who whilst under 'an unpleasant shell fire' in the Uluguru Mountains' managed to uncover one (Loveridge, 1925; p.764). This specimen, the only caecilian he collected during this time, was initially identified as *B. boulengeri* (Loveridge, 1925), but was later described as the species *B. uluguruensis* (Barbour & Loveridge, 1928) once further samples were collected on his second visit to the Ulugurus in 1926.

Loveridge's several expeditions to East Africa took him to; the Ulugurus and Usambaras (1926); Southern Highlands (1929-30); Kenya and Tanzania (1932); Uganda (1939); Malawi and Mozambique (1948-9) among many others. As a result of Loveridge's surveys, the knowledge of the herpetological fauna was greatly increased, doubling the numbers of amphibians known since Nieden's 1912 publication, which included caecilians, a group Loveridge showed great interest in:

'imagine my joy on seeing a squirmy mass of slimy caecilians' (Loveridge, 1947; p.31). During this period of exploration Loveridge described two more *Boulengerula* species; *B. changamwensis* (Loveridge, 1932) from lowland Changamwe in Kenya; *B. taitanus* (Loveridge, 1935) from the Taita Hills, in Kenya (see Fig.6.1) part of the Eastern Arc Mountains. Loveridge distinguished his species mainly on colouration and annular counts.

Figure 6.1.

Pictures in life of *Boulengerula boulengeri* (left) and *Boulengerula taitanus* (right). Scale unknown.

In addition to describing new caecilian species, Loveridge also made significant amendments to caecilian taxonomy. During Loveridge's time in Kenya he made collections of caecilians from the Tana River, an area he interpreted as including the holotype localities of the three caeciliid species that had been previously reported from this region, *Dermophis gregorii* (Boulenger, 1894), *Boulengerula denhardti* (Nieden, 1912), and *Bdellophis unicolor* (Boettger 1913). Loveridge's material included only a single species of caecilian, and based on a comparison of his material with published reports for the three named species, Loveridge (1936) concluded that all three names were synonymous, with the oldest name, *D. gregorii*, having priority. Later, Parker (1941) partitioned African and Neotropical *Dermophis*, and designated *gregorii* the holotype species of the strictly African genus *Schistometopum*. He and subsequent workers, except Wilkinson *et al.* (2004) see below, accepted Loveridge's view that both *B. unicolor* and *B. denhardti* were junior synonyms of *S. gregorii* (Taylor, 1968; Nussbaum and Hinkel, 1994; Nussbaum and Pfrender, 1998).

Taylor (1968) reviewed the genus *Boulengerula*, recognising the four species described by Tornier (1897), Barbour and Loveridge (1928) and Loveridge (1932; 1935), but removing three of the species (*B. changamwensis, B. taitanus,* and *B. uluguruensis*) to a newly designated genus *Afrocaecilia,* with *B. boulengeri* remaining as the sole species of *Boulengerula*. Taylor (1968) reasoned that the absence of splenial teeth and fusion of the tip of the tongue to the gum in the species *B. changamwensis, B. taitanus,* and *B. uluguruensis* were such distinct morphological differences that distinction at the generic level was warranted. Nussbaum and Hinkel (1994) concluded that Taylor's split between these groups was unjustified and 'artificial' and they preferred to recognise a single genus *Boulengerula* as originally conceived (Nussbaum and Hinkel, 1994; p.754). Their justification for this was primarily based on an analysis of the phylogenetic relationships (using 17 morphological characters) of *Boulengerula* that contradicted Taylor's scheme. Their analysis showed that the genera *Boulengerula* and *Afrocaecilia* are possibly paraphyletic, and therefore did not support Taylors (1968) concept of *Afrocaecilia*.

In addition to reviewing the genus, Nussbaum and Hinkel (1994) provided a description of a new species *B. fisheri* from the cloud forests of Rwanda. *B. fisheri* is a long slender species, with a high number of primary annuli (186), compared to the other species: *B. boulengeri* (124-134); *B. changamwensis* (140-148); *B. uluguruensis* (128-144); and *B. taitanus* (137-144). Nussbaum and Hinkel (1994) also provided an updated key to the species, principally based on annular counts, colouration, and presence and absence of teeth series. Distribution data were also extended for certain species, *B. uluguruensis* was shown to occur in the Ngurus (based on Emmrich, 1994), for the species *B. changamwensis* referral of specimens extended the distribution from Changamwe to the Shimba Hills in coastal SE Kenya (NMK L/1887) to a disjunct population 1320km south in the Shire Highlands in Malawi (BMNH 92.12.31.45). Lastly, a Masters thesis by the Danish herpetologist Martin Vestergaard (1994) also extended the distribution of *B. boulengeri* based on collections he made throughout the Usambaras, showing the presence of the species in the West Usambara Mountains, but with the suggestion that this population might be specifically distinct.

Following a re-examination of the holotype of *B. denhardti,* Wilkinson *et al.* (2004) confirmed Nieden's (1912) assessment that this form represented a distinctive species of *Boulengerula,* contra to Loveridge's (1936) assessment that the species

was a junior synonym of *Schistometopum gregorii*. Accordingly, Wilkinson *et al.* (2004) resurrected *B. denhardti* from synonymy. They also showed that the morphological data used by Nussbaum and Hinkel (1994) were insufficient to resolve relationships with any confidence, or evaluate the *Afrocaecilia* concept. No taxonomic changes were suggested by Wilkinson *et al.* (2004), however their analysis left open questions regarding the systematics and taxonomic classification of this group.

The goals of this study were to estimate the phylogeny of *Boulengerula* using 12S, 16S and *cytb* and elucidate the history of biogeographic diversification in *Boulengerula* in the Eastern Arc Mountains. Specifically, I used phylogenetic data to evaluate (1) species boundaries, based on the most complete survey to date of *Boulengerula* populations from Kenya and Tanzania (2) taxonomic classifications proposed by various authors (Taylor, 1968; Nussbaum and Hinkel, 1994) based on morphology (3) the pattern of speciation in the group; both the timing and sister group relationships, which may allow an inference on the biogeographic history of the group and how this correlates with the climatic and geological history of the EAM and coastal region of Kenya and Tanzania.

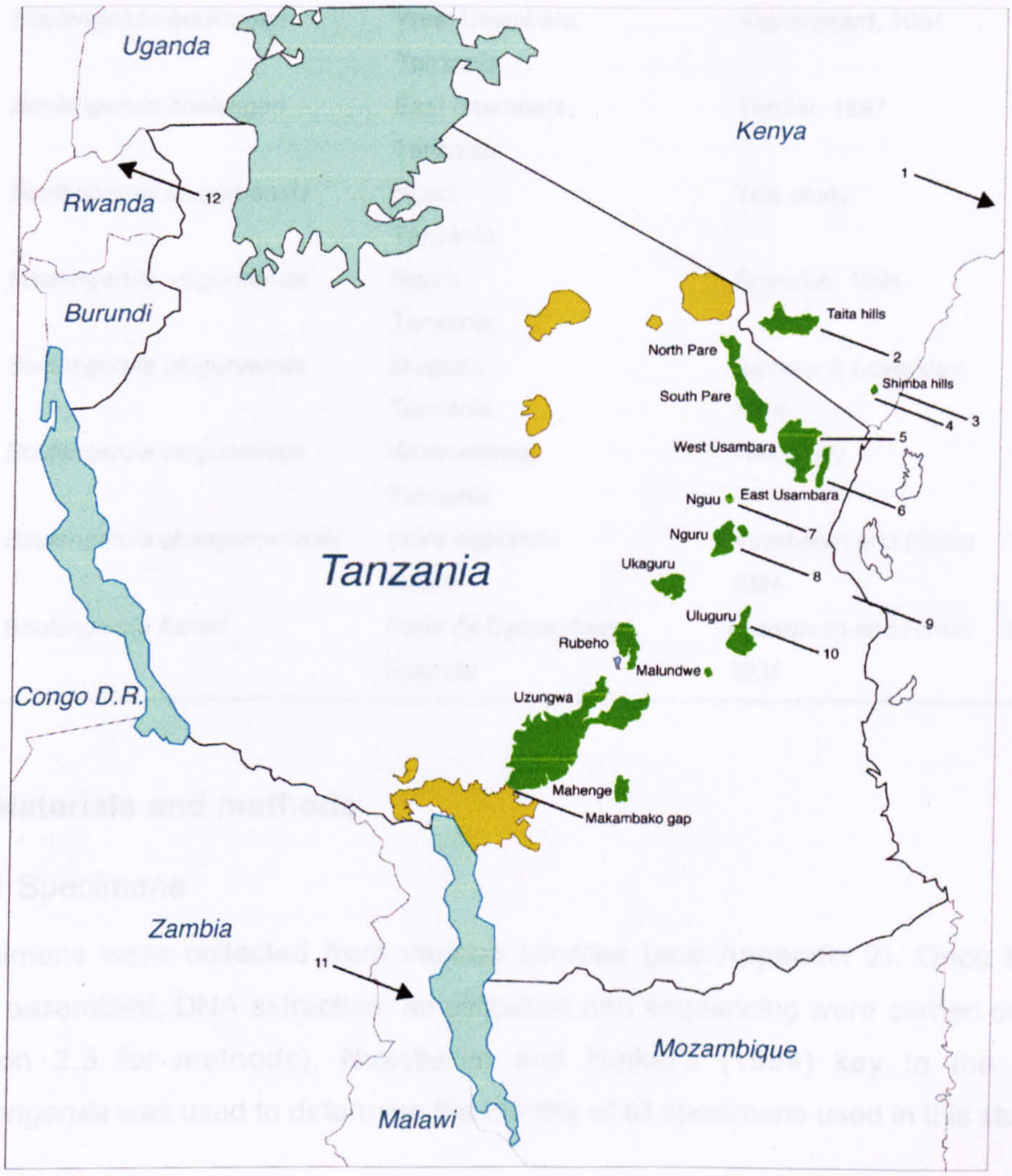

Figure 6.2

Distribution of the caecilian *Boulengerula* in East Africa (see Table 6.1 for details).

Table 6.1.

List of the occurrence of *Boulengerula* species in East Africa and sampled in this study. With 59% of populations sampled.

	Species	Locality	Reference	Sampled
1	*Boulengerula denhardti*	Tana river, Kenya	Nieden, 1912 Wilkinson et al. 2004	X
2	*Boulengerula taitanus*	Taita hills, Kenya	Loveridge, 1935	√
3	*Boulengerula changamwensis*	Changamwe, Kenya	Loveridge, 1932	√
4	*Boulengerula changamwensis*	Shimba hills, Kenya	Nussbaum and Hinkel 1994	X

5	*Boulengerula boulengeri*	West Usambara, Tanzania	Vestergaard, 1994	√
6	*Boulengerula boulengeri*	East Usambara, Tanzania	Tornier, 1897	√
7	*Boulengerula uluguruensis*	Nguu, Tanzania	This study.	X
8	*Boulengerula uluguruensis*	Nguru, Tanzania	Emmrich, 1994	√
9	*Boulengerula uluguruensis*	Uluguru, Tanzania	Barbour & Loveridge, 1928.	√
10	*Boulengerula uluguruensis*	Kazizumbwe, Tanzania	This study	√
11	*Boulengerula changamwensis*	Shire highlands, Malawi	Nussbaum and Hinkel 1994	X
12	*Boulengerula fisheri*	Forêt de Cymandungo, Rwanda	Nussbaum and Hinkel 1994	X

6.3 Materials and methods

6.3.1 Specimens

Specimens were collected from various sources (see Appendix 2). Once tissues were assembled, DNA extraction, amplification and sequencing were carried out (see section 2.3 for methods). Nussbaum and Hinkel's (1994) key to the genus *Boulengerula* was used to determine the identity of all specimens used in this study.

Table 6.2

Boulengerula and outgroups sequenced in this study.

Sequence number	Specimens	Species	Locality	Forest Reserve
T6	N/a	*Boulengerula boulengeri*	Unknown	Unknown
T9	B 8	*Boulengerula taitanus*	Taita Hills, Kenya	Wundanyi
T161	MW 1024	*Boulengerula taitanus*	Taita Hills, Kenya	Wundanyi
T162	MW 1026	*Boulengerula taitanus*	Taita Hills, Kenya	Wundanyi
T163	MW 1021	*Boulengerula taitanus*	Taita Hills, Kenya	Wundanyi
T164	MW 1019	*Boulengerula taitanus*	Taita Hills, Kenya	Wundanyi
T165	MW 390	*Boulengerula boulengeri*	East Usambara	Msyuzi Scarp FR
T166	MW 392	*Boulengerula boulengeri*	East Usambara	Amani-Kwamkoro FR
T171	MW 399	*Boulengerula boulengeri*	East Usambara	Amani-Kwamkoro FR
T173	MW 1015	*Boulengerula taitanus*	Taita Hills, Kenya	Wundanyi
T201	KMH 21450	*Boulengerula uluguruensis*	Uluguru	Mkungwe FR
T202	KMH 21463	*Boulengerula uluguruensis*	Uluguru	Mkungwe FR
T203	KMH 21480	*Boulengerula uluguruensis*	Uluguru	Mkungwe FR
T225	KMH 21270	*Boulengerula boulengeri*	East Usambara	Nilo FR

T243	KMH 23345	*Boulengerula uluguruensis*	Coastal Forest	Kazizumbwe FR
T277	MW 1899	*Boulengerula uluguruensis*	Nguru Mountain	Nguru South FR
T278	MW 1956	*Boulengerula uluguruensis*	Nguru Mountain	Nguru South FR
T279	MW 1984	*Boulengerula boulengeri*	West Usambara	Mazumbai FR
T280	MW 2331	*Boulengerula boulengeri*	West Usambara	Mazumbai FR
T299	MW 1901	*Boulengerula uluguruensis*	Nguru Mountain	Nguru South FR
T300	MW 1903	*Boulengerula uluguruensis*	Nguru Mountain	Nguru South FR
T301	MW 1907	*Boulengerula uluguruensis*	Nguru Mountain	Nguru South FR
T302	MW 1909	*Boulengerula uluguruensis*	Nguru Mountain	Nguru South FR
T305	MW 2336	*Boulengerula boulengeri*	West Usambara	Mazumbai FR
T434	MW 3132	*Boulengerula boulengeri*	West Usambara	Lushoto
T436	MW 3208	*Boulengerula boulengeri*	West Usambara	Ambangula FR
T437	MW 3217	*Boulengerula boulengeri*	West Usambara	Ambangula FR
T439	MW 3268	*Boulengerula uluguruensis*	Uluguru	Uluguru North
T445	MW 3174	*Boulengerula taitanus*	Taita Hills	Ngangao FR
T475	JM 150	*Boulengerula boulengeri*	East Usambara	Shambangeda, nr Amani FR
T476	MW 3777	*Boulengerula changamwensis*	Changamwe	Kenya
T482	JM 849	*Boulengerula taitanus*	Taita Hills	Kasigau FR
T483	JM 794	*Boulengerula taitanus*	Taita Hills	Mbololo FR
T484	JM 966	*Boulengerula uluguruensis*	Uluguru	Tandai Village,
T487	JM228	*Boulengerula taitanus*	Taita Hills	Chawia FR
T490	HM 1	*Boulengerula changamwensis*	Coastal Kenya	Changamwe
T491	HM 51	*Boulengerula* new sp.	Taita Hills	Sagala
T492	HM 52	*Boulengerula* new sp.	Taita Hills	Sagala
n/a	UTA 38889	*Herpele squalostoma*	Cameroon	Mundemba
n/a	UTA 51487	*Dermophis mexicanus*	Guatemala	Izabal, Morales
T438	MW 3225	*Schistometopum gregorii*	Coastal Tanzania	Bagamoyo
n/a	MW 331	*Gegenophis ramaswami*	India	Thenmalai

6.3.2 Phylogenetic analyses

Phylogenetic analyses were carried out as detailed in section 2.7.1. Only one alignment was used to investigate relationships among species of *Boulengerula*. Following analyses by Wilkinson, *et al.* (2003) which showed *Herpele* and *Boulengerula*, possibly form a sister group, the species *Herpele squalostoma* was designated as an outgroup and used to root trees for preliminary analyses. Following these analyses, additional taxa were then included to calibrate molecular clock estimations; *Gegeneophis ramaswamii*, *Dermophis mexicanus* and *Schistometopum gregorii*. These taxa were then used as the outgroup for further analyses. The relationships recovered from these analyses did not differ from preliminary analyses, and are presented in the results section.

6.3.3 Molecular divergence estimates

Molecular divergence dates between specific clades were estimated by adding a number of taxa that provided calibration points (see section 2.5 and 5.2.3 for precise details and approaches for these calibrations).

6.4 Results

6.4.1 Phylogeny

6.4.1.1 Data Quality and Details

Partial 12S, 16S and *cytb* data were collected for all samples apart from T6, T166 and T483 for which I was unable to amplify the first part of *cytb*. Randomly permuted data were shown to be significantly different from the *Boulengerula* dataset (P< 0.001). No significant base composition differences occur across all taxa analysed (chi-squared tests for homogeneity, $P = 1$). The incongruence length difference test showed no significant incongruence between each gene (P= 0.72). Separate and combined analyses were carried out which resulted in a few minor differences, but overall the phylogeny was similar between all analyses, whether different data partitions were combined or analysed separately, which is as expected with the ILD test results. For *cytb*, 12S and 16S branch lengths for each data partition show similar rates of molecular evolution (Fig. 6.4a-c). Analysis of each *cytb* codon indicated 3rd position to show only marginally greater transition/transversion rate than other positions (see Fig. 5.4d-f). Saturation plots were calculated for each gene partition and *cytb* codon position. These plots indicate some saturation in the data (summarised in Fig. 6.3g), as shown by considerable overlap in points of transitions and transversions. Furthermore, fitting linear and power regression lines to a plot of transitions and transversions also indicated saturation is potentially a problem for this data set (not shown; linear r^2=0.878, power r^2=0.9135).

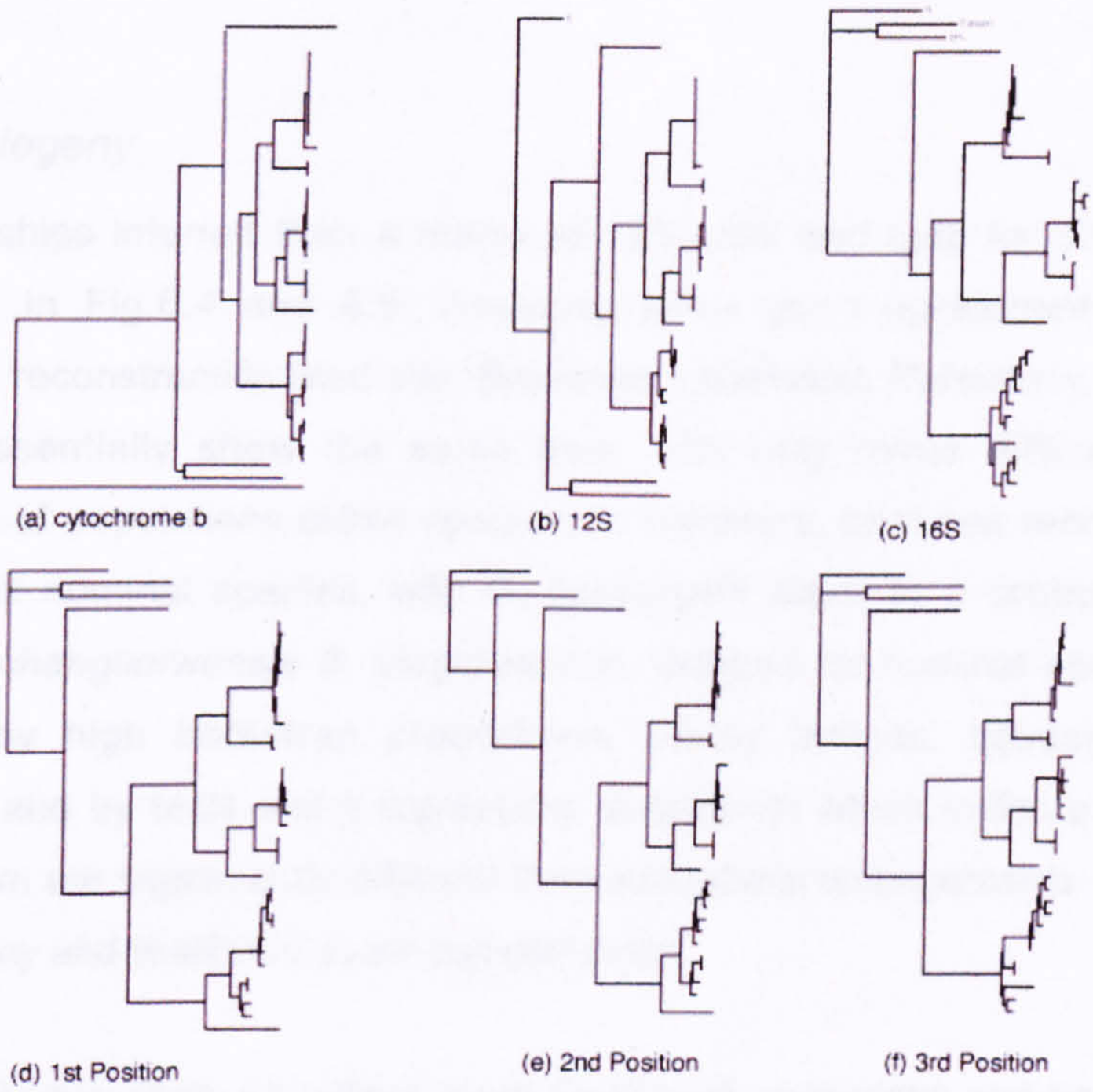

Figure 6.3.

(a-f) Branch length comparisons of different data partitions (g) Plot of substitution of transversions and transitions, indicating levels of saturation, with transitions in blue, and transversions in purple, r^2 value for transitions ($r^{2=}$0.9611) and transversions ($r^{2=}$0.954).

The alignment consisted of 37 taxa (33 *Boulengerula*) and 1445 characters (see Table 6.3).

Table 6.3.

Details of character informativeness for the full alignment of 37 taxa.

	Cytb				12S rRNA	16S rRNA	Total
	All positions	Position 1	Position 2	Position 3			
Constant	466	160	161	145	141	165	771
Variable-uninformative	96	30	36	30	53	29	178
Parsimony informative	223	72	64	87	119	153	495
Total	785	262	261	262	313	347	1445

6.4.1.2 Phylogeny

The relationships inferred from a matrix of 12S, 16S and *cytb* for 37 samples are summarised in Fig.6.4 and 6.5. Analyses show good agreement between all phylogenetic reconstruction methods; Bayesian, Likelihood, Parsimony and Distance which all essentially show the same tree, with only minor differences in the arrangement of populations within species. In summary, analyses recovered clades formed by all nominal species, with *B. boulengeri* sister to a group including *B. taitanus (B. changamwensis B. uluguruensis).* Support for nominal species is good as judged by high bootstrap proportions, decay indices, bayesian posterior probabilities and by tests using topological constraints which indicate all clades of species shown are significantly different from suboptimal arrangements (as shown in both parsimony and likelihood score comparisons).

For the *B. taitanus* clade; all optimal trees (likelihood, parsimony and bayesian) show the resolution of populations: Sagalla (Kasigau (Mbololo, Chawia (Wundanyi, and Ngangao)))). However, among the 11 haplotypes sampled, convincing support is shown only for the Clades including: (1) Sagalla, (Kasigau (Mbololo, Chawia (Wundanyi, and Ngangao) (2) Kasigau (Mbololo, Chawia (Wundanyi, and Ngangao). There is very little sequence difference among populations within the (Mbololo, Chawia (Wundanyi, and Ngangao)) clade, which is why resolution is so poor. Considerable differences are shown between Sagalla and the rest of the Taita populations. For the *B. uluguruensis* clade, all trees show a basal split between Uluguru/Coastal Tanzania and Nguru populations and this is well supported. Within Uluguru populations, the lowland semi-deciduous forest populations of Mkungwe form a clade with the coastal Tanzanian population (Kazizumbwe), but this is only moderately supported and there is little sequence divergence among all *B. uluguruensis* populations (excluding Nguru populations). The main Uluguru montane forest block populations (Tandai and Uluguru North) form a weakly supported clade. Nguru populations of *B. uluguruensis* are shown to form a well-supported clade (samples all from one locality- Komboro in Nguru South FR), which is highly divergent from the Uluguru/Coastal Tanzania clade. One sample for *B. changamwensis* (from SE Kenya) is included, this is strongly supported as the sister group to *B. uluguruensis* clade.

The clade including samples of populations of *B. boulengeri* is the only part of the *Boulengerula* tree that shows conflicting results among methods, however these differences are minimal. Parsimony recovers the sample T165 as sister to all other *Boulengerula boulengeri* samples, whereas Likelihood and Bayesian reconstructions recover T165 within an otherwise West Usambara clade (T437, T436, T305, T279).

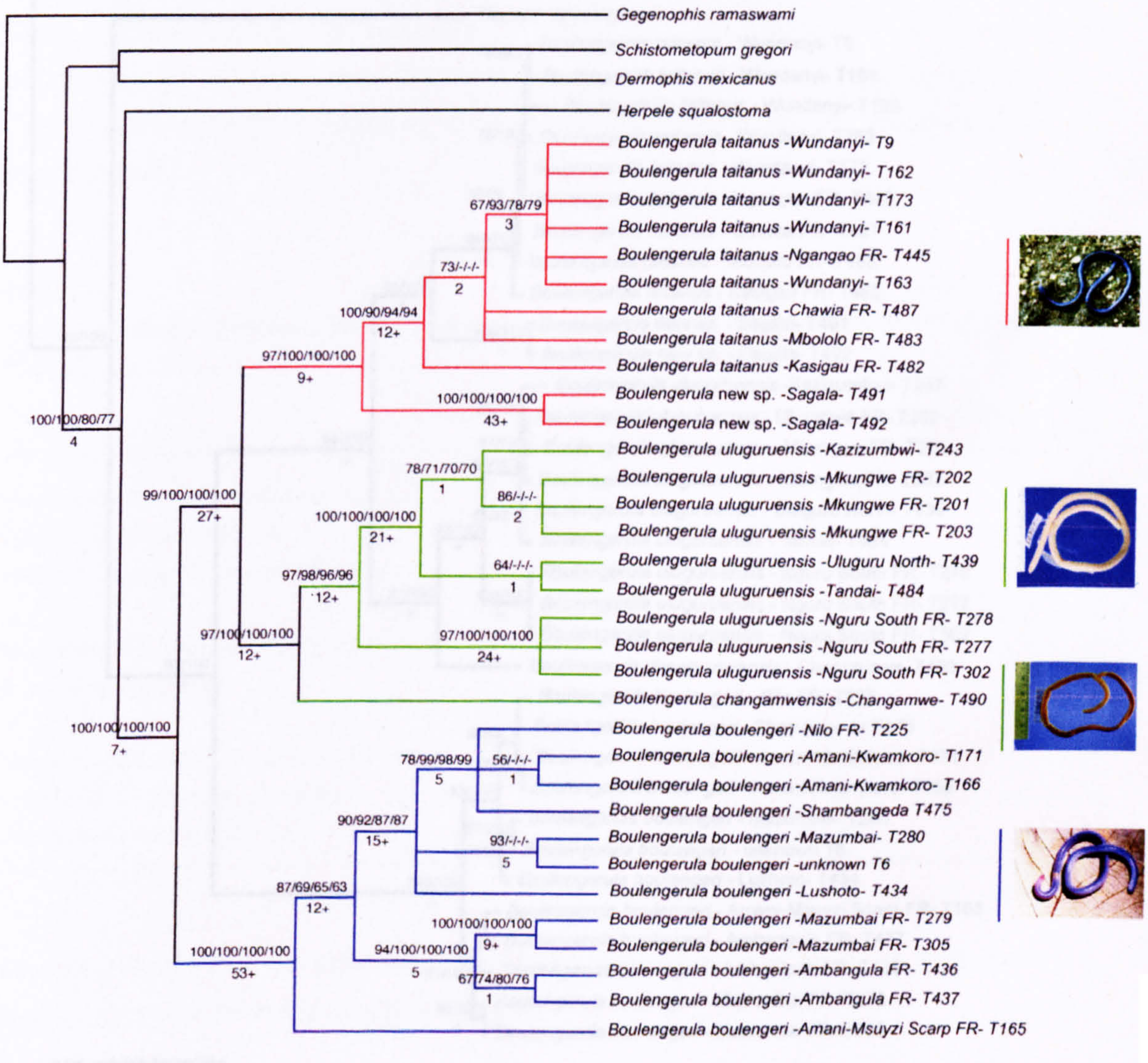

Figure 6.4.

Strict consensus of 2160 MPTs, tree length 1612. Bootstrap proportions shown above branches for parsimony, ML distance, and log-det distance. Decay Index values are shown below along with Templeton test result (+= significant P=<0.05).

There are uncertain relationships among many of the populations because there is only limited divergence. Support for the main splits within *B. boulengeri* is generally good; this includes the monophyly of *B. boulengeri* from Amani FR and Nilo FR

(T225, T171, T166, T475; in East Usambara); and from Mazumbai and Ambangula (T437, T436, T305, T279; in West Usambara).

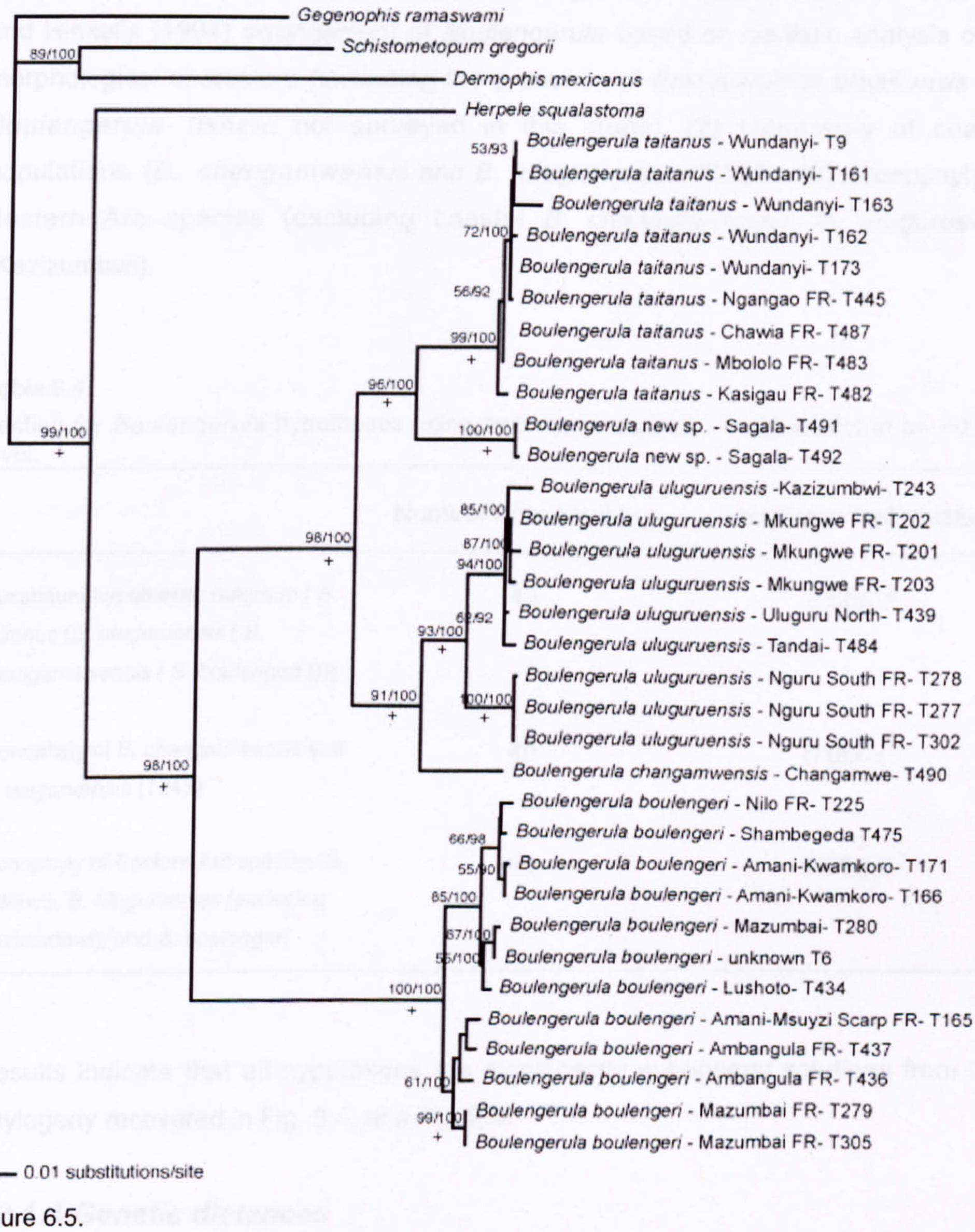

Figure 6.5.

Maximum likelihood tree (LnL= 9150.18654), GTR+I+G model selected from Modeltest. Base frequencies estimated at 0.33100, 0.23630, 0.15750, and 0.27520 for A, C, G and T respectively, substitution rates = 3.5006 4.4277 2.9227 1.1268 14.6852, and the proportion of invariant sites set at 0.3702 and a gamma distribution shape parameter of 1.2118. Values on branches show bootstrap proportions for maximum likelihood and Bayesian posterior probabilities. Bremer support values are shown below, and SH test results (+= significant at P= <0.05).

6.4.1.3 Hypotheses

Comparisons were made between the optimal phylogeny and constrained suboptimal hypotheses. Trees were constrained to investigate two hypotheses: (1) Nussbaum and Hinkel's (1994) arrangement of *Boulengerula* based on cladistic analysis of 17 morphological characters (excluding the presence of *Brasilotyphlus braziliensis* and *Boulengerula fisheri*, not surveyed in this study). (2) Monophyly of coastal populations (*B. changamwensis* and *B. uluguruensis* (T243). (3) Monophyly of Eastern Arc species (excluding coastal *B. changamwensis, B. uluguruensis* (Kazizumbwi).

Table 6.4.
Testing for *Boulengerula* hypotheses using parsimony analysis, *= significant at p= <0.005 level.

	Number of extra steps	Templeton test p value
Nussbaum hypothesis: outgroup (*B. taitanus* (*B. uluguruensis* (*B. changamewensis* (*B. boulengeri*))))	43	0.0002*
Monophyly of *B. changamwensis* and *B. uluguruensis* (T243)	40	0.0003*
Monophyly of Eastern Arc species: *B. taitanus*, *B. uluguruensis* (excluding Kazizumbwi), and *B. boulengeri.*	43	0.0002*

Results indicate that all hypotheses are significantly suboptimal solutions from the phylogeny recovered in Fig. 6.4. and Fig. 6.5.

6.3.1.4 *Genetic distances*

Pairwise distances were calculated for an exemplar of each geographical population of *Boulengerula.* In addition, the geographic distances between each sample was calculated using GPS co-ordinates (see section 2.1.2.3). These results are given in Table 6.5. The values were used for evaluating the presence of divergent lineages within and between mountain populations.

Table 6.5.

Summary of the geographical distance (km) (above the diagonal) and % genetic distances (12S, 16S and *cytb* combined) between *Boulengerula* populations (below the diagonal), using exemplars. Geographical distances provided by Neil Cox (Conservation International), apart from samples 17-20 which were approximated.

	1	2	3	4	5	6	7	8	9	10	11	12
1. *Herpele squalostoma*	-	-	-	-	-	-	-	-	-	-	-	-
2. *B. taitanus* (Wundanyi) T9	20.7%		4.8	392.0	308.7	389.0	394.2	168.3	193.3	193.3	157.9	152.4
3. *B. taitanus* (Ngangao) T445	20.6%	0.1%		396.1	311.5	392.4	397.6	172.7	197.5	197.5	162.0	156.0
4. *B. uluguruensis* (Kazizumbwe) T243	21.5%	11.7%	11.7%		170.4	99.2	111.8	224.4	198.8	198.8	234.2	244.4
5. *B. uluguruensis* (Nguru) T278	21.1%	12.0%	12.0%	5.4%		100.8	99.5	180.6	160.2	160.2	174.7	164.2
6. *B. uluguruensis* (Mkungwe) T203	20.9%	11.1%	11.2%	0.6%	4.6%		13.2	235.9	210.4	210.4	237.7	237.0
7. *B. uluguruensis* (Uluguru North) T439	21.3%	10.9%	10.9%	0.6%	4.3%	0.7%		243.9	218.7	218.7	244.8	242.8
8. *B. boulengeri* (Nilo) T225	22.4%	18.6%	18.7%	18.7%	17.9%	18.3%	18.3%		26.2	26.2	19.7	46.2
9. *B. boulengeri* (Amani) T171	22.2%	18.5%	18.7%	18.6%	17.9%	18.3%	18.2%	0.7%		0	36.7	56.3
10. *B. boulengeri* (Amani) T166	21.1%	17.9%	17.9%	17.5%	16.1%	16.9%	17.0%	0.7%	0.3%		36.7	56.3
11. *B. boulengeri* (Mazumbai) T280	22.9%	18.2%	18.4%	18.2%	17.7%	18.0%	17.9%	2.1%	2.0%	1.8%		26.7
12. *B. boulengeri* (Lushoto) T434	22.6%	18.2%	18.2%	18.1%	17.7%	17.8%	17.8%	2.0%	1.9%	1.3%	1.3%	
13. *B. boulengeri* (Mazumbai) T279	21.8%	18.7%	18.6%	18.3%	17.8%	17.8%	17.7%	4.3%	4.2%	3.3%	3.9%	3.5%
14. *B. boulengeri* (Ambangula) T436	22.0%	18.7%	18.7%	18.4%	17.8%	17.8%	17.9%	4.2%	4.2%	3.1%	4.1%	3.8%
15. *B. uluguruensis* (Tandai) T484	21.0%	11.1%	11.1%	0.9%	4.6%	1.1%	0.7%	17.9%	17.8%	16.5%	17.5%	17.3%
16. *B. changamwensis* (Changamwe) T490	20.6%	11.5%	11.3%	7.9%	7.5%	7.7%	7.9%	18.7%	18.6%	17.5%	18.6%	18.6%
17. *B.* new sp. (Sagalla) T492	21.7%	8.5%	8.4%	11.7%	12.5%	12.0%	11.7%	18.6%	18.4%	17.4%	17.9%	18.1%
18. *B. taitanus* (Chawia) T487	21.1%	0.4%	0.4%	11.0%	11.9%	11.3%	11.1%	18.5%	18.4%	17.8%	18.1%	18.1%
19. *B. taitanus* (Kasigau) T482	20.9%	1.2%	1.2%	11.9%	12.3%	11.4%	11.0%	18.7%	18.6%	17.4%	18.2%	18.0%

Table 6.5 (continued)

	13	14	15	16	17	18	19
1. *Herpele squalostoma*	-	-	-	-	-	-	-
2. *B. taitanus* (Wundanyi) T9	157.9	186.6	394	155.0	45	15	85
3. *B. taitanus* (Ngangao) T445	162.0	190.5	397.6	159.5	45	20	90
4. *B. uluguruensis* (Kazizumbwe) T243	234.2	207.1	111.8	332.3	385	390	330
5. *B. uluguruensis* (Nguru) T278	174.7	145.5	99.5	323.3	300	305	250
6. *B. uluguruensis* (Mkungwe) T203	237.7	206.7	13.2	368.8	390	385	335
7. *B. uluguruensis* (Uluguru North) T439	244.8	213.7	10.0	378.4	395	390	320
8. *B. boulengeri* (Nilo) T225	19.7	35.6	243.9	142.8	170	165	110
9. *B. boulengeri* (Amani) T171	36.7	25.1	218.7	163.8	190	185	105
10. *B. boulengeri* (Amani) T166	36.7	25.1	218.7	163.8	190	185	105
11. *B. boulengeri* (Mazumbai) T280	0	31.1	244.8	151.9	155	160	100
12. *B. boulengeri* (Lushoto) T434	26.7	38.0	242.8	171.4	150	150	95
13. *B. boulengeri* (Mazumbai) T279		31.1	244.8	151.9	155	160	100
14. *B. boulengeri* (Ambangula) T436	1.6%		213.7	178.2	185	180	120
15. *B. uluguruensis* (Tandai) T484	17.3%	17.5%		378.4	395	390	320
16. *B. changamwensis* (Changamwe) T490	18.4%	18.3%	8.3%		150	155	140
17. *B.* new sp. (Sagalla) T492	18.3%	18.6%	11.7%	11.7%		30	40
18. *B. taitanus* (Chawia) T487	18.6%	18.6%	10.7%	11.4%	8.2%		60
19. *B. taitanus* (Kasigau) T482	18.4%	18.5%	10.8%	11.6%	8.3%	0.7%	

6.4.2 Molecular divergence estimates

6.4.2.1 Consistency of calibration estimates

Calibration points for caecilian molecular dating estimates were evaluated in the previous chapter, reference to this section is suggested (section 5.4.2.1).

6.4.2.2 Absolute time estimates for *Boulengerula*

Estimation of divergence times was carried out using both Penalized likelihood (PL) and Langley-Fitch (LF) methods. The data set showed evidence of rate variation and therefore LF estimates should be interpreted cautiously. However, both divergence estimation methods show considerable overlap in estimates, with LF methods showing marginally more recent estimates. The two estimates are shown in Table 6.6.

Table 6.6.

Absolute divergence times in Myr. for clades within *Boulengerula*. Refer to Fig.6.7 for precise position of nodes.

Most recent common ancestor (MRCA)	Estimation method	
	Penalized Likelihood	Langley-Fitch
1. B. boulengeri (B.uluguruensis, B. changamwensis, B. taitanus)	78.95	64.85 (58.92-71.24)
2. B. boulengeri (refer to node)	11.34	11.86 (8.17-15.86)
3. B. boulengeri (refer to node)	8.53	8.02 (6.65-10.99)
4. B. boulengeri (refer to node)	2.36	1.59 (1.18-3.41)
5. B. boulengeri (refer to node)	1.75	1.44 (0.89-3.09)
6. B. boulengeri (refer to node)	1.62	1.04 (0.33-3.43)
7. B. boulengeri (refer to node)	10.45	9.93 (6.74-12.40)
8. B. boulengeri (refer to node)	8.24	7.91 (5.01-11.19)
9. B. boulengeri (refer to node)	6.31	6.13 (4.24-10.50)
10. (B. changamwensis, B. taitanus, B. uluguruensis)	45.10	37.74 (32.96-42.78)
11. B. taitanus (B. changamwensis, B.uluguruensis)	26.81	24.21 (20.29-28.64)
12. B. uluguruensis (refer to node)	2.69	3.11 (1.97-4.68)
13. B. uluguruensis (refer to node)	15.59	13.16 (7.24-16.67)
14. B. taitanus (refer to node)	25.48	20.32 (17.47-24.22)
15. B. taitanus (refer to node)	3.11	2.62 (0.26-4.60)
16. B. taitanus (refer to node)	1.60	1.35 (0.74-2.57)
17. B. taitanus (refer to node)	1.16	0.98 (0.10-1.64)

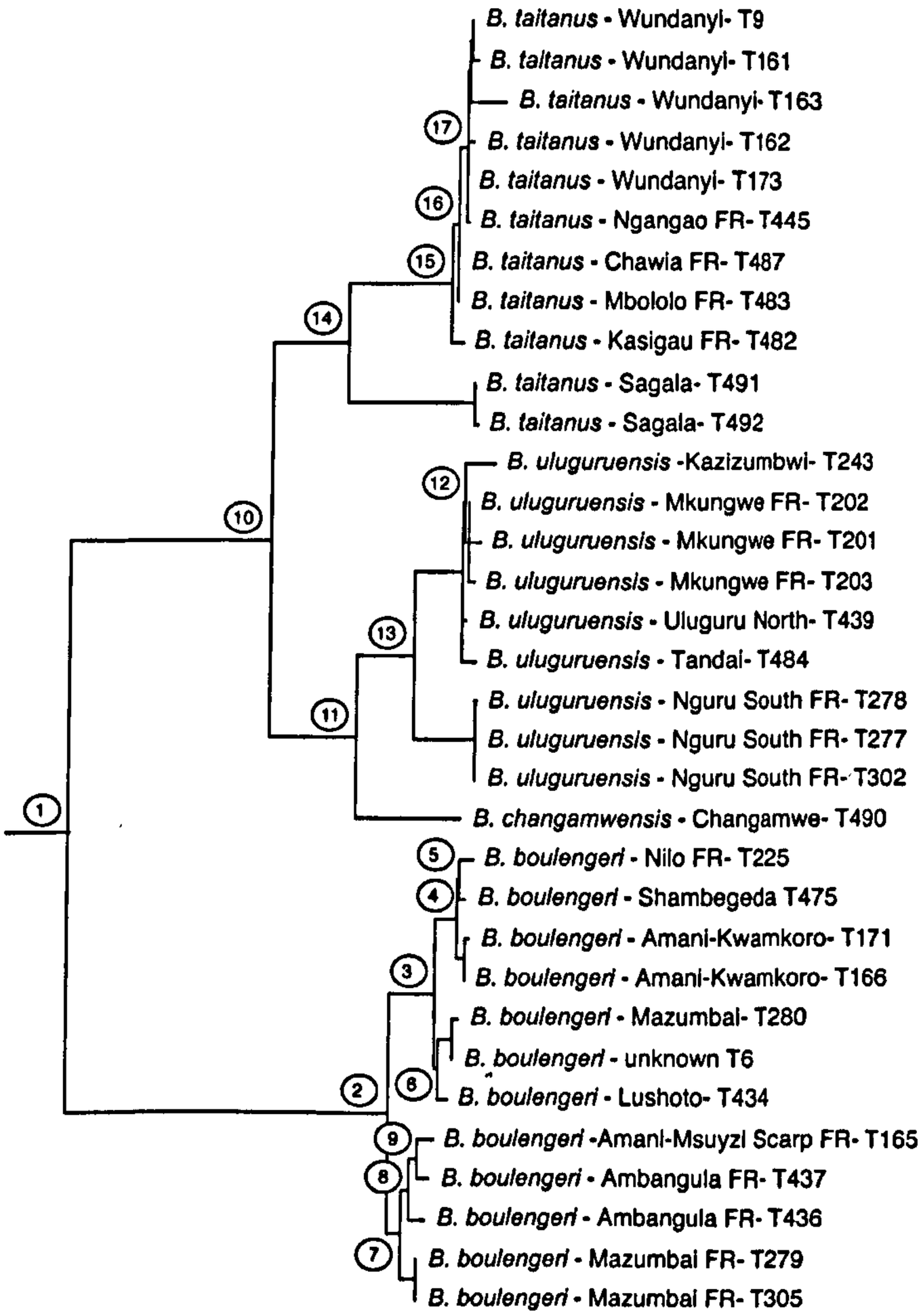

Figure 6.6

Phylogeny of *Boulengerula*, with nodes calculated for molecular divergence using r8s (see table 6.6).

6.5 Discussion

6.5.1 Phylogeny

6.5.1.1 Relationships among species of *Boulengerula*

Analyses of ~1.5kb/per sample of mitochondrial sequence data presented here allows the first robust estimate of species relationships within *Boulengerula* and therefore a quantitative assessment of the various proposed schemes for the classification of the genus. The optimal phylogeny recovered in the analyses is shown in Fig.6.4 and 6.5 and summarised in Fig.6.7a). This phylogeny is strongly

supported in all analyses and differs significantly from suboptimal arrangements. The data strongly rejects Nussbaum and Hinkel's (1994) morphological phylogeny and therefore by implication questions the taxonomic action that Nussbaum and Hinkel made in rejecting Taylor's (1968) separation of *Afrocaecilia* from *Boulengerula*. Clades recovered in this analysis directly correspond to the groups recognised by Taylor: *Afrocaecilia* (*B. changamwensis, B. taitanus, B. uluguruensis,*) and *Boulengerula* (*B. boulengeri*).

Although Taylor's (1968) classification cannot be rejected based on the optimal molecular tree, is it appropriate to recognise two distinct genera, *Afrocaecilia* and *Boulengerula*? Essentially, the question focuses on whether the differences between these two groups are substantial enough to warrant recognition at generic level. However this is a highly subjective aspect of biological higher classifications. It has been widely acknowledged that biological classifications suffer from a lack of standardization across groups and a criterion for what constitutes certain hierarchies, such as a genera, is not clear (de Queiroz and Gauthier, 1992). Given this ambiguity, it is difficult to conclusively judge whether *Boulengerula* species should be recognised in two groups or not.

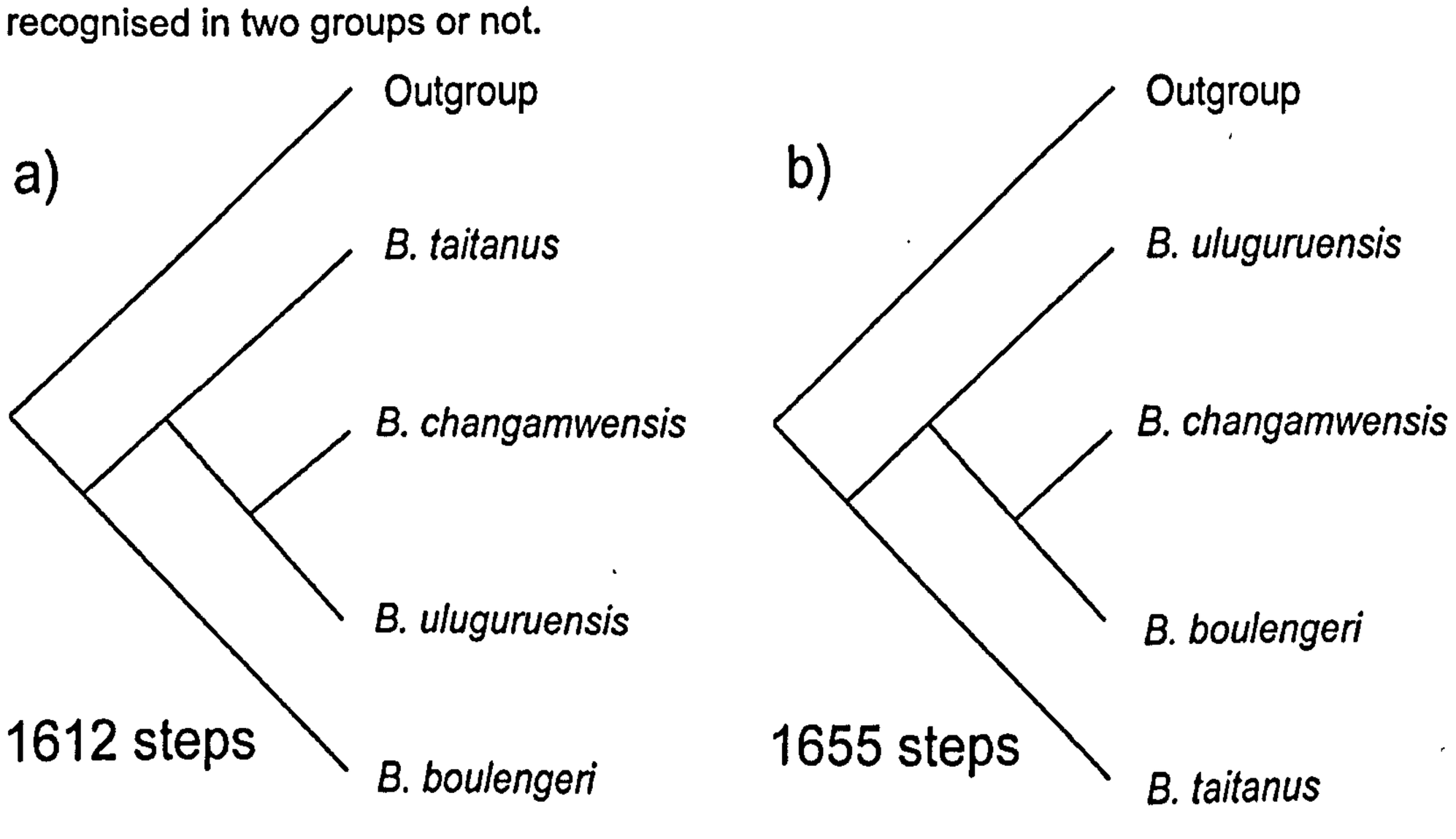

Figure 6.7
Comparison of hypotheses with tree scores, a) Optimal phylogeny recovered in analyses b) Constrained subpotimal phylogeny as proposed in Nussbaum and Hinkel (1994).

Avise and Johns (1999) suggested molecular divergences might be a good tool for standardisation of taxonomic ranks, and although ranks differ between groups they

can be used to gauge differences, if not on a broad scale but within groups (such as amphibians). Large molecular divergences between the two 'groups' *Boulengerula* and '*Afrocaecilia*' point to the possible recognition at the generic level. Divergences of around ~18% are exhibited between *Boulengerula boulengeri* and *'Afrocaecilia'* (*B. taitanus, B. uluguruensis, B. changamwensis*) which is substantial, even at the generic level. When these values are compared to other caecilians (Gower *et al.* 2005), amphibians (Graybeal, 1993), and other vertebrates (de Queiroz and Lawson, 1994; Avise and Johns, 1999) the differences would be recognised as being distinctive at the generic level. Indeed, even the differences between *B. taitanus* and *B. uluguruensis/ B. changamwensis* clade are substantial (~10-12%), and if compared to widely divergent caecilian genera (e.g. *Uraeotyphlus* and *Ichthyophis* sp. 14%) it might be tempting to consider these two groups at generic level also. Perhaps this can be best decided by comparing morphological differences between closely related, but uncontroversially distinct genera of other caeciliids. Morphologically there is a poor understanding of the diversity in *Boulengerula*, however, preliminary investigations of phallus morphology has indicated some distinct differences between Taylor's (1968) *Afrocaecilia* and *Boulengerula* groups (see Fig. 6.7; Müller *et al.* 2005). The phallodeum has been shown to have diagnostic value (Gower and Wilkinson, 2002), and Müller *et al.* (2005) suggest that differences in the degree of development in tuberosities on the phallus might be a putative synapomorphy for *Afrocaecilia*. Further morphological and molecular work will be necessary to fully ascertain the relationships and taxonomic ranks of these groups, however it seems that the current recognition of one genus *Boulengerula* does not fully convey the taxonomic diversity of the group.

A strongly supported relationship is shown between *B. changamwensis* and *B. uluguruensis,* which are also morphologically very similar, known to differ only in the lengths of teeth rows, and the presence of a diastema in the vomerine tooth series. *B. changamwensis* is thought to occur in SE Kenya (Changamwe and Shimba Hills) and Malawi (Nussbaum and Hinkel, 1994; Malonza and Muller, 2004), and has been associated with coastal lowland amphibian fauna (Poynton, 2000b), whereas *B. uluguruensis*, prior to this study, was known to occur only in the EAM, in the Ulugurus and Ngurus (Nussbaum and Hinkel, 1994). It was surprising therefore to sample a population identified as *B. uluguruensis* from the Tanzanian lowland coastal forest Kazizumbwe. Sequences of this population confirmed this identity, as it grouped with *B. uluguruensis* populations to the exclusion of *B. changamwensis*. To further assess

this optimal topology and suboptimal alternatives, trees were constrained to include a coastal clade (Changamwe and Kazizumbwe samples). Templeton tests ($p < 0.0003$) do not require attributing the difference (40 steps) between the MPTs and the best trees consistent with coastal monophyly to random sampling error, and therefore data indicates strongly that *B. uluguruensis* also occurs in coastal lowlands, bisecting the distribution of *B. changamwensis* as currently conceived.

The data allow a preliminary evaluation of the likely extension of *B. changamwensis* from Kenya to Malawi to be made. Given the substantial distance between populations of SE Kenya and Malawi (1,430km), the differentiation between Malawi and Tanzanian fauna (Howell, 1993) and the apparent absence of *B. changamwensis* in coastal Tanzania (intermediate in location between these two disjunct populations), it is likely that the Malawi population may represent a presently undescribed species. The species *B. fisheri* and *B. denhardti* were not sampled in this study, but based on morphology, and their distribution it is likely they are affiliated with *B. uluguruensis* and *B. changamwensis*.

Boulengerula boulengeri

The type species *B. boulengeri* was described based on collections from Amani in the East Usambaras, and the species is known to occur throughout the East Usambaras (Johansson *et al.* 1988). Recently the distribution of *B. boulengeri* has been extended to the West Usambara (Drewes, pers.comm.; Vestergaard, 1994). Vestergaard (1994) believed the West Usambara populations were distinguishable from *B. boulengeri,* though closely related. He outlined this in his masters thesis, suggesting there were differences in colour and annular counts. A description of this putative species was not completed, and in the literature *B. boulengeri* has been reported to occur in both East and West Usambara (Burgess *et al.* 1998a). In light of this preliminary work, surveys throughout the Usambaras were carried out to cover the widest geographical coverage in the now fragmented forest components of each mountain block. From the first field season, surveys provided samples from the geographical extremes of the East Usambaras (Amani NR and Nilo FR) and north east of the West Usambara, (Mazumbai FR) the locality where Vestergaard had collected samples of his proposed new species (see Fig. 6.8 and 6.9). Preliminary morphological and molecular work appeared to support Vestergaard (1994) and his designation of a new species of *Boulengerula*. Measurements showed a greater

mean number of annuli and vertebrae in West Usambara populations (Loader, unpublished). Furthermore, the molecular phylogeny showed a clear separation between East and West populations (clades A and D in Fig. 6.8), with genetic distances of 3.3% (see Fig. 6.8 for summary of phylogeny, with blue *B. boulengeri and* red 'new' *Boulengerula* species), together considered significant enough to warrant the description of a new species.

Figure 6.8

Relationships in *Boulengerula boulengeri* recovered from ML analysés. EU = East Usambara, WU = West Usambara, tree symbol means collected in forest, brown circle in agricultural away from forest, both symbols implies collected near to forest in agriculture (see text for full explanation).

Following the acquisition and sequencing of samples from different forest reserves (Ambangula, Lushoto) and habitats (agricultural plots of land) it was found that certain West Usambara haplotypes nest in an East Usamabara clade, and vice versa (see Fig. 6.8 clades B and C). These 'rogue' haplotypes, as I will call them, blurred the previously straightforward differences between East and West populations, pointing to significant genetic heterogeneity and uncertain population affinities in *B. boulengeri*. Support for these relationships is only moderate and only minimal divergence is shown (1-2% between A and B, and 1-2% for C and B). If the phylogenies represent distinct phylogeographic patterns, which may not necessarily be the case, then there appears to have been recent exchanges between both mountain blocks, in corresponding directions between each lineage.

It is also noteworthy that the 'rogue' populations are derived from samples collected in agricultural areas, and these cluster within forest populations from opposite mountain blocks. Perhaps this pattern is co-incidental, a result of the patchiness in sampling, however the pattern may also be the indicative of other processes. Are there any reasonable explanations for such genetic patterns between two areas? Outside of any biogeographical explanation, which will be discussed later, the patterns could be indicative of processes that give rise to specific genetic patterns, such as stochastic lineage sorting events (Avise, 1994). The localities may still represent two distinct genetic populations, even with no gene flow, but the sharing of haplotypes could be the result of a shared genetic diversity appearing in the ancestral gene pool. The chances of a mitochondrial haplotype in the ancestral gene pool surviving through the generations of population has been suggested to depend on the effective population size and the number of generations (Avise, 1994). Another possible explanation is that certain populations sampled in this study have undergone introgression, and now have mitochondrial genomes originating in neighbouring species. The incorporation of genes of one species into another is a well-documented phenomenon and, if undetected, can prevent the retrieval of the true evolutionary relationships (Alves, *et al.* 2003). This is especially likely when phylogenies are inferred using just mitochondrial or nuclear genes but not a combination of both, the situation in the current study.

Overall the relationships recovered suggest large genetic and morphological heterogeneity in the species *Boulengerula boulengeri* in the Usambaras. Whether the variation observed can be resolved into distinct clades, that are morphologically and molecularly congruent, and by implication represent distinct lineages, is currently unclear. Further sampling is necessary, and once achieved, among other phylogenetic reconstruction methods, nested clade analysis (Templeton, 1998) may prove useful for understanding genetic differentiation among populations. Molecular systematics provides a powerful tool for investigating relationships and species level questions, but an uncritical approach can lead to incorrect taxonomic conclusions. The evolutionary history of the Usambaras probably needs more samples before any well-founded conclusions can be reached. Informative nuclear markers also need to be included in future studies, which would allow a better interpretation of evolutionary relationships between species.

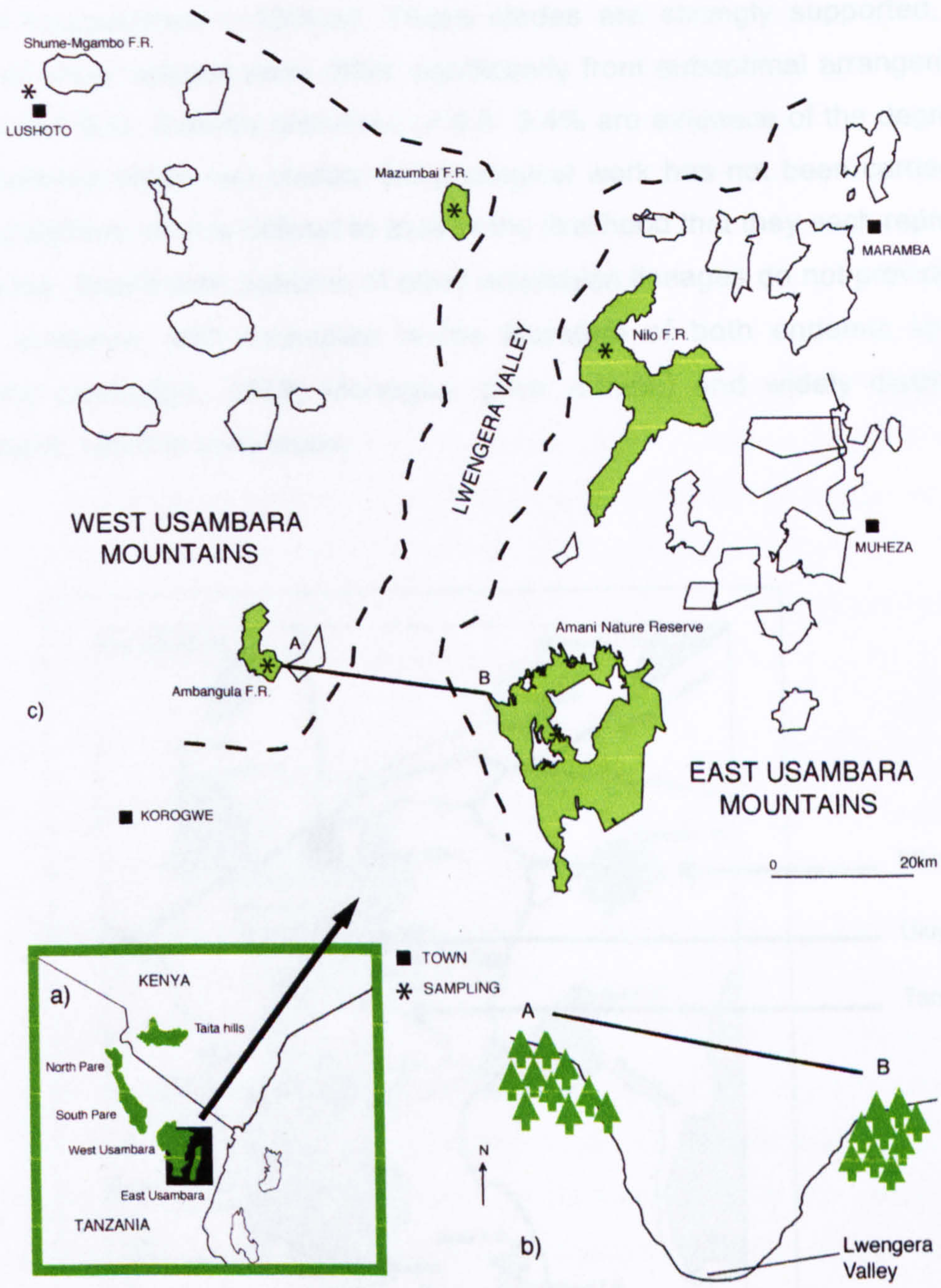

Figure 6.9

a) Schematic map of Northern Eastern Arc Mountains. b) Cross section of Usambara Mountains, with distribution of tropical evergreen forest at high altitudes. c) Schematic map of Usambara Mountains and forest reserves. Asterix indicates sampling areas for *Boulengerula*, and filled squares indicate towns. Based on maps in Iverson (1991), Goodman *et al.* (1995), and Frontier (2002).

Boulengerula uluguruensis

The unpigmented caecilian *B. uluguruensis* is known to occur in the Uluguru, Nguru Mountains, and the coastal forest Kazizumbwe. The phylogeny here recovers two distinct clades, one including Uluguru and Kazizumbwe populations, and a second including populations from the Ngurus, despite the Ulugurus being equidistant from

Ngurus and Kazizumbwe (~100km). These clades are strongly supported, and topology tests show optimal trees differ significantly from suboptimal arrangements (see Fig.6.4 and 6.5). Genetic distances of 4.3- 5.4% are evidence of the degree of difference between these two clades. Morphological work has not been carried out on these populations, so it is difficult to assess the likelihood that they each represent distinct species. Distribution patterns of other amphibian lineages do not provide any supporting evidence, with examples in the literature of both endemic species (Barbour and Loveridge, 1928; Menegon, pers. comm.) and widely distributed species (Howell, 1993) in both areas.

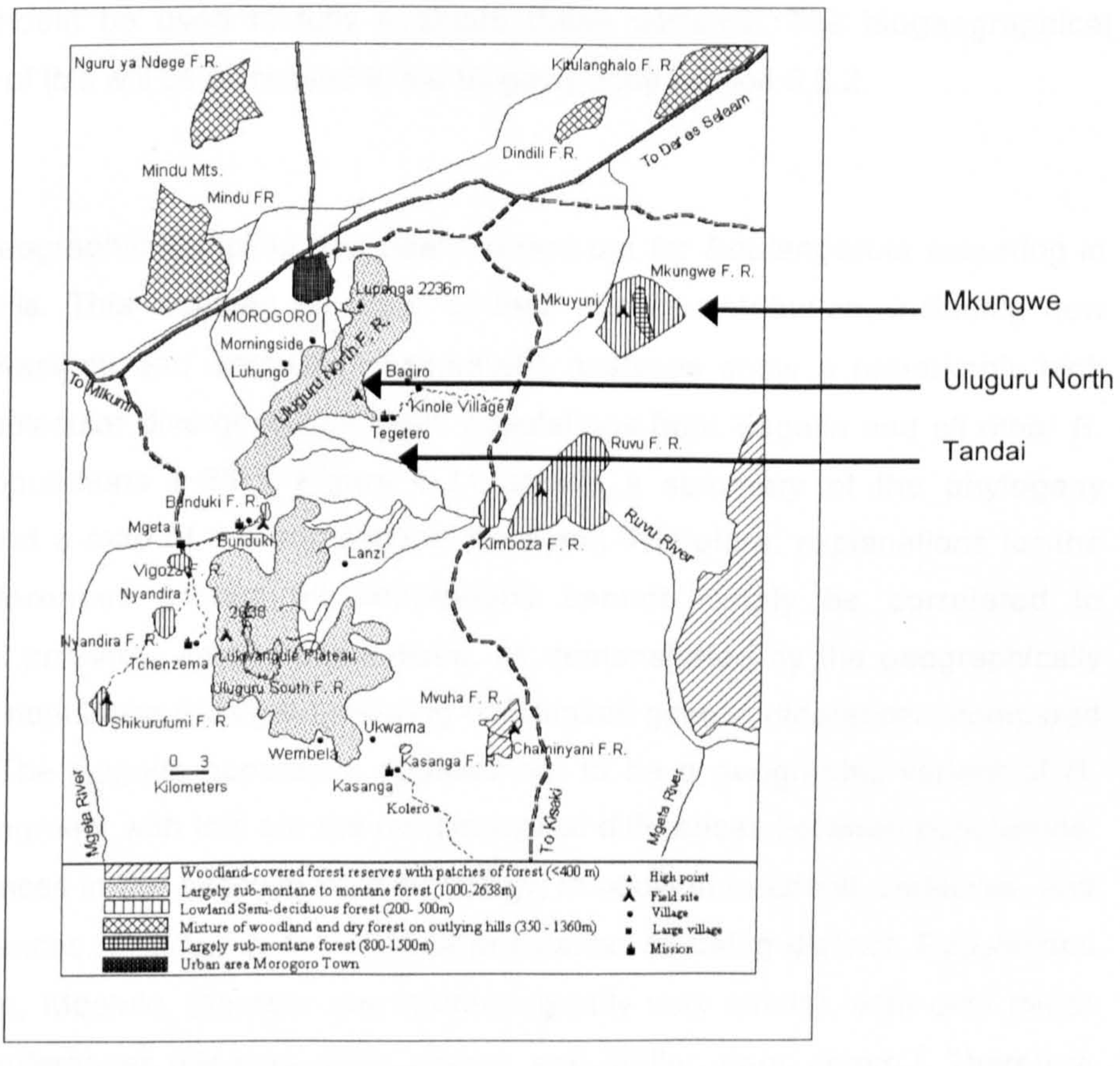

Figure 6.10.

Map of the Uluguru Mountains, provided by Nike Doggart and Andy Perkin.

Within the *B. uluguruensis* clade, which includes samples from Ulugurus and coastal Kazizumbwe, there is significant genetic heterogeneity between two subclades (T201, T202, T203, T243 and T484, T439), which is moderately supported. One clade corresponds to caecilians collected in lowland deciduous forests (eg. Mkungwe FR in the Ulugurus and coastal forests of Kazizumbwe) and the second to the main Uluguru block in agricultural sites (Tandai and Uluguru North) (see Fig.6.10). The lowland clade includes samples (Mkungwe and Kazizumbwe) that are geographically more distant from each other than any of the other pairs of samples (Kazizumbwe and Mkungwe are 100km apart, whereas Mkungwe and Uluguru North are 13km apart). The geographical variation therefore might be associated with ecological similarities among these populations rather than geographic distances. Denser sampling should be used to fully evaluate these patterns. The biogeographical significance of this will be discussed in the biogeography section 6.5.2.

B. taitanus

Thorough geographic sampling has been carried out for *Boulengerula* occurring in the Taita Hills. This includes all areas of their known distribution, including new records in Kasigau and Sagalla. Phylogenetic analyses show a remarkably high degree of molecular divergence between populations from Sagalla and all other *B. taitanus* populations (~8%). Figure 6.11 shows a summary of the phylogeny recovered and a map of the populations surveyed. Therefore, explanations for the genetic differences among all populations cannot simply be correlated to geographical proximity among populations, as demonstrated by the geographically most distant population (Kasigau) showing only limited genetic differences, compared to Sagalla. The Sagalla population appears not to be a geographic variant of *B. taitanus*. Congruent with this are the morphological differences between populations: gross differences in the phallodeum morphology, in addition to annuli, vertebrae, and colour differences, distinguish the Sagalla population as being distinct. Populations from Kasigau, Mbololo, Dawidia are morphologically very similar, with only minor colouration differences (Measey, pers. comm. and Müller, pers. comm.). Therefore there are consistent taxonomic conclusions from both morphology and molecular data, which suggest the Sagalla population represents a new and distinct species (Müller *et al.* 2005).

Excluding the Sagalla population, relationships among all *B. taitanus* populations show congruence with geographical proximity among populations, with the most

distant and isolated population showing greatest differences (e.g. Kasigau). Inclusive relationships are shared between populations derived from the main Taita block Dawida, with Mbololo and Kasigau lineages consecutively outside this clade (see Fig. 6.11). However, divergence among these lineages is very limited and this part of the tree is only moderately resolved. Further resolution of these lineages using quickly evolving genes, such as control region (Presswell, 2002), may further elucidate the relationships and geographical correlates between populations.

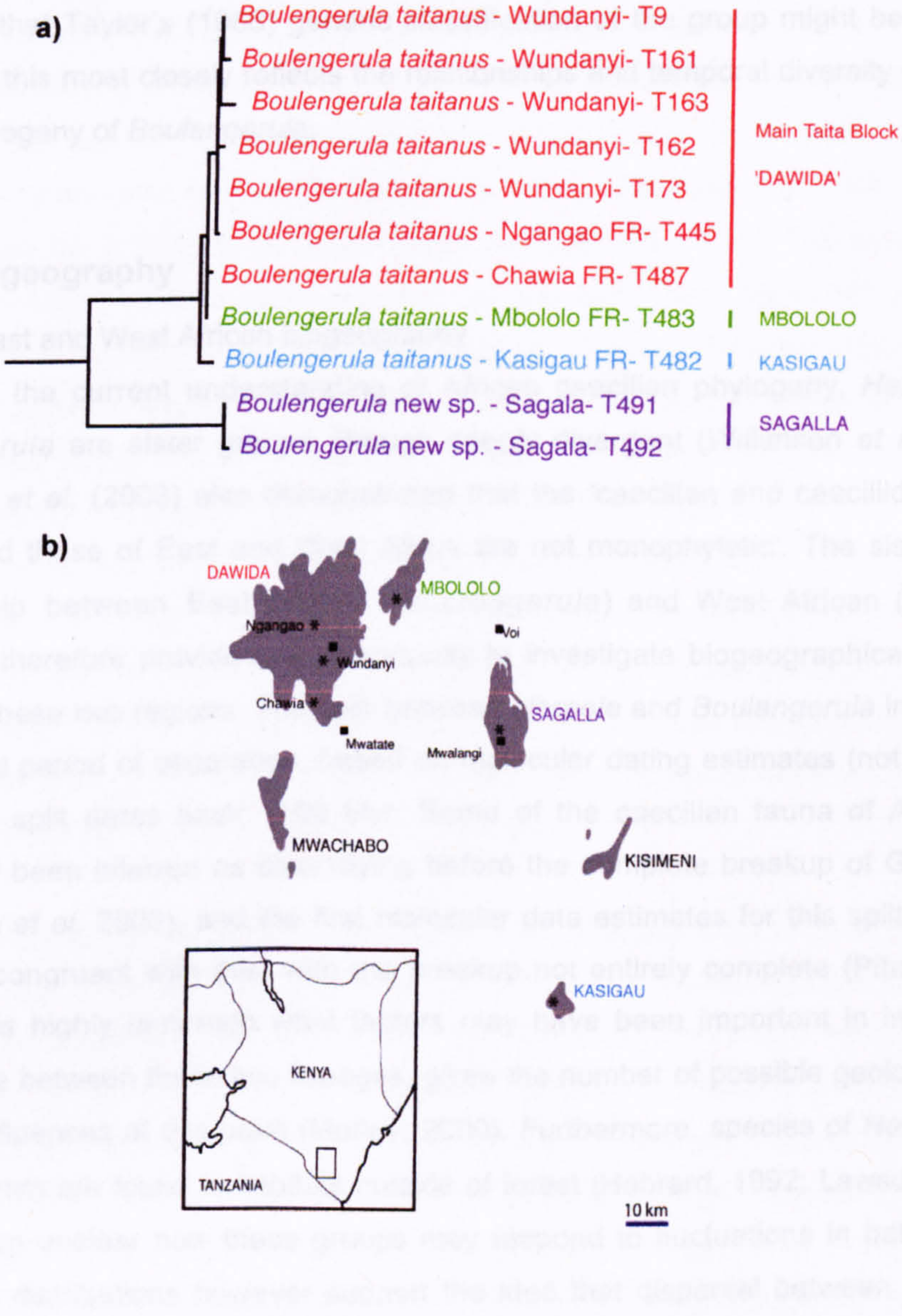

Figure 6.11

a) Phylogeny of *B. taitanus*, with b) schematic map of the Taita Hills. The inset map shows the position of the Taita Hills in Kenya. The map shows all mountainous areas above 1000m. Names written in capital letters refer to mountains, others to towns. Asterix indicates sampling areas for *Boulengerula*. Map kindly provided by Hendrik Müller.

Conclusions

The study provides the first molecular test of relationships within the genus *Boulengerula* and the patterns of diversification. The results also provide an essential basis for future reviews of the taxonomy of the group and its classification. With caveats concerning incomplete sampling (*B. fisheri*, *B. denhardti*, and Malawi populations of *B. changamwensis*) the mitochondrial relationships indicate the presence of at least one cryptic and presently undescribed species. Data also suggests that Taylor's (1968) generic classification of the group might be the most useful, as this most closely reflects the relationships and temporal diversity recovered in the phylogeny of *Boulengerula*.

6.5.2 Biogeography

6.5.2.1 East and West African biogeography

Based on the current understanding of African caecilian phylogeny, *Herpele* and *Boulengerula* are sister groups, though deeply divergent (Wilkinson *et al.* 2003). Wilkinson *et al.* (2003) also demonstrated that the 'caecilian and caeciliid fauna of Africa, and those of East and West Africa are not monophyletic'. The sister group relationship between East African (*Boulengerula*) and West African (*Herpele*) caeciliids therefore provides an opportunity to investigate biogeographical patterns between these two regions. The split between *Herpele* and *Boulengerula* indicates a substantial period of separation, based on molecular dating estimates (not shown in table) the split dates back ~100 Myr. Some of the caecilian fauna of Africa has previously been inferred as diversifying before the complete breakup of Gondwana (Wilkinson *et al.* 2003), and the first molecular date estimates for this split provided here are congruent with this, with the breakup not entirely complete (Pitman *et al.* 1993). It is highly uncertain what factors may have been important in influencing divergence between these two lineages, given the number of possible geological and climatic influences at this point (Morley, 2000). Furthermore, species of *Herpele* and *Boulengerula* are found in habitats outside of forest (Hebrard, 1992; Lawson, 1993), so it is also unclear how these groups may respond to fluctuations in habitat. The dates and distributions however support the idea that dispersal between East and West Africa has been limited since the Miocene (Lovett, 1993a; Matthee *et al.* 2004), which corresponds with the palynological and distribution data (e.g. Grimshaw, 2001).

6.5.2.1 Biogeography of Eastern Arc Mountains and coastal lowlands

The genus *Boulengerula* is found almost throughout the EAM and the lowlands of East Africa, biogeographically distinct elements of East Africa (Poynton, 2000b). The relationships between *Boulengerula* species of both lowlands and highlands is therefore of general interest in studies of East Africa biogeography. Phylogenetic evidence suggests the two biogeographic areas are not monophyletic (both monophyletic EAM and coastal forest were shown to be significantly suboptimal, see Hypotheses 6.3.2.3). Furthermore, coastal lineages are nested within montane clades, with varying divergence. There are two implications from these results; firstly, although the coastal fauna and flora appears to have an archaic history, as suggested by endemic species (Burgess *et al.* 1998b; Matthee *et al.* 2004), the origin of these species is likely to be more recent than that of the EAM. The basal position of Eastern Arc species is good evidence for this and is well supported from other studies indicating the disparity in the species richness between these forests (Burgess, 1998b). Furthermore, contact between these areas appears to have occurred on more than one occasion, based on branch lengths between *B. changamwensis*, *B. uluguruensis* and populations of *B. uluguruensis* from Mkungwe and Kazizumbwe, which show disproportionate differences (7% and 0.6% respectively). Molecular divergence estimates further corroborate this evidence by indicating at least ten times amount of difference between these splits (PL estimates: 26.81Myr and 2.65Myr respectively). Even if molecular dates are incorrect, a highly improbable disparity in molecular rates between lineages would have to compensate for such a substantial differences between lineages.

These results suggest, for *Boulengerula* at least, that the EAM is most probably a refuge for the more recently diversified coastal fauna, because of the position of coastal species/populations on the *Boulengerula* tree. This might reflect a more general pattern, as there are other examples of species that have populations in both areas, such as the forest gecko *Cnemaspis barbouri* (Uluguru, Nguru, and Coastal Forests), dwarf bufonid *Mertensophryne micranotis* (East Usambara, Coastal Forests), and closely related species within the genus *Saintpaulia* (EAM and coastal) (Howell, 1993; Lindquist and Albert, 2001). Testing the phylogeographic patterns of these populations will be important for understanding the diversification of the coastal fauna and flora. The data outlined here are consistent with the hypothesis that both the EAM and coastal forests share a biogeographic history, where presumably multiple contacts between areas have been made (Burgess *et al.* 1998b).

6.5.2.2 Biogeography of the isolated, fragmented mountains of the EA

Between Mountains

Based on the geological history in the EAM of fragmentation and isolation between mountain blocks, it would be predicted that the closer geographical proximity of a mountain would be coupled with closer phylogenetic relationships between species occurring in these mountains. From the optimal trees, only the relationships between the Ngurus and Ulugurus appear to be congruent with this. Surprisingly, Taita Hills species show closer phylogenetic affinity, and by implication biogeographical affinity, to the more geographically distant Ulugurus and Ngurus, to the exclusion of the geographically closer Usambaras. Using dating estimates the tempo of these diversifications can be investigated, with both the congruent and incongruent geographic relationships showing informative patterns. The incongruent relationships between mountain blocks show consistently deep divergences, substantial enough to pre-date the timing of the fragmentation of the Eastern Arc, which is shown for splits between the Usambaras and Taita Hills (~59-71Myr); and Uluguru/Nguru- Taita Hills (~32-42Myr). If molecular dates are correct, for these speciation patterns, the data allow the rejection of the hypothesis that fragmentation of the Eastern Arc corresponds to speciation events. Even if the dating estimates are incorrect, the relative disparity between these two splits (nearly twice as much) does not fit with geological data, which suggest a period of rapid fragmentation in the region (Lovett, 1993a; Griffiths, 1993) around 20Myr. It is uncertain what biogeographical processes influenced the splits. The relationship between Nguru and Uluguru populations, the only congruent geographic relationship also reveals interesting biogeographical patterns. The split shows divergences estimated between 7-16Myr, which might be contemporaneous with fragmentation of the EAM (suggested to occur between 10-14Myr). The correspondence might be co-incidental, but the timing and spatial congruence suggests a possible link. Further sampling of populations and nuclear genes will be necessary to improve estimates dates.

Moreau (1966) and Loveridge (1937) proposed an ancient origin for montane forests of East Africa, because of some striking sister group relationships between montane forest species of East and West Africa, areas separated for at least 20Myr. Surprisingly little quantitative data has been collected in the seventy years since these early speculations (see Burgess *et al.* 1998a). Molecular divergence times between monophyletic EAM clades including *B. taitanus- B. boulengeri* and *B. uluguruensis*, if correct, suggest a prolonged period of isolation in the EAM. The

presence of monophyletic Eastern Arc taxa suggests that taxa have been present in these forests since these splits, and provides some of the first quantitative support for the archaic age of Eastern Arc taxa and the habitats they occupy.

Within Mountains

Considerable genetic heterogeneity is exhibited in populations restricted to single mountain blocks. How significant these patterns are for understanding the high species diversity in the EAM is unclear, however consistent patterns of fragmentation between closely distributed populations within mountains would appear to be have significant implications for the formation of new species. Within the Taita Hills, extraordinary levels of genetic differences are shown between the fragmented isolated forests reserves, which do not all simply correspond with geographic distance. Excluding Sagalla, Fig. 6.11. shows the relationships between populations and a map of their distribution. The results indicate limited genetic heterogeneity between populations, based on isolated localities scattered in the Taita Hills (Chawia, Ngangao, and Mbololo), some nearly 60kms away (Kasigau). Evidence shows that geographically more isolated areas (Kasigau and Mbololo) are more divergent, indicating a positive correlation between distance and genetic differences. These results suggest recent contact between these areas have been made, though more recently in closer localities, presumably at a time when forest was more widely distributed than today (Hamilton, 1988). The molecular dates for all these clades correspond to periods when climatic conditions could have plausibly connected forest in these localities. However, populations from Sagalla, isolated, but not geographically the most distance, show deep divergence patterns suggesting prolonged isolation. Substantial dates are given for this separation, ranging from 17-25Myr. Regardless of whether the estimates are correct, considerable divergence is exhibited in this population, which differ considerably from all other Taita localities sampled. These results appear to be congruent with the limited biogeographic data on other groups in the Taita Hills. Brooks *et al.* (1998) indicated Sagalla as being home to species not present in other Taita blocks, suggesting the distinctiveness of avian fauna in the area. Furthermore, the similarity between Kasigau, Dawida, and Mbololo blocks is shown by the population homogeneity of new species of; millipedes (Vandenspiegel, 2001), spiders (Warui and Jocqué, 2002), and galagos (Perkin *et. al.* 2002) found in the Taita Hills. The data provides an insight into the remarkable fauna of the Taita Hills. Further phylogeographic studies of other groups are

encouraged to elucidate common biogeographic patterns between the fragmented forests of this region.

Herpetologically, the Usambaras have distinctive elements, with a number of endemics restricted to single mountain blocks; for example for the West Usambara (*Arthroleptis tanneri, Callulina kiswamsitu, Nectophrynoides vestergaardi*) and East Usambara (*Nectophrynoides frontieri, Callulina kreffti)* (Menegon *et al.* 2004; de Sa *et al.* 2004). Further taxonomic studies on other amphibian groups are likely to distinguish more populations as being distinct (Poynton, pers. comm.). The presence of single site endemics suggests a period of isolation between these habitats, separated by the Lwengera Valley (see Fig. 6.9). Significant genetic heterogeneity is exhibited between populations of *Boulengerula boulengeri* from both East and West Usambaras, which if interpreted as being indicative of population divergence, show that both populations from the East and West Usambaras have made contact on more than one occasion. The phylogenetic tree presented provides a complex picture of the interrelationships, with three distinct splits. The first oldest split corresponds to splitting between East and West Usambara (8-15Myr), with consecutive speciation events within each mountain block between East and West mountain blocks more recently (6-10Myr and 4-10Myr), perhaps dispersals between East and West Usambaras. Furthermore there is considerable genetic heterogeneity within clades in each population that may suggest substantial fragmentation within the Usambara Mountains. Although currently fragmented, the forests were, until very recently, believed to be continuous, so it is uncertain whether the heterogeneity maybe the result of fragmentation or simply reflective of other processes. Sampling throughout the range of distribution will be necessary to assess this.

The expansion and contraction of forests between these closely aligned mountain blocks is likely to produce complex patterns, particularly in species able to disperse through marginal habitats, such as caecilians. Fig. 6.12 outlines a possible scenario that could account for the patterns observed in *Boulengerula boulengeri* in the Usambaras. The diagram shows how, previously separated populations might have come into contact, and left patterns similar to those shown in the phylogeny. These scenarios are conjectured and quantitative data will be needed to assess such hypotheses. Multiple contacts between the East and West Usambaras species have been suggested. Gravlund (2002) showed recent divergence patterns in the forest snake *Crotaphopeltis*, with the West Usambara clade nested within an East

Usambara clade showing 0.7% difference in NADH 2 and *cytb* sequences of ~1.5kb in length. Older divergences are reflected in the Microhylid *Callulina*, (de Sá *et al.* 2004) which showed both substantial molecular (7.5%) and morphological differences that are distinguished at species level. Differences cannot be discounted as reflecting differing dispersal abilities (between frogs and snakes) in response to the same geographic events. Substantial differences (ten times) however imply a history of multiple contacts between these regions.

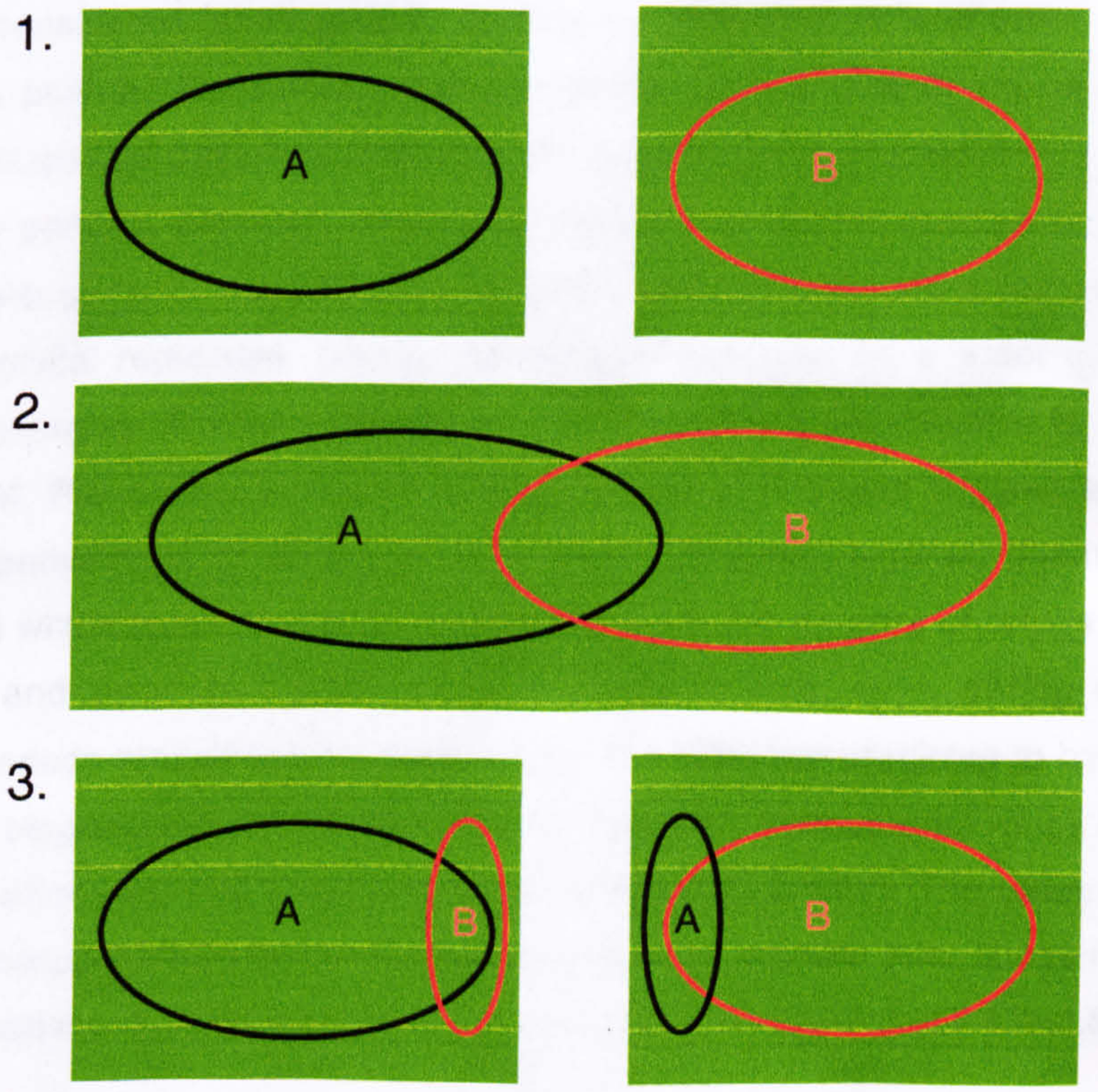

Figure 6.12

Schematic diagram showing the splitting and rejoining of habitats and its consequences on populations A and B (habitats in shown in green for time periods 1-3). (1) Disjunct distributed species A and B (2) Extension of habitat, brings populations spatially in contact (3) Retreat of habitat, populations of each species become separated and spatially closer to other population.

Conclusions

The patterns of diversification in *Boulengerula* suggest complex histories involving both allopatric isolation among refugial areas (i.e. Ngurus and Ulugurus) and patterns of dispersal, albeit over different spatial and temporal scales seemingly corresponding to the fragmentation of Eastern Arc mountains and many fluctuating climatic periods during the last 10Myr and other uncertain ancient influences. Given

the number of complex geological and climatic events that have occurred during these periods it is easy to correlate biogeographical events with splits in a phylogenetic tree. For this reason, only cautious inferences should be drawn. However, evidence shows that speciation events do seem to occur at points in time when climates are fluctuating. For example, 53% of all the splits (as calculated from PL estimates) between populations of *Boulengerula* occur at periods associated to fluctuating climatic periods (2-6Myr; 6-9Myr) in the last 40Myr. If Langley-Fitch S-units are considered (analogous to confidence intervals), 70% of splits correspond with these periods. Considering this constitutes around 20% of the last 40 million years, perhaps this pattern is indicative of elevated levels of speciation during these fluctuating periods, although divergence patterns of extinct species are unknown. More general patterns are difficult to decipher, because there are a limited number of biogeographical replicates. Denser sampling of lineages on a wider geographical scale is necessary in order to gauge if these phenomena are common to all lineages in the EAM. Preliminary evidence from a number of lineages suggest a prolonged period of persistence of clades in EAM areas (Ulugurus, Ngurus, Usambaras, and Taita Hills) which supports the claims that the EAM are an area of refuge for ancient lineages, and perhaps the longevity of these forests, even during climatically unstable periods maintained the biodiveristy. The EAM are predicted to have become significant biogeographical refuges when the position of equatorial Africa reached its current position, and the mountains were uplifted (30-20Myr). The dates from splits between monophyletic Eastern Arc taxa significantly predate this. These results may lead to questions regarding the persistence of these forests during the last 50 Myr.

Chapter Seven

Biogeography of the Eastern Arc Mountains

'The derivation of the Eastern Arc fauna is both long and complex. This biogeographical complexity is well illustrated by the fact that in addition to species found throughout Africa and those confined to eastern Africa, there are also genera and species linking the Eastern Arc with Guineo-Congolian forest block further to the west, the forested areas of Madagascar, and South East Asia. The presence of this wide variety of relationships, comprising relics as well as members of recently diversified groups, makes this area extremely interesting for studies of evolution and biogeography.' Burgess *et al.* (1998a).

7.1

Biogeographic relationships among the mountains of the Eastern Arc have been difficult to quantitatively elucidate, despite a long historical interest in this highly biodiverse region. The origin and determinants of EAM biodiversity have been speculated upon, with attention focused on a history of prolonged persistence of forest with periodic fragmentation. Isolation and persistence in montane habitats should generate specific phylogenetic patterns, providing a means of testing hypotheses explaining the causal determinants of diversity in the EAM. However, investigating biogeographic patterns in the EAM has been difficult, because phylogenetic relationships have been so poorly studied. Data collected in this study provide the first broadscale phylogenetic survey of independently derived amphibian lineages found in the EAM, and this presents an opportunity to address previously intangible biogeographic problems. Molecular clock approaches are used to estimate divergence times and these are coupled with analytical biogeographic approaches and published phylogenies to assess spatial and temporal congruence of divergences among independent lineages. Elucidation of biogeographic patterns in the EAM may also have broader implications for understanding diversification in rainforests.

7.2 Introduction

'On the premise that major environmental changes can drive evolutionary events, the rough coincidence in time between major geological, environmental and evolutionary changes may not be trivial'
Adamson and Williams (1987; p.597).

Two central questions in biology are why are there so many living things and why are they distributed where they are? In an attempt to answer these, biogeographers have searched for correlations between environmental changes and the diversification of organisms. Large-scale changes, such as the fragmentation of continental plates, are believed to have had a significant influence on the evolution of organisms. Recent work has provided compelling evidence that environmental processes have influenced diversification (for review see Hewitt, 1996; 2004). For example, analytical biogeographical approaches have shown how the break up of the supercontinent Pangea influenced dinosaurian diversification (Upchurch *et al.* 2002), and changes in climate have influenced plant diversity in the Amazonian basin (Pennington *et al.* 2004). In the past, correlations between environmental change and evolutionary events were made in a 'narrative', 'descriptive' way, and were therefore somewhat speculative. This was primarily a consequence of the unavailability of appropriate data and methods that allow evaluation of explicit hypotheses within a statistical framework. Recent molecular methods have greatly advanced our knowledge of diversification (Hewitt, 2004) because explicit assessments of both temporal and spatial relationships are now possible. The methods are now in place to address a plethora of both large and small-scale biogeographic problems, which, given good sampling of organisms throughout their ranges, can test long-standing hypotheses about their diversification. The accumulation of data on diversification allows not only an understanding of evolutionary history, but also the assessment of broader questions, such as 'why are there so many species in the tropics?' (Hewitt, 2004)

7.2.1 Eastern Arc Biogeography

The forests of the Eastern Arc are thought to be ancient, and to have persisted throughout severe climatic fluctuations and fragmentation (Lovett 1993a). This environmental stability coupled with isolation of the component mountains are believed to have had an important influence on the evolution of forest species and

high levels of species endemism (Hoffman, 1993; Fjeldså and Lovett 1997; Lovett 1993a; Gravlund 2002; Roy, 1997; Howell, 1993; Burgess *et al.* 1998a). Numerous amphibian species are endemic to the Eastern Arc Mountains (Howell, 1993), and are considered by Poynton (2000; 2003b) cool-adapted and forest associated, with many species not known to occur below the 400m boundary between lowland and montane forest (Poynton *et al.* 1998). Because of these apparent ecological limitations, the ability of most amphibians to disperse across the dry habitats found between mountains in the Eastern Arc would appear to be limited. Consequently, as has been proposed for other animal and plant species (e.g. Loveridge, 1937; Lovett, 1993a), the distribution of amphibians is thought to reflect both the long history of the forests but also periods of isolation of the fragmented mountain blocks.

The hypothesis that environmental changes have influenced diversification in EAM amphibians has thus far been based primarily on distribution data, which are not optimal for addressing such questions. The aim of this chapter is to investigate the likely influences that environmental changes have had on the diversification of amphibians, using primarily their evolutionary history and current patterns of distribution. Because amphibians are sensitive to habitat changes, more general conclusions on the biogeographic history of the region can also be inferred. The geographic history of the EAM has been summarised (see section 1.5.1), and Fig.7.1 outlines the general sequence of events that are believed to have occurred in the history of the region.

The early origins of the EAM are poorly understood (see Fig.7.1a) (Lovett, 1993a); forest cover in the region was likely to have fluctuated periodically, due to a low lying East African topography. Following the formation of the rift valley by 20 Mya (see Fig.7.1b), the Eastern Arc Mountains, along with other mountains in East Africa (e.g. Mount Kahuzi (Biega) of Congo, Mount Elgon, and Ruwenzori Mountains) became uplifted (Cahen and Snellling, 1984) and would have attracted higher precipitation (orographic rainfall) and therefore supported moist forest habitats. Some authors have suggested that certain mountain regions may have originated substantially earlier (Cahen and Snelling, 1984). Once the EAM became uplifted, forest habitats are thought to have persisted in montane regions, with areas surrounding the mountain 'islands' having forest habitats (see Fig.7.1b lighter green areas) fluctuating in size in response to climatic changes in East Africa. Over time, other mountain regions in the vicinity of the EAM originated; the southern highlands originating about

~15-10Mya (see Fig.7.1c) and the volcanic mountains, Kilimanjaro more recently 1-2Mya (see Fig.7.1d) (Davenport, pers.comm.).

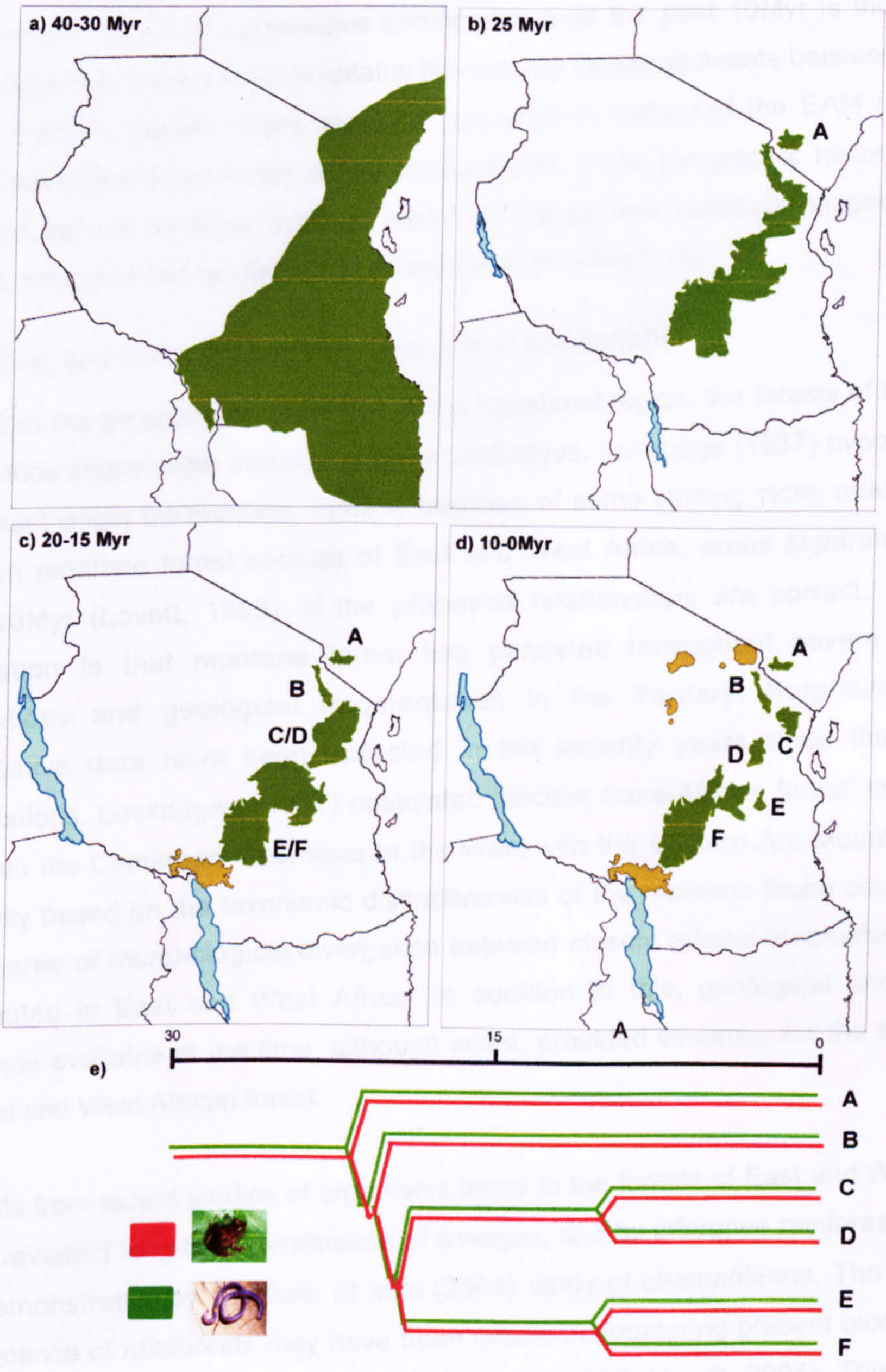

Figure 7.1

Hypothetical model of the geographical history of the EAM at a) 40-30 Myr b) 20-15 Myr c) 10 Myr d) 5-0 Myr. Fragmentation of Eastern Arc forest, shaded in dark green. Volcanic areas shown in brown. e) Hypothetical chronogram (in Myr), of amphibian lineages showing correspondence in their speciation patterns with consecutive isolation of areas in the EAM.

Throughout the history of the forests of East Africa, forest is believed to have persisted, though extending or contracting in response to climatic changes. An overall climatic trend of progressive aridification over the past 10Myr is thought to have isolated the fragmented mountains limiting any dispersal events between blocks (Lovett, 1993a). Based on the proposed geographic history of the EAM outlined, specific biological hypotheses would be predicted. If the geographic history of the EAM corresponds to those outlined (see Fig.7.1a-d), then particular biogeographic patterns, temporal and spatial would be expected (see Fig.7.1e)

7.2.2 East and West African montane forest relationship

Located in the geographical extremes of the equatorial region, the forests of East and West Africa share some interesting biotic similarities. Loveridge (1937) hypothesised an ancient origin for montane forests, because of some striking close relationships between montane forest species of East and West Africa, areas separated for at least 20Myr (Lovett, 1993). If the proposed relationships are correct, then the implication is that montane forest has persisted throughout severe climatic fluctuations and geological fragmentation in the Tertiary. Surprisingly little quantitative data have been collected in the seventy years since these early speculations. Loveridge's (1937) postulated 'ancient trans-African forest' connection between the Cameroon Mountains in the West with the Eastern Arc Mountains was primarily based on the taxonomic distinctiveness of the montane fauna coupled with the degree of morphological divergence between closely related amphibian species distributed in East and West Africa. In addition to this, geological and climatic evidence available at the time, although scant, provided evidence for the separation of East and West African forest.

Results from recent studies of organisms found in the forests of East and West Africa have revealed long-term persistence of lineages, and by inference rainforest habitats, as demonstrated by Matthee *et al.'s* (2004) study of chamaeleons. The long-term persistence of rainforests may have been critical in structuring present biodiversity in montane African rainforests (Fjeldså and Lovett, 1997; Hewitt, 2004). This is further demonstrated by the prevalence of other deeply divergent endemic monophyletic EAM taxa (Loader *et al.* 2004; Wilkinson *et al.* 2003). However, because of the limited number of studies, more general patterns are difficult to decipher. Evidence from the taxonomic distinctiveness of species and genera restricted to the montane regions of East and West Africa also do not contradict the hypothesis that long term

persistence of forest habitats has occurred, and suggest that East and West African regions have been separated for a prolonged period (e.g. Gauld and Underwood, 1986; Largen and Drewes, 1991; Largen, 1997). The large mammal fauna of Africa shows more recent patterns of divergence between East and West African regions (e.g. Hamilton, 1988; Kingdon, 1989; Pitra *et al.* 2002) probably due to their greater dispersal ability. Denser sampling of lineages on a wider geographical scale is necessary to gauge whether deeply divergent forest clades are common to all lineages, and if they are paralleled in other African montane regions, such as Ethiopia. Given the geographic history of the region, it would be anticipated deep divergences between East and West African montane taxa would be recovered (see Fig. 7.2a). Data conflicting such a hypothesis would show recent divergence between forest-restricted East and West African taxa, as shown in Fig.7.2b. An evaluation of new phylogenetic data should allow a fresh perspective on this long-standing question in African biogeography.

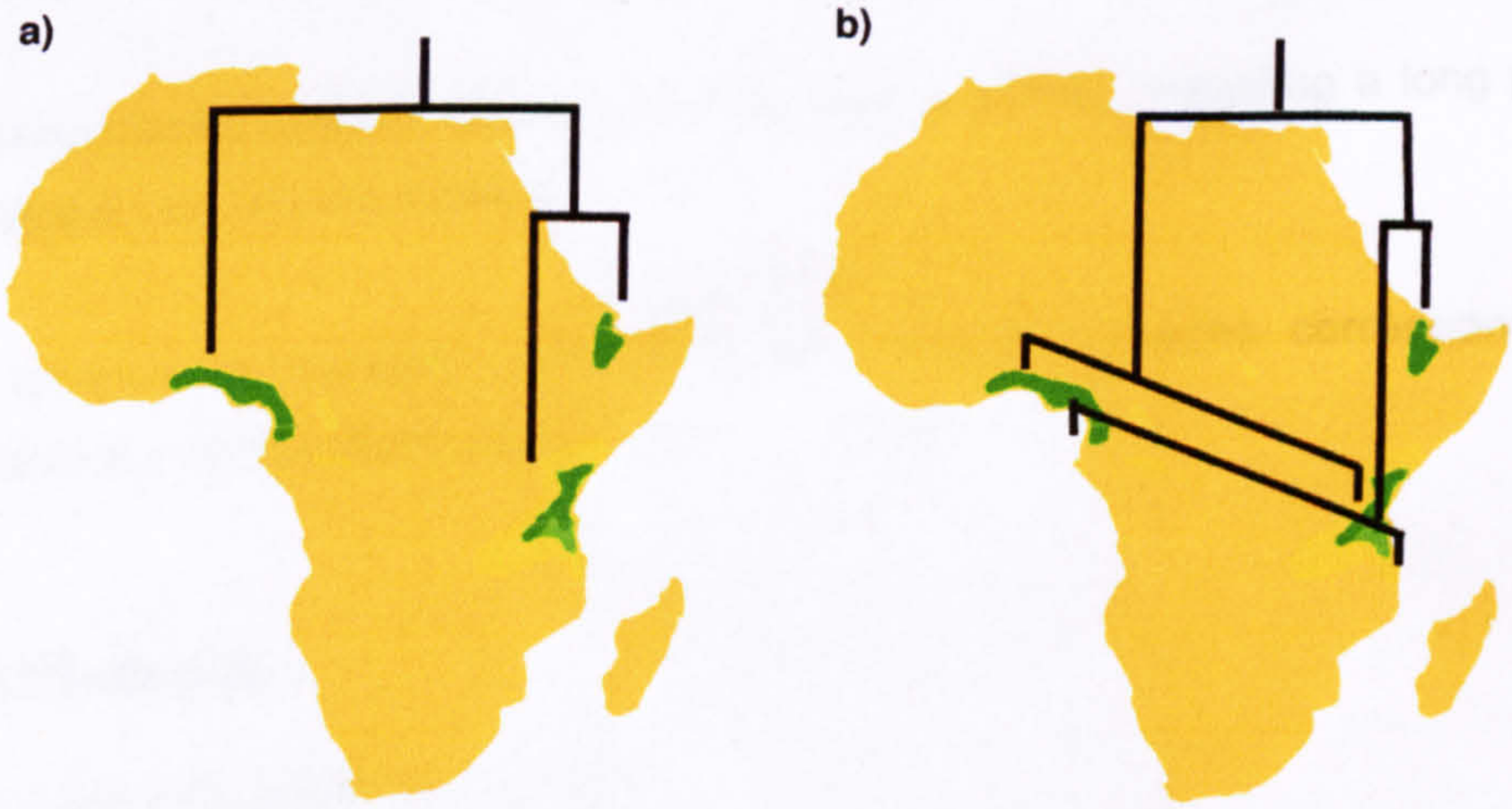

Figure 7.2.

Hypothetical relationships among lineages in the montane forests of Ethiopia, Eastern Arc and West Africa. (a) Deep divergence between East and West Africa (b) Shallow genetic divergence between East and West Africa.

7.2.4 Hypotheses

(1) The phylogenetic relationships of amphibians and other groups reflect the fragmentation history of the EAM.

(2) If congruent area relationships are recovered, area relationship(s) are temporally congruent with the initial uplift and eventual fragmentation of the EAM.

(3) Congruent area relationships in Cladistic are consistent with nestedness patterns recovered in parsimony analysis of endemicity and phenetic analyses of similarity indices. Congruence between methods is repeated in all EAM amphibian groups and provides further evidence for a common biogeographic history.

(4) Discordance in temporal and spatial relationships is correlated with dispersal ability.

(5) There is a strong correlation both temporally and spatially between area relationships and major geographic events, e.g. uplift of the EA mountains in the late Miocene.

(6) Divergences among monophyletic EA taxa are deep, reflecting a long period of geological and climatic stability.

(7) Divergence between East and West African lineages correspond to the separation of these biogeographic regions ~20 Mya.

7.3 Methods

7.3.1 Descriptive Biogeography

7.3.1.1 Matrix construction

A presence/absence data matrix was constructed for the distribution of amphibians in the Eastern Arc Mountains, Southern Highlands, Coastal forest, and Malawi where "0" coded for absence, "1" for presence, and "?" for uncertain records (refer to Appendix 4). Up to date species descriptions were incorporated where possible, including unpublished descriptions (Channing *et al.* in prep.; Menegon *et al.* in prep). Approximately 16 new records were also added based on unpublished fieldwork and newly collected material held at the Natural History Museum. Two matrices were compiled, the first included records of all amphibians occurring in the Eastern Arc Mountains, based on a number of different sources (Howell, 1993; Loader *et al.*

2004a; Menegon *et al.* 2003; Menegon, pers. comm.; Poynton, pers. comm.; Mariaux, pers. comm.; Doggart *et al.* 2004; Doggart *et al.* in press; Loader, field data Appendix 1; Emmrich, 1994). The second matrix was based on the first but with the removal of most species occurring below 400 metres or associated with non-forest habitats. The aim of the descriptive biogeographical analyses is to understand the relationships between forested mountains of the Eastern Arc, and therefore excluding amphibians associated with the lowland fauna or higher altitiude but non-forest habitats was necessary. Determining whether a species is truly dependent upon forest habitats is often difficult because of a lack of ecological, physiological and behavioural studies of African amphibians (Howell, 2000). Data on this aspect of the study is particularly lacking and more data is required which would greatly enhance future evaluations. Further studies will be necessary to assess to what degree the current ecological classifications are accurate.

The use of 400m as the boundary between highland and lowland species was based on the subtraction patterns of the amphibian fauna in East Africa. Poynton (1990) initially suggested the differentiation between highland and lowland fauna occurred at 300m, but recent evidence, based on amphibian distribution in East Usambara (Poynton *et al.* submitted) suggests that the zone is better delimited at 400m as shown by characteristic changes in bufonid genera and the flora (Burgess and Clarke, 2000). Species occurring below 300m and/or occurring in non-forest habitats were removed from the second matrix, though exceptions were made as follows: *Bufo brauni, Boulengerula boulengeri, Hoplophryne rogersi, Callulina kreffti, Arthroleptides martiensseni* and *Scolecomorphus vittatus* are predominantly found in montane habitats and associated with the afromontane fauna (Poynton, 1998), but a few were collected at about 200m. The presence of these species at slightly lower elevation than predicted is likely to be the influence of microgeographical differences between forest reserves. The records are based from specimens collected at Mtai Forest Reserve, Kambai Forest Reserve, and Kwamgumi Forest Reserve, all eastward facing escarpments of significant altitude (within the East Usambaras) that attract increased levels of precipitation (see Fig.7.3). In comparison, the forests reserves of Manga, Mlinga, Longuza, Mgambo, Magrotto are generally much more low lying, constituting the scattered remnants of lowland forest and do not attract similar amounts of precipitation. These abiotic factors might influence the composition of assemblages in the fragmented forest reserves.

To assess if the data show any hierarchical structure, a permutation tail probability test (PTP) was carried out (Faith and Cranston, 1991). PTPs evaluate whether a matrix is more hierarchically structured than randomly permuted data. Below the chosen cutoff value (<0.05), parsimony PTP rejects the null hypothesis that the data have no more phylogenetic structure than expected by chance alone. Failure to pass the test suggests the data are not suitable for phylogeny reconstruction or any analogous methods, such as PAE. Faith & Cranston's (1991) PTP was determined with parsimony analyses of 100 randomisations of the data using PAUP.

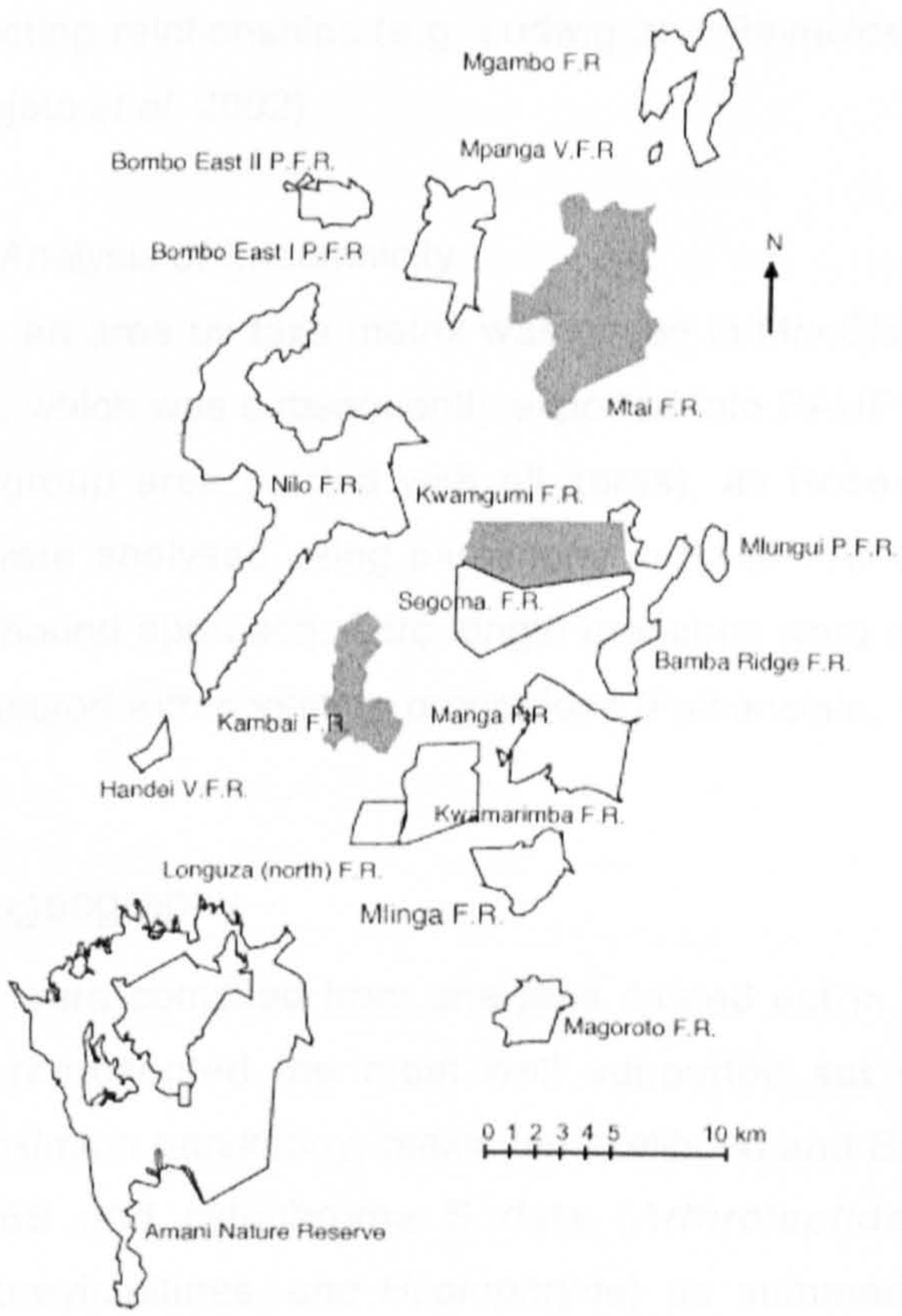

Figure 7.3

Geographical arrangement of the forest reserves in the East Usambara Mountains, shaded areas show submontane forests at lower elevations (~200-400m).

7.3.1.2 Similarity Indices

The data matrix was constructed based on species distribution as shown in Appendix 4, which was then imported into Community Analysis Package (CAP) (1999). Analyses of similarity between communities are divided into two stages: First, an appropriate similarity or difference index must be chosen, such as Jaccard's and Sorenson's Indices. These are simple and reliable measures of the extent to which two sites share species in common (Southwood and Henderson, 2000). Secondly, in

an attempt to graphically represent the differences (e.g. Jaccard Index), a hierarchical clustering method can be used to determine relationships between sites. Clustering methods usually join the shortest branches (smallest difference) of the dendrogram first and the two largest branches last. Examples of such algorithms include single linkage, Wards linkage, and average linkage approaches (also known as Unweighted Pair Group Method, UPGMA). UPGMA methods are the most commonly used numerical classification methods in community ecology analyses (Krebs, 1999), though there are minor differences between the clustering methods used for reconstructing relationships (e.g. Ludwig and Reynolds, 1988; Turpie *et al.* 2000; Ramanamanjato *et al.* 2002).

7.3.1.3 Parsimony Analysis of Endemnicity

To perform a PAE, an area by taxa matrix was coded in MacClade (the same matrix as used in 7.3.1.3), which was subsequently exported into PAUP (Appendix 4). Using a hypothetical outgroup area (coded with all zeros), as Rosen and Smith (1998) suggested, trees were analysed using parsimony in PAUP. All analyses were done with a branch and bound approach. Zero length branches were suppressed. Support for clades was measured with bootstrap proportions (Felsenstein, 1985).

7.3.2 Cladistic Biogeography

Taxon cladograms were compiled from analyses carried out in Chapters 3-6. Most cladograms used represented the most well supported set of relationships as estimated using maximum parsimony, maximum likelihood and Bayesian analyses of combined 12S, 16S and cytochrome B data (*Arthroleptides, Boulengerula, Scolecomorphus*, brevicipitines, *and Hoplophryne*) as summarised in Fig.7.4.a-f. Because cladistic biogeographic analyses need fully bifurcating trees, where there was incongruence in a tree topology between methods of analyses, the most fully resolved trees were used, usually derived from likelihood or Bayesian analyses. The relationships among brevicipitines and *Scolecomorphus* were obtained from likelihood analyses and Bayesian methods, all other trees were from analytical results that were congruent among all methods. Additionally, phylogenies from the literature were included; Snakes (*Crotaphopeltis;* Gravlund, 2002), Chamaeleons (*Rhampholeon;* Matthee *et al.* 2004), Birds (*Andropadus;* Roy, 1997; *Nectarina;* Bowie *et al.* 2004), and Angiosperms (*Saintpaulia;* Lindqvist and Albert, 2001; *Lobelia;* Knox and Palmer, 1998) as summarised in Fig.7.4.g-m. Fully resolved

likelihood trees were utilised instead of consensus parsimony trees, as is the case for *Rhampholeon* (Matthee et al. 2004). For all area cladograms, multiple accessions of a single clade from the same area of endemism were collapsed to a single area terminal.

Two data sets were subjected to 'tree reconciliation analysis' (TRA: Page, 1995 and references therein) in order to test for the presence of repeated spatial relationships. The first data set consisted of amphibian phylogenies, generated in this study. The second data set included all available phylogenies from the literature in addition to the amphibian phylogenies. A General Area Cladogram (GAC) was obtained using Component 2.0 (see Appendix 5 for file executed in Component 2.0). There were no widespread taxa (i.e. taxa occurring in two or more of the areas), so there was no need to consider either assumptions 1 or 2 of Nelson & Platnick (1981) or assumption 0 of Zandee and Roos (1987). Analytically, this made searching for the GAC simpler.

The GAC was obtained using a heuristic nearest neighbour interchange (NNI) branch-swapping algorithm. The criterion of minimisation was 'duplication events' (i.e. the number of times the taxa had to unnecessarily split to accommodate the topology of the putative GAC). When multiple GACs were recovered, a Nelson's consensus of them was calculated; this finds the largest clique of (non-conflicting) area components. For assessing the robustness of the GAC, it was randomly permuted 10,000 times in TreeMap 1.0 (Page, 1993) and then reconciled with the taxon trees; for each permutation, the duplication figure was also recorded. The p-value for the GAC was obtained from this distribution (Page, 1993).

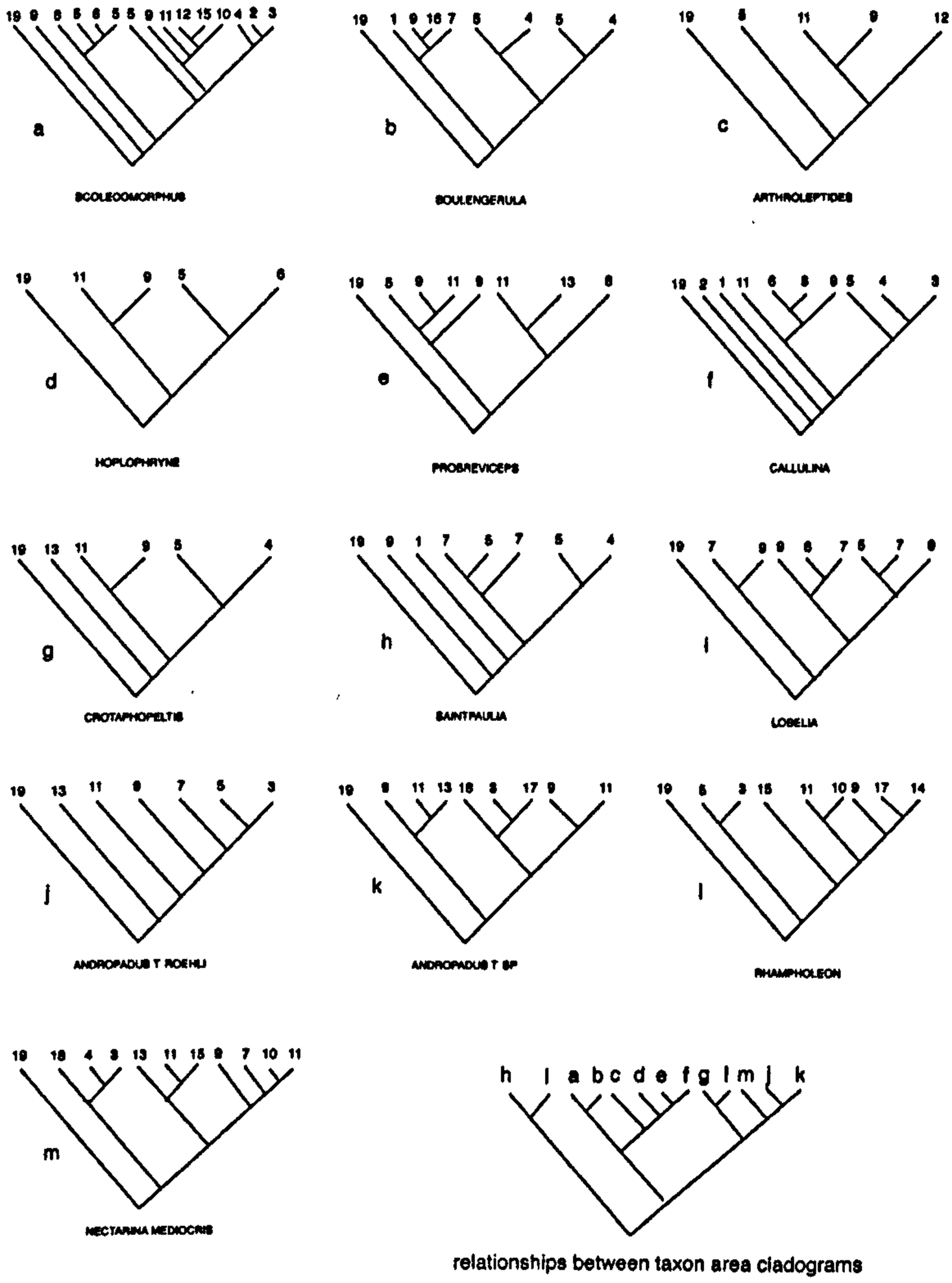

Figure 7.4.

Phylogenies used in cladistic biogeography, a-f amphibian phylogenies generated here and g-m non-amphibian phylogenies from the literature. Numbers refer to areas and letters to phylogenies. Relationships between taxon area cladograms are summarised (bottom right) which is necessary for Component 2.0 block. Areas were coded using the following convention, 1 Taita Hills, 2 North Pare, 3 South Pare, 4 West Usambara, 5 East Usambara, 6 Nguu, 7 Nguru, 8 Ukaguru, 9 Uluguru, 10 Rubeho, 11 Udzungwa, 12 Mahenge, 13 Southern highlands, 14 Malawi, 15 Mozambique, 16 Coastal Forest, 17 Ruwenzori, and 18 Volcanic mountains.

7.3.3 Temporal Data

Data from genetic pairwise distance and likelihood dating estimates were compiled into tables. For molecular dating estimates generated by r8s, the confidence values from *S*-values provide the range in which temporal data is shown to be incongruent or not. In addition, comparisons between lineages showing significantly different rate

variation (e.g. *Scolecomorphus*) were evaluated conservatively. Rate variation between lineages was calculated with the computer program r8s. Using the differences in rate variation, comparisons of temporal congruence and incongruence could be better evaluated by accounting for rates of molecular evolution in lineages. In addition to comparing molecular dates from r8s, pairwise genetic distances were compared between each lineage for each EA mountain population to assess the correspondence between genetic diversity and geographic distance. Further comparisons were also made with published temporal data. Molecular date estimates were taken directly from Gravlund (2002) and Matthee *et al.* (2004) and compared.

7.4 Results

7.4.1 Descriptive Biogeography

7.4.1.1 Similarity indices

The PTP test (p= >0.05) did not reject the null hypothesis that the data show no more hierarchical structure than randomly permuted data. Analysis of the relationships among EAM species assemblages, using similarity indices are summarised in Fig. 7.5-7.10. The dendrograms show the pairwise similiarities among each area combination, using two similarity indices (Jaccard's Index, Fig.7.4, 6 and 7.8; Sorenson's Index, Fig.7.5, 7 and 7.9) and three clustering alogirthms (Wards Clustering method, Fig.7.4 and 7.5; Average Linkage Method, Fig.7.6 and 7.7; Single Linkage Method, Fig.7.8 and 7.9).

The results of hierarchical classification are presented in the form of tree diagrams, or dendrograms. These dendrograms are useful not only for displaying groupings, but also the degree of difference between groups (Branch lengths). The results show a general congruence between different clustering methods and indices. Differences are confined to the placement of certain areas (e.g. Taita Hills and Ukaguru between Average and Wards clustering algorithms; Malawi between Single and Average/Wards clustering methods). The greatest differences between methods are: Average and Wards dendrograms consistently recover two main groups (1) Eastern Arc 'clade' (2) Rubeho, Mahenge, Coastal Forest, and Southern Highlands, whereas single Linkage clustering recovered (1) Eastern Arc 'clade' (same as Average/Wards cluster, excluding Taita Hills and Pares) (2) Southern Highlands sister to this group (3) Malawi and Pares sister group to 2. (4) All other areas successively sister to this

group. In most dendrograms close relationships are generally shown by areas in close proximity, e.g. Malawi (North and South), Pares (North and South), Nguu and Ngurus, Uluguru and Udzungwa, Southern Highlands and Mahenge/Malawi. The EAM areas Rubeho and Mahenge do not nest with other EAM, but cluster with Malawi, Southern Highlands, Mahenge, and Coastal Forests. Closer similarity between areas, however, is not always directly proportional to geographical proximity; Uluguru and Udzungwa show the greatest faunal similarity despite being separated by ~190km. Most importantly, the mountains of the EA do not form a single group. Overall, there are similarities between clustering methods, but there is some ambiguity in the relationships recovered among certain areas.

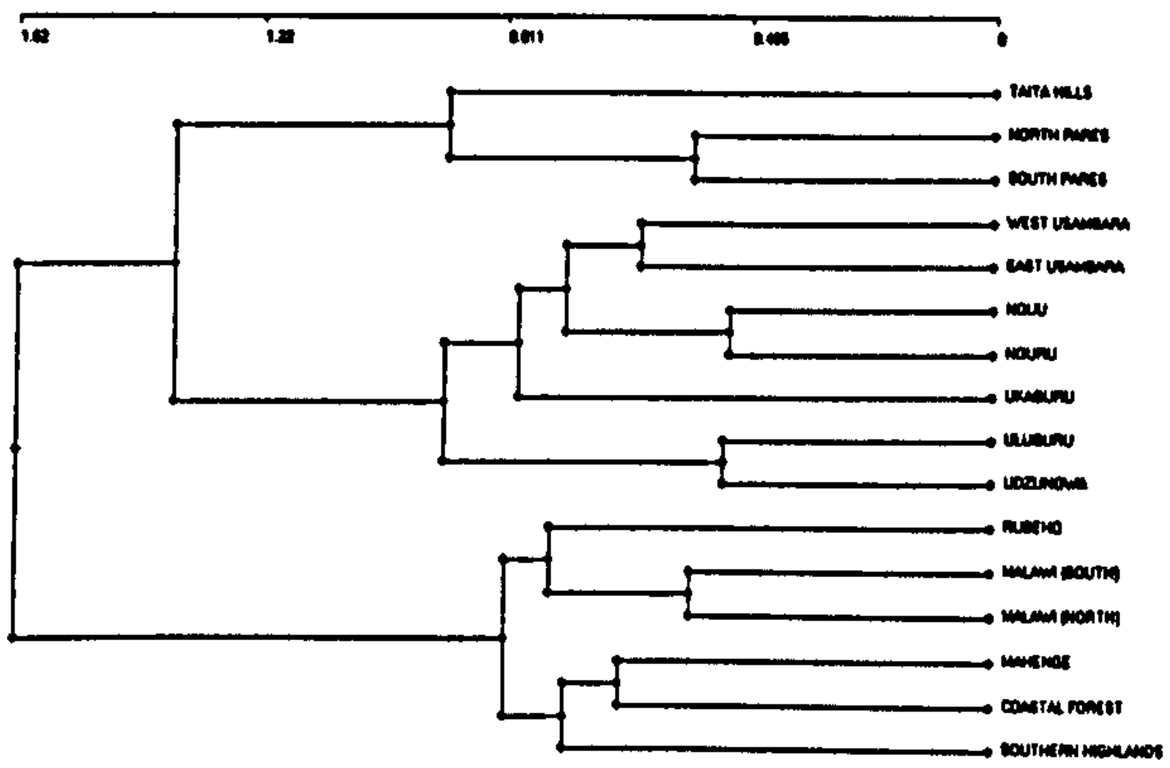

Figure 7.5
Cluster analysis of Jaccard's similarity co-efficient for 16 forests in EAM using Ward's agglomerative method.

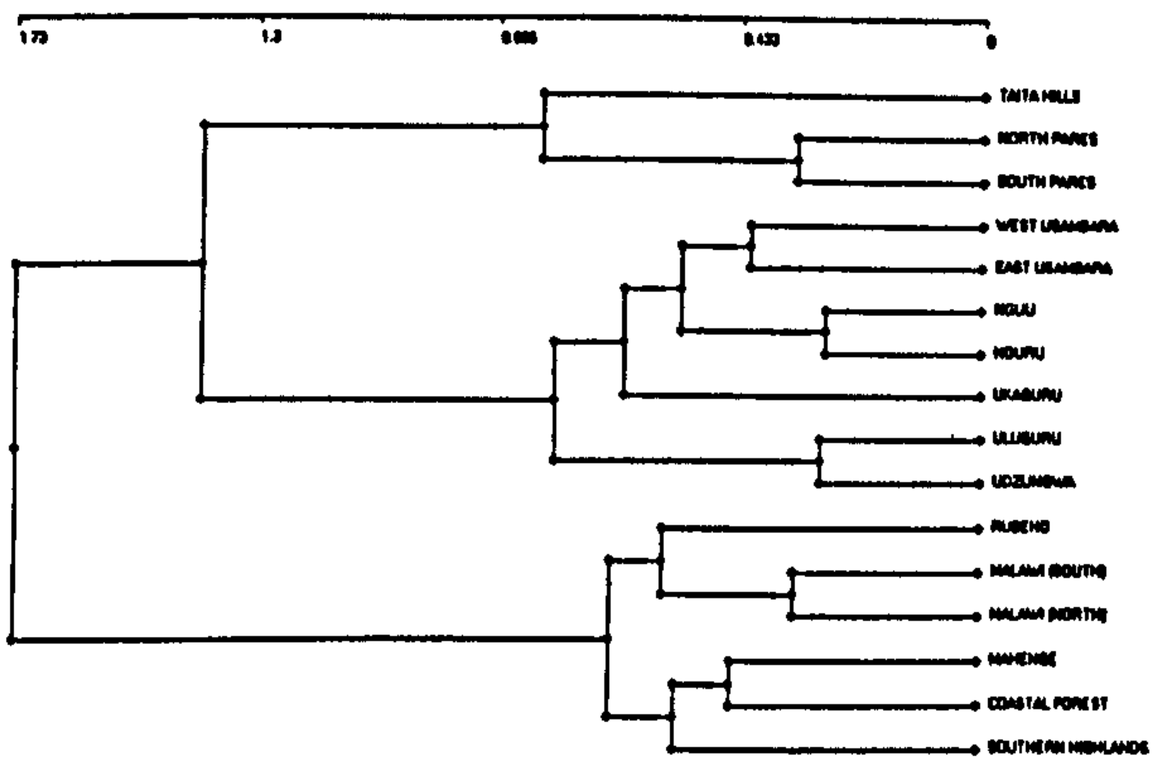

Figure 7.6
Cluster analysis of Sorenson's similarity co-efficient for 16 forests in EAM using Ward's agglomerative method.

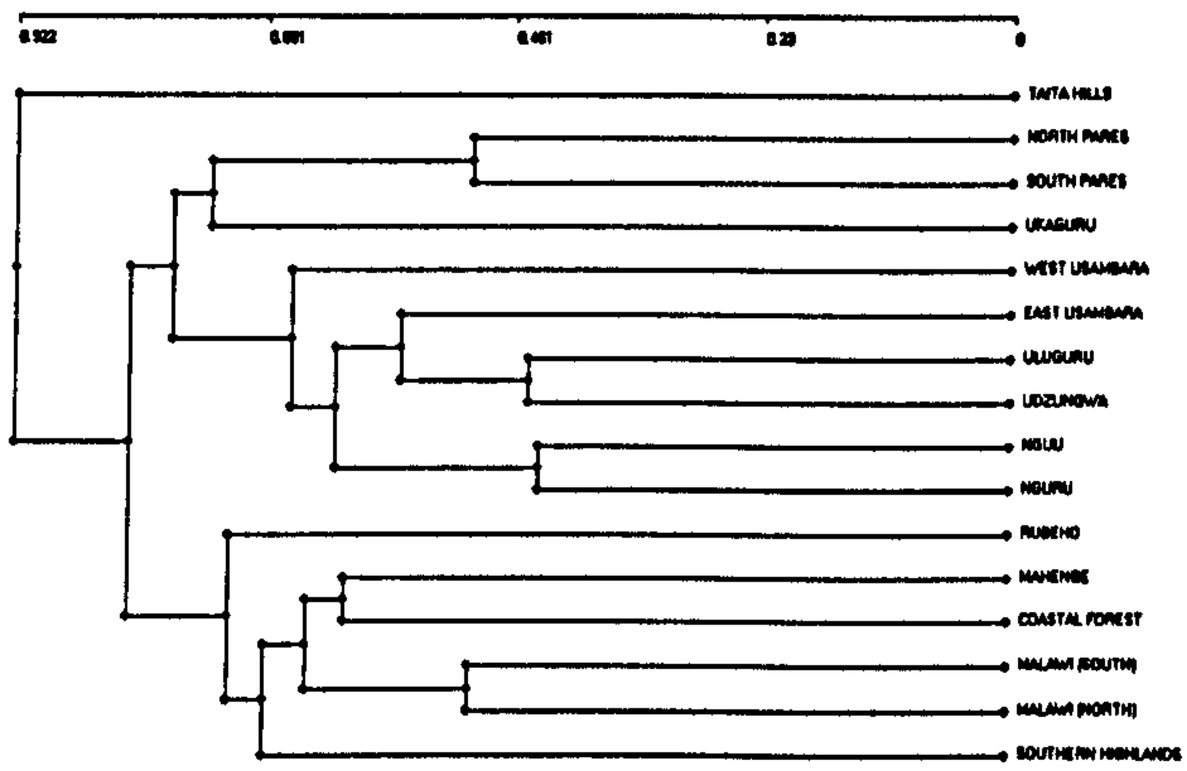

Figure 7.7
Cluster analysis of Jaccard's similarity co-efficient for 16 forests in EAM using average linkage agglomerative method.

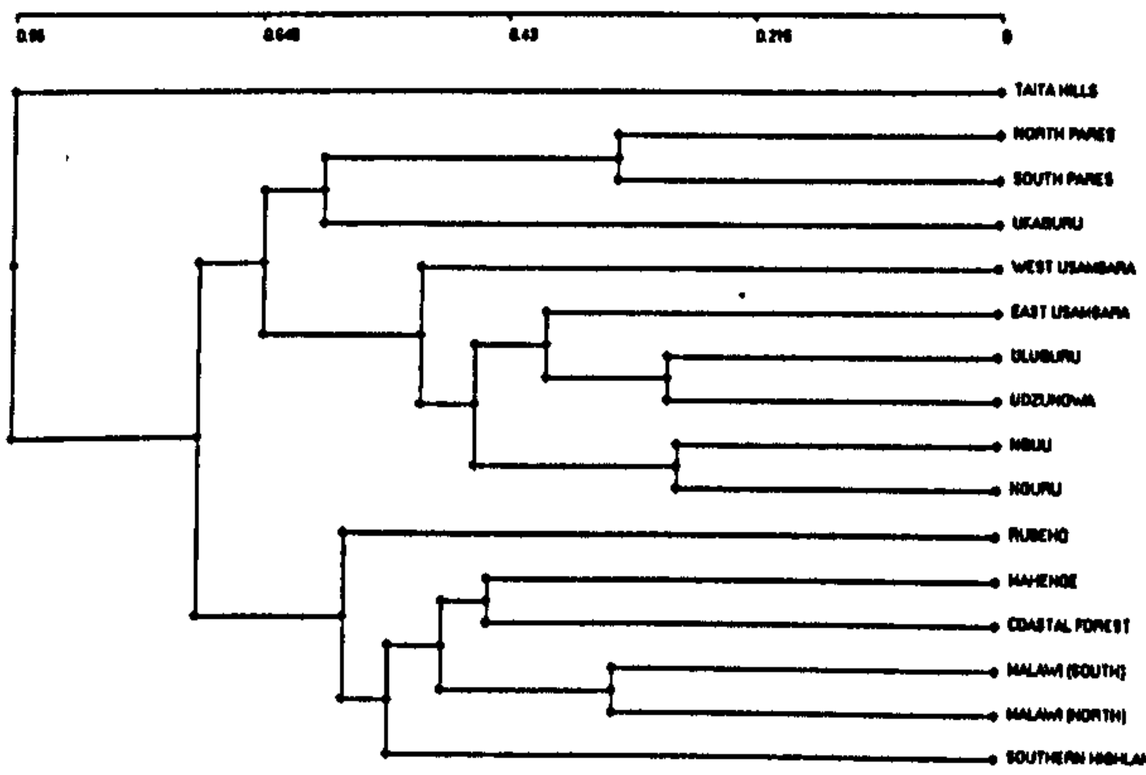

Figure 7.8
Cluster analysis of Sorenson's similarity co-efficient for 16 forests in EAM using average linkage agglomerative method.

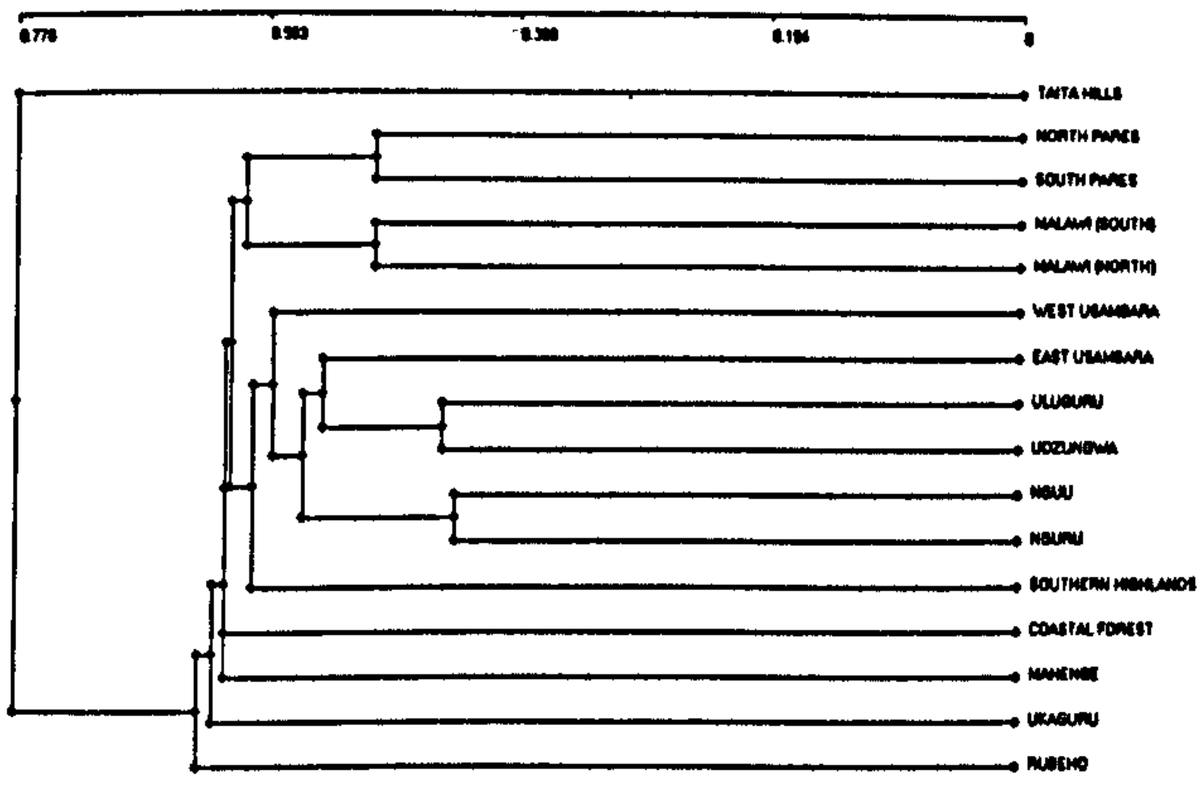

Figure 7.9
Cluster analysis of Jaccard's similarity co-efficient for 16 forests in EAM using single linkage agglomerative method.

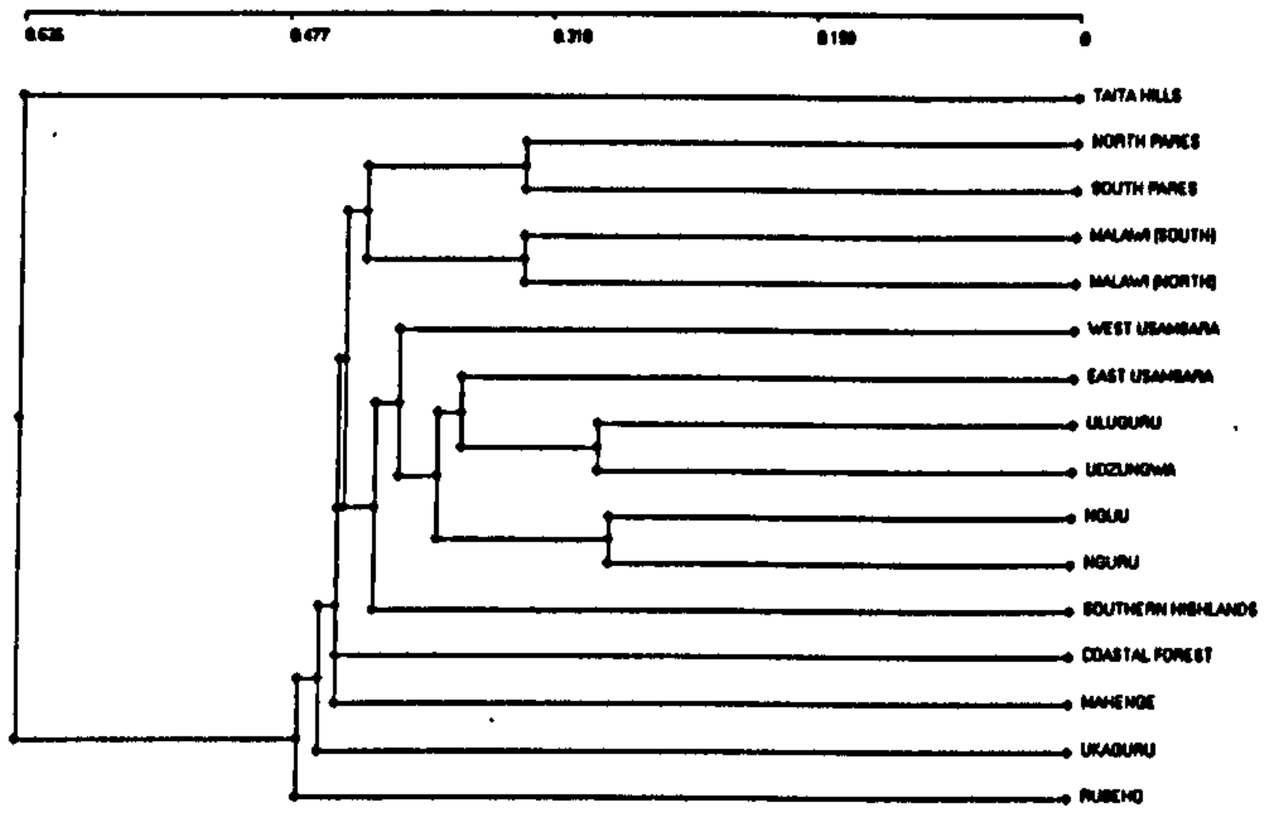

Figure 7.10
Cluster analysis of Sorenson's similarity co-efficient for 16 forests in EAM using single linkage agglomerative method.

7.4.1.2 Parsimony Analysis of Endemnicity

For 16 areas and 60 taxa, PAE yielded 30 most parsimonious area cladograms, with 107 steps, consistency index of 0.561, and retention index of 0.598. Of the 60 characters, 28 were parsimony uninformative and 32 are parsimony informative. Analyses were carried out with parsimony uninformative characters included and excluded. No significant differences were noted from these two analyses. A majority rule consensus tree is shown in Fig. 7.11a and bootstrap proportions given in Fig. 7.11b, with only proportions >50% shown. Note that phylogenetic terminology is used to describe the relationships in PAE (clades, monophyly), denoting groupings between areas.

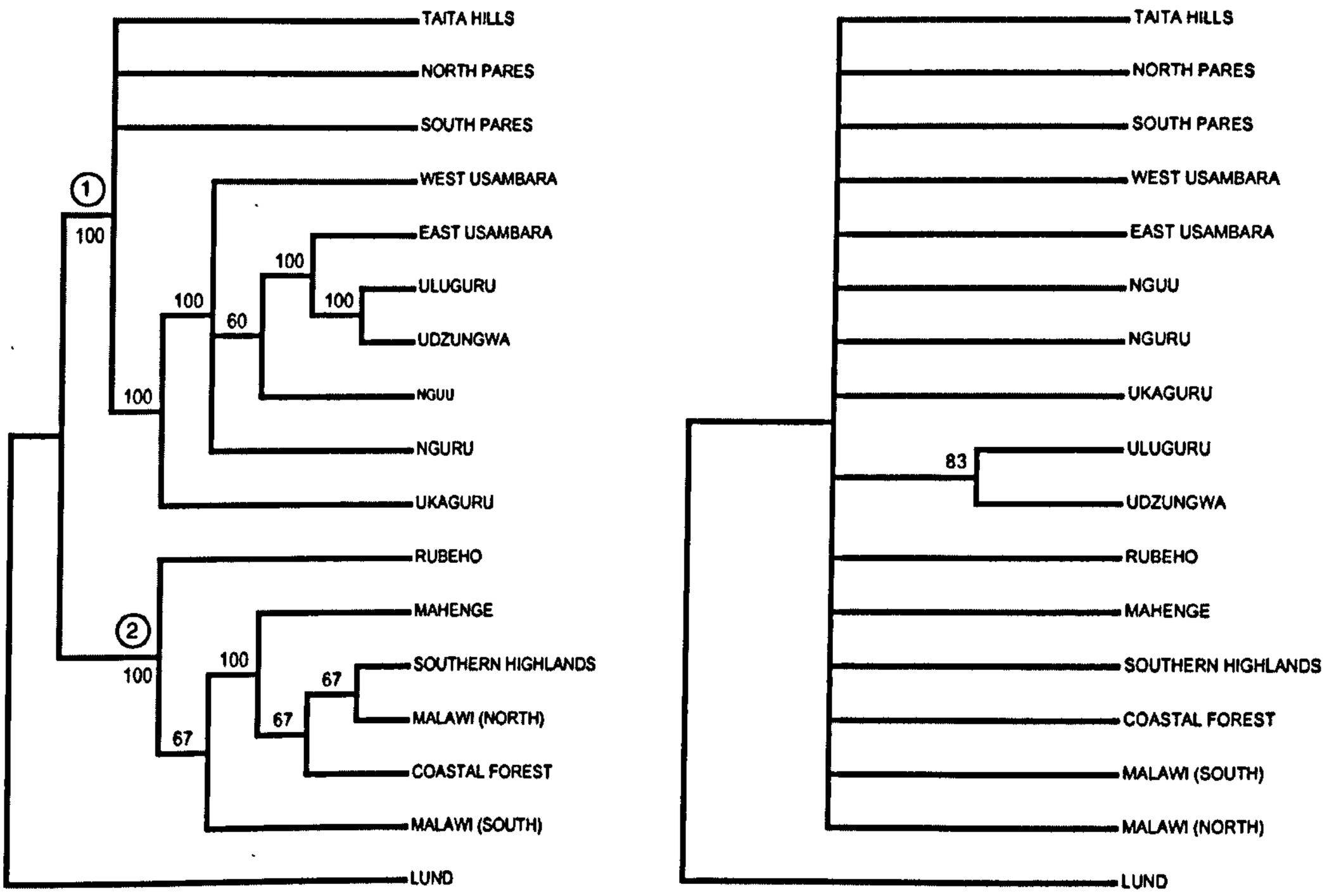

Figure 7.11
Parsimony Analysis of Endemnicity reconstructions of forest dependent species a) Majority rule b) Bootstrap analysis. Circled numbers refer to 1) EAM clade 2) mixed area clade.

The majority rule consensus shows groupings of all Eastern Arc Mountain areas, apart from Rubeho and Mahenge. The split between these two clades (marked 1 and 2; see Fig.7.11a) are poorly supported, with bootstrap values of less than 50 for both clades. Northern EAM areas are basally positioned within EAM clade (Taita Hills, North Pares, South Pares), though this is very poorly supported in bootstrap analyses. Within clade 1, southern areas show geographically discordant patterns of relationships, for example, East Usambara shares closer relationships with Nguu, Uluguru and Udzungwa than West Usambara. However, all the relationships within clade 1 are poorly supported. For clade 2, Malawi areas- North and South, are polyphyletic, in addition EAM areas, Rubeho and Mahenge are also paraphyletic. Almost all relationships in the PAE tree are poorly supported, apart from Uluguru + Udzungwa, which is moderately supported. Overall, analyses show that there is a poor resolution of relationships among EAM area amphibian assemblages.

7.4.1.3 Are there congruent patterns of amphibian assemblage relationships between PAE and similarity index methods?

There appears to be congruence between relationships recovered in PAE and similarity index results. Based on the reconstructions using PAE (based on majority rule consensus tree) and similarity indices, nestedness patterns showed similarity in the close relationship shared between some amphibian assemblages (1) Uluguru and Udzungwa (2) East and West Usambara and (3) Nguru and Nguu (4) close relationship of Rubeho and Mahenge to non-EAM areas (5) Non-monophyly of EAM. However, even in the examples given, the relationships are very poorly supported (see Fig.7.11b). Only the close relationship between Uluguru and Udzungwa is supported in PAE analyses, which is consistently recovered by all similarity methods. The lack of robust resolution is also highlighted by similarity measures, using Single Linkage clustering methods, which show proportionally similar degrees of difference among many of the sites (see Fig.7.9 and 7.10). Thus, although there are differences in amphibian assemblages shared between areas (in dendrograms recovered from similarity measures), the poor resolution between these suggests that there is only limited hierarchical structure.

7.4.2 Cladistic Biogeography

The results of a TRA analysis are shown in Fig.7.12. Each data set was analysed separately.

7.4.2.1 Amphibians only

Component analysis was run on six amphibian phylogenies (see Appendix 5 for blocks). The maximum allowable 1000 GACs were recovered by Component (the program does not allow any more). Based on the 1,000 GACs, a Nelson consensus was constructed which is summarised in Fig.7.12a.

This topology was taken as the GAC, and was statistically evaluated using TreeMap 1.0. The GAC has a duplication value of 18 and the topology has a p-value of 0.54, which is non-significant. In an attempt to investigate solution space more effectively, because nearest neighbour interchange methods (NNI) are liable to get trapped on 'islands', a subtree pruning and re-grafting branch swapping algorithm was used (more rigorous, but with increased computation time). This however produced exactly the same 1000 GAC's and Nelson consensus as recovered in NNI analysis.

7.4.2.2 All phylogenies

Component analysis was run on all thirteen phylogenies (see Appendix 5 for blocks). Similar to the last result, a maximum allowable 1000 GACs were recovered. The Nelson consensus of these is shown in Fig.7.12b.

This topology was then imported into TreeMap 1.0 as the GAC. The GAC has a duplication value of 38 and the consensus has a p-value was 0.81 which again is non-significant.

7.4.2.3 Overview of Cladistic biogeographical results

Analysis of both datasets indicates statistically non-significant results, which means that biogeographic inferences from the GAC in Fig.7.12 are not supported. However, the randomisation test carried out in TreeMap 1.0 cannot be interpreted as an absence of vicariance patterns in the EAM, as randomisation tests are 'asymmetrical' (Upchurch *et al.* 2002: p.614), which means an absence of evidence of a signal, not absence of repeated area relationships.

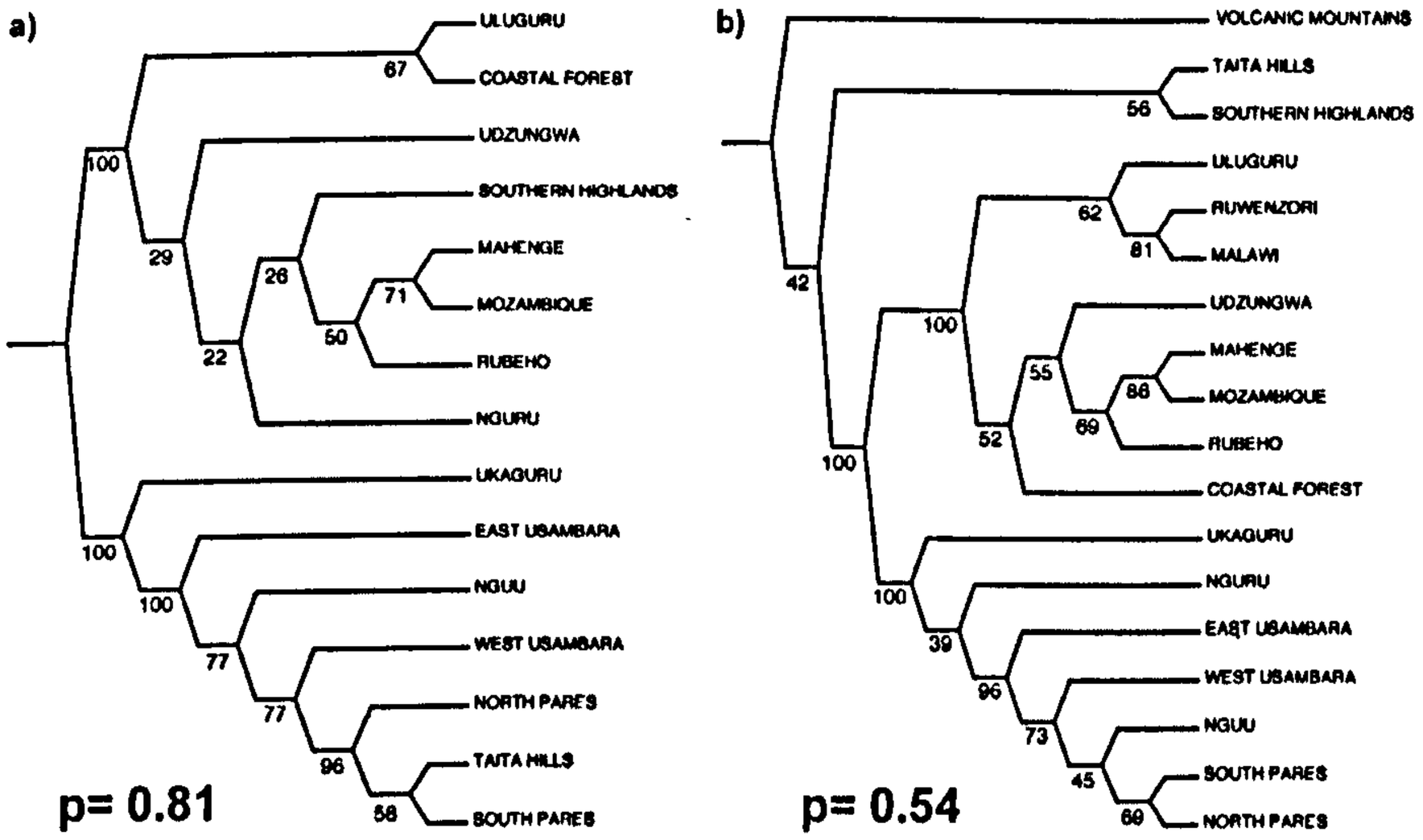

Figure 7.12

Nelson consensus of 1000 General Area cladograms recovered in Component analysis a) Amphibians only b) All phylogenies included. Values on branches denote proportion of non-conflicting area components. P values are shown, both highly insignificant.

7.4.2.4 Congruent patterns of spatial relationships between descriptive and cladistic biogeographical methods?

An overview of the biogeographic relationships recovered in cladistic and descriptive analyses is presented in Fig.7.13. The figure presents commonalities shared between the two approaches, one based on the phylogenetic relationships of amphibians and the second on the distribution of amphibians. There is some similarity seen between approaches, the close biogeographic relationships shared between northern located EAM (Pares and Taita Hills), the placement of Rubeho and Mahenge (EAM) in a group of forests located outside of the EAM (Malawi, Coastal Forest, and Southern Highlands). Despite these similarities there are predominantly discordant biogeographic relationships including the of grouping of Nguru + Nguu; Uluguru + Udzungwa among others. Overall, the congruent area relationships are difficult to quantitatively or qualitatively evaluate, because robust area relationships cannot be inferred from either data set, as shown by lack of support in TreeMap for general area cladograms, and bootstrap results in PAE. Thus I am led to conclude that there is no statistical support for amphibian area relationships in the EAM across methods.

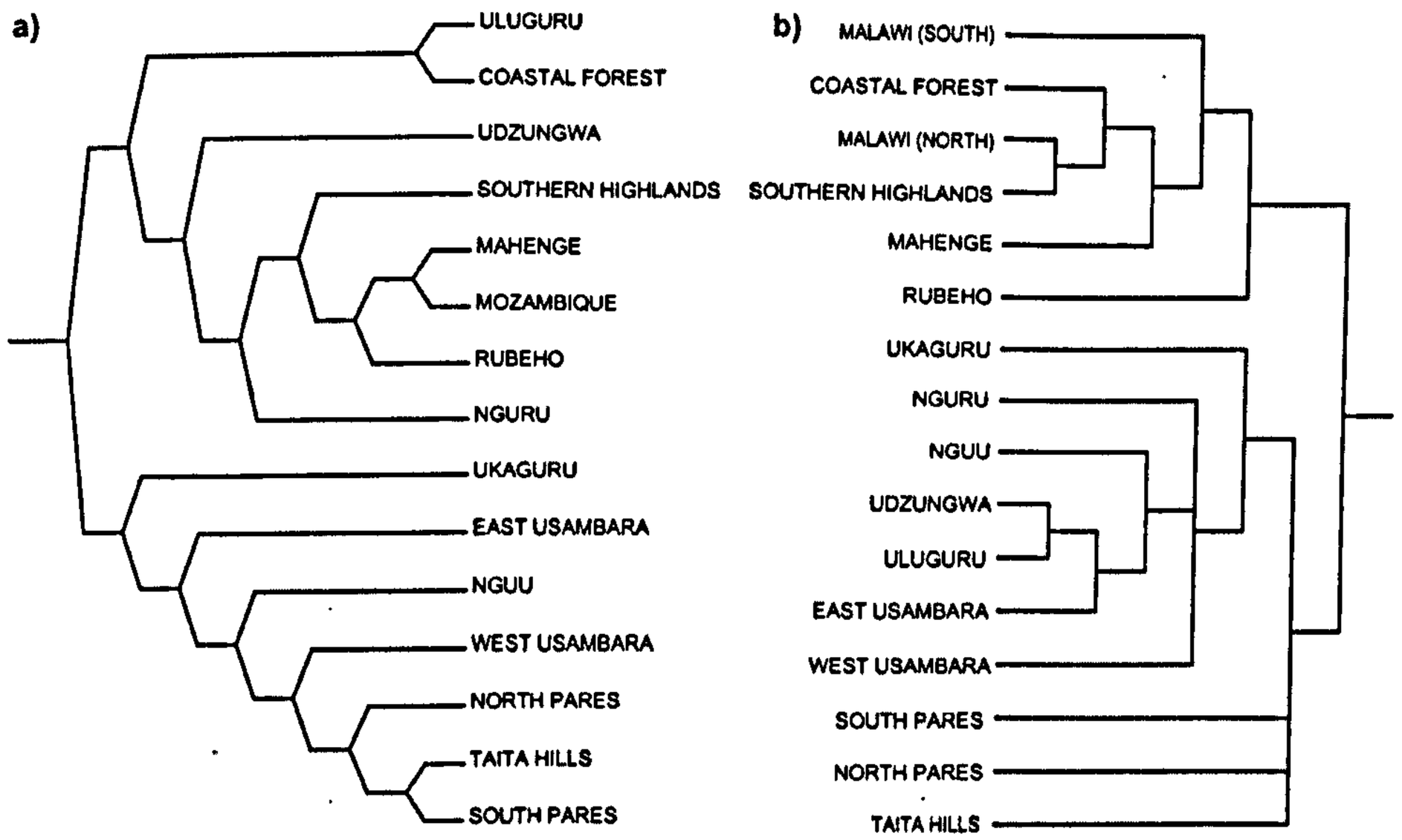

Figure 7.13

a) General Area Cladogram recovered in Component analysis for amphibian phylogenies b) Majority rule tree recovered in PAE analysis.

7.4.3 Temporal Data

Sequence divergence patterns can provide a test of whether species are affected by the same causal processes. In addition (assuming endemicity) they provide evidence for either the relative or absolute length of time that lineages may have occurred in a region, and therefore whether lineages are recent or relict diversifications. Temporal patterns are therefore critical in evaluating the biogeographic history of an area. The following results provide a synthesis of all molecular dating estimates and divergence levels among phylogenies estimated in this study and taken from the literature.

7.4.3.1 Temporal comparisons within EAM

Genetic pairwise differences were compiled (Table 7.2) for all Eastern Arc Mountain lineages analysed in Chapters 3-6. In addition geographic distances were calculated for each mountain block. Molecular rate variation comparisons were made between all lineages, indicating a homogenous rate between microhylids, *Boulengerula* and *Arthroleptides*. However, *Scolecomorphus* mtDNA was shown to evolve more rapidly, roughly three times as fast (see Table 7.1). The largest degree of variation (standard deviation) was exhibited in African microhylid rates, which would be expected given the large degree of phylogenetic diversity in this group.

Table 7.1.
Summary of rate variation (substitutions per site per unit time)

	Rate Variation (mean)	Standard Deviation
African Microhylids	0.001023	0.0002474
Scolecomorphus	0.003016	0.0001687
Boulengerula	0.001096	0.0000026
Arthroleptides	0.0009387	0.000008491

Molecular divergence estimates were compiled and compared among each mountain block (see Table 7.3; upper diagonal). Temporal congruence was then evaluated (see Table 7.3 lower diagonal) using absolute time estimates. Incongruent temporal estimates are marked in Table 7.3. Where temporal congruence could not be rejected, i.e. there was significant overlap in absolute time confidence limits (s-values), this was marked in Table 7.3. Of the 18 comparisons made in the Table 7.3, 12 (66%) show differences that cannot reject temporal congruence, but 6 (33%) allow rejection of temporal congruence. Table 7.3 provides evidence based on the temporal relationships from lineages, which reject the hypothesis that all speciation events between mountain populations show temporally congruent divergences.

Table 7.2.

Upper diagonal shows pairwise divergence of lineages between each mountain block. Lower diagonal shows geographical distance between each mountain block.

	1	2	3	4	5	6	7	8	9	10	11	12
1. Taita Hills	-	9.5%	8.8%	8.2%	18.6% 9.7%	18.2% 8.0%	12.0%	7.8%	11.1% 7.8%		8.2%	
2. North Pares	90	-	5.3% 10.6%	5.2% 10.6%	5.5% 8.9% 9.2%	9.5% 8.9%		7.8% 9.4%	14.2% 6.7% 9.5%	5.4%	6.6% 9.0%	5.8%
3. South Pares	115	70	-	5.3% 4.7%	5.6% 9.0%	9.1% 7.4%		8.2% 7.2%	14.4% 7.2% 7.1%	5.9%	7.3% 7.1%	6.5%
4. West Usambara	160	155	85	-	2.0% 4.3% 4.5% 7.7% 7.5% 7.5% 0.8%	6.4% 7.7%	17.7%	7.4% 6.2%	17.9% 14.8% 6.2% 6.2% 8.0%	5.2%	5.4% 6.3% 7.4%	5.1%
5. East Usambara	195	190	120	40	-	7.3% 7.5% 6.3%	17.9%	7.7% 6.7% 7.2% 9.7%	18.3% 14.2% 14.6% 5.9% 9.3% 7.2% 8.6% 2.7% 8.1% 7.8%	5.3% 7.6%	5.8% 8.4% 7.3% 10.2% 2.9% 10.0% 7.5% 7.8%	5.1% 8.5% 7.8%
6. Nguu	255	200	140	130	125	-		0.2%	0.8% 14.6% 9.3%	7.6%	1.0% 8.4% 10.0%	8.5%
7. Nguru	310	260	200	175	160	55	-		4.3%			
8. Ukaguru	365	300	250	240	230	115	71	-	8.7% 0.6% 9.7% 9.3%	7.5%	8.2% 0.9% 5.4% 9.2%	8.9%
9. Uluguru	395	355	290	245	220	155	95	110	-	14.6% 5.8%	15.3% 6.8% 1.1% 9.8% 9.5% 8.4% 1.6% 0.6% 3.9% 0.5%	14.8% 6.6% 0.5% 1.5%
10. Rubeho	420	350	305	305	290	175	130	60	145	-	5.0%	4.5%
11. Udzungwa	540	480	430	410	395	290	240	180	190	130	-	6.3% 0.5% 1.3%
12. Mahenge	645	590	535	505	480	395	340	290	260	255	130	-

(*Boulengerula* (Red), *Scolecomorphus* (Green), *Callulina* (Indigo), *Probreviceps* (Plum), *Spelaeophyrne* (Black) *Hoplophryne* (Brown) *Arthroleptides* (Yellow) and *Crotaphopeltis* (Light Blue)

Table 7.3.

Upper diagonal shows divergence estimates (in Myr) between areas. Temporal congruence is shown in Lower diagonal based on results from Table 7.X and upper diagonal in this table.

	1	2	3	4	5	6	7	8	9	10	11	12
1. Taita Hills	-				64.85 (58.92-71.24)		37.74 (32.96-42.78)		37.74 (32.96-42.78)			
2. North Pares		-	11.95 (10.37-13 46)	12.94 (10.42-14.33)	13.78 (12.42-15 33)							
3. South Pares			-	11.50 (8.90-13.64) 12.94 (10.42-14 33)	13.78 (12.42-15.33) 18.00 (12.58-23.42)							
4. West Usambara			†	-	13.78 (12.42-15.33)							
5. East Usambara			†		-	29.18 (22.00-37.31) 1.01 (0.81-1.28)	64.85 (58.92-71.24)	15.23 (13.28-18.06)	19.88 (15.17-25.32) 22.58 (19.86-25.82) 64.85 (58.92-71.24)		19.88 (15.17-25.32) 40.23 (25.86-48.59) 12.03 (9.46-15.21) 22.58 (19.86-25 82)	19.88 (15.17-25.32)
6. Nguu			†		†	-		0.53 15.23 (13.28-18.06)	1.96		40.23 (25.86-48.59) 3.43 (2.33-4 37)	
7. Nguru							-		13.16 (7.24-16.67)			
8. Ukaguru			†	†	†			-	1.96		3.43 (2.33-4.37) 22.92 (17.92-28 65)	
9. Uluguru	†		†		§				-		2.55 (1.69-2.76) 3.43 (2.33-4.37) 12.03 (9.46-15.21)	3.65 (2.76-4.81) 1.39 (1.13-3.08)
10. Rubeho										-	7.55 (4.75-10.35)	13.28 (11.78-16.27) 14 51
11. Udzungwa			†	†	§	§		§	§		-	3.65 (2.76-4.81) 0.47 (0.30-1.48) 14 51
12. Mahenge										†	§	-

§ - Molecular clock incongruence (no overlap in LF estimates)
† - Temporal congruence cannot be rejected (no significant difference in % difference or LF estimates)

7.4.3.2 Temporal comparisons between EAM and other regions

Molecular clock estimates and genetic pairwise differences between Eastern Arc Mountain taxa and species/populations occurring south of EAM, Coastal Lowlands, and West Africa are shown in Table 7.4. Patterns indicate a substantial period of isolation between regions South of Mahenge and Udzungwa, i.e. Rungwe and Mozambique. The correspondence between molecular clock estimates from the frog lineage *Probreviceps* and snake lineage *Crotaphopeltis*, indicate a common biogeographic history. Comparisons between coastal and lowland forests indicate temporally incongruent patterns, as shown by recent divergence between southern coastal forests/lowland forest (Kazizumbwe and Kilombero) and more archaic to northern, Kenyan coastal forests (Changamwe) in *Boulengerula.*

Temporal comparisons indicate a broad spectrum of divergence dates between EAM and West Africa lineages, divergences ranging from 12.27 - 99.25 Mya. The patterns of divergence seem to correspond with dispersal capability, as shown by tolerance to a diverse array of habitats. For amphibians not restricted to forest habitats (*Hemisus* and *Phrynomantis)*, species pairs from East and West Africa are shown to have diverged more recently, 30-45Mya and 12-25Mya respectively. In contrast, (generally) forest restricted amphibian pairs; *Arthroleptides/ Petropedetes*, *Boulengerula/ Herpele*, and *Scolecomorphus/ Crotaphatrema* show much deeper divergences of 40-100Mya. The lower bound of temporal estimates for forest restricted species significantly predate the separation of East and West African forests, (around 20Mya of Lovett, 1993a). Data presented by Matthee *et al.* (2004) also show molecular clock estimates (18 – 35Mya) that indicate similar levels of divergence. The data permit the conclusion that if molecular clock estimates are correct, dispersal between forest restricted amphibian species between East and West Africa has been limited since the Miocene. Even if dates are incorrect, forest restricted species show substantially greater divergences than non-forest amphibians.

Table 7.4.

Pairwise divergences of EA lineages (*Boulengerula* (Red), *Scolecomorphus* (Green), *Probreviceps* (Plum), *Phrynomantis* (Black) *Hemisus* (Brown) *Arthroleptides* (Indigo) *Spelaeophryne* (Grey) *Rhampholeon* († Dark Red) and *Crotaphopeltis* (Light Blue)) between regions in Africa.

	South of EAM		Coastal or Lowland Forest			West Africa
	Rungwe, Southern Highlands	Mozambique	Kazizumbwe	Changamwe	Kilombero Valley	
Eastern Arc Mountains: Genetic distance and molecular date estimates	3.8% 12.50Myr (9.25-15.89) *18.6% ß 22.8Myr (8.4 -37.2)	5.9% 11.01Myr (9.46 -12.86)	0.6% 3.11Myr (1.97 - 4.68)	7.7% 24.21Myr (20.29 - 28.64)	0.5% 0.47Myr (0.30 - 1.48)	20.7% 99.25Myr 20.2% 87.81Myr (82.56 - 93.98) 5.2% 17.67Myr (12.27 - 24.73) 12.7% 36.34Myr (29.74 - 44.88) 14.3% 50.12Myr (40.16 - 60.17) †?% 26.19 (18.7-33.68)
Range	3.8-18.6% 9.25 - 37.2 Myr	As above	As above	As above	As above	5.2- 20.7% 12.27 - 99.25 Myr

* divergences based on *cytb* and ND2, ß estimates based on molecular clock rates, see Gravlund (2002), †divergences based on estimates in Matthee *et al.* (2004).

7.4.3.3 Correlations between genetic and geographical distances within East Africa amphibian lineages

Genetic heterogeneity among populations should theoretically increase with greater geographical distances if there is no barrier to interbreeding, for example one would expect hypothetical populations A and B separated by 10km to be closer genetically than to population C which is 100km equidistant from both A and B. However, if there are barriers to contact between populations, such as a mountain between A and B, then there is likely to be biases in the degree of genetic similarity between populations. Genetic pairwise differences were compared with the geographical distance for each combination of area and within-lineage difference (exemplars of each mountain block). Fig.7.14 shows a positive correlation between increasing

genetic distance and geographical distance (r^2 value= 0.4864), although it is not perfectly linear, explaining only 48% of the variation. Therefore, although genetic distances are likely to increase with greater geographical distance, there is not a direct linear relationship, which might indicate more complex biogeographic processes are evident.

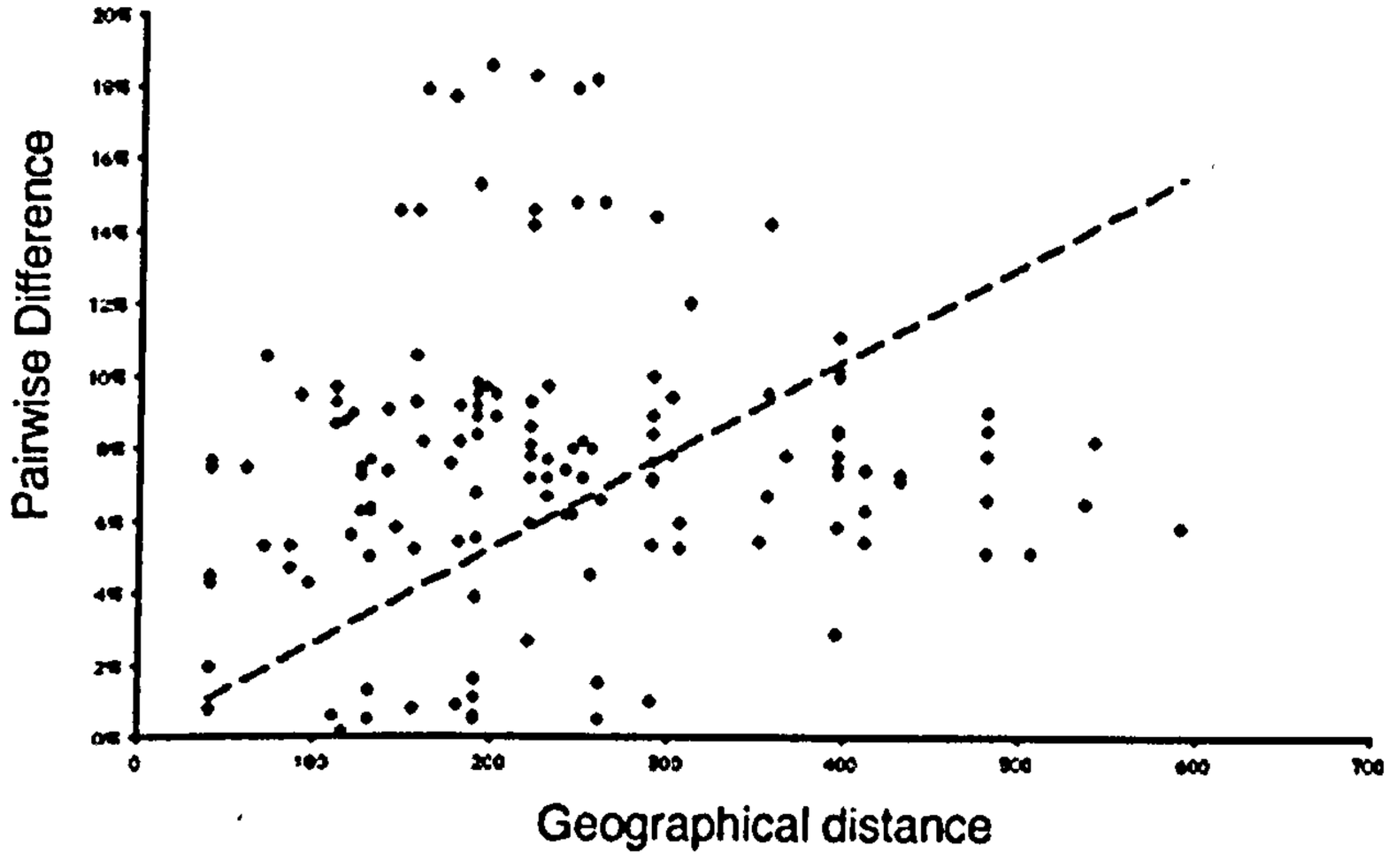

Figure 7.14
Graph showing the correlation between genetic distance (% pairwise difference) and geographical distance (in km) based on exemplars presented in Table 7.2 (r^2= 0.4864).

7.4.3.4 Persistence estimates

Divergence time estimates between monophyletic Eastern Arc Mountain taxa are shown in Table 7.5. EAM clades allow biogeographic interpretations to be made, in particular the persistence of forest habitats for forest-restricted species. For example, a parsimonious biogeographic interpretation of a clade distributed in the EAM (see Fig.7.15a) would be that all ancestors, from the initial split between taxa, would be distributed in the EAM area, otherwise a less parsimonious long dispersal event is needed to explain the pattern. In contrast, a clade incorporating species distributed outside the EAM makes biogeographic interpretations ambiguous (Fig.7.15b). The presence of forest-dependent clades is consistent with the interpretation that the habitat has also persisted for as long as the clade, therefore providing a proxy for investigating the former distribution and persistence of forest. Molecular clock results indicate that in all montane lineages a substantial period of persistence is inferred, ranging from 15- 70 Mya, with four of the six lineages showing temporally congruent divergence between 25- 48 Myr, averaging approximately 42 Mya.

Lindqvist and Albert (2001) have suggested the 'basal' position of a species and the area, or areas they occur in might be indicative of the geographical origin of a clade, termed 'ancestral areas'. Ancestral areas for basal clades in lineages however are difficult to interpret, because single areas cannot be unambiguously assigned as an ancestral area (see *Probreviceps* clade as an example Fig. 4.12). Furthermore, conclusions are likely to be highly sensitive to extinction processes and range expansions (Losos and Glor, 2003). Current distribution of a species is not necessarily a reliable indicator of the previous range of a species. In this study therefore ancestral areas were not inferred, but areas that were absent from basal positions on trees were identified (see Table 7.5). Areas not found in basal nodes include; Rubeho, Nguu, West Usambara, and South Pares. The absence of these areas might be indicative of a more recent origin of the forest.

Table 7.5.
Molecular divergence estimates (Mya) between basally positioned EAM forest-restricted clades.

	Arthroleptides	*Hoplophryne*	*Callulina*	*Probreviceps*	*Scolecomorphus*	*Boulengerula*
Molecular date estimates	19.88 (15.17-25.32)	40.23 (25.86-48.59)	40.62 (33.34-48.61)	44.94 (37.62-53.07)	43.45 (38.56-47.72)	64.85 (58.92-71.24)
Eastern Arc Ancestral Area Origin	East Usambara or Mahenge	Udzungwa or Uluguru	North Pare	Uluguru, Ukaguru or Udzungwa	Uluguru	Taita Hills, Usambara, Nguru or Uluguru

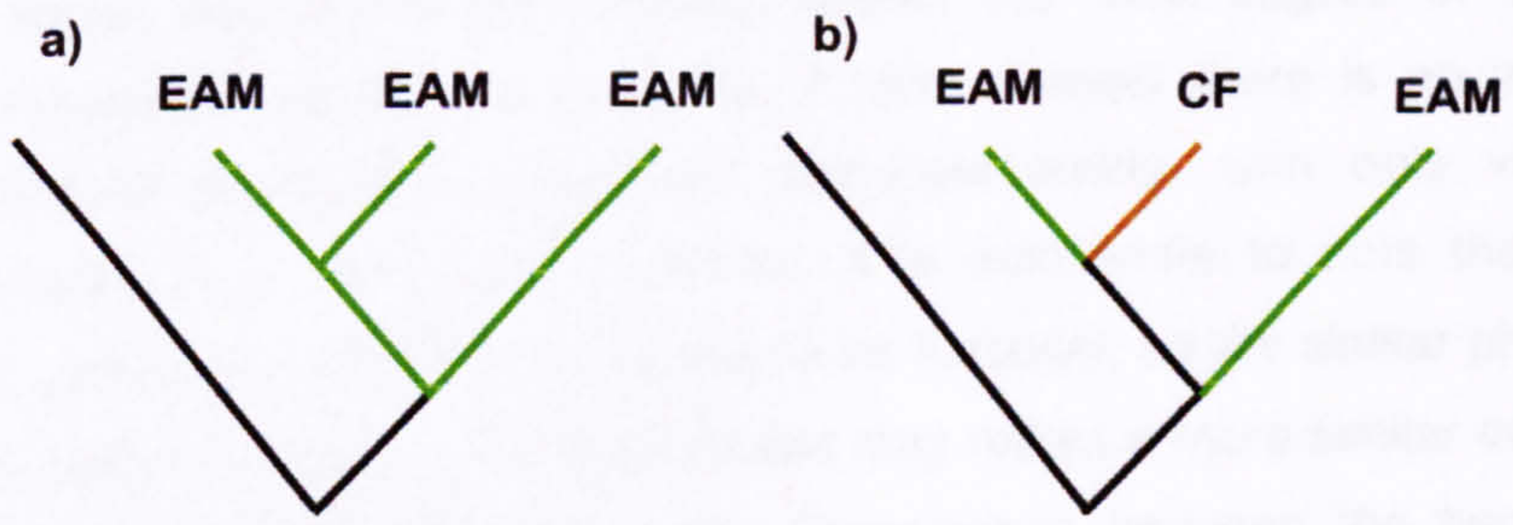

Figure 7.15
Use of phylogenies to infer persistence of montane forest a) Monophyly of EAM taxa b) Non-monophyly of EAM taxa. Green infers montane forest, brown infers lowland forest, CF= Coastal Forest and Black refers to ambiguous distribution and therefore uncertain habitat of ancestors.

7.4.3.5 Dispersal ability of study taxa

Taxonomic groups in this study were chosen primarily for their best potential to reconstruct forest habitats, thereby limiting any potential confounding effects. All taxa selected were 'forest restricted', which in this study was best determined by their current occurrence only in forests, and a constrained altitudinal distribution, as Poynton (1998a) outlined. However, in general, more detailed data were unavailable that might better evaluate the ecology of the chosen species (Howell, 1993; 2000). In addition to the use of altitudinal data for selecting lineages, specific biological characteristics make a distribution outside forest for certain taxa highly improbable. For example, none of the amphibian lineages chosen in this study is believed to be an open site breeder. Therefore, because lineages utilized in this study have restrictive habitat requirements, it is unlikely that any minor differences in biological characteristics between lineages can account for temporal incongruence. A rudimentary exploration of data was carried out to assess, qualitatively, whether lineages showed decreased divergence proportional to dispersal ability. For each amphibian lineage, ranges of altitudinal distribution and habitats were compiled (see the ranked Table 7.6). In addition, chronograms of each lineage, which were ranked according to dispersal ability, are compared in Fig.7.16a,b.

Comparisons were made separately for frogs and caecilians, because it is unclear how geographic patterns would influence the diversification of these divergent animals. In frogs, there appear to be consistent associations between phylogenetic divergence and dispersal ability. The brevicipitine lineage *Spelaeophryne*, which is found in forest and non-forest habitats, shows the least degree of divergence between mountain populations (see Fig. 7.16b). Overall there is an increase in phylogenetic diversity with presumed dispersal ability, with only very minor inconsistencies (e.g. *Callulina*). However, it is worthwhile to note that 'basally' positioned populations of *Callulina* appear to be fossorial, so the similar phylogenetic diversity between *Callulina* and *Probreviceps* may reflect a more similar ecology and life history than the data implied here. Comparison between the two caecilian lineages (*Scolecomorphus* and *Boulengerula*) also appear to show consistent relationship between phylogenetic diversity and dispersal ability (see Fig. 7.16a). The genus *Boulengerula* shows characteristics that imply it would be a poorer disperser than *Scolecomorphus*, in that it is more subterranean and is oviparous (Gower *et al.* 2004).

Table 7.6.

Table showing ecological and reproductive characteristics of lineages sampled in this study for frogs and caecilians.

		Lineages	Altitudinal range	Habitats collected	Breeding	Ecological Niche
Frogs						
Dispersal ability (1) poor (5) good	1	*Hoplophryne*	2100-355m	LF, SMF, MF, AZ	Specialized Tadpoles: develop in tree holes.	Cryptic and forest floor
	2	*Probreviceps*	2100-420m	RF, LF, SMF, MF, AZ	Presumed to be a direct developer.	Cryptic and forest floor
	3	*Callulina*	1900-180m	LF, SMF, MF, PF	Presumed to be a direct developer.	Arboreal and forest floor
	4	*Arthroleptides*	1900-180m	FE, PF, RF, LF, SMF	Specialized Tadpoles: develop in river torrents	Forest floor and stream
	5	*Spelaeophryne*	1000m-290m	FE, RF, LF	Presumed to be a direct developer.	Cryptic and floor dwelling
Caecilians						
As above	1	*Boulengerula*	2100m-180m	PF, LF, SMF, MF, A	Direct developer.	Subterranean
	2	*Scolecomorphus*	2100m-190m	AZ, PF, FE, LF, SMF, MF, A	Viviparous	Subterranean & forest floor

Habitat Abbreviations: FE (Forest Edge), PF (Plantation Forest), LF (Lowland Forest), RF (Riverine Forest), SMF (Submontane Forest), MF (Montane Forsest), AZ (Alpine Zone) and Agriculture (A).

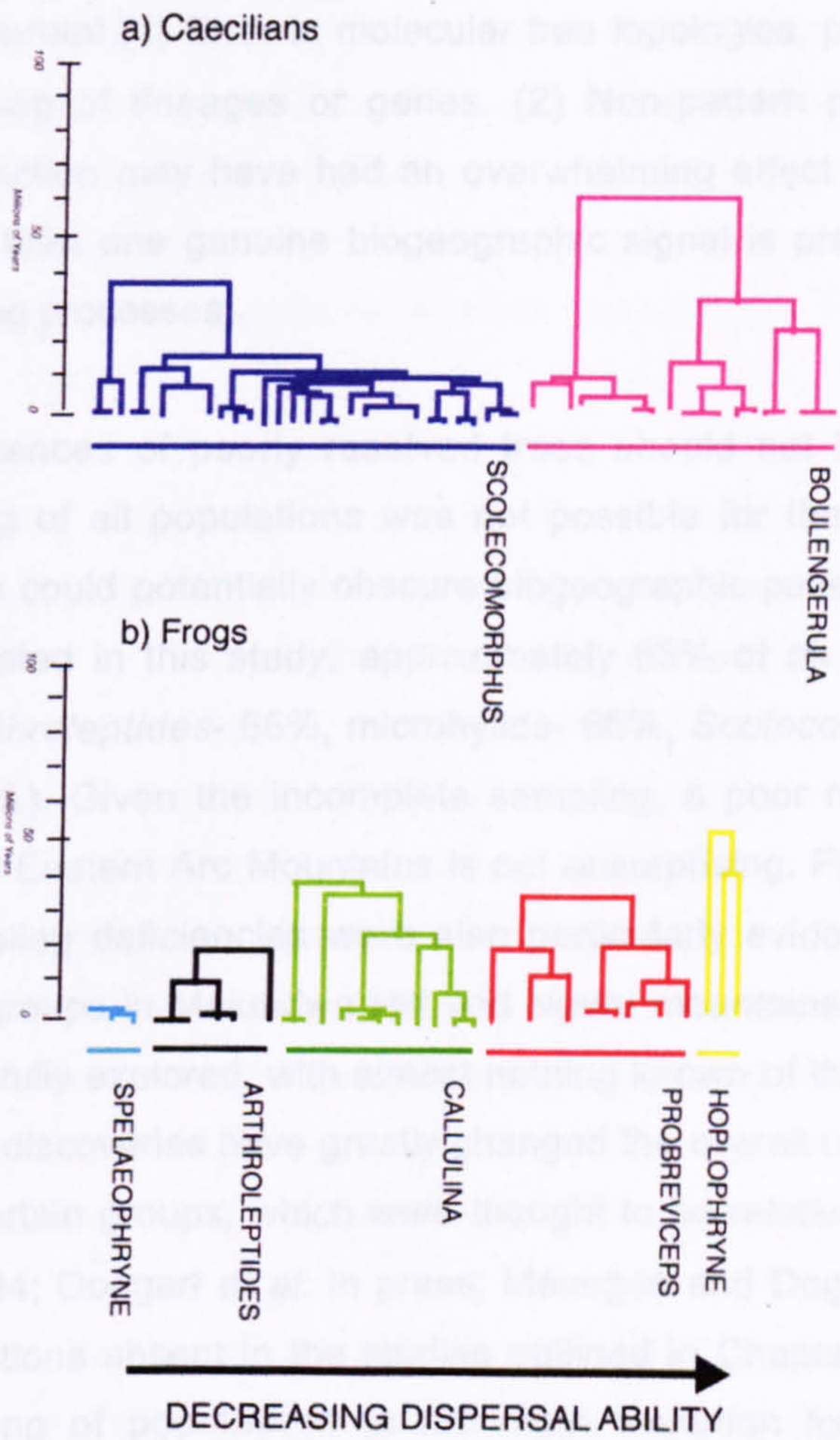

Figure 7.16

Chronogram of a) caecilians b) frogs sampled in this study, showing phylogenies ranked with presumed decreasing dispersal ability left to right.

7.5 Discussion

7.5.1 Hypotheses

7.5.1.1 Are there significant area relationship(s) in the EAM recovered using cladistic methods?

Data from cladistic biogeographic analyses of amphibians show non-significant area relationships within the Eastern Arc Mountains. Furthermore, with the addition of phylogenies from other groups, area relationships continue to be non-significant. The statistical failure of the cladistic biogeographic analyses of the EAM might be the result of a number of potential influences, though it is difficult to assess which factor

might be most important (1) Error in molecular tree topologies, perhaps the result of inadequate sampling of lineages or genes. (2) Non-pattern processes, such as dispersal and extinction may have had an overwhelming effect on biogeographical patterns (3) More than one genuine biogeographic signal is present (vicariance or vicariance mimicking processes).

The potential influences of poorly resolved trees should not be underestimated. Thorough sampling of all populations was not possible for the amphibian groups investigated, which could potentially obscure biogeographic patterns. For amphibian phylogenies estimated in this study, approximately 63% of all known populations were sampled (*Arthroleptides*- 66%, microhylids- 66%, *Scolecomorphus*- 62% and *Boulengerula*- 59%). Given the incomplete sampling, a poor resolution of spatial relationships in the Eastern Arc Mountains is not unsurprising. Furthermore, notable geographical sampling deficiencies were also particularly evident in this study for populations in all groups in Malundwe Hill and Nguru mountains. Many parts of the EAM are yet to be fully explored, with almost nothing known of the Nguu, or Rubeho Mountains. Recent discoveries have greatly changed the overall understanding of the diversification of certain groups, which were thought to be relatively well understood (Dineson *et al.* 1994; Doggart *et al.* in press; Menegon and Doggart, in prep.). The inclusion of populations absent in the studies outlined in Chapters 3-6 is a priority. Incomplete sampling of populations is the main limitation for resolving spatial relationships in this study.

Amphibians are generally intolerant of habitat changes (e.g. Inger and Voris, 1991; Biju and Bossuyt, 2003). It is therefore unlikely that species, particularly those restricted to forest habitats, would be able to disperse between mountains separated by savanna regions. Therefore, forest restricted species would only be able to move between mountain blocks if suitable forest corridors existed at some past time(s). Based on the data, it is difficult to evaluate how likely the discordant area relationships are the product of dispersals (see also below). Given the restrictive nature of study taxa, dispersal through forest corridors and/or depressed forest belts during glacial fluctuations would be the only likely mechanism. Glacial fluctuations are thought to have impacted the East African region significantly (e.g. Hamilton, 1988), which would have promoted habitat expansion and contraction periodically, and perhaps increased the likelihood of dispersal, in contrast to a static environment which would not have permitted dispersal between areas for forest restricted taxa.

Temporal data indicate consistently that between many areas are punctuated by more than one speciation event, with both ancient and recent divergences. Such evidence indicates that at least partially, the biogeographic history has been influenced by dispersal, which may have affected the resolution of biogeographic analyses focussed on vicariance patterns (see also Section 7.5.1.5).

It is difficult to evaluate, based on the evidence at hand, if there is a superposition of biogeographic patterns, which may obscure more recent relationships. However, the large degree of divergence exhibited between monophyletic taxa (see Temporal results), suggests there is a substantial period of time in which different geographic 'pattern processes' (vicariance, or vicariance mimicking processes) may have influenced amphibian diversification. From a probabilistic perspective, considering the large time span that taxa have persisted in the region, it is likely that more than one biogeographic event has influenced the relationships recovered, possibly with vicariant patterns decaying over time. Without an appropriate method for time slicing molecular data, in contrast to fossil data (Grande, 1985; Upchurch *et al.* 2002), it is unlikely that untangling two biogeographic signals is possible using current methods. Overall the TRA analysis provided no clear evidence for a common area relationship.

Reiterating the conclusion that Lavin *et al.* (2001) made on the failure of modern biogeographic approaches to reconstruct vicariance patterns, perhaps the 'rarity' of groups that have not been influenced by 'extinction, dispersal and sympatric speciation' may confound current analytical, vicariant approaches. Future studies of the EAM might focus on event based biogeographic methods, which use other biogeographic processes (dispersal) to explain incongruent area relationships, such as those implemented in the program DIVA (Ronquist, 1997). Conceivably, the inclusion of more data (phylogenies) would also permit a greater understanding of the biogeographic relationships in the EAM, though this is uncertain and no previous study provides a precedent for this. The findings presented here underline the intrinsically complex nature of biogeographic studies spanning long periods of time.

7.5.1.2 Are area relationships temporally congruent in the EAM?

Understanding patterns of speciation between lineages allows hypotheses about changing climates and fragmentation to be investigated. Potentially, divergence events may show spatially congruent patterns that occurred at different times, but

without temporal divergence estimates this would be difficult to determine. Besides fossils, molecular clock divergence times are our only means of understanding the changes of faunas and floras through time (see Chapter 2.6.4.2). Despite the well-documented controversy surrounding the calculation of absolute time estimates, molecular data have provided important evidence concerning the tempo and mode of evolution (Kumar and Hedges, 1998; 2004; Hedges *et al.* 1996; 2004). Area relationships recovered in this study are not robustly resolved, but the amphibian phylogenies show some congruent area relationships, which allow an assessment of a common history between pairs of areas.

As outlined in the results section, temporal data reject the hypothesis that all divergence events between mountains show congruent temporal divergences. Of the 18 comparisons made, 60% allow the rejection of the hypothesis of temporal congruence. Taking a conservative approach for assessing temporal congruence, 33% of comparisons unambiguously reject divergence events as being co-temporal between lineages. For example, comparing population samples between Uluguru and Udzungwa Mountains, temporal data show divergences of 2.55 Mya (1.69-2.76) for *Arthroleptides yakusini*; 3.43 Mya (2.33-4.37) *Callulina* sp.; 12.03 Mya (9.46-15.21) for *Probreviceps macrodactylus;* 13.28 Mya (11.78-16.27) for *Scolecomorphus* sp. Based on these molecular date estimates, speciation patterns show temporal congruence between the species pairs *Arthroleptides* + *Callulina* and *Probreviceps* + *Scolecomorphus* but not among them. Evidence therefore suggests at least two contacts between Uluguru and Udzungwa Mountains, rejecting the hypothesis that one causal event (vicariant or dispersal event) has occurred between the two areas. For example, it has been speculated that once the EAM became fragmented, each mountain would have became totally isolated, with only a dispersal events for forest restricted species possible during wet periods when forest may have connected each mountain block. The data suggest more than one geographic event, which may correspond to fragmentation and dispersal events, or simply multiple dispersal events.

Temporal congruence between lineages is supported in a greater proportion of comparisons made, which could support a single geographic event influencing diversification of amphibians in the EAM. However these comparisons are based on only a limited number of replicates, and therefore do not provide a stringent biogeographic test. Overall, the implications are that divergence events have

occurred over a prolonged period, with no single cause, (such as the fragmentation of the EAM), likely to have influenced diversification patterns in amphibians. Given the small number of replicates, comparison of more amphibian lineages will undoubtedly provide a more thorough test of temporal congruence. Based on the results here, increased sampling of lineages will probably show added complexity to the temporal and spatial biogeographic history of the EAM. Evidence presented here cannot reject the hypothesis that fragmentation has been important in structuring the biodiversity in the EAM.

7.5.1.3 Are area relationships consistent with nestedness patterns recovered in descriptive methods?

Analysis of data based on the distribution of amphibian species shows congruent patterns between methods (PAE vs. similarity indices, and clustering methods), although the data are weak, as shown by poor bootstrap results. Both the taxonomy and distribution of EAM amphibian species is poorly understood (Howell, 1993; Poynton, 2004; Poynton *et al.* submitted), thus any evaluation or comparison between PAE and similarity analyses may be limited, certainly for the more poorly studied mountains. It is clear that evidence presented in this thesis suggests the presence of many new species with different distributions to those currently recognised, with the genus *Callulina* a prime example. Furthermore, it is likely that such taxonomic problems will be repeated in other amphibian groups found in the EAM. Based on preliminary studies of certain groups; e.g. large arthroleptids (Poynton, pers. comm.) *Nectophrynoides* (Menegon *et al.* unpublished), *Afrixalus uluguruensis* and *Hyperolius spinigularis* (Clarke, pers. comm.) current taxonomic understanding severely underestimates taxonomic richness of EAM amphibians. New species and genera will continue to be described (Channing and Stanley, 2002; Menegon, pers. comm.), which will change current understanding of species and their distribution.

A fuller understanding of species distribution throughout the EAM is also likely to impact on descriptive biogeographic analyses. Faunal and floral inventories of all Eastern Arc blocks remain incomplete, and further study in every Eastern Arc block would likely discover additional new species and new distribution records in all but the best-known groups, such as mammals. Surveys of all Eastern Arc Mountains are necessary, but certain areas have historically attracted more interest, such as the East Usambara, which has led to survey effort biases. Burgess *et al.* (in press)

investigated such biases, using Isango's (2001) list of Eastern Arc publications as a proxy for survey effort. Based on this measure, Burgess *et al.* (in press) found a correlation between survey effort and the biodiversity ranking of the Eastern Arc blocks. These findings give an indication of the sampling inequalities in the EAM and point to the limitations of biogeographic analyses based on incomplete distribution data. Perhaps the low statistical support for relationships obtained in descriptive analyses, reflect the uneven sampling of amphibians in the EAM. Overall it is clear that determining species taxonomy and their distribution based on current understanding is difficult. Prior to the investigations outlined here, which have refined taxonomic understanding of certain groups and the distribution of species, our perception of amphibian diversity in eastern Tanzania grossly underestimated species numbers and diversity within each mountain block. As a consequence of these, the data matrix was probably incorrectly coded in places, which potentially obscured patterns in these analyses.

In addition to problems with incomplete or incorrectly coded data matrix as a result of insufficient information on distributions or incorrect taxonomic classifications, there are methodological problems in reconstructing the biogeographic history of an area using species distributions. As has already been outlined (see section 2.6.2), descriptive approaches use data that are unable to confidently evaluate biogeographic patterns. Upchurch (2004) forcefully argued, against using 'descriptive' approaches, stating such methods 'should never be used to assess the historical relationships between biotas and/or geographic areas' even in the case when 'scarcity of data prevents us from applying appropriate methods'. Upchurch, (2004) insists that instead of using data derived from species distributions for investigating biogeographical problems when species phylogenies are unavailable, it would be better to focus attention on collecting data we lack. The descriptive biogeographic results presented here are ambiguous and it is difficult to assess their utility. However, even with an improved or even 'complete' data set, understanding the biogeographic complexity of the EAM based on distribution data is not likely to be a fruitful mode of research, taking into account the ages, ecology and differing dispersal capabilities of amphibians distributed in the forests.

7.5.1.4 Are temporal or spatially discordant relationships correlated with dispersal ability?

'Environmental tracking of ranges of species in accordance with climatic changes is likely to involve complex lagging, governed by the spreading and retracting rate of each species' (Poynton, 1999; p.513).

For species able to tolerate a wide range of habitats (e.g. some birds and mammals), or able to disperse long distances (e.g. birds), the footprints of climatic and geological changes are less likely to affect diversification patterns. Based on the fact that biological characteristics of taxa influence temporal and spatial biogeographic patterns (Hewitt, 2004), it is pertinent to consider whether taxa in this study have notably different biological characteristics (e.g. dispersal ability) that might explain the discordant temporal and spatial patterns recovered. Even within groups with restrictive habitats, such as amphibians, there is a spectrum of ecological differences among species (Austin *et al.* 2004), as shown by restrictive breeders (e.g. *Arthroleptides*) and habitat generalists (e.g. hyperoliids). For inferring changes in forest distribution it is sensible to use species closely associated with forest, so that their ancestors can be inferred as occurring in similar types of habitats. Such prerequisites for a study are not always possible, mainly because the habitat requirements of an extant or extinct organism cannot always be easily inferred.

Generally, a correlation between dispersal ability and phylogenetic diversity was recovered from comparisons made in this study (see section 7.4.3.5), which is not entirely unsurprising given the impact dispersal has on diversification of organisms (Vermeij, 1991). However, the poor understanding of the ecology of lineages and how these affect dispersal ability forbids any clear understanding. For example, in comparisons of the two-caecilian lineages, (*Scolecomorphus* and *Boulengerula)* a consistent relationship between phylogenetic patterns and dispersal ability is recovered, with the presumed poorer disperser *Boulengerula* showing patterns of longer isolation. In support of this, Gower *et al.* (2004) recently evaluated the distribution and abundance of *Scolecomorphus vittatus* and *Boulengerula boulengeri* and concluded *B. boulengeri* was primarily a subterranean burrower while *S. vittatus* spends more time at the surface. These findings could be used as evidence of *S. vittatus* being a better disperser. In addition, Gower *et al.* (2004) outlined other biological characteristics that might have a significant impact on dispersal ability, e.g.

reproductive biology. However it might be premature to make such broad comparative conclusions on dispersing ability. For example, recent surveys of *Boulengerula* and *Scolecomorphus* in the EAM (Loader, pers. obs.) show both species show a large range of ecological tolerance to non-forest mountain habitats, and therefore it is unclear how this may affect dispersal ability. Despite the finding that dispersal ability influences diversification patterns in the amphibians utilized in this study, the degree of incongruence, both spatially and temporally, means that it is unlikely that dispersal can explain fully the many incongruent biogeographic patterns recovered.

Findings based on cladistic biogeographical analyses and temporal data suggest that speciation events are temporal and spatially incongruent in amphibians. This is further corroborated by phylogenies of other groups. Temporal and spatial incongruence between lineages cannot be easily explained by proportional differences in dispersal ability. Therefore the biogeographic history of EAM is likely to be the result of more than one single event, such as fragmentation that might produce a clear vicariant signal. More detailed sampling of populations will need to assess how robust this hypothesis is. The sampling intensity in this study precludes any strong conclusions. Perhaps a greater understanding of ecology, physiology, and genetic diversity may alter perceptions of this in the future. Future studies should attempt to better quantify both quantitative and qualitatively the ecology of the Eastern Arc amphibian fauna and thereby evaluate more effectively the diversification of these lineages. This can be achieved by traditional ecological approaches or more sophisticated GIS techniques (Raxworthy *et al.* 2003). Austin *et al.* (2004: p.814) suggested that differences in phylogenetic diversity may be 'partially attributable to generation times, or metabolic rates', dispersal ability clearly influences biogeographic patterns but to what extent it alters phylogeny of closely related species is unclear at present. Correlations between habitats and species distribution have been examined using geo-reference data (Raxworthy *et al.* 2003). Such data could potentially better constrain biogeographical analyses and their conclusions.

7.5.1.5 Is there a strong temporal and spatial correlation between area relationships and geographic events in the EAM?

Recent biogeographic studies have shown that a comparison of phylogenetic patterns can provide evidence that current distributions are the result of particular fragmentation events, such as the movement of continental land-masses (e.g. Upchurch *et al.* 2002; Gower *et al.* 2002; Bossuyt *et al.* 2004). Furthermore, with the availability of molecular divergence estimates, the ages of the events, such as isolating barriers, can be assessed and correlations made between geographical history and the evolutionary diversification of lineages. The use of more than one phylogenetic lineage allows the testing of more general biogeographic patterns, because the same biogeographic barrier might be expected to influence independent lineages in a similar way.

Determining causal events from spatial data remains difficult without resolved trees. The main spatial process thought to have impacted on the EAM is the rapid uplifting of mountains in the Miocene and it would be expected that spatial relationships would be recovered which would correspond to the fragmentation of a previously continuous forest. It is unclear what the pattern and sequence of fragmentation between mountains is (Griffiths, 1993), and so it can only be assumed that closely adjoined mountains would, in general, show closer area relationships from a process of fragmentation. Despite the fact that area relationships obtained from cladistic and descriptive biogeographic approaches were shown to be non-significant, an analysis to evaluate if closer area relationships (based on the GAC recovered from Fig.7.13a) were positively correlated with geographical distance was carried out. Figure 7.17b shows a positive correlation between geographical distance and distance of the GAC between areas (with an r^2 of 0.51). This might be indicative of a history of fragmentation. However, the area relationships recovered in this analysis were shown to be non-significant, and even if the GAC was shown to be significant, correspondence between area relationships and geographical distances could be the result of other vicariant mimicking processes (Hunn and Upchurch, 2002). Overall, the spatial data remain problematic and require the gathering of more taxon area cladograms to assess whether the pattern of incongruence is common to all amphibian lineages in the EAM.

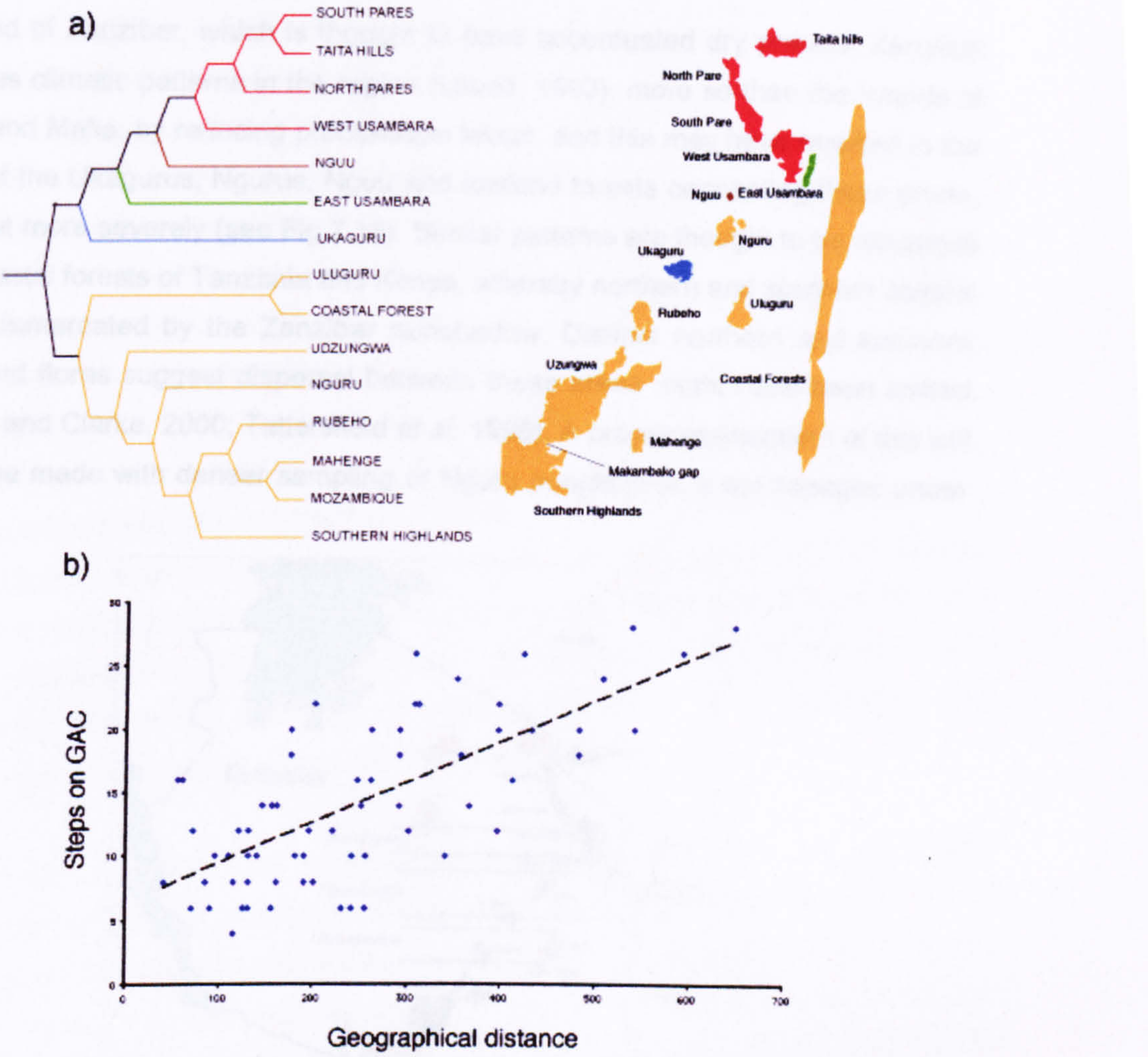

Figure 7.16.

a) Spatial relationships recovered in TRA analysis, with map of Eastern Arc Mountains. Colours correspond to clades and geographical areas. b) Graph showing the geographical distance between areas against the number of steps between areas in the General Area Cladogram (r^2= 0.51).

Significant area relationships were not recovered in any of the analyses carried out. However, there appears to be a consistent separation between southern and northern EA areas (see Fig.7.17a). This phylogenetic pattern implies the presence of a biogeographic barrier between northern (Taita Hills, Pares, Usambaras) and southern (Ulugurus, Rubeho, Udzungwa, Mahenge) EAM regions, with the separation between northern and southern regions around the Ngurus. This separation of Eastern Arc fauna (shown also in the non-significant GAC, Fig.7.16) has previously been suggested (e.g. Bowie *et al.* 2004). The area around the Ngurus is believed to be a significant barrier to dispersal because of its position in line with

the island of Zanzibar, which is thought to have accentuated dry phases. Zanzibar influences climatic patterns in the region (Lovett, 1993), more so than the islands of Pemba and Mafia, by reducing precipitation levels, and this may have resulted in the forests of the Ukagurus, Ngurus, Nguu and lowland forests connecting these areas, drying out more severely (see Fig.7.18). Similar patterns are thought to be influential in the coastal forests of Tanzania and Kenya, whereby northern and southern coastal forests, demarcated by the Zanzibar rainshadow. Distinct northern and southern faunas and floras suggest dispersal between these areas might have been limited (Burgess and Clarke, 2000; Tattersfield *et al.* 1998). A proper assessment of this will need to be made with denser sampling of Nguru populations in the lineages under study.

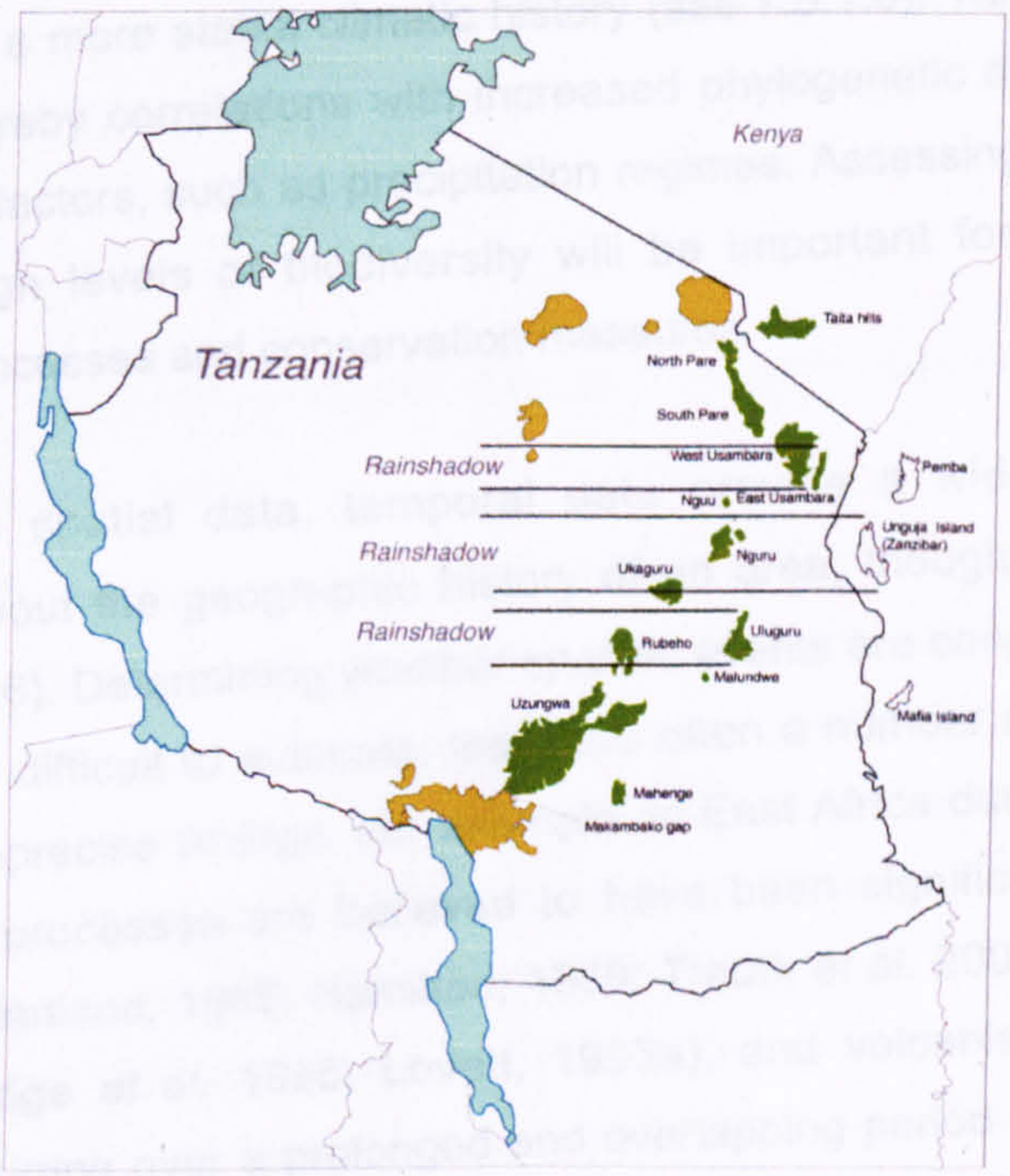

Figure 7.18

Map of the Eastern Arc Mountains showing rainshadows produced from adjacent areas (Zanzibar, Ulugurus, and East Usambaras)

The proximity of coastal islands relative to the EAM is not the only influence on the climatic circulation in East Africa that might have implications for understanding biogeographic patterns. The effect of distance each mountain is to the coast has clear influences, with increasing distance correlated decreased precipitation levels. Burgess *et al.* (in press) have identified a correlation between greater distance to coast and lower biodiversity levels. Another factor influencing climatic conditions is

the topography of land adjacent to the coast, in some areas the coast is obscured by other mountains. The Ukagurus and Rubehos have reduced rainfall, in part the result of the longer distance to the coast, and the barrier of the Ulugurus (Fig. 7.18) condensing much of the moist air. Regions influenced by the effects of rainshadows, such as the Rubehos, receive reduced levels of precipitation, and likely experience more severe arid phases. It is interesting to note that the Rubehos, along with the Nguu, West Usambara, and South Pares show consistently more recent levels of divergences in amphibians than other EAM areas, regions that have reduced precipitation levels. Conversely, mountains in close proximity to the coast, and not obscured by other mountains, such as the East Usambara and Ulugurus show biogeographic patterns consistent with greater and deeper phylogenetic diversity, correlating with a more stable climatic history (see 7.5.1.6). This should be tested in the future, whereby correlations with increased phylogenetic diversity can be made against abiotic factors, such as precipitation regimes. Assessing the determinants for maintaining high levels of biodiversity will be important for understanding both evolutionary processes and conservation measures.

In contrast to spatial data, temporal data provide a wider scope for testing hypotheses about the geographic history of an area, though not uncontroversially (see section 2.6). Determining whether specific events are congruent with divergence events can be difficult to evaluate, there are often a number of possible geographic events with imprecise timings. For example, in East Africa during the Tertiary period a number of processes are believed to have been significant, including climatic changes (Williamson, 1985; Hamilton, 1988; Trauth *et al.* 2005), formation of the rift valley (Partridge *et al.* 1995; Lovett, 1993a), and volcanism (Lovett, 1993a) all generally occurring over a prolonged and overlapping period of time. Molecular data from this study provide a number of examples that show an apparent correspondence with the uplifting of mountains in the early Miocene period (~25-10 Myr). The lineages *Arthroleptides, Scolecomorphus, Callulina, Probreviceps, Boulengerula,* and *Hoplophrye* all have examples of branching patterns that correspond to this time. Only *Spelaeophryne*, with its more widespread habitat distribution, is shown to have more recent divergence patterns. Of particular note is the rapid diversification of *Scolecomorphus*, where in a relatively short period of time many lineages appear to show rapid allopatric divergence between mountains, which corresponds precisely with the proposed final phases of the fragmentation of the EAM (Partridge *et al.* 1995).

More recent climatic fluctuations, such as the humid episode 3.3-3.4 Mya (Williamson, 1985; Trauth *et al.* 2005) and Pleistocene fluctuations (0-1Mya) are marked by divergence patterns in most lineages of *Arthroleptides, Scolecomorphus, Callulina, Probreviceps* and *Boulengerula*. The association between divergence and various geographic events (uplift and climatic changes) might be coincidental, but the timing does not reject a possible link. Further sampling of populations and nuclear genes will be necessary to fully clarify these dating estimates. In addition, a greater understanding of the geological and geographical changes that have taken place on the African mainland may assist in interpreting biogeographic patterns more critically. Taking both the spatial data and temporal data together, this study identifies that there are a number of different processes that have influenced the diversification of the EA amphibian fauna. This complex biogeographic history of this region offers a challenging area of future study.

7.5.1.6 Are phylogenetic lineages deeply divergent between monophyletic taxa in the Eastern Arc?

Investigating the endemic ranid genera in southern mountain ranges of India, Roelants *et al.* (2004) found 'long term' evolutionary history based on proportionally large divergences in amphibian molecular phylogenies. Drawing on comparisons around the globe, they mentioned similar areas of high amphibian endemism that have also experienced a prolonged period of isolation, including Island faunas (e.g. New Zealand's leiopelmatid frogs; sooglossid frogs of the Seychelles; Madagascar's mantelline frogs), and climatically isolated regions (e.g. heleophrynid frogs of South Africa). Missing from these examples are the climatically isolated Eastern Arc amphibian fauna of Tanzania and Kenya (e.g. dwarf bufonids, microhylids and caecilians), which is believed to be highly diversified, and thought to have persisted for a long period of time (Howell, 1993). It is likely that the absence of the EAM as an example of an isolated amphibian fauna in Roelants *et al's* (2004) paper is a consequence of the relatively poor understanding of the mountain fauna. Only very limited phylogenetic evidence has been used to address questions on the EAM, none of which has been based on amphibian lineages, an ideal indicator group (Avise, 2000). Matthee *et al.* (2004) and Gravlund (2002) provided some recent evidence for the long-term residence of forest restricted species; with *Rhampholeon* and crotaphopeltids in the Eastern Arc estimated to have peristed for ~20-30Myr, though

the patchy geographical sampling provided only a limited test of this. Most research has concentrated on distributional data to infer isolation and persistence (e.g. Bruhl, 1997; Poynton, 1999).

Bruhl (1997 p.223) used the distribution of flightless insects to investigate patterns of forest distribution in Africa. Bruhl (1997) suggested a 'clearer view of former distribution of montane habitats' could be inferred because of the inability of flightless insects to disperse long distances 'which could obscure common biogeographical patterns'. Based on the taxonomic distinctiveness of insect lineages between certain mountains throughout equatorial Africa, and the absence of taxa in other mountains, Bruhl (1997) suggested persistence of forest in the Eastern Arc Mountains and the likely drying of forests in other regions, such as Mount Kenya. Recent evidence has supported this, as shown by the close phylogenetic relationships between species in the EAM, and Madagscar (Vandenspiegel, 2001; Warui and Jocqué, 2002; Huber, 2004; Tattersfield *et al.* 1998), Seychelles (Johanson and Williassen, 1997) and South East Asia (Dineson *et al.* 1994; Burgess, *et al.* 1998a), which suggests the persistence of lineages since the breakup of Madagascar and Africa (~130Myr).

Based on a number of different sources of evidence, palaeontological, phylogenetic, and distributional, the EAM have been predicted to show divergence patterns of forest persistence dating back some 25Myr. This precise date is thought to correspond with the uplift of EAM during the rifting of East Africa, which attracted orographic rainfall providing the necessary humid environment to support rainforest habitats. Prior to this rifting and the exclusive climate this afforded, the forests of the EAM are thought to have been present, though their occurrence temporally and spatially would likely have fluctuated dramatically. The African climate has been shown to have fluctuated dramatically, long periods of aridification between 50-30 Mya (Retallack *et al.* 1990; Cerling *et al.* 1992; Morley, 2000) promoted grass-dominated habitats, and rainforest habitats would have likely been restricted to small refuges (Hamilton, 1988).

Molecular dating estimates support (from lineages representing four currently recognised amphibian families: Scolecomorphidae, Caeciilidae, Petropeditidae, and Microhylidae) the long-term persistence of EAM clades. Forest dependent amphibians sampled in this study (excluding *Spelaeophryne*) all show long branch lengths, which when calibrated to estimate molecular divergence times show a

proportionally long period of persistence in monophyletic EAM taxa, an average of ~42Myr (15-71Myr). A minimum period of persistence is calculated at 15Myr (minimum s-value estimate) for the *Arthroleptides* lineage. If the molecular clock estimates are correct, then the results consistently indicate archaic persistence, pre-dating substantially the rifting of East Africa (~25Myr). That the dates nearly double the predicted period of persistence of forest in the EAM may seem surprising, but there appears to be some geological evidence that may support this. Cahen and Snelling (1984) suggested some higher peaks in the EAM might be older than initially predicted, for example, the Ulugurus may have been a significantly sized mountain around 37Mya, which might have attracted orographic rainfall and therefore forest habitats would be likely to have persisted.

The dates estimated for the origin of the EAM by Cahen and Snelling (1984) corresponds more closely with the temporal data of basal amphibian clades sampled in this study. In addition, four of the six lineages sampled in this study indicate the Ulugurus as a possible ancestral area (*Scolecomorphus uluguruensis* being a prime example), which provides further evidence for the ancient history of this region. The generally more basal position of Uluguru species is also supported in phylogenies of other EAM groups, for example *Lobelia morogoroensis* (Knox and Palmer, 1998) *Saintpaulia goetzeana* and *Saintpaulia pusilla* (Lindqvist and Albert, 2001), *Andropadus neumanni* (Roy, 1997), *Helicopsyche stoltzei* (Johanson and Williassen, 1997) and *Crocidura* (Stanley, pers. comm.). Based on these preliminary findings it might be reasonable to assume that the Ulugurus may have served as an early refuge for ancient forest fauna of the EAM. Knox and Palmer (1998) similarly suggested for giant lobelias that the Ulugurus might be an ancestral area in the EAM, arriving as a colonist from the Asia/Pacific region. Attempts to infer ancestral areas, based on modern distributions and phylogenies of extant species, however are considered difficult to interpret (Losos and Glor, 2003). Range expansions particularly can confuse reconstructing ancestral areas, particularly in areas dominated by climatic process promoting forest persistence, and periodic expansion and contraction (Morley, 2000). Furthermore, sampling of coastal, central African areas may show that EA taxa are not monophyletic and thereby render the reconstructions ambiguous. The sampling of more lineages with similar distributions over a wider area would be an appropriate step to evaluate patterns. There remain many difficulties in investigating the geography of speciation (Losos and Glor, 2003).

Stuart (1981) investigated correspondence between geologically older mountains and levels of avian biodiversity in the EAM, and he proposed the highly biodiverse forests of Usambaras and Ulugurus are derived from geologically older mountains and that the maintenance of these habitats was likely to have influenced biodiversity patterns. Stuart (1981) noted that it is not necessarily correct to assume that older mountain ranges have older forests, because forest palaeo-history is likely to be more dependent on climatic changes than on geology. Amphibian lineages sampled in this study indicate that almost all mountain blocks in the EAM have patterns of long-term persistence of lineages (see Table 7.6), with only the South Pares, West Usambara, and Mahenge showing more recent patterns (within 10 Myr). Whether this reflects a more general pattern of species persistence in these mountain blocks or is a specific pattern of the lineages sampled is difficult to currently evaluate, because there are few geological data on the specific origin of each mountain in the EAM. As in several other continents, biodiversity in Africa is concentrated in the tropical forests along the equator. Opposing hypotheses explaining tropical forest biodiversity are the ancient, stable nature of these environments (Fisher, 1960; Fjeldså & Lovet 1997) or their fluctuating history in association with global climate change, whereby forest taxa are repeatedly confined to periodically isolated refugia (Haffer, 1969). Amphibian lineages sampled in this study seem to bear the genetic footprints of both recent climatic fluctuations and of the long-term persistence of rainforests.

The results provide a preliminary insight into more general patterns of diversification in the EAM, perhaps repeated in other groups and elsewhere in tropical forests around the globe. Fjeldså and Lovett (1997) suggested that the distribution patterns of plant and bird species implied that persistence was important in maintaining biodiversity in montane African rainforests, which was supported by phylogenetic surveys of Matthee *et al.* (2004) and Gravlund (2002). It is likely that without the presence of suitable forest habitats, such as the EAM, the ancient endemic amphibian fauna as determined here would have perished. Clearly persistence has been critical in structuring the biodiversity of the EAM. Persistence of habitats is thought to have been critical in other regions (see Knapp and Mallet, 2003 for review). For example, in Asia, Roelants *et al.* (2004) suggested the persistence of favourable climatic conditions, analogous to those outlined in the EAM, served as a refuge for old lineages of amphibians. A more precise investigation into the rates of diversification, and how they vary over time, across geographic boundaries and among taxonomic groups needs to be undertaken to understand the processes of

persistence more effectively in Africa (in methods and approaches outlined by Barraclough and Nees, 2001).

The results outlined here identify a valuable reservoir of amphibian evolutionary history in the EAM of Kenya and Tanzania. Further sampling of more populations and lineages should clarify whether these ancient divergences reflect persistence in the region as a whole. Consistent patterns in other animal and plant groups appear to support the theory that the long persistence of habitats has been critical in structuring the biodiversity of the EAM (e.g. Lovett *et al.* 2005). The data support the status of the EAM as a biodiversity hotspot, and lends further claim to the recognition of the EAM as a World heritage site (Lovett, 1988). It is difficult to evaluate how the EAM compares to other regions in Africa, such as West Africa and Ethiopia, because there is a real lack of phylogenetic data of amphibians from these areas. It might be predicted that the other forested African mountains, which are of different ages and surrounded by different habitats, will show proportionally different biodiversity levels. Such hypotheses need to be tested with molecular phylogenies and good ecological and distributional data. Studies will also be necessary to evaluate how this ancient endemic diversity compares with other regions in the world and thereby evaluate conservation efforts more effectively (Sechrest *et al.* 2002). Considering the small area of the spatially fragmented EAM forests (5,011 km^2 in area of which only 1,560km^2 is primary, mature forest), the ancient fauna of the EAM is highly threatened by increasing levels of deforestation (Newmark, 2002). If prioritising areas for conservation in the EAM is necessary, then based on the heterogeneity in phylogenetic diversity between mountains, certain areas deserve greater attention for conservation efforts, e.g. ancestral areas (Ulugurus, Taita, Usambaras) (e.g. Moritz and Faith, 1998; Moritz *et al.* 2001; Moritz, 2002).

7.5.1.7 Are there deep divergences between faunas in East and West Africa?

If the molecular clock estimates are correct, then the separation between East and West African forest amphibians (see Table 7.4) is consistent with the hypothesis that dispersal has been restricted between East and West. However, it is unlikely that the separation of forests during the Miocene uplift was the causal factor for the split, as demonstrated by the significantly large divergence estimates (>25Myr). Which causal factors are associated with these deeper splits (in Scolecomorphidae, *Boulengerula*/*Herpele* and *Arthroleptides*/*Petropedetes*), is very unclear given the

poor understanding we have of this period in Africa. Matthee *et al.* (2004) suggested the separation may have been important in the diversification of the dwarf chameleons, and the dates they provide are certainly more contemporaneous with Miocene uplift than the amphibians outlined in this study. A better understanding of African geography will be necessary for an evaluation of possible causal factors of speciation in amphibians sampled here. More recent divergence estimates are shown for non-forest amphibians separated by the same geographical barriers, given their tolerance to savanna habitats its not surprising that *Hemisus* and *Phrynomantis* are able to disperse more recently between East and West African regions. This provides further compelling evidence that may support the hypothesis that forest species are strongly restricted to either Eastern or Western forests in Africa, and are unable to disperse freely.

7.5.1.8 Biogeographical relationship between coastal fauna and EAM

The biogeographic history of the EAM is believed to be highly complex (see above). It has been speculated that the turbulent biogeographic history has had a direct influence on the evolutionary history of the organisms in the EAM (Lovett, 1993). The coastal lowland forests of Eastern Africa, stretching from the Tana River in Kenya to the inselberg forests of Mozambique (see Fig. 6.13), are also believed to have a prolonged history, associated with fluctuations in climate. The presence of single site endemic species considered 'relicts' (Burgess *et al.* 1998b) supports the idea that they have persisted for a relatively long period. Matthee *et al.'s* (2004) study of the pigmy chamaeleon *Rhampholeon* lent support to this hypothesis, demonstrating that the coastal fauna is characterised by old lineages, stretching back 30 Myr and diversifying around 10-12 Mya. Over the past 30 Myr, the coastal region is thought to have been submerged on several occasions, with the last complete inundation around ~12 Mya (Zachos *et al.* 2001). The consequences of such flooding would have been periodic clearing of coastal forests, species would have either retreated to refuges (possibly the EAM) or become extinct. Recent climatic fluctuations, such as in the Pleistocene, are thought to have had a less catastrophic effect, only restricting forests to localised patches (Prell *et al.* 1980), which maybe the reason for the persistence of relict species, tracing back 10-12 Mya in the coastal region (Mathee, *et al.* 2004). The EAM have been postulated to be a refuge for the coastal forests, with a few species common to both used as evidence (Howell, 1993), but the details of this interaction are poorly understood (Burgess *et al.* 1998b).

The coastal forests of East Africa (see Fig. 6.13) are now mainly small isolated patches running adjacent and close to the coast line (Burgess, and Clarke, 2000), but there are some areas of overlap with the Eastern Arc Mountains (eg. East Usambara and Ulugurus), which has significant biogeographical implications. Forests in the Eastern Arc Mountains are altitudinually stratified, whereby different forest types lie in close proximity along the sharp topographical conformities between the mountains and the coastal plateau. For example, the forests of the East Usambara show marked changes between montane, submontane, and lowland forest (see Fig. 6.13). At lower elevations the sub-montane forest grades in species composition and physiognomy to that of the lowland Coastal Forests. Thus there is no hard altitudinal boundary between these two forest types (Lovett *et al.* 2001), but instead a continuum between the Eastern Arc and coastal forest types.

Despite the 'continuum' between submontane, lowland and coastal forests, there appears to be a clear difference in the faunas (Burgess and Clarke, 2000). This is shown by the remarkable amphibian species turnover between the lowland and montane forests (e.g. Loveridge, 1937; Poynton, 2003; Loader *et al.* 2004b), and this appears to be indicative of a more general biogeographic pattern, seen also in other groups (e.g. birds: Stuart, 1991). For example, the lowland amphibian fauna of East Usambara shows greater similarity with coastal forests than montane regions (Poynton, unpublished data). Only a few species are truly shared between lowland and highland regions, which suggest a long-standing difference between these areas. The reasons for these differences are very uncertain, but it is believed that the answer lies in the comparative age of the two areas, with the EAM containing long-term resident species, as shown by some distinct taxonomic differences (microhylid and dwarf bufonid fauna).

Molecular data based on lineages with distributions in both the EAM and coastal lowlands suggest a prolonged history, as shown by the long-branch connecting *B. changamwensis* (~20 Myr). This species could be considered as further evidence of an 'ancient relict' lowland fauna, congruent with Matthee *et al.'s* (2004) findings for *Rhampholeon*. This finding may change in the future with sampling of other coastal and montane species, such as *B. denhardti* and *B. fisheri*. More recent fluctuations, and presumably colonization events between forested regions of EAM and coastal areas are also evident (e.g. *Boulengerula uluguruensis* and *Spelaeophryne methneri*) which may correspond with the retreat and expansion of lowland forest habitats over

the past few millions years (Clarke and Karoma, 2000). Furthermore, truly montane species do not occur in lowland habitats (e.g. *Hoplophryne, Callulina,* and *Probreviceps*). The rarity of species with distributions in both lowland and highland areas might be the result of the specialization of species into montane and lowland habitat niches associated with their long periods of environmental stability.

Campbell and Duellman (2000) discussed similar issues, with reference to the amphibian fauna of central America. They noted the 'severe impediments' of living in montane habitats, most significantly the problems amphibians face with breeding in fast flowing streams. Plethodontid salamanders are a good example of a dramatic radiation of specialized amphibian breeders in the montane tropics. A number of amphibian species in the EAM show distinctive breeding strategies. For example, the semi-terrestrial tadpole of *Arthroleptides*, has well-developed limbs and digital pads that allows them to cling on steep humid rock faces (Drewes *et al.* 1989). Some of these specializations may have restricted the endemics to a narrow range of potential niches and prevented their subsequent dispersion outside these mountain ranges. Perhaps dispersal from lowland to highland habitats, which have been long occupied by a highly adapted amphibian assemblage, would be difficult. It presumably would also be difficult for montane taxa to disperse to the lowlands. The ecological and physiological implications for making a transition from lowland to highland habitats are probably considerable and of great significance to the evolutionary diversification in the area. Further data will be needed to investigate the relationships between lowland and highland fauna areas and the influence of abiotic and biotic factors.

7.5.1.9 Biogeographical relationship between Southern Highlands and the EAM.

The Southern Highlands have a highly complex geological history, similar in respect to the Eastern Arc Mountains, with its geological origins over 400 Mya. Over the past 290 Myr it is thought that the Southern Highlands have been subjected to an assortment of different faulting processes and volcanism in the region (e.g. Lake Nyasa rifting, Miocene uplifiting, late tertiary volcanics ~7 Mya e.g. Mt Rungwe; and recent Quaternary volcanics). Essentially the Southern Highlands are very old, with much younger volcanics (e.g. Mt Rungwe). Very little detailed study has been made of this region, despite its interesting affiliations with both the EAM and the Nyasa mountains (Davenport, in prep.). Some amphibian genera and species that

characterise the montane fauna of the EAM are found distributed in this region, and provide good evidence for the close biogeographic history shared between these regions; e.g. *Nectophrynoides viviparous, Scolecomorphus kirkii, Probreviceps rungwensis,* and *Arthroleptis reichei.* Recent study of the snake *Crotaphopeltis* (Gravlund, 2002) has shown an interesting biogeographic pattern of prolonged separation of the Eastern Arc and Southern Highlands populations of the species *C. tornieri,* despite being morphologically very similar. Gravlund (2002) placed emphasis on the Makambako Gap, an arid area separating the regions (see Fig.7.19), which he believed may have restricted dispersal between the Udzungwa/Mahenge mountain escarpment and the Southern Highlands. The timing of the separation between populations of *C. tornieri* was estimated to be 8-37 Myr, and Gravlund (2002) suggested the gap might be a significant barrier for other lineages.

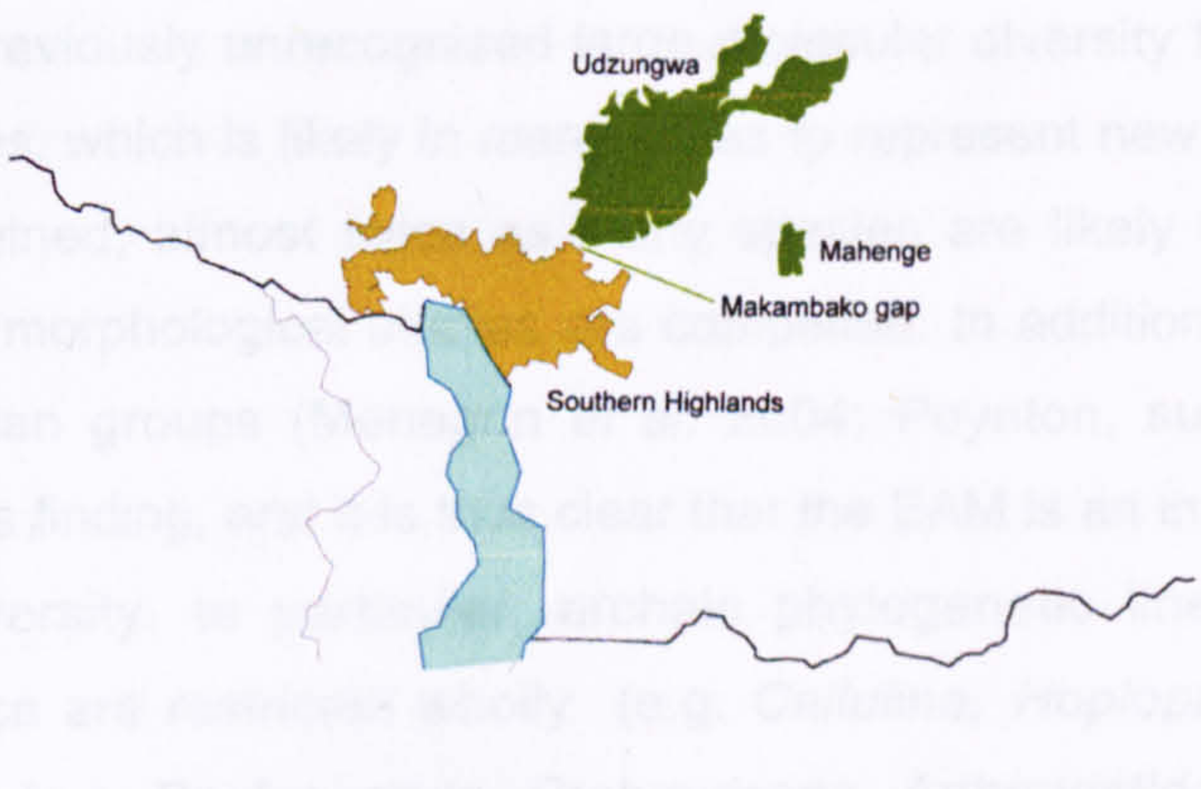

Figure 7.18
Detailed schematic map of the Southern EAM and the Southern Highlands in Tanzania.

Using the species *Probreviceps rungwensis*, distributed in both the Southern Highlands and EAM, it was possible to evaluate if the Southern Highlands and the Udzungwa mountains do show patterns of long-term isolation in amphibians. Estimates based on molecular clock methods indicate that the populations of *P. rungwensis* have been separated for ~10 Mya, which coincides with date estimates provided by Gravlund (2002). It is possible that the diversification of the amphibian and reptile lineages could have resulted from different biogeographic events, but the data cannot reject the hypothesis that divergence events were temporally incongruent. More biogeographic replicates are necessary to fully scrutinize this finding, to test whether these two amphibian and reptile examples reflect a general biogeographic pattern.

Chapter Eight

Conclusions

The EAM have long been recognised as a centre of high species diversity and endemism (e.g. Loveridge, 1925; Barbour and Loveridge, 1928; Howell, 1993). Despite this, only limited understanding of the factors that have been responsible for generating this diversity have been investigated? Findings presented here, principally using amphibians as indicators for elucidating patterns of diversification, have added substantially to understanding the likely factors that have been influential in promoting the rich diversity in this African biodiversity hotspot. As outlined in each of chapters 3-6, a previously unrecognised large molecular diversity for all investigated amphibian lineages, which is likely in many cases to represent new species. In nearly all lineages examined, almost twice as many species are likely to be recognised, once subsequent morphological studies are completed. In addition, preliminary data on other amphibian groups (Menegon *et al.* 2004; Poynton, submitted) appears congruent with this finding, and it is thus clear that the EAM is an important repository of amphibian diversity. In particular, archaic phylogenetic lineages, not found elsewhere in Africa are restricted wholly (e.g. *Callulina, Hoplophryne*) or partially (e.g. *Scolecomorphus, Boulengerula, Probreviceps, Arthroleptides*) to the EAM. Considering the small area of the EAM, the region is evidently one of the most important areas in Africa and perhaps globally for amphibians.

This high level of amphibian endemism and diversity is thought to be the result of a geographic history in which the EAM underwent periods of fragmentation and isolation. In the case of the EAM, a dynamic geographic history appears to have yielded equivalently complex diversification patterns in amphibians. It is probable, because amphibians are generally intolerant of ecological change, that these patterns that appear to be evident in other groups, reflect a common pattern. Spatial and temporal data complied in this study have provided the first comprehensive test of this, using seven amphibian lineages.

A comparison of phylogenetic trees from amphibians distributed in the EAM rejects the presence of congruent area relationships. Furthermore, with the addition of other groups (birds, chameleons, and plants) non-significant area relationships are also

recovered. Because there are some sampling inequalities in each lineage, biogeographical patterns might be obscured. However, because incongruent spatial (cladistic and descriptive) and temporal (molecular dating) relationships are recovered in this analysis, multiple (some non-pattern making) events (fragmentation and climatic change) seem to have influenced the biogeographic history of the EAM. Relationships between each EA mountain and areas located outside of the EAM offer some interesting insights. Not surprisingly, there is a correlation, although only weak, between geographic distance and genetic pairwise distance/steps on the GAC, which indicates a closer (historical) connection between geographically closer areas. A consistent separation is also seen between northern and southern EAM regions in most amphibian phylogenies (also shown in the non-significant GAC), whereby dispersal between these areas is less frequent, which is perhaps indicative of there being significant barriers to dispersal, as previously suggested (e.g. Bowie *et al.* 2004). Generally deep levels of divergence occur between EAM taxa and sister groups/populations distributed outside of the EAM, which lends further support to the suggestion that EAM is an area of a prolonged and isolated history.

Temporal data show that divergences have occurred repeatedly between areas and lineages, rejecting the possibility that areas have been fragmented and then completely isolated. Instead of a complete isolation, it is more probable that the more recent climatic changes have provided routes for dispersal between mountains. Evidence from molecular dates of amphibians indicates a more recent history of divergence in certain taxa that may correspond with changes in the Pliocene and Pleistocene. Despite the lack of a vicariant biogeographic pattern, a correspondence between the isolation of mountain populations and the uplift and final rapid separation of mountains is indicated in one or two examples using molecular dates, most clearly in the caecilian genus *Scolecomorphus*. The geographic process of rapid uplift might be less likely to produce clear vicariant patterns than slower processes and perhaps might be the reason why fragmentation patterns are not recovered in these analyses. It would be worthwhile testing whether this pattern of diversification is repeated in other groups and areas where similar geographic histories have occurred.

Molecular data presented in this study have enhanced biogeographic interpretations. Using molecular data, determining whether speciation between lineages is synchronous, and corresponds to known geographic events has been possible.

However, despite the clear importance of including time in biogeographic studies (Hunn and Upchurch, 2001), this study has identified the weakness inherent in biogeographic approaches; resolving complex spatial patterns and the inability of data to distinguish between dispersal and vicariance processes. Prospects for remedying these problems appear, at least partially, possible with an advancement of some methods (Donoghue and Moore, 2003). Time slicing as recently formalised by Hunn and Upchurch (2001) is an approach for resolving complex spatial patterns in dated fossil species. Upchurch and Hunn (2002; p.613) found time slicing particularly effective at alleviating complex dinosaurian spatial biogeographic relationships, and suggested it was 'a key step in detection of ancient biogeographic patterns'. A logical extension of the time slicing method could be the utilisation of dated molecular trees instead of fossils. There are a number of theoretical hurdles that will need to be jumped before this approach could be effectively used on molecular data. For example, it would be difficult to infer the spatial distribution of lineages back in time with only extant species as the reference to this (Losos and Glor, 2003). This study also demonstrates the need for detecting dispersal and vicariant processes in phylogenies, which is currently difficult, and in some people's opinions are impossible to uncover (Ebach *et al.* 2002). The use of molecular dated trees with approaches such as DIVA, which optimise spatial relationships using both vicariance and dispersal events could be important (e.g. Donoghue and Moore, 2003), though criticisms have been made of these approaches (Ebach *et al.* 2002). Other important developments include using coalescence analysis, for determining the direction of colonization events (Hewitt, 1996), which perhaps might indicate the probability of vicariance or dispersal processes (Losos and Glor, 2003). Clearly more methodological work is required, and this study identifies areas where it might be most useful to invest time in developing methods, especially with the continuing emphasis of molecular data in systematics and biogeography. Furthermore, and perhaps most critically, better historical abiotic data will be necessary so hypotheses can be better formulated and tested to investigate the possible influences that the geographic history of the EAM has had on species diversity.

Dispersal ability is clearly important in interpreting biogeographical patterns, especially when there are clear differences between the lineages being compared, e.g. comparing birds and flightless insects. In the case of this study, differences in dispersing, as quantified by habitat preferences, were shown to have an influence on diversification. Despite this apparent influence, it seems unlikely that diversifications

between lineages might be temporally congruent once lineage specific differences are taken account of, as demonstrated by the magnitude of difference in temporal estimates. A better explanation would be that each amphibian lineages is influenced by more than one causal event between areas in the EAM, obscuring biogeographic patterns.

Comparisons between forest restricted, and non-forest restricted species indicate that at least for forest species in the EAM, their ability to migrate to other regions has been limited, both to closely positioned areas (e.g. Southern Highlands) or further away (e.g. West African forests). The causal processes for these are poorly understood but appear to be intimately associated to the formation and subsequent isolation of mountains that have excluded, or limited dispersal between areas at different times. Testing the degree of isolation between different animals groups would be a logical next step to evaluate how strongly the processes of isolation and persistence, as outlined here, have shaped the diversification of all organisms in the region.

Different hypotheses have been used to explain global tropical forest biodiversity (Knapp and Mallet, 2002), the ancient, stable nature of these environments (Fisher 1960; Fjeldså and Lovett 1997) or their fluctuating history in association with global climate change, whereby forest taxa are repeatedly confined to isolated refugia (Haffer, 1969). In the absence of any phylogenetic evidence, Burgess, *et al.* (1998a) emphasised that the number of endemics provided good evidence that persistence and isolation were important in structuring the biodiversity of the EAM. An important aspect of the evolutionary diversification of EA amphibians, as outlined in this study, appears to be the prolonged persistence of forest. Dates suggest a history substantially predating the Miocene uplift (~40 Mya), which indicates that forest habitats are likely to have persisted for a prolonged period, extending the early evolution of the EAM further back than previously anticipated. This has important implications for understanding the origin and maintenance of African biodiversity.

The climatic history of Africa has been marked by periods of severe aridification. Recently, throughout the dry phases in Africa's Tertiary history, areas such as the South African succulent region, Ethiopian Highlands, EAM and West African montane region are thought to have been essential in providing stable environments for maintaining biodiversity. Prior to the formation of these mountains (e.g. formation

of the rift valley) good evidence (Morley, 2000) suggests that there were no mountains of moderate altitude in equatorial Africa that could confer highly localised climates for forests. Therefore, during periods of aridification (Morley, 2000), large areas of equatorial Africa, usually covered in forest, would have severely dried out. Based on temporal data on the diversification of forest amphibians in the EAM, this study suggests either East African climate was stable enough for supporting forests even in low lying areas (in small refuges), or perhaps mountains of significant altitude and size were present. It is difficult to assess which hypothesis is more likely based on the limited evidence. Because there is more convincing evidence that the African climate did suffer from severe arid periods (Morley, 2000) and that low lying forest would have dried out as a consequence of this, the presence of mountainous areas, such as progenitors of the EAM is a more probable hypothesis. Considering that we have only a very limited understanding of the geological and climatic history of Africa, the finding that phylogenetic evidence might show contradictory patterns is not surprising. Evolutionary relationships of amphibians (and other groups) also consistently recover certain areas as having lineages placed at the basal split of trees, and perhaps these areas (Ulugurus, East Usambaras) may have been significant topographical features, in a pre-Miocene African landscape. Testing the archaic age of these areas would be useful using lineages as proxies, it would also focus attention on the geology of the region.

Habitat and lineage persistence is clearly an important process in explaining current biodiversity patterns in the EAM. Evidence outlined in this study provides the first quantitative test of one the four biodiversity hotspots in Africa, and supports the prediction that highly biodiverse areas show prolonged history. Testing whether this is more general pattern in Africa would be interesting for future understanding of the origin and maintenance of biodiversity in Africa. Persistence seems to have also influenced phylogenetic diversification in other tropical rainforests on the globe (Roelants *et al.* 2004), which may imply a common relationship between biological diversity and geographic history. These patterns contrast distinctly with diversification patterns in temperate regions, where more recent changes, corresponding to relatively recent glacial fluctuations, seem to be more influential (Hewitt, 2004). Comparing and contrasting these different areas will undoubtedly prove important for establishing global understanding of the origin and maintenance of biodiversity.

In light of the phylogenetic evidence presented here, the human disturbance of pristine habitats in the EAM is of great concern (Brooks *et al.* 2002; Burgess et al. 2002). Disturbance of pristine habitats stresses natural populations in complex ways, in particular those with highly adaptive life history strategies such as seen in the torrent frog *Arthroleptides*. Amphibians are believed to be especially prone to changes in habitats (Stuart *et al.* 2004; Gardner, 2001) and it is a critically important task to maintain rich forest environments for the continued preservation of these species (Burkey, 1995). Many of the species described in this thesis are endemic to single mountain blocks, some of which are severely threatened, as exemplified by the recent gold mining pressure in the Usambaras (Doggart *et al.* 2004). The restricted ranges of some species (e.g. *Nectophrynoides* and *Callulina)* make them particularly at risk when faced by habitat loss and environmental pressures (the recent decline of *N. asperginis* being a case in point). The forests of the EAM are still relatively poorly known (Burgess *et al.* 1998), so the task for future researchers is to inventorise the unexplored areas, and new funding incentives are planning to achieve this (CEPF, 2002). This is important for determining conservation priorities within the EAM. Long-term management policies will be needed to guarantee the preservation of these fascinating forests before they disappear. The EAM represent a unique biological heritage (Burgess *et al.* 2004), as well as an important water catchment for much of Tanzania. The loss of the Eastern Arc forests would be devastating to all concerned.

Appendix 1. Specimens collected during fieldwork 2001-2002.

Specimen number	Species	Date	Arc locality	Region	Forest reserve/Specific Locality	GPS South	GPS East	DNA sample
MW 01805	*Ptychadena anchietae*	10/6/01	COASTAL FOREST	KIBAHA COASTAL REGION	RAS KUTANI	S06 56'05.0"	E039 39'05.0"	NONE
MW 01806	*Ptychadena anchietae*	10/6/01	COASTAL FOREST	KIBAHA COASTAL REGION	RAS KUTANI	S06 56'05.0"	E039 39'05.0"	NONE
MW 01807	*Xenopus muelleri*	10/5/01	COASTAL FOREST	KIBAHA COASTAL REGION	RAS KUTANI	S06 56'05.0"	E039 39'05.0"	NONE
MW 01808	*Ptychadena anchietae*	10/5/01	COASTAL FOREST	KIBAHA COASTAL REGION	RAS KUTANI	S06 56'05.0"	E039 39'05.0"	NONE
MW 01809	*Phrynobatrachus mababiensis*	10/5/01	COASTAL FOREST	KIBAHA COASTAL REGION	RAS KUTANI	S06 56'05.0"	E039 39'05.0"	NONE
MW 01813	*Hyperolius parkeri*	10/5/01	COASTAL FOREST	KIBAHA COASTAL REGION	RAS KUTANI	S06 56'05.0"	E039 39'05.0"	MW 01814
not taken	*Mabuya maculilabris*	10/6/01	COASTAL FOREST	KIBAHA COASTAL REGION	RAS KUTANI	S06 56'05.0"	E039 39'05.0"	MW 01812
MW 01811	*Lygodactylus capensis*	10/6/01	COASTAL FOREST	KIBAHA COASTAL REGION	RAS KUTANI	S06 56'05.0"	E039 39'05.0"	MW 01810
MW 01815	SNAKE	10/7/01	COASTAL FOREST	KIBAHA COASTAL REGION	RAS KUTANI	S06 56'05.0"	E039 39'05.0"	MW 01816
MW 01817	*Kassina maculata*	10/7/01	COASTAL FOREST	KIBAHA COASTAL REGION	RAS KUTANI	S06 56'05.0"	E039 39'05.0"	MW 01818
MW 01819	*Hyperolius tuberilinguis*	10/7/01	COASTAL FOREST	KIBAHA COASTAL REGION	RAS KUTANI	S06 56'05.0"	E039 39'05.0"	MW 01820
MW 01821	*Hyperolius parkeri*	10/7/01	COASTAL FOREST	KIBAHA COASTAL REGION	RAS KUTANI	S06 56'05.0"	E039 39'05.0"	NONE
MW 01823	*Nectophrynoides tornieri*	10/10/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01822
MW 01824	*Nectophrynoides tornieri*	10/10/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01825
MW 01826	*Breviceps mossambicus*	10/10/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01827
MW 01828	*Afrixalus fornasini*	10/10/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01829
MW 01830	*Leptopelis vermiculatus*	10/10/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01831
MW 01832	*Leptopelis vermiculatus*	10/10/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01833
MW 01834	*Afrixalus uluguruensis*	10/10/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01835
MW 01836	*Afrixalus uluguruensis*	10/10/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01837
MW 01838	*Afrixalus uluguruensis*	10/10/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01839
MW 01840	*Afrixalus uluguruensis*	10/10/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01841
MW 01842	*Scolecomorphus sp.*	10/11/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01843
MW 01844	*Arthroleptides martiennseni*	10/10/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01845
MW 01846	*Scolecomorphus sp.*	10/11/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01847
MW 01848	*Breviceps mossambicus*	10/11/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01849
MW 01850	*Speleophryne methneri*	10/11/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01851

Specimen	Species	Date	Arc Locality	Region	Forest Reserve	GPS South	GPS East	DNA Sample
MW 01852	*Arthroleptides martiennseni*	10/10/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01853
MW 01854	*Arthroleptides martiennseni*	10/10/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01855
MW 01856	*Hemisus marmoratum*	10/11/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01857
JM	*Rhinotyphlops mucrosa*	10/12/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01858
JM	*Cnemaspis africanna*	10/9/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01859
MW 01860	*Hyperolius puncticulatus*	10/11/01	MAHENGE MOUNTAINS	MOROGORO	SALI F.R.	S08 57'57.4"	E036 41'17.9"	MW 01861
MW 01862	*Scolecomorphus sp.*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	MW 01863
MW 01864	*Scolecomorphus sp.*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	MW 01865
MW 01866	*Scolecomorphus sp.*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	MW 01867
MW 01868	*Scolecomorphus sp.*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	MW 01869
MW 01870	*Scolecomorphus sp.*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	MW 01871
MW 01872	*Scolecomorphus sp.*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	MW 01873
MW 01874	*Scolecomorphus sp.*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	MW 01875
MW 01876	*Scolecomorphus sp.*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	MW 01877
MW 01878	*Scolecomorphus sp.*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	MW 01879
MW 01880	*Scolecomorphus sp.*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	MW 01881
MW 01882	*Scolecomorphus sp.*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	MW 01883
MW 01884	*Boulengerula uluguruensis*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	MW 01885
MW 01886	*Boulengerula uluguruensis*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	MW 01887
MW 01888	*Boulengerula uluguruensis*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	MW 01889
MW 01890	*Boulengerula uluguruensis*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	MW 01891
MW 01892	*Scolecomorphus sp.*	10/13/01	NGUU MOUNTAINS	TANGA	NGUU/KANGA	S05 01' 00.0"	E037 25' 60.0"	NONE
MW 01893	*Nectophrynoides viviparus*	10/18/01	RUBEHO MOUNTAINS	DODOMA	MAFWEMIRO F.R.	S06 56'26.6"	E036 35'13.7"	MW 01894
MW 01895	*Nectophrynoides viviparus*	10/18/01	RUBEHO MOUNTAINS	DODOMA	MAFWEMIRO F.R.	S06 56'26.6"	E036 35'13.7"	MW 01896
MW 01897	*Scolecomorphus sp.*	10/18/01	RUBEHO MOUNTAINS	DODOMA	MAFWEMIRO F.R.	S06 58'51.9	E036 36'51.6"	MW 01898
MW 01899	*Boulengerula uluguruensis*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01900
MW 01901	*Boulengerula uluguruensis*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01902
MW 01903	*Boulengerula uluguruensis*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01904
MW 01905	*Boulengerula uluguruensis*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01906
MW 01907	*Boulengerula uluguruensis*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01908

Specimen	Species	Date	Arc Locality	Region	Forest Reserve	GPS South	GPS East	DNA Sample
MW 01909	*Boulengerula uluguruensis*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01910
MW 01911	*Boulengerula uluguruensis*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01912
MW 01913	*Boulengerula uluguruensis*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01914
MW 01915	*Boulengerula uluguruensis*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01917	*Boulengerula uluguruensis*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.9	E037 31'43.1"	MW 01916
MW 01918	*Boulengerula uluguruensis*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01919
MW 01920	*Boulengerula uluguruensis*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01921
MW 01922	*Boulengerula uluguruensis*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01923
MW 01924	*Boulengerula uluguruensis*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01925	*Boulengerula uluguruensis*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01926	*Natriceteres vaerigata*	10/20/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'29.0	E037 30'40.5"	MW 01927
MW 01928	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01929	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01930	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01931	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01932	*Cnemaspis barbouri*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'29.0	E037 30'40.5"	MW 01933
MW 01934	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01935
MW 01936	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01937
MW 01938	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01939	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01940
MW 01941	*Leptopelis uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'29.0	E037 30'40.5"	MW 01942
MW 01943	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01944	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01945	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01946
MW 01947	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01948	*Nectophrynoides tornieri*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'29.0	E037 30'40.5"	MW 01949
MW 01950	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01951	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01952	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01953	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE

Specimen	Species	Date	Arc Locality	Region	Forest Reserve	GPS South	GPS East	DNA Sample
MW 01954	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01955	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01956	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01957
MW 01958	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01959
MW 01960	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01961	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	MW 01962
MW 01970	*Arthroleptis stenodactylus*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'29.0	E037 30'40.5"	MW 01969
MW 01963	*Boulengerula uluguruensis*	10/22/01	NGURU MOUNTAINS	MOROGORO	NGURU SOUTH F.R.	S06 02'51.0	E037 31'43.1"	NONE
MW 01964	*Phrynobatrachus krefftii*	10/27/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.1	E038 30'12.9"	MW 01965
MW 01966	*Cnemaspis africana*	10/27/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.2	E038 30'12.9"	MW 01967
MW 01968	*Callulina kreffti*	10/27/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.3	E038 30'12.9"	MW 01971
MW 01972	*Callulina kreffti*	10/27/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.4	E038 30'12.9"	MW 01973
MW 01974	*Hyperolius puncticulatus*	10/27/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.5	E038 30'12.9"	MW 01975
MW 01976	*Afrixalus uluguruensis*	10/27/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.6	E038 30'12.9"	MW 01977
MW 01978	*Afrixalus uluguruensis*	10/27/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.7	E038 30'12.9"	MW 01979
MW 01980	*Leptopelis vermiculatus*	10/27/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.8	E038 30'12.9"	MW 01981
MW 01982	*Leptopelis vermiculatus*	10/27/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.9	E038 30'12.9"	MW 01983
MW 01984	*Boulengerula boulengeri*	10/27/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.10	E038 30'12.9"	MW 01985
MW 01986	*Leptopelis parkeri*	10/28/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.11	E038 30'12.9"	MW 01987
MW 01988	*Leptopelis parkeri*	10/28/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.12	E038 30'12.9"	MW 01989
MW 02331	*Boulengerula boulengeri*	10/28/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.13	E038 30'12.9"	MW 02332
MW 02333	*Cnemaspis africana*	10/28/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.14	E038 30'12.9"	MW 02334
MW 02335	*Boulengerula boulengeri*	10/28/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.15	E038 30'12.9"	MW 02336
MW 02337	*Arthroleptis tanneri*	10/28/01	WEST USAMBARAS	TANGA	MAZUMBAI F.R.	S04 48'45.16	E038 30'12.9"	MW 02338
N/A	*Chamaeleo dilepis*	4/22/02	COSTAL FOREST	KENYA	ARABUKO-SOKOKE	S03 18'12.1"	E040 00'22.9"	MW 03038
N/A	*Rhampholeon kersteni*	4/22/02	COSTAL FOREST	KENYA	ARABUKO-SOKOKE	S03 18'12.1"	E040 00'22.9"	MW 03039
KMH23346	*Scolecomorphus sp.*	4/28/02	EAST USAMBARA	TANGA	MGAMBO FR	S04 47'39.4"	E038 48'49.8"	MW 03040
MW 03041	*Lygodactylus sp.*	5/4/02	UKAGURU MOUNTAINS	MOROGORRO	IKWAMBA FR	S06 21'14.4"	E036 57'53.1"	MW 03042
MW 03043	*Hyperolius puncticulatus*	5/4/02	UKAGURU MOUNTAINS	MOROGORRO	IKWAMBA FR	S06 21'14.4"	E036 57'53.1"	MW 03044
MW 03045	*Scolecomorphus sp.*	5/4/02	UKAGURU MOUNTAINS	MOROGORRO	IKWAMBA FR	S06 21'14.4"	E036 57'53.1"	MW 03046

Specimen	Species	Date	Arc Locality	Region	Forest Reserve	GPS South	GPS East	DNA Sample
MW 03048	*Scolecomorphus sp.*	5/4/02	UKAGURU MOUNTAINS	MOROGORRO	IKWAMBA FR	S06 21'14.4"	E036 57'53.1"	MW 03049
MW 03050	*Callulina kreffti*	5/4/02	UKAGURU MOUNTAINS	MOROGORRO	IKWAMBA FR	S06 20'31.5"	E036 58'57.8"	MW 03051
MW 03052	*Callulina kreffti*	5/4/02	UKAGURU MOUNTAINS	MOROGORRO	IKWAMBA FR	S06 20'31.5"	E036 58'57.8"	MW 03053
MW 03054	*Scolecomorphus sp.*	5/4/02	UKAGURU MOUNTAINS	MOROGORRO	IKWAMBA FR	S06 21'14.4"	E036 57'53.1"	MW 03055
MW 03056	*Bufo gutturalis*	5/4/02	UKAGURU MOUNTAINS	MOROGORRO	IKWAMBA FR	S06 21'14.4"	E036 57'53.1"	MW 03057
MW 03058	*Probreviceps sp.*	5/5/02	UKAGURU MOUNTAINS	MOROGORRO	IKWAMBA FR	S06 21'14.4"	E036 57'53.1"	MW 03059
MW 03061	*Typhlops sp.*	5/5/02	UKAGURU MOUNTAINS	MOROGORRO	IKWAMBA FR	S06 21'14.4"	E036 57'53.1"	MW 03062
MW 03063	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	MW 03064
MW 03065	*Callulina sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	MW 03066
MW 03067	*Callulina sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	MW 03068
MW 03069	*Phrynobatrachus*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03070	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	MW 03071
MW 03072	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	MW 03073
MW 03074	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	MW 03075
MW 03076	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	MW 03077
MW 03078	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	MW 03079
MW 03080	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03081	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03082	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03083	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03084	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03085	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03086	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03087	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03088	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03089	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03090	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03091	*Callulina sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	MW 03092

Specimen	Species	Date	Arc Locality	Region	Forest Reserve	GPS South	GPS East	DNA Sample
MW 03093	*Callulina sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03094	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03095	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03096	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03097	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03098	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03099	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03100	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03101	*Callulina sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	MW 03102
MW 03103	*Callulina sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03104	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	MW 03105
MW 03106	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03107	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03108	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03109	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03110	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03111	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03112	*Scolecomorphus sp.*	5/10/02	NORTH PARE MTS.	KILIMANJARO	KINDOROKO FR	S03 43'43.5"	E037 39'16.1"	
MW 03114	*Scolecomorphus sp.*	5/13/02	NGUU MOUNTAINS	MOROGORRO	NGUU FR	S05 01' 00.0"	E037 25' 60.0"	MW 03113
MW 03115	*Scolecomorphus sp.*	5/13/02	NGUU MOUNTAINS	MOROGORRO	NGUU FR	S05 01' 00.0"	E037 25' 60.0"	MW 03116
MW 03117	*Scolecomorphus sp.*	5/13/02	NGUU MOUNTAINS	MOROGORRO	NGUU FR	S05 01' 00.0"	E037 25' 60.0"	MW 03118
MW 03119	*Scolecomorphus sp.*	5/13/02	NGUU MOUNTAINS	MOROGORRO	NGUU FR	S05 01' 00.0"	E037 25' 60.0"	MW 03120
MW 03121	*Scolecomorphus sp.*	5/13/02	NGUU MOUNTAINS	MOROGORRO	NGUU FR	S05 01' 00.0"	E037 25' 60.0"	MW 03122
MW 03123	*Scolecomorphus sp.*	5/13/02	NGUU MOUNTAINS	MOROGORRO	NGUU FR	S05 01' 00.0"	E037 25' 60.0"	MW 03124
MW 03125	*Scolecomorphus sp.*	5/13/02	NGUU MOUNTAINS	MOROGORRO	NGUU FR	S05 01' 00.0"	E037 25' 60.0"	
MW 03126	*Scolecomorphus sp.*	5/13/02	NGUU MOUNTAINS	MOROGORRO	NGUU FR	S05 01' 00.0"	E037 25' 60.0"	
MW 03127	*Scolecomorphus sp.*	5/13/02	NGUU MOUNTAINS	MOROGORRO	NGUU FR	S05 01' 00.0"	E037 25' 60.0"	
MW 03128	*Scolecomorphus sp.*	5/13/02	NGUU MOUNTAINS	MOROGORRO	NGUU FR	S05 01' 00.0"	E037 25' 60.0"	

Specimen	Species	Date	Arc Locality	Region	Forest Reserve	GPS South	GPS East	DNA Sample
MW 03129	*Scolecomorphus sp.*	5/13/02	NGUU MOUNTAINS	MOROGORRO	NGUU FR	S05 01' 00.0"	E037 25' 60.0"	
MW 03130	*Scolecomorphus sp.*	5/13/02	NGUU MOUNTAINS	MOROGORRO	NGUU FR	S05 01' 00.0"	E037 25' 60.0"	
MW 03131	*Scolecomorphus sp.*	5/13/02	NGUU MOUNTAINS	MOROGORRO	NGUU FR	S05 01' 00.0"	E037 25' 60.0"	
MW 03132	*Boulengerula sp.*	5/13/02	WEST USAMBARA	TANGA	LUSHOTO	S04 19' 60.0"	E037 52' 00.0"	MW 03133
MW 03134	*Boulengerula sp.*	5/13/02	WEST USAMBARA	TANGA	LUSHOTO	S04 19' 60.0"	E037 52' 00.0"	MW 03135
MW 03136	*Boulengerula sp.*	5/13/02	WEST USAMBARA	TANGA	LUSHOTO	S04 19' 60.0"	E037 52' 00.0"	MW 03137
MW 03138	*Boulengerula sp.*	5/13/02	WEST USAMBARA	TANGA	LUSHOTO	S04 19' 60.0"	E037 52' 00.0"	
MW 03139	*Boulengerula sp.*	5/13/02	WEST USAMBARA	TANGA	LUSHOTO	S04 19' 60.0"	E037 52' 00.0"	
MW 03140	*Boulengerula sp.*	5/13/02	WEST USAMBARA	TANGA	LUSHOTO	S04 19' 60.0"	E037 52' 00.0"	
MW 03141	*Scolecomorphus sp.*	5/14/02	SOUTH PARE MTS.	KILIMANJARO	CHOME FR	S04 18' 35.0"	E037 54' 37.0"	MW 03142
MW 03143	*Scolecomorphus sp.*	5/14/02	SOUTH PARE MTS.	KILIMANJARO	CHOME FR	S04 18' 35.0"	E037 54' 37.0"	MW 03144
MW 03145	*Scolecomorphus sp.*	5/14/02	SOUTH PARE MTS.	KILIMANJARO	CHOME FR	S04 18' 35.0"	E037 54' 37.0"	MW 03146
MW 03147	*Scolecomorphus sp.*	5/14/02	SOUTH PARE MTS.	KILIMANJARO	CHOME FR	S04 18' 35.0"	E037 54' 37.0"	
MW 03148	*Scolecomorphus sp.*	5/14/02	SOUTH PARE MTS.	KILIMANJARO	CHOME FR	S04 18' 35.0"	E037 54' 37.0"	
MW 03149	*Scolecomorphus sp.*	5/14/02	SOUTH PARE MTS.	KILIMANJARO	CHOME FR	S04 18' 35.0"	E037 54' 37.0"	
MW 03150	*Scolecomorphus sp.*	5/14/02	SOUTH PARE MTS.	KILIMANJARO	CHOME FR	S04 18' 35.0"	E037 54' 37.0"	
MW 03201	*Scolecomorphus sp.*	5/14/02	SOUTH PARE MTS.	KILIMANJARO	CHOME FR	S04 18' 35.0"	E037 54' 37.0"	
MW 03202	*Scolecomorphus sp.*	5/14/02	SOUTH PARE MTS.	KILIMANJARO	CHOME FR	S04 18' 35.0"	E037 54' 37.0"	MW 03203
MW 03204	*Scolecomorphus sp.*	5/14/02	SOUTH PARE MTS.	KILIMANJARO	CHOME FR	S04 18' 35.0"	E037 54' 37.0"	
MW 03205	*Scolecomorphus sp.*	5/14/02	SOUTH PARE MTS.	KILIMANJARO	CHOME FR	S04 18' 35.0"	E037 54' 37.0"	
MW 03206	*Scolecomorphus sp.*	5/14/02	SOUTH PARE MTS.	KILIMANJARO	CHOME FR	S04 18' 35.0"	E037 54' 37.0"	
MW 03207	*Scolecomorphus sp.*	5/14/02	SOUTH PARE MTS.	KILIMANJARO	CHOME FR	S04 18' 35.0"	E037 54' 37.0"	
MW 03208	*Boulengerula boulengeri*	5/15/02	WEST USAMBARA	TANGA	AMBANGULA FR	S05 03' 97.0"	E038 24' 63.0"	MW 03209
MW 03210	*Nectophrynoides sp.*	5/15/02	WEST USAMBARA	TANGA	AMBANGULA FR	S05 03' 97.0"	E038 24' 63.0"	MW 03211
MW 03212	*Boulengerula boulengeri*	5/15/02	WEST USAMBARA	TANGA	AMBANGULA FR	S05 03' 97.0"	EO38 24' 63.0"	MW 03213
MW 03214	*Boulengerula boulengeri*	5/15/02	WEST USAMBARA	TANGA	AMBANGULA FR	S05 03' 97.0"	E038 24' 63.0"	
MW 03215	*Callulina kisiwamsitu*	5/15/02	WEST USAMBARA	TANGA	AMBANGULA FR	S05 03' 97.0"	E038 24' 63.0"	MW 03216
MW 03217	*Boulengerula boulengeri*	5/15/02	WEST USAMBARA	TANGA	AMBANGULA FR	S05 03' 97.0"	E038 24' 63.0"	MW 03218

Specimen	Species	Date	Arc Locality	Region	Forest Reserve	GPS South	GPS East	DNA Sample
MW 03219	*Boulengerula boulengeri*	5/15/02	WEST USAMBARA	TANGA	AMBANGULA FR	S05 03' 97.0"	E038 24' 63.0"	
MW 03220	*Boulengerula boulengeri*	5/15/02	WEST USAMBARA	TANGA	AMBANGULA FR	S05 03' 97.0"	E038 24' 63.0"	MW 03221
MW 03222	*Boulengerula boulengeri*	5/15/02	WEST USAMBARA	TANGA	AMBANGULA FR	S05 03' 97.0"	E038 24' 63.0"	
MW 03223	*Boulengerula boulengeri*	5/15/02	WEST USAMBARA	TANGA	AMBANGULA FR	S05 03' 97.0"	E038 24' 63.0"	
MW 03224	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03225	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	MW 03226
MW 03227	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03228	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03229	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	MW 03240
MW 03230	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03231	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	MW 03239
MW 03232	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03233	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03234	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03235	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03236	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	MW 03241
MW 03237	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03238	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	MW 03242
MW 03244	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03245	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03246	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03247	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03248	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03249	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03250	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03251	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03252	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03253	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	

Specimen	Species	Date	Arc Locality	Region	Forest Reserve	GPS South	GPS East	DNA Sample
MW 03254	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03255	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03256	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03257	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03258	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03259	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03260	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03261	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03262	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03263	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03264	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03265	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03266	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03267	*Schistometopum gregorii*	5/18/02	BAGAMOYO	KIBAHA COASTAL REGION	RUVU FERRY	S06 28' 32.5"	E038 49' 22.3"	
MW 03268	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	MW 03269
MW 03270	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	MW 03271
MW 03272	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	MW 03273
MW 03274	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	MW 03275
MW 03276	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03277	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03278	*Scolecomorphus sp.*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	MW 3279
MW 03280	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03281	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03282	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03283	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03284	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03285	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03286	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	

Specimen	Species	Date	Arc Locality	Region	Forest Reserve	GPS South	GPS East	DNA Sample
MW 03287	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03288	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03289	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03290	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03291	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03292	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03293	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03294	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03295	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03296	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03297	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03298	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03299	*Typhlops uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	MW 03300
MW 03301	*Typhlops uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	
MW 03302	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	MW 03303
MW 03304	*Boulengerula uluguruensis*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 56' 49.0"	E037 43' 16.0"	MW 03305
MW 03306	*Leptopelis parkeri*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 54' 66.0"	E037 40' 47.0"	MW 03307
MW 03308	*Nectophrynoides minutus*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 54' 66.0"	E037 40' 47.0"	MW 03309
MW 03310	*Nectophrynoides minutus*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 54' 66.0"	E037 40' 47.0"	MW 03311
MW 03312	*Hyperolius puncticulatus*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	6S0 54' 66.0"	E037 40' 47.0"	MW 03313
MW 03314	*Nectophrynoides minutus*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 54' 66.0"	E037 40' 47.0"	MW 03315
MW 03316	*Nectophrynoides minutus*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 54' 66.0"	E037 40' 47.0"	MW 03317
MW 03317	*Nectophrynoides minutus*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 54' 66.0"	E037 40' 47.0"	MW 03319
MW 03318	*Arthroleptis xenodactyloides*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 54' 66.0"	E037 40' 47.0"	
MW 03319	*Leptopelis parkeri*	5/22/02	ULUGURU MOUNTAINS	MOROGORRO	ULUGURU NORTH	S06 54' 66.0"	E037 40' 47.0"	

Appendix 2. DNA extractions

Specimen No.	Species	DNA number	Locality	Forest Reserve	Molecular accession number	Preservation
1	*B. boulengeri*	RAN 31531	Unknown	Unknown	T6	E
2	*Scolecomorphus* sp.	RAN 31529	Unknown	Unknown	T7	E
3 Black 8	*B. taitanus*	Black 8	Taita Hills	Wundanyi	T9	E
4 SG 5700	*S. vittatus*	SG 5700	West Usambaras	Ambangula	T17	E
5 SG 5589	*Scolecomorphus* sp.	SG 5589	East Usambaras	4.5 km SE of Amani	T20	E
6 KMH 12895	*S. vittatus*	MW 01800	East Usambaras	Kambai	T124	IMS
7 BM 1994.857	*S. vittatus*	MW 01801	East Usambaras	Manga	T125	IMS
8 KMH 12829	*S. vittatus*	MW 01802	East Usambaras	Kambai	T126	IMS
9 BM 1983.46	*P. macrodactylus loveridgei*	MW 01803	Uluguru	Lupanga Peak	T127	IMS
10 BM 1986.604	*P. macrodactylus loveridgei*	MW 01804	Udzungwa	Sanje	T128	IMS
11 BM 1980.266	*S. vittatus*	MW 01990	Uluguru	Near Morningside	T129	IMS
12 BM 1980.267	*S. vittatus*	MW 01991	Uluguru	Morningside	T130	IMS
13 A & H	*S. vittatus*	MW 01992	Ukaguru	Mamiwa-Kisara	T131	F
14 KMH 7706	*S. vittatus*	MW 01993	Nguru Mountains	Nguru South	T132	IMS
15 KMH 11865	*S. vittatus*	MW 01994	East Usambaras	Longuza	T133	IMS
16 KMH 14406	*S. vittatus*	MW 01995	East Usambaras	Mtai	T134	IMS
17 KMH 11868	*S. vittatus*	MW 01996	East Usambaras	Magrotto	T135	IMS
18 BM 1994.532	*S. vittatus*	MW 01997	East Usambaras	Bamba Ridge	T136	IMS
19 BM 1986.605	*P. macrodactylus loveridgei*	MW 01998	Udzungwa	Sanje	T137	IMS
20 BM 1986.603	*P. macrodactylus loveridgei*	MW 01999	Udzungwa	Mufindi, Kigogo	T138	IMS
21 BM 1986.601	*P. macrodactylus loveridgei*	MW 02000	Udzungwa	Chita	T139	IMS
22 BM 1976.300	*P. macrodactylus loveridgei*	MW 02001	Uluguru		T140	IMS
23 BM 1976.301	*P. macrodactylus loveridgei*	MW 02002	Uluguru		T141	IMS
24 KMH 11307	*P. macrodactylus macrodactylus*	MW 02003	East Usambaras	Magrotto	T142	IMS
25 KMH 11318	*P. macrodactylus macrodactylus*	MW 02004	East Usambaras	Magrotto	T143	IMS
26 KMH 11318	*P. macrodactylus macrodactylus*	MW 02004	East Usambaras	Magrotto	T144	IMS
27 KMH 14531	*P. macrodactylus macrodactylus*	MW 02005	East Usambaras	Mtai	T145	IMS
28 KMH 14516	*P. macrodactylus macrodactylus*	MW 02006	East Usambaras	Mtai	T146	IMS
29 KMH 16072	*P. macrodactylus macrodactylus*	MW 02007	East Usambaras	Amani-Sigi	T147	IMS
30 KMH 16360	*P. macrodactylus macrodactylus*	MW 02008	East Usambaras	Amani-Sigi	T148	IMS

Specimen No.	Species	DNA number	Locality	Forest Reserve	M. Number	Preservation
31 CAM 973	*P. macrodactylus rungwensis*	MW 02009	Udzungwa	Mufindi, Kigogo	T149	F
32 CAM 969	*P. macrodactylus rungwensis*	MW 02010	Udzungwa	Mufindi, Kigogo	T150	F
33 DCMOYER 698	*P. macrodactylus rungwensis*	MW 02011	Udzungwa	Mastswe Village	T151	F
34 CAM 826	*Probreviceps uluguruensis*	MW 02012	Uluguru	Uluguru North, Camp 2	T152	F
35 CAM 876	*Probreviceps uluguruensis*	MW 02013	Uluguru	Uluguru North, Camp 2	T153	F
36 KMH 15600	*P. macrodactylus loveridgei*	MW 02014	Udzungwa	Kihansi Lower Falls	T154	F
37 KMH 15190	*P. macrodactylus loveridgei*	MW 02015	Udzungwa	Kihansi Lower Falls	T155	F
38 UDSM 1147	*P. macrodactylus loveridgei*	MW 02016	Udzungwa	Lulanda	T156	F
39 KMH 7721	*P. macrodactylus macrodactylus*	MW 02017	Nguru Mountains	Nguru South	T157	F
40 KMH 18974	*P. macrodactylus rungwensis*	MW 02018	Udzungwa	West Kilombero Scarp	T158	IMS
41 NMZB 16547	*Probreviceps rhodesianus*		Zimbabwe	Stapleford	T159	F
42 UFS 5662	*Scolecomorphus sp.*		Unknown		T160	E
43 MW 01024	*B. taitanus*	MW 01024	Taita Hills	Wundanyi	T161	E
44 MW 01026	*B. taitanus*	MW 01026	Taita Hills	Wundanyi	T162	E
45 MW 01021	*B. taitanus*	MW 01021	Taita Hills	Wundanyi	T163	E
46 MW 01019	*B. taitanus*	MW 01019	Taita Hills	Wundanyi	T164	E
47 MW 00390	*B. boulengeri*	MW 00390	East Usambaras	Msyuzi Scarp	T165	E
48 MW 00392	*B. boulengeri*	MW 00392	East Usambaras	Amani-Kwamkoro	T166	E
49 MW 00397	*B. boulengeri*	MW 00397	East Usambaras	Amani-Kwamkoro	T167	E
50 MW 00938	*B. boulengeri*	MW 00938	East Usambaras	Amani-Kwamkoro	T168	E
51 RAN 31529	*Scolecomorphus spp.*	RAN 31529	Unknown		T169	E
52 RAN 31530	*Scolecomorphus spp.*	RAN 31530	Unknown		T170	E
53 MW 00399	*B. boulengeri*	MW 00399	East Usambaras	Amani-Kwamkoro	T171	E
54 MW 00901	*S. vittatus*	MW 00901	East Usambaras	Amani-Kwamkoro	T172	E
55 MW 01015	*B. taitanus*	MW 01015	Taita Hills	Wundanyi	T173	E
56 KMH 22480	*Scolecomorphus spp.*	MW 02052	Udzungwa	West Kilombero Scarp	T174	E
57 KMH 22703	*Scolecomorphus spp.*	MW 02053	Udzungwa	West Kilombero Scarp	T175	E
58 KMH 22168	*Scolecomorphus spp.*	MW 02054	Udzungwa	West Kilombero Scarp	T176	E
59 KMH 22716	*Scolecomorphus spp.*	MW 02055	Udzungwa	West Kilombero Scarp	T177	E
60 KMH 22724	*Scolecomorphus spp.*	MW 02056	Udzungwa	West Kilombero Scarp	T178	E
61 KMH 22041	*Scolecomorphus spp.*	MW 02057	Udzungwa	West Kilombero Scarp	T179	E
62 KMH 19330	*P. macrodactylus macrodactylus*	MW 02058	East Usambaras	Amani	T180	IMS
63 KHM 19329	*P. macrodactylus macrodactylus*	MW 02059	East Usambaras	Amani	T181	IMS

Specimen No.	Species	DNA number	Locality	Forest Reserve	M. Number	Preservation
64 KMH 19152	*P. macrodactylus rungwensis*	MW 02060	Udzungwa	West Kilombero Scarp	T182	E
65 KMH 22702	*P. macrodactylus loveridgei*	MW 02061	Udzungwa	West Kilombero Scarp	T183	E
66 KMH 22067	*P. macrodactylus loveridgei*	MW 02062	Udzungwa	West Kilombero Scarp	T184	E
67 KMH 22720	*P. macrodactylus loveridgei*	MW 02063	Udzungwa	West Kilombero Scarp	T185	E
68 KMH 22060	*P. macrodactylus rungwensis*	MW 02064	Udzungwa	West Kilombero Scarp	T186	E
69 KMH 22067	*P. macrodactylus loveridgei*	MW 02065	Udzungwa	West Kilombero Scarp	T187	E
70 CAS 168810	*S. vittatus*	CAS 168810	West Usambaras		T188	E
71 CAS 168812	*S. vittatus*	CAS 168812	West Usambaras		T189	E
72 CAS 168597	*P. macrodactylus macrodactylus*	CAS 168597	East Usambaras	Amani	T190	E
73 CAS 168560	*P. macrodactylus macrodactylus*	CAS 168560	East Usambaras	Amani	T191	E
74 CAS 168598	*P. macrodactylus macrodactylus*	CAS 168598	East Usambaras	Amani	T192	E
75 CAS 168599	*P. macrodactylus macrodactylus*	CAS 168599	East Usambaras	Amani	T193	E
76 CAS 168559	*P. macrodactylus macrodactylus*	CAS 168559	East Usambaras	Amani	T194	E
77 CAS 168811	*S. vittatus*	CAS 168811	West Usambaras		T195	E
78 CAS 168596	*P. macrodactylus macrodactylus*	CAS 168596	East Usambaras	Amani	T196	E
79 KMH 25021	*S. cf. uluguruensis*	MW02066	Uluguru	Ulluguru South	T197	E
80 KMH 25024	*S. uluguruensis*	MW02067	Uluguru	Uluguru North	T198	E
81 KMH 25000	*S. uluguruensis*	MW02068	Uluguru	Uluguru North	T199	E
82 KMH 25009	*S. uluguruensis*	MW02069	Uluguru	Uluguru North	T200	E
83 KMH 21450	*B. uluguruensis*	MW02070	Uluguru	Mkungwe	T201	E
84 KMH 21463	*B. uluguruensis*	MW02071	Uluguru	Mkungwe	T202	E
85 KMH 21480	*B. uluguruensis*	MW02072	Uluguru	Mkungwe	T203	E
86 KMH 21570	*P. ulugurensis*	MW02073	Uluguru	Uluguru South	T204	E
87 KMH 21577	*P. ulugurensis*	MW02074	Uluguru	Uluguru South	T205	E
88 KMH 21575	*P. ulugurensis*	MW02075	Uluguru	Uluguru South	T206	E
89 KMH 21461	*P. macrodactylus loveridgei*	MW02076	Uluguru	Mkungwe	T207	E
90 KMH 21532	*P. macrodactylus loveridgei*	MW02077	Uluguru	Kasanga	T208	E
91 KMH 21475	*P. macrodactylus loveridgei*	MW02078	Uluguru	Mkungwe	T209	E
92 CAS 159945	*S. vittatus*	MW02079	North Pare	Ugweno	T210	F
93 CAS 193112	*S. vittatus*	MW02080	North Pare	Ugweno	T211	E
94 CAS 159946	*S. vittatus*	MW02081	North Pare	Ugweno	T212	F
95 CAS 159950	*S. vittatus*	MW02082	North Pare	Ugweno	T213	F
96 CAS 159951	*S. vittatus*	MW02083	North Pare	Ugweno	T214	F

Specimen No.	Species	DNA number	Locality	Forest Reserve	M. Number	Preservation
97 CAS 159947	*S. vittatus*	MW02084	North Pare	Ugweno	T215	F
98 CAS 159952	*S. vittatus*	MW02085	North Pare	Ugweno	T216	F
99 CAS 159949	*S. vittatus*	MW02086	North Pare	Ugweno	T217	F
100 CAS 196374	*P. macrodactylus loveridgei*	MW02087	Udzungwa	Kihansi Lower Falls	T218	F
101 CAS 193118	*P. macrodactylus macrodactylus*	MW02088	Nguru Mountains	Nguru South	T219	F
102 CAS 162682	*Probreviceps rhodesianus*	MW02089	Zimbabwe	Stapleford	T220	F
103 CAS 202676	*Probreviceps uluguruensis*	MW02090	Uluguru		T221	E
104 R 96036	*Scolecomorphus vittatus*	MW02091	Udzungwa	Kilanzi Kihungulu	T222	E
105 R 96037	*Scolecomorphus vittatus*	MW02092	Udzungwa	Kilanzi Kihungulu	T223	E
106 R 96035	*Scolecomorphus vittatus*	MW02093	Udzungwa	Kilanzi Kihungulu	T224	E
107 KMH 21270	*B. boulengeri*		East Usambaras	Nilo	T225	E
108 KMH 21262	*S. vittatus*		East Usambaras	Nilo	T226	E
109 KMH 23333	*S. vittatus*		East Usambaras	Nilo	T227	E
110 KMH 21263	*S. vittatus*		East Usambaras	Nilo	T228	E
111 KMH 23327	*B. boulengeri*		East Usambaras	Nilo	T229	E
112 KMH 19131	*B. boulengeri*		East Usambaras	Nilo	T230	E
113 R 0265	*S. vittatus*	MW02095	East Usambaras	Nilo	T231	E
114 R 096037	*Scolecomorphus spp.*		Udzungwa	Kilanzi Kihungulu	T238	E
115 R 99014	*Scolecomorphus spp.*		Udzungwa	Kilanzi Kihungulu	T239	E
116 R 96035	*Scolecomorphus spp.*		Udzungwa	Kilanzi Kihungulu	T240	E
117 R 96036	*Scolecomorphus spp.*		Udzungwa	Kilanzi Kihungulu	T241	E
118 R 96028	*Scolecomorphus spp.*		Udzungwa	Kilanzi Kihungulu	T242	E
119 KMH 23345	*B. cf. uluguruensis*		Coastal Forest	Kazizumbwi	T243	E
120 KMH 23344	*S. vittatus*		East Usambaras	Nilo	T244	E
121 KMH 23136	*P. macrodactylus macrodactylus*		East Usambaras	Nilo	T245	E
122 KMH 23137	*P. macrodactylus macrodactylus*		East Usambaras	Nilo	T246	E
123 KMH 21399	*P. macrodactylus macrodactylus*		East Usambaras	Nilo	T247	E
124 KMH 23121	*P. macrodactylus macrodactylus*		East Usambaras	Nilo	T269	E
125 KMH 23125	*P. macrodactylus macrodactylus*		East Usambaras	Nilo	T270	E
126 MW 01842	*Scolecomorphus sp.*	MW 01843	Mahenge Mountains	Sali	T271	E
127 MW 01846	*Scolecomorphus sp.*	MW 01847	Mahenge Mountains	Sali	T272	E
128 MW 01862	*Scolecomorphus sp.*	MW 01863	Nguu Mountians	Handeni side	T273	F
129 MW 01886	*B. uluguruensis*	MW 01887	Nguu Mountians	Handeni side	T274	F

Specimen No.	Species	DNA number	Locality	Forest Reserve	M. Number	Preservation
130 MW 01888	*B. uluguruensis*	MW 01889	Nguu Mountians	Handeni side	T275	F
131 MW 01897	*Scolecomorphus sp.*	MW 01898	Rubeho Mountains	Mafwemiro	T276	E
132 MW 01899	*B. uluguruensis*	MW 01900	Nguru Mountains	Nguru South	T277	E
133 MW 01956	*B. uluguruensis*	MW 01957	Nguru Mountains	Nguru South	T278	E
134 MW 01984	*B. boulengeri*	MW 01985	West Usambaras	Mazumbai	T279	E
135 MW 02331	*B. boulengeri*	MW 02332	West Usambaras	Mazumbai	T280	E
136 MW 01826	*Breviceps mossambicus*	MW 01827	Mahenge Mountains	Sali	T281	E
137 MW 01844	*Arthroleptides yakusini*	MW 01845	Mahenge Mountains	Sali	T282	E
138 MW 01848	*Breviceps mossambicus*	MW 01849	Mahenge Mountains	Sali	T283	E
139 MW 01850	*Spelaeophryne methneri*	MW 01851	Mahenge Mountains	Sali	T284	E
140 MW 01852	*Arthroleptides yakusini*	MW 01853	Mahenge Mountains	Sali	T285	E
141 MW 01854	*Arthroleptides yakusini*	MW 01855	Mahenge Mountains	Sali	T286	E
142 MW 01864	*Scolecomorphus sp.*	MW 01865	Nguu Mountians	Handeni side	T287	F
143 MW 01866	*Scolecomorphus sp.*	MW 01867	Nguu Mountians	Handeni side	T288	F
144 MW 01868	*Scolecomorphus sp.*	MW 01869	Nguu Mountians	Handeni side	T289	F
145 MW 01870	*Scolecomorphus sp.*	MW 01871	Nguu Mountians	Handeni side	T290	F
146 MW 01872	*Scolecomorphus sp.*	MW 01873	Nguu Mountians	Handeni side	T291	F
147 MW 01874	*Scolecomorphus sp.*	MW 01875	Nguu Mountians	Handeni side	T292	F
148 MW 01876	*Scolecomorphus sp.*	MW 01877	Nguu Mountians	Handeni side	T293	F
149 MW 01878	*Scolecomorphus sp.*	MW 01879	Nguu Mountians	Handeni side	T294	F
150 MW 01880	*Scolecomorphus sp.*	MW 01881	Nguu Mountians	Handeni side	T295	F
151 MW 01882	*Scolecomorphus sp.*	MW 01883	Nguu Mountians	Handeni side	T296	F
152 MW 01884	*B. uluguruensis*	MW 01885	Nguu Mountians	Handeni side	T297	F
153 MW 01890	*B. uluguruensis*	MW 01891	Nguu Mountians	Handeni side	T298	F
154 MW 01901	*B. uluguruensis*	MW 01902	Nguru Mountains	Nguru South	T299	E
155 MW 01903	*B. uluguruensis*	MW 01904	Nguru Mountains	Nguru South	T300	E
156 MW 01907	*B. uluguruensis*	MW 01908	Nguru Mountains	Nguru South	T301	E
157 MW 01909	*B. uluguruensis*	MW 01910	Nguru Mountains	Nguru South	T302	E
158 MW 01968	*Callulina kisiwamsitu*	MW 01971	West Usambaras	Mazumbai	T303	E
159 MW 01972	*Callulina kisiwamsitu*	MW 01973	West Usambaras	Mazumbai	T304	E
160 MW 02336	*B. boulengeri*	MW 02335	West Usambaras	Mazumbai	T305	E
161 KMH 22148	*Arthroleptides yakusini*	KMH 22148	Udzungwa	West Kilombero Scarp	T306	E
162 KMH 12708	*S. vittatus*	KMH 12708	East Usambaras	Kambai	T307	E

Specimen No.	Species	DNA number	Locality	Forest Reserve	M. Number	Preservation
163 KMH 22148	*Arthroleptides yakusini*	KMH 22148	Udzungwa	West Kilombero Scarp	T414	E
164 KMH 21533	*Arthroleptides yakusini*	KMH 21533	Uluguru	Kasanga	T415	E
165 KMH 21535	*Arthroleptides yakusini*	KMH 21535	Uluguru	Kasanga	T416	E
166 KMH 21215	*Arthroleptides martiennseni*	KMH 21215	East Usambaras	Nilo	T417	E
167 KMH 23365	*Arthroleptides martiennseni*	KMH 23365	East Usambaras	Nilo	T418	E
168 KMH 21188	*Arthroleptides martiennseni*	KMH 21188	East Usambaras	Nilo	T419	E
169 KMH 22723	*Hoplophryne ulugurensis*	KMH 22723	Uzungwa	West Kilombero Scarp	T420	E
170 KMH 7743	*P. macrodactylus macrodactylus*	KMH 7743	Nguru Mountains	Nguru South	T421	F
171 KMH 22994	*Arthroleptides martiennseni*	MW 03034	East Usambaras	Kwamgumi	T422	E
172 KMH 23534	*Callulina kreffti*	MW 03035	East Usambaras	Nilo	T423	E
173 KMH 23364	*Hoplophryne rogersi*	MW 03036	East Usambaras	Nilo	T424	E
174 KMH 21555	*Callulina kreffti*	MW 03037	Uluguru Mountains	Shikurufumi	T425	E
175 MW 03050	*Callulina kreffti*	MW 03051	Ukaguru Mountains	Ikwamba	T426	E
176 MW 03054	*Scolecomorphus sp.*	MW 03055	Ukaguru Mountains	Ikwamba	T427	E
177 MW 03058	*Probreviceps sp.*	MW 03059	Ukaguru Mountains	Ikwamba	T428	E
178 MW 03065	*Callulina sp.*	MW 03066	North Pare	Kindoroko	T429	E
179 MW 03070	*Scolecomorphus sp.*	MW 03071	North Pare	Kindoroko	T430	E
180 MW 03072	*Scolecomorphus sp.*	MW 03073	North Pare	Kindoroko	T431	E
181 MW 03101	*Callulina sp.*	MW 03102	North Pare	Kindoroko	T432	E
182 MW 03114	*Scolecomorphus sp.*	MW 03113	Nguu Mountians	Handeni side	T433	E
183 MW 03132	*B. boulengeri*	MW 03133	West Usambaras	Lushoto	T434	E
184 MW 03141	*Scolecomorphus sp.*	MW 03142	South Pares	Chome	T435	E
185 MW 03208	*B. boulengeri*	MW 03209	West Usambaras	Ambangula	T436	E
186 MW 03217	*B. boulengeri*	MW 03218	West Usambaras	Ambangula	T437	E
187 MW 03225	*Schistometopum gregorii*	MW 03226	Bagamoyo	Ruvu Ferry	T438	E
188 MW 03268	*B. uluguruensis*	MW 03269	Uluguru	Uluguru North	T439	E
189 MW 03278	*Scolecomorphus sp.*	MW 03279	Uluguru	Uluguru North	T440	E
190 KMH 23346	*S. vittatus*	MW 03040	East Usambaras	Mgambo	T441	E
191 MW 03048	*Scolecomorphus sp.*	MW 03049	Ukaguru Mountains	Ikwamba	T442	E
192 MW 03115	*Scolecomorphus sp.*	MW 03116	Nguu Mountians	Handeni side	T443	E
193 MW 03143	*Scolecomorphus sp.*	MW 03144	South Pares	Chome	T444	E
194 MW 03174	*B. taitanus*	MW 030175	Taita Hills	Ngangao	T445	E
195 MW 03197	*Callulina sp.*	MW 03198	Taita Hills	Ngangao	T446	E

Specimen No.	Species	DNA number	Locality	Forest Reserve	M. Number	Preservation
196 MW 03215	*Callulina kisiwamsitu*	MW 03216	West Usambaras	Ambangula	T447	E
197 KMH 22478	*Callulina sp.*	MW 03321	Uzungwa	West Kilombero Scarp	T448	E
198 KMH 19141	*P. macrodactylus rungwensis*	MW 03323	Uzungwa	West Kilombero Scarp	T449	IMS
199 KMH 19158	*P. macrodactylus rungwensis*	MW 03324	Uzungwa	West Kilombero Scarp	T450	IMS
200 R 258	*Scolecomorphus kirkii*		Malawi	Namadzi	T451	F
201 MS 023	*Callulina kreffti*	MW 03326	South Pare	Chome	T452	IMS
202 L	*Phrynomantis bifasciatus*	MW 03327	Ubani Mbuga	Mkomazi	T453	IMS
203 S	*Phrynomantis bifasciatus*	MW 03328	Ubani Mbuga	Mkomazi	T454	IMS
204 MW 01856	*Hemisus marmoratum*	MW 01857	Mahenge Mountains	Sali	T455	E
205 BM1983.45	*Callulina kreffti*	MW 03329	Nguru Mountains	Maskati Mission	T456	F
206 MW 03202	*Scolecomorphus sp*	MW 03203	South Pare	Chome	T457	E
207 RdS5862	*Arthroleptides yakusini*	RdS5862	Uluguru	Uluguru North	T458	E
208 RdS5946	*Arthroleptides martiennseni*	RdS5946	East Usambaras	Amani	T459	E
209	*Callulina kreffti*		East Usambaras	Kwamgumi	T460	IMS
210 KMH 21451	*Speleophryne methneri*	KMH 21451	Uluguru	Mkungwe	T461	E
211 BM 1975.770	*Probreviceps rhodesianus*	BM 1975.770	Zimbabwe	Stapleford	T462	F
212	*Balebreviceps hillmani*		Ethiopia	Bale Mountains	T463	F
213 MW 03044	*Hyperolius puncticulatus*	MW 03044	Ukaguru Mountains	Ikwamba	T464	E
214 MW 01837	*Afrixalus uluguruensis*	MW 01837	Mahenge Mountains	Sali FR	T465	E
215 MW 02338	*Arthroleptis tanneri*	MW 02338	West Usambaras	Mazumba	T466	E
216 MW 03830	*Callulina kreffti*	MW 03830	Nguu Mountians		T467	E
217 MW 03831	*Hoplophryne rogersi*	MW 03831	Nguu Mountians		T468	E
218 MW 03832	*Scolecomorphus sp.*	MW 03832	Udzungwa Mts.	Udzungwa Scarp	T469	E
219 Red Label	*Phrynomantis microps*	Red Label	Ivory Coast	Comoe	T470	E
220 Yellow Label	*Hemisus sudanensis*	Yellow Label	Ivory Coast	Comoe	T471	E
221 MW 3851	*Probreviceps rungwensis*	TD 4638	SouthERN Highlands	Rungwe	T472	F
222 MW 3852	*Spelaeophryne methneri*	KMH 24289	Kilombero Valley		T473	E
223 MW 3853	*Arthhroleptis stenodactylus*		East Usambaras	Amani	T474	E
224 JM 150	*Boulengerula boulengeri*	JM 150	East Usambaras	Shambangeda village, Amani	T475	E
225 NMK L/1887	*Boulengerula changamwensis*	MW 3777	Shimba Hills		T476	F
226 JM 849	*Boulengerula taitanus*	JM 849	Taita Hills	Kasegau	T482	E
227 JM 794	*Boulengerula taitanus*	JM 794	Taita Hills	Mbololo	T483	E
228 JM 966	*Boulengerula uluguruensis*	JM 966	Uluguru	Tandai Village	T484	E

Specimen No.	Species	DNA number	Locality	Forest Reserve	M. Number	Preservation
229 WTS 4085	*Probreviceps cf. rungwensis*	WTS 4085	Ukaguru Mountains	Mamiwa-Kisara	T485	F
230 WTS 4177	*Probreviceps cf. rungwensis*	WTS 4177	Ukaguru Mountains	Mamiwa-Kisara	T486	F
231 JM228	*Boulengerula taitanus*	JM228	Taita Hills	Chawia FR	T487	E
232 WTS 4194	*Probreviceps cf. rungwensis*	WTS 4194	Ukaguru Mountains	Mamiwa-Kisara	T488	F
233 WTS 3985	*Probreviceps cf. rungwensis*	WTS 3985	Ukaguru Mountains	Mamiwa-Kisara	T489	F
234 HM	*Boulengerula changamwensis*	HM	Changamwe		T490	E
235 HM	*Boulengerula taitanus*	HM	Taita Hills	Sagala FR	T491	E
236 HM	*Boulengerula taitanus*	HM	Taita Hills	Sagala FR	T492	E
237 Ni197	*Scolecomorphus sp*	Ni197	Mozambique		T493	E
238 UTA 38889	*Herpele squalostoma*	DPL 2888	SW Pr., Cameroon	Mundemba	-	E
239 UTA 51487	*Dermophis mexicanus*	ENS 8543	Guatemala	Izabal, Morales, Sierra de Caral, Carreter	-	E
240 UTA 51667	*Crotaph. tchabalmbaboensis*	DPL 5251	Cameroon	Mount Tchabal Mbabo	-	E
241 MW 331	*Gegenophis ramaswami*	MW 333	India	Thenmelai	-	E
242	*Schistometopum thomense*	RAN 31500	San Thome Island		-	E
243 TD1	*Probreviceps rungwensis*		Southern Highlands	Rungwe	T494	E

Appendix 3. Sequences obtained from Genbank.

Species	12s	16s	Cytochrome b
Pipa parva	AY333652	AY333690	AY341743
Hymenochirus boettgeri	AY341634	AY341726	AY341744
Petropedetes parkeri	AY341628	AY341724	AY341738
Mantidactylus sp. ZSM 652_2000	AY341585	AY341585	AY341731
Mantidactylus wittei	AY330904	AY263275	AY263303
Boophis sp. ZSM 685_2000	AY341610	AY341716	AY341733
Boophis tephraeomystax	AF215163	AF215334	AF249070
Heterixalus tricolor	AY330900	AY341725	AY341740
Tachycnemis seychellensis	AY341629	AY454395	AY341739
Kaloula taprobanica	AF249004	AF249057	AF249085
Scaphiophryne brevis	AF026357	AF026377	-
Scaphiophryne gottlebei	AF215144	AF215385	-
Microhyla sp. FB-2000	-	AF249060	AF249081

Appendix 4. Species distribution in the Eastern Arc Mountains, southern highlands, coastal forests and Malawi.

Genus	*Species*	Habitat	TH	NP	SP	WU	EU	NGU	NGR	UKA	RUB	ULU	MAL	UDZ	MA	SH	CF	ML-N	ML-S
FROGS																			
Arthroleptis	*affinis*	F					1	1	1	1	1	1		1					
Arthroleptis	*francei*	F																	1
Arthroleptis	*nikeae*	F									1								
Arthroleptis	*reichei*	F												1		1		1	
Arthroleptis	*stenodactylus*	F	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1
Arthroleptis	*tanneri*	F				1													
Arthroleptis	*xenodactylus*	F					1												
Arthroleptis	*xenodactyloides*	F			1		1	1	1		1	1		1	1		1	1	1
Schoutedenella	*xenochirus*															1		1	
Bufo	*brauni*	F				1	1					1		1					
Bufo	*gutturalis*					1	1	1	1	1	1	1		1	1	1	1		1
Bufo	*lindneri*																1		
Bufo	*maculatus*						1								1				
Bufo	*nyikae*																	1	
Bufo	*reesi*														1				
Bufo	*steindachneri*							1									1		
Bufo	*taitanus*		1													1	1	1	1
Bufo	*urunguensis*	F														1			
Bufo	*uzunguensis*													1		1			
Churamita	*maridadi*	F								1									
Nectophrynoides	*asperginis*	F												1					
Nectophrynoides	*cryptus*	F										1							
Nectophrynoides	*frontieri*	F					1												
Nectophrynoides	*laevis*	F										1							

Genus	Species	Habitat	TH	NP	SP	WU	EU	NGU	NGR	UKA	RUB	ULU	MAL	UDZ	MA	SH	CF	ML-N	ML-S
Nectophrynoides	*minutus*	F								1	1	1							
Nectophrynoides	*poyntoni*	F												1					
Nectophrynoides	*pseudotornieri*	F										1							
Nectophrynoides	*tornieri*	F					1	1	1			1		1	1	1			
Nectophrynoides	*new sp. ukaguru 1*	F								1									
Nectophrynoides	*new sp. ukaguru 2*	F								1									
Nectophrynoides	*new sp. uluguru 1*	F										1							
Nectophrynoides	*vestergaardi*	F				1													
Nectophrynoides	*viviparus*	F									1	1		1		1			
Nectophrynoides	*wendyae*	F												1					
Mertensophryne	*micranotis*		1				1					1					1		
Schismaderma	*carens*						1									1	1	1	1
Stephopaedes	*howelli*																1		
Stephopaedes	*loveridgei*														1				
Stephopaedes	*usambarae*						1												
Hemisus	*marmoratus*		1				1					1			1		1		
Afrixalus	*brachycnemis*						1							1	1				
Afrixalus	*fornasinii*						1		1		1	1		1	1		1		
Afrixalus	*fulvovittatus*											1							
Afrixalus	*morerei*													1		1			
Afrixalus	*sylvaticus*						1										1		
Afrixalus	*uluguruensis*	F				1	1	1	1			1		1	1				
Afrixalus	*P and B*						1										1		
Hyperolius	*argentovittatus*															1			
Hyperolius	*argus*						1										1		
Hyperolius	*kihangensis*	F												1					
Hyperolius	*goetzi*													1					
Hyperolius	*glandicolor*		1																

Genus	Species	Habitat	TH	NP	SP	WU	EU	NGU	NGR	UKA	RUB	ULU	MAL	UDZ	MA	SH	CF	ML-N	ML-S
Hyperolius	*kivuensis*													1					
Hyperolius	*mariae*						1					1					1?		
Hyperolius	*mitchelli*						1	1				1			1		1		
Hyperolius	*minutissimus*													1		1			
Hyperolius	*nasutus*						1										1		
Hyperolius	*parkeri*																1		
Hyperolius	*pictus*													1		1			
Hyperolius	*sp. 'puncticulatus'*	F			1	1	1	1	1	1	1	1		1	1	1	1	1	1
Hyperolius	*pusillus*						1										1		
Hyperolius	*pseudoargus*						1							1					
Hyperolius	*rubrovermiculatus*	F															1		
Hyperolius	*spinigularis*	F				1	1	1				1		1					1
Hyperolius	*tannerorum*	F				1													
Hyperolius	*tornieri*	? uncertain										1							
Hyperolius	*tuberilinguis*						1								1		1		
Kassina	*maculata*						1										1		
Kassina	*senegalensis*														1	1	1		
Leptopelis	*argenteus*						1										1		
Leptopelis	*barbouri*	F					1		1	1				1		1			
Leptopelis	*concolor*																1		
Leptopelis	*flavomaculatus*	F					1	1				1		1	1	1	1		
Leptopelis	*parkeri*	F			1	1	1					1		1					
Leptopelis	*uluguruensis*	F				1	1	1	1			1							
Leptopelis	*vermiculatus*	F				1	1	1	1	1		1		1		1			
Leptopelis	*bocagii*													1					
Leptopelis	*parbocagii*																		
Breviceps	*fichus*															1			
Breviceps	*mossambicus*															1	1		

Genus	Species	Habitat	TH	NP	SP	WU	EU	NGU	NGR	UKA	RUB	ULU	MAL	UDZ	MA	SH	CF	ML-N	ML-S
Callulina	*kreffti*	F	1	1	1		1	1	1	1		1		1			1		
Callulina	*kisiwamsitu*	F				1													
Hoplophryne	*rogersi*	F					1	1											
Hoplophryne	*uluguruensis*	F							1			1		1					
Parhoplophryne	*usambarica*	F					1												
Phrynomantis	*bifasciatus*																1		
Probreviceps	*macrodactylus*	F					1		1			1		1					
Probreviceps	*new sp. ukaguru*	F								1									
Probreviceps	*rungwensis*	F												1		1			
Probreviceps	*uluguruensis*	F										1							
Spelaeophryne	*methneri*	F?										1		1	1	1	1		
Arthroleptides	*martiensseni*	F				1	1												
Arthroleptides	*yakusini*	F							1			1		1	1				
Phrynobatrachus	*acridoides*						1								1		1		
Phrynobatrachus	*krefftii*	F		1	1?	1	1												
Phrynobatrachus	*mababiensis*						1										1		
Phrynobatrachus	*natalensis*						1	1			1	1			1		1		
Phrynobatrachus	*minutus'*						1										1		
Phrynobatrachus	*parvulus*	F									1			1			1		
Phrynobatrachus	*ukingensis*	F														1	1	1	
Phrynobatrachus	*rungwensis*	F														1		1	
Phrynobatrachus	*uzungwensis*	F						1				1		1					
Phlyctimantis	*keithae*													1					
Pyxicephalus	*edulis*																1		
Xenopus	*muelleri*						1										1		
Ptychadena	*anchietae*		1				1	1							1				
Hildebrandtia	*ornata*																1		
Ptychadena	*grandisonae*													1					

Genus	Species	Habitat	TH	NP	SP	WU	EU	NGU	NGR	UKA	RUB	ULU	MAL	UDZ	MA	SH	CF	ML-N	ML-S
Ptychadena	*shillukorum*																1		
Ptychadena	*mascareniensis*															1	1		
Ptychadena	*mossambica*																1		
Ptychadena	*oxyrhynchus*						1					1							
Ptychadena	*porosissima*													1					
Ptychadena	*uzungwensis*													1					
Hylarana	*galamensis*																1		
Rana	*angolensis*	F?	1		1		1					1		1		1	1	1	1
Strongylopus	*fuellerborni*										1			1		1			
Tomopterna	*tuberculosa*															1			
Chiromantis	*xerampelina*						1					1			1		1		
Chiromantis	*petersi*																		
CAECILIANS																			
Boulengerula	*boulengerula*	F				1	1												
Boulengerula	*changamwensis*																1		1?
Boulengerula	*denhardti*																1		
Boulengerula	*taitanus*	F	1																
Boulengerula	*niedeni*	F	1																
Boulengerula	*uluguruensis*	F						1	1			1					1		
Schistometopum	*gregorii*																1		
Scolecomorphus	*kirkii*	F							1		1	1		1	1	1		1	1
Scolecomorphus	*vittatus*	F		1	1	1	1	1		1		1							
Scolecomorphus	*uluguruensis*	F										1							

Appendix 5. Component Analysis

#NEXUS- AMPHIBIANS ONLY COMPONENT ANALYSIS

begin taxa;
dimensions ntax= 15;
taxlabels TH SP NP WU EU NGU NGR UK UL RUB UD MAH SH MOZ CF;
endblock;

begin distribution;
ntax= 46;
range
a2 : UL,
a3 : UK,
a4 : EU,
a5 : NGU,
a6 : EU,
a7 : EU,
a8 : UL,
a9 : UD,
a10 : MAH,
a11 : MOZ,
a12 : RUB,
a13 : WU,
a14 : SP,
a15 : NP,
b2 : TH,
b3 : UL,
b4 : CF,
b5 : NGR,
b6 : EU,
b7 : WU,
b8 : EU,
b9 : WU,
c2 : EU,
c3 : UD,
c4 : UL,
c5 : MAH,
d2 : UD,
d3 : UL,
d4 : EU,
d5 : NGU,
e2 : EU,
e3 : UL,
e4 : UD,
e5 : UL,
e6 : UD,
e7 : SH,
e8 : UK,
f2 : SP,
f3 : TH,
f4 : UD,
f5 : NGU,
f6 : UK,
f7 : UL,
f8 : EU,
f9 : WU,
f10 : NP;

tree associate = (((a2, ((a3, (a4, (a5, a6))), (a7, ((a8, (a9, (a12, (a10, a11)))), (a13, (a14, a15)))))),((b2, (b5, (b3, b4))), ((b6, b7), (b8, b9)))), ((c2, (c5, (c3, c4))), (((d2, d3), (d4, d5)),(((e5, (e2, (e3, e4))), (e8, (e6, e7))), (f2, (f3, ((f4, (f7, (f5, f6))), (f8, (f9, f10)))))))));
endblock;

#NEXUS – ALL TAXA COMPONENT ANALYSIS

begin taxa;
dimensions ntax= 18;
taxlabels TH SP NP WU EU NGU NGR UK UL RUB UD MAH SH MAL MOZ CF RUW VM;
endblock;

begin distribution;
ntax= 98;
range
a2 : UL,
a3 : UK,
a4 : EU,
a5 : NGU,
a6 : EU,
a7 : EU,
a8 : UL,
a9 : UD,
a10 : MAH,
a11 : MOZ,
a12 : RUB,
a13 : WU,
a14 : SP,
a15 : NP,
b2 : TH,
b3 : UL,
b4 : CF,
b5 : NGR,
b6 : EU,
b7 : WU,
b8 : EU,
b9 : WU,
c2 : EU,
c3 : UD,
c4 : UL,
c5 : MAH,
d2 : UD,
d3 : UL,
d4 : EU,
d5 : NGU,
e2 : EU,
e3 : UL,
e4 : UD,
e5 : UL,
e6 : UD,
e7 : SH,
e8 : UK,
f2 : SP,
f3 : TH,
f4 : UD,
f5 : NGU,
f6 : UK,
f7 : UL,
f8 : EU,
f9 : WU,
f10 : NP,
g2 : SH,
g3 : UD,
g4 : UL,
g5 : EU,
g6 : WU,
h2 : UL,
h3 : TH,
h4 : NGR,
h5 : EU,
h6 : NGR,

h7 : EU,
h8 : WU,
i2 : NGR,
i3 : UL,
i4 : UL,
i5 : UK,
i6 : NGR,
i7 : EU,
i8 : NGR,
i9 : UL,
j2 : SH,
j3 : UD,
j4 : UL,
j5 : NGR,
j6 : EU,
j7 : NP,
k2 : UL,
k3 : UD,
k4 : SH,
k5 : VM,
k6 : NP,
k7 : RUW,
k8 : UL,
k9 : UD,
l2 : EU,
l3 : NP,
l4 : MOZ,
l5 : UD,
l6 : RUB,
l7 : UL,
l8 : RUW,
l9 : MAL,
m2 : VM,
m3 : WU,
m4 : NP,
m5 : SH,
m6 : UD,
m7 : MOZ,
m8 : UL,
m9 : NGR,
m10 : RUB,
m11 : UD
;
tree associate = (((h2, (h3, ((h6, (h4, h5)), (h7, h8)))), ((i2, i3), ((i4, (i5, i6)), (i9, (i7, i8))))),((((a2, ((a3, (a4, (a5, a6))), (a7, ((a8, (a9, (a12 (a10, a11)))), (a13, (a14, a15)))))), ((b2, (b5, (b3, b4))), ((b6, b7), (b8, b9)))), ((c2, (c5, (c3, c4))), (((d2, d3), (d4, d5)), (((e5, (e2, (e3, e4))), (e8, (e6, e7))), (f2, (f3, ((f4, (f7, (f5, f6))), (f8, (f9, f10)))))))), (((g2, ((g3, g4), (g5, g6))), ((l2, l3), (l4, ((l5, l6), (l7, (l8, l9)))))), (((m2, (m3, m4)), ((m5, (m6, m7)), (m8, (m9, (m10, m11))))), ((j2, (j3, (j4, (j5, (j6, j7))))), ((k2, (k3, k4)), ((k5, (k6, k7)), (k8, k9))))))))
;

endblock;

Appendix 6. Papers published

- Muller, H, Measey, GJ, **Loader, SP**. and Malonza, P.K. 2005. A new species of *Boulengerula* Tornier (Amphibia: Gymnophiona: Caeciliidae) from an isolated mountain block of the Taita Hills. Zootaxa 1004: 37-50.

- Gower DJ, **Loader, SP.**, Wilkinson, M. and Moncrieff, C. 2004. Niche separation and comparative abundance of *Boulengerula boulengeri* and *Scolecomorphus vittatus* (Amphibia: Gymnophiona) in East Usambara forest, Tanzania. African J. Herpetology 53 (2): 183-190.

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- Gower DJ, **Loader, SP** and Wilkinson M. 2004. Assessing the conservation status of soil dwelling vertebrates: insights from the discovery of *Typhlops uluguruensis* (Reptilia: Serpentes: Typhlopidae). Systematics and Biodiversity 2 (1): 79-82.

- Wilkinson, M., **Loader, SP**., Muller, H., and Gower D.J. 2004. Taxonomic status and phylogenetic relationships of *Boulengerula denhardti* (Amphibia: Gymnophiona: Caeciliidae). Mitt. Mus. Nat.kd. Berl., Zool. Reihe 80 (1) 41-51. NOT INCLUDED

- **Loader, SP**., Poynton, J., and Mariaux, J. 2003. Herpetofauna of the Mahenge Mountains, Tanzania: A window on African biogeography. African Zoology 39 (1) 71-76.

- Wilkinson, M, **Loader, SP.**, Gower, DG, Sheps, JA and Cohen, BL. 2003. Phylogenetic Relationships of African Caecilians (Amphibia: Gymnophiona): Insights from Mitochondrial rRNA Gene Sequences. African Journal of Herpetology 52 (2): 83-92.

- Wilkinson, M, and **Loader, SP**. 2003. Worms of wisdom. BBC Wildlife, March edition. NOT INCLUDED

- **Loader, SP**, Gower, DG, and Wilkinson, M. 2003. Caecilians: mysterious amphibians of the Eastern Arc Mountains (Arc Journal, July 2003).

- **Loader, SP**, Wilkinson, M, Gower, DJ, and Msuya, C. 2002. A remarkable juvenile *Scolecomorphus vittatus* (Amphibia: Gymnophiona) from the Pare Mountains, Tanzania. (Journal of Zoology 259: 93-101).

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1004

A new species of *Boulengerula* Tornier (Amphibia: Gymnophiona: Caeciliidae) from an isolated mountain block of the Taita Hills, Kenya

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Abstract

A new species of East African caeciliid caecilian, *Boulengerula niedeni* spec. nov. (Amphibia: Gymnophiona) is described based on a series of 11 specimens from Sagalla Hill, an isolated mountain block of the Taita Hills in southern Kenya. It differs from all other *Boulengerula*, except *B. taitanus* (Loveridge), in being pigmented with whitish marked annular grooves and from *B. taitanus* by its distinctive brownish colouration, an exposed sphenethmoid, a higher mean number of annuli and vertebrae, and different phallus morphology.

Key words: caecilians, taxonomy, herpetology, Eastern Arc Mountains, East Africa, phallus morphology, systematics

Introduction

The caecilian fauna of East Africa currently comprises seven species of caeciliids and three species of scolecomorphids. As currently conceived (Wilkinson *et al.* 2004) the genus *Boulengerula* comprises six species, which makes it the most speciose of all African caecilian genera. With the exception of one or two lowland forms, most of the East African caecilian species inhabit the forested mountains of the Eastern Arc, a recognized biodiversity hotspot of global importance (e.g. Myers *et al.* 2000). In 1935, Loveridge described *B. taitanus* from Mt. Mbololo in the Taita Hills complex, Kenya (Fig. 1). This species became subsequently known to also inhabit Dawida, i.e. the main Taita Hills block (e.g. Glaser 1984; Hebrard *et al.* 1992; Gaborieau & Measey 2004; Measey 2004). Recently collected caecilians from Sagalla Hill, an isolated mountain block of the Taita Hills range in the Eastern Arc, differ substantially from *B. taitanus* and all other members of *Boulengerula* in their colouration, phallodeal characters and other morphological features. Here we describe these caecilians as a new species.

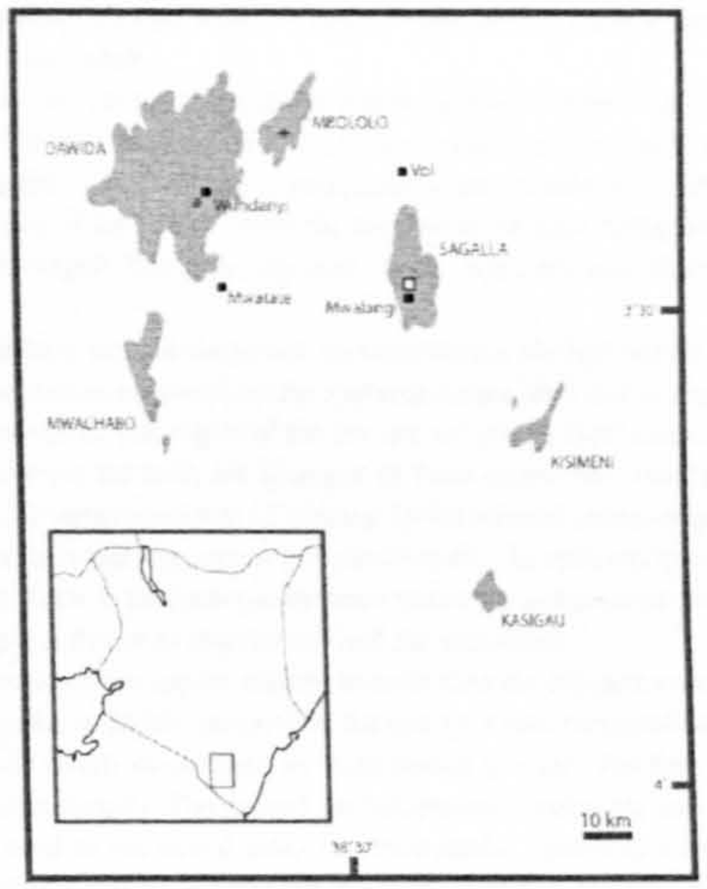

FIGURE 1. Map of the Taita Hills Formation. The inset map shows the position of the Taita Hills in Kenya. The map shows all mountainous areas above 1000m. The open square marks the type locality of *Boulengerula niedeni* and asterisks mark localities of *B. taitanus* used for comparison. Towns are marked with a square. Names written in capital letters refer to mountains, others to towns.

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***Boulengerula niedeni* sp. nov.**
(Figs. 2, 3, Table 1)

Holotype: NMK A/4294 National Museums of Kenya, Nairobi, Kenya. A mature female collected approximately 100m behind the matatu (bus) terminus at Mwalangi shopping centre, Sagalla Hills, Taita-Taveta District, Kenya, by Hendrik Müller and David Mwaghania, 23 January 2004. The specimen was dug up from underneath a banana plant on a local shamba (small agricultural holding) at approximately 1080 m above sea level (S03°30.76', E38°34.59').

Paratypes: 10 additional specimens collected 23.01.2004 (BMNH2005.10–11, NMK A/4295) by Hendrik Müller and David Mwaghania and 26.04.2004 (NMK A/4298/1–7) by Patrick K. Malonza from the same locality as the holotype. All specimens were dug up from shambas, mostly in soil underneath bananas or under decomposing organic debris.

Diagnosis: A medium to large *Boulengerula* with the sphenethmoid exposed between the frontals. It differs from all other members of the genus, except *B. taitanus*, in being strongly pigmented, which gives it a brownish appearance in life and dark slate in preservative, with whitish annular grooves. It differs from *B. taitanus* in its distinctive brownish rather than bluish-black colouration, the sphenethmoid being exposed between the frontals, a higher mean number of vertebrae and annuli, and different phallus morphology with broad, flange-like tuberosities of the dorsolateral ridges that are in close proximity dorsomedially.

Description of the holotype: Morphometric and meristic data are given in Table 1. The holotype is in good condition generally. It is slightly dehydrated, which results in a somewhat wrinkled appearance with the ventral side being partly concave. Three patches of artefactual dark discolouration are present on the ventral side of the head and anterior body. There are two midventral longitudinal incisions, the first (13 mm) beginning 24 mm in front of the vent and the second (18 mm) 67 mm in front of the vent.

The natural shape of the body is cylindrical to subcylindrical and appears slightly dorsoventrally compressed (more so in preservative). The body is almost uniform in width, except for the anterior 30 mm where the body gently narrows towards the head. The body also narrows slightly in its last 25 mm towards the vent, from where the body narrows abruptly towards the terminus.

In dorsal view, the head is parabolic, tapering from the first nuchal groove towards the nares, with a slight bulging in the region of the tentacles. Anterior to the nares, it terminates in a rounded but narrow snout tip. In lateral view, the top of the head is almost straight and tapers gently towards the level of the tip of the lower jaw, from where it tapers strongly towards the tip of the snout. The snout is bluntly rounded and has its apex on a horizontal line halfway between naris and tentacle in lateral view. The underside of the snout (rostrum) is slightly concave above the margin of the upper lip. In ventral view, the tip of the lower jaw is more broadly rounded than the tip of the snout. The distance between the jaw angle and the top of the head (at the level of the jaw angle) is slightly

greater (but less than one and a half times) than the distance between the jaw angle and the ventral side of the head.

The eyes are not visible and no differentiation in skin pigmentation indicates their position. There is no conspicuous depression or elevation in the region where the eyes would be expected to lie.

The tentacles are short and globular. The tentacular apertures are almost perfectly circular and raised, resulting in an elevated rim around the tentacular orifice. They are encircled by a dark ring and are positioned slightly ventrolaterally on the head. In ventral view, the tentacular apertures are more clearly visible than in dorsal view, where they just about reach the outline of the head. In lateral view, the tentacle is much closer to the margin of the upper lip than to the top of the head and lies on an imaginary straight line between the naris and the jaw angle. The tentacular aperture is positioned almost half way between the anteriormost margin of the mouth and the angle of the jaw, but slightly closer to the anteriormost margin of the mouth.

The nares are very small, subcircular to kidney shaped, and less than 0.1 mm in diameter. They sit at the anterior rim of a very shallow, oval to subcircular depression of 0.26 mm length. In lateral view, the naris is positioned almost equidistant from the upper side, underside, and apex of the rostrum, with the distance to the apex being the smallest and to the underside the largest. The nares are also visible anteriorly and dorsally but not ventrally.

The choanae have an oval shape and no conspicuous choanal valves are visible. The lateral rim of the choana is formed by the vomeropalatine shelf and is slightly raised. The medial rim is not raised. The angles of the jaw are not cut, so tooth counts are approximations. In the holotype, the teeth are arranged in three series. We count 24 premaxillary-maxillary teeth, 22 vomeropalatine, 18 dentary. Of the splenial series, only a single pedicel is present on the right side, the crown being broken off. No traces of splenials were found on the left side. There is no diastema between vomerine and palatine teeth. The flat and featureless tongue is free at its anterior end and not pigmented.

The two nuchal collars appear slightly broader than the adjacent areas. At 2.2 mm the second nuchal collar is slightly longer than the first (1 9 mm, measured laterally). The two nuchal collars are clearly demarcated by three nuchal grooves. The first nuchal groove is largely incomplete dorsally. The second nuchal groove is complete and has a small, forward pointing bend on the dorsal side. The third nuchal groove is interrupted ventrally, with the gap slightly shifted towards the left side. Ventrally, the first and second nuchal grooves are straight, whereas the open ends of the third groove are bent slightly backwards, mirroring the posterior groove of the first annulus. The first nuchal collar has a short transverse groove on the dorsal side, which is only very faintly indicated. A clearly marked, albeit very narrow crease is present on the ventral side. The second nuchal collar also bears a transverse groove, which is much broader than the one on the first collar and clearly indicated. Lateral transverse grooves are present on neither collar.

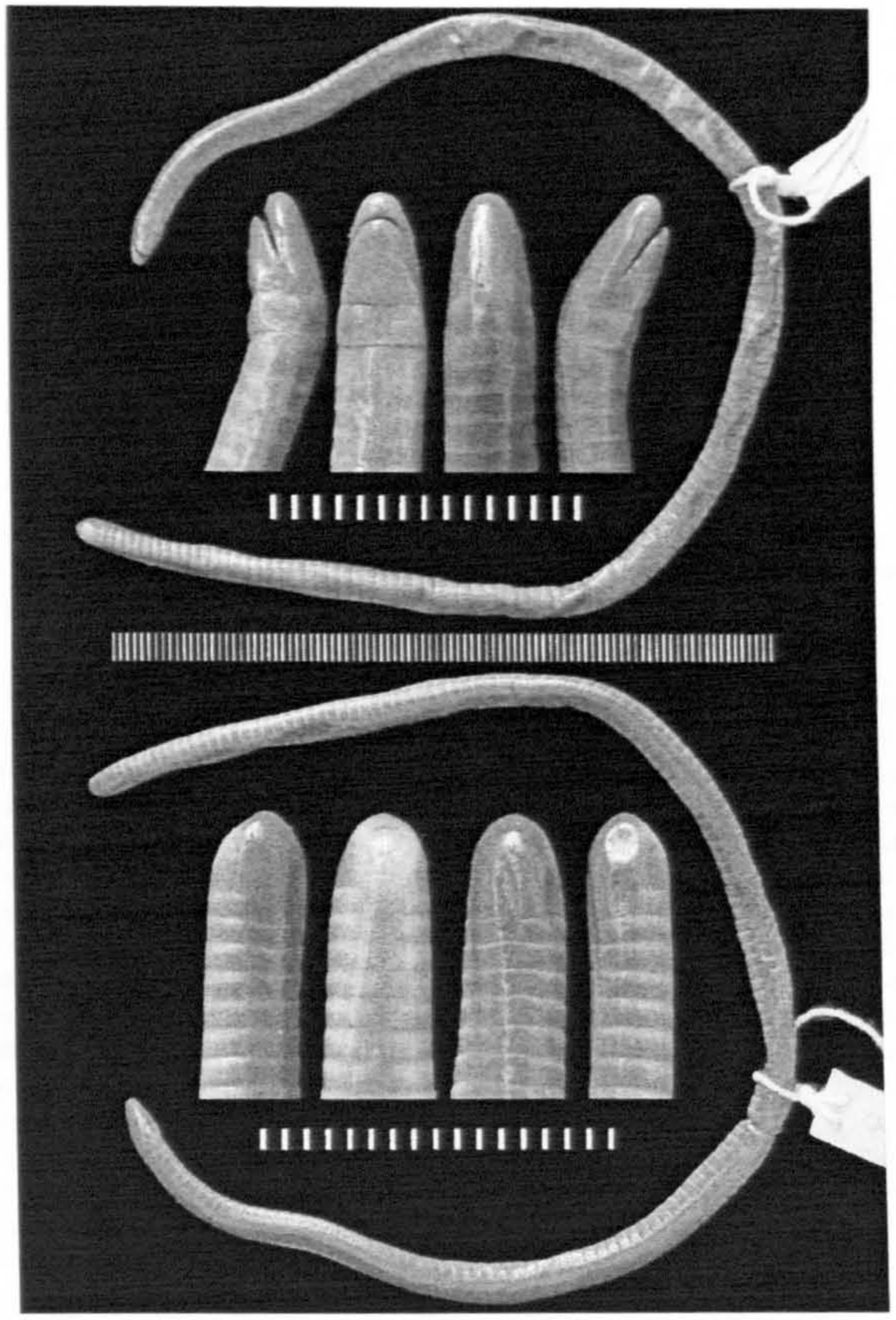

FIGURE 2. Holotype of *Boulengerula niedeni* (NMK A/4294), showing the ventral and dorsal side of the whole specimen. The head and body terminus are shown enlarged in dorsal, ventral and lateral views. Scales are in mm.

TABLE 1. Morphometric and meristic data for holotype and paratypes of *Boulengerula niedeni*. All measurements are in mm and were made to the nearest 0.1 mm under a binocular microscope with ocular micrometers, except for length and circumference, which were measured with a ruler and a piece of thread and a ruler, respectively. Tooth counts marked with an asterisk are approximations.

	NMK A/4294	BMNH 2005.10	BMNH 2005.11	NMK A/4298/7	NMK A/4298/6	NMK A/4298/2	NMK A/4298/3	NMK A/4298/4	NMK A/4298/1	NMK A/4298/5
Sex	f	m	f	indet.	indet.	m	m	j	m	j
Total length	275	228	216	253	245	213	201	121	196	92
Number of primary annuli	144	142	140	143	141	142	142	143	138	-
Head length	5.5	6.0	5.2	5.8	5.4	5.5	4.8	3.6	4.8	3.4
Distance between snout tip and angle of jaws	4.0	4.2	3.7	3.3	4.0	3.9	3.4	2.6	3.5	2.3
Distance between tip of lower jaw and first nuchal groove	4.6	5.0	4.3	5.0	4.6	4.3	3.6	3.0	4.1	2.5
Distance between tip of lower jaw and angle of jaws	3.0	3.4	2.9	3.4	3.2	2.7	2.3	1.8	2.5	1.4
Length of first nuchal collar	1.8	2.0	1.6	2.0	2.1	1.6	1.7	1.3	1.5	1.3
Head width at first nuchal groove	4.0	4.8	3.7	4.6	4.3	4.0	4.2	2.9	3.9	2.5
Head width at angle of jaws	3.8	4.3	3.4	4.1	4.0	3.8	3.5	2.9	3.4	2.4
Distance between external nares	1.5	1.5	1.5	1.5	1.4	1.5	1.4	1.1	1.3	1.0
Distance between tentacles	3.1	3.5	2.9	3.1	3.4	3.1	2.9	2.2	2.7	2.0
Distance between external naris and tentacle	1.6	1.8	1.6	1.8	1.6	1.5	1.3	1.1	1.3	0.9
Distance between tentacle and margin of upper lip	0.3	0.4	0.4	0.3	0.4	0.4	0.4	0.2	0.4	0.3
Distance between external naris and first nuchal groove	5.2	5.6	4.8	5.4	5.3	5.0	4.3	3.3	4.6	3.3
Distance between external naris and angle of jaws	3.6	4.0	3.3	3.6	3.6	3.4	3.0	2.1	3.3	2.2
Distance between tentacle and tip of snout	2.1	2.2	1.9	2.3	2.0	1.8	1.7	1.3	1.5	1.3
Distance between tentacle and angle of jaws	1.8	2.1	1.8	1.8	1.8	1.8	1.5	1.3	1.9	1.2
Distance between snout tip and anterior margin of mouth	1.0	0.9	0.9	1.1	0.8	1.1	0.8	0.7	0.8	0.8
Width at midbody	4.7	4.8	4.8	4.8	6.3	4.2	4.5	3.0	4.3	2.6
Body width at level of vent	2.9	2.9	2.8	3.1	3.7	2.9	3.0	2.2	3.4	2.0
Distance from vent to body terminus	0.8	0.4	0.8	0.8	0.5	0.9	0.7	0.6	0.9	0.3
Circumference at midbody	17	16	12	16	19	18	15	10	13	10
Premaxillary-maxillary teeth	24	23	24	24	25	25	26	22	21	-
Vomeropalatine teeth	22	17*	18	-	19*	-	-	-	-	-
Dentary teeth	18	19	21	21*	20*	20*	18*	-*	17*	-
Splenial teeth	1	2	2	-	-	-	-	-	-	-
Number of vertebrae	150	145	148	152	146	151	150	150	150	146

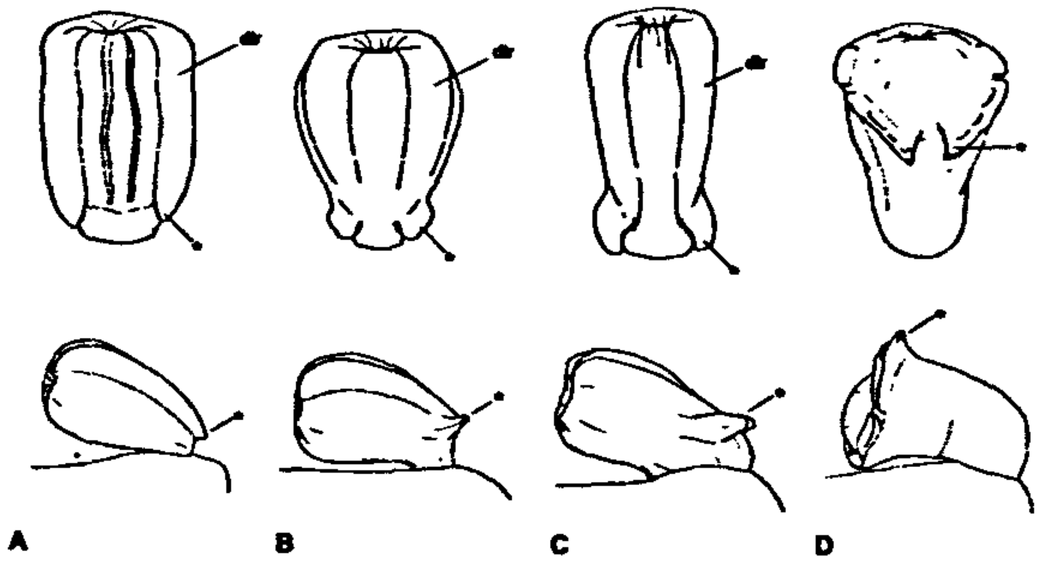

FIGURE 3. Phallus of (A) *Boulengerula boulengeri*, (B) *B. uluguruensis*, (C) *B. taitanus*, and (D) *B. niedeni*, in dorsal (top row) and lateral view. Not to scale. The horns and tuberosities referred to in the text are marked by an asterisk (*), dlr – dorsolateral longitudinal ridge.

In preservative, the annuli are marked by whitish grooves. These markings are most pronounced laterally and ventrolaterally but are lacking on the dorsal side. There are 144 primary annuli. With the exception of the anterior sixth of the body, annular grooves are mostly incomplete dorsally. The last seven annuli prior to the second to last annulus are also well demarcated by dorsally complete annular grooves. Most annuli are interrupted at the ventral midline, except in the anterior and posterior fifths of the body. The body terminus is capped by a terminal shield, which bears no annular grooves posterior to the vent. The annular grooves, separating the terminal shield from the last annulus are short, incomplete dorsally and ventrally, and only visible in lateral and ventral view. The penultimate annulus is also incompletely separated ventrally and dorsally from the last annulus, although to a far lesser extent than the last is from the terminal shield. There are no indications of a subdivision of the primaries by secondary annulation on any part of the body. No attempt was made to search for scales.

The body terminus is formed by a terminal shield of 3.5 mm length (measured laterally). It is rounded in dorsal view and ends in a very bluntly pointed tip. In lateral view, the terminal shield appears slightly thicker than the adjacent part of the body, although this could be an artefact of preservation. The body tapers strongly from anterior of the vent towards the terminal apex. It tapers more strongly from the venter, where the terminal shield is almost planar and angled upwards at about 60 degrees towards the terminal apex, whereas the dorsal side is evenly rounded. The transverse vent lies within a poorly defined, transverse oval disc of about 1.9 by 2 6 mm, 0.8 mm in front of the body terminus. The vent is somewhat irregular within the disc, having a pattern of six anterior and seven posterior denticles, of which the anteromedial is the largest. The anterior denticles appear duplicated in a second internal row, which suggest that the cloaca is slightly prolapsed.

The colour in life is brownish on the dorsum with a clear pinkish-reddish tinge. The ventrum is light brown to pinkish and appears somewhat translucent. The annuli are indicated by white markings, which are faint anteriorly and more pronounced towards the body terminus, giving the flanks a lighter appearance. The head also has a pinkish colouration that only slightly contrasts with the rest of the body. In preservative, the colour has faded to grey dorsally and to light grey ventrally. The flanks are still lighter coloured and give the impression of broad ventrolateral stripes, although the ventral border is not very well defined.

The specimen is a mature female with developing ovarian eggs. Five eggs are visible through the second incision (from the vent) at the ventral side of the specimen. The diameter of one egg is 3.0 x 1.6 mm.

Etymology: This species is named for Fritz Nieden, herpetologist at the Zoologisches Museum Berlin during the early 1910s, for his many contributions to the taxonomy of African amphibians and reptiles. The specific name is a noun in the genitive case.

Additional information from paratypes

Colouration.—Adult specimens of *B. niedeni* exhibit no variation in their colouration and show no obvious differences to the colouration of the holotype as described earlier. The three juvenile specimens however, differ markedly in their colouration from the adult specimens in our sample. All juveniles are unpigmented on the ventral and lateral sides and only possess a dark and narrow dorsal band. Loveridge (1935) and Nussbaum and Hinkel (1994) reported the young of *B. taitanus* to be pink in life. Nussbaum and Hinkel (1994) further reported that the dark colouration of *B. taitanus* develops ontogenetically from a darker, middorsal band, which intensifies and gradually broadens. It appears therefore as if in *B. niedeni* too the adult pigmentation develops some time after hatching, presuming that *B. niedeni* is direct-developing as is known for *B. taitanus* (Nussbaum & Hinkel 1994).

Oral cavity, teeth, and skull morphology.—To gain a better understanding of the morphology of the oral cavity, we cut the jaws of one of the paratypes (BMNH 2005.10). In this specimen, the choanae are oval and about 7 mm x 3.5 mm. The smallest distance between them is 11 mm. The lateral margin of the choana is raised and formed by the gums of the vomeropalatine tooth shelf. The medial rim is not raised. Clearly developed choanal valves are not visible. The teeth are arranged in four series. The premaxillary-maxillary (PMM) series traces a broad arc across the midline anteriorly and extends backwards to the jaw angle. It is continuous except for a slight gap on each side behind the

anterior four to five teeth. These gaps are not strictly symmetrical but might demarcate the border between premaxillary and maxillary teeth. The vomerine teeth form a short arc, even broader anteriorly than in the PMM, and end well before the anterior rim of the choanae. The palatine teeth are separated from the vomerine teeth by substantial diastemata. The palatal series begins just behind the anterior rim of the choana and extends to about one tooth position (0.1 mm) behind the posterior-most PMM teeth. Whereas the right side of the palatine series contains eight teeth and is apparently complete, there are no teeth on the left side except what appears to be the posterior-most tooth. It seems as if the teeth are being replaced as a group here (see Taylor 1968). The dental teeth form a continuous arc that extends backwards to the jaw angle. The splenial series consists of a single pair of teeth anteriorly, almost completely hidden within the gums.

The PMM teeth slightly increase in size from the anterior to the 3rd or 4th position, from where they then gradually decrease posteriorly. The vomerine teeth are larger than the palatine teeth and are about the same size as posterior PMM teeth, although the vomerine teeth appear to be more slender. The palatine teeth again are more slender than vomerine teeth. The dentary series has the largest teeth, which increase in size from anterior towards the 4th or 5th position, behind which they are smaller and gradually decrease posteriorly. The splenial teeth are the smallest of all series. The vomerine, palatine, and splenial teeth are bicusped, whereas the PMM and dentary teeth are monocusped. The tongue is free anteriorly but does not cover the splenial teeth. It is unpigmented and gently rounded anteriorly. The anterior part of the tongue is flat and featureless but some longitudinal plicae are present posteriorly.

Another paratype (BMNH2005.11) shows a similar intraoral morphology to BMNH2005.10 except for the diastemata between vomerine and palatine teeth. This specimen exhibits a morphology intermediate between BMNH 2005.10 and the holotype, in that only moderate diastemata are present.

For a preliminary examination of skull characteristics the skin covering the right side of the head was reflected in BMNH 2005.10. In this specimen, the slender, dorsal part of the sphenethmoid is exposed in the skull roof and separates the frontals. The orbital region is completely covered by bone and no trace of an eye is visible.

Phallus morphology.—The terminology for the description of phallus morphology follows Gower and Wilkinson (2002). The phallus is fully or partially everted in four specimens (BMNH 2005.10, NMK A/4298/1, NMK A/4298/2, NMK A/4298/3). There are no obvious longitudinal ridges on the phallus. However, there are large, flange like tuberosities near the apex of the phallus. These are bipartite with a smaller ventrolateral flange and a larger dorsolateral flange that increases in size towards the dorsal side of the phallus, resembling a collar like structure (see Fig. 3D). In lateral view, the flanges are inclined diagonally to almost vertically along the longitudinal axis of the phallus. The dorsolateral flanges have serrated edges and converge towards the dorsal side of the phallus and terminate in pointed, horn-like structures that point dorsally and are only separated by a narrow

gap. Besides these main flanges, smaller, pointed or plate-like, serrated accessory protuberances are present and seem to be associated with the dorsolateral ridges. The extent to which the phallus ornamentation, i.e serrations and accessory protuberances, is developed is variable among the specimens.

Body terminus and vent.—The number of cloacal denticles varies in our sample from five anterior and six posterior denticles to six anterior and seven posterior. The largest denticles are usually the one or two medial anterior denticles. A keel on the terminal shield is weakly indicated in some of the specimens but very conspicuously developed in one of the juvenile paratypes (NMK A/4298/5).

Phylogenetic relationships and comparison with other *Boulengerula*

Boulengerula niedeni fits the diagnosis of ***Boulengerula*** as currently conceived (Nussbaum & Hinkel 1994). Compared to other members of the genus, ***B. niedeni*** is readily distinguished by its distinctive colouration. Whereas ***B. taitanus*** is a dark coloured form, most other ***Boulengerula*** are either unpigmented and appear pink in life, including ***B. changamwensis***, ***B. fischeri***, ***B. uluguruensis***, and (presumably) ***B. denhardti***, or show a light bluish grey colouration, like ***B. boulengeri***. When comparing ***B. niedeni*** with other ***Boulengerula*** species, the closest relative appears to be ***B. taitanus***, based on overall phenetic similarity and close geographic proximity of their distributions.

To assess morphological differences between ***B. niedeni*** and ***B. taitanus***, we compared our sample of ***B. niedeni*** against a sample of 58 ***B. taitanus*** ranging from 55 mm total length to 345 mm (see Appendix). Although at the upper end, the range of the number of annuli of our sample of ***B. niedeni*** falls within the range of ***B. taitanus***, with 138-144 compared to 124-147 respectively. The mean values for the number of annuli however, are well separated at 141.67 (SD 1.80) for ***B. niedeni*** and 135.53 (SD 4.67) for ***B. taitanus***. When comparing the number of vertebrae (counted from x-rays) in both samples, ***B. niedeni*** also shows a higher mean number of vertebrae with 149.00 (SD 2.14) as compared to 142.07 (SD 5.20) in ***B. taitanus***, although overlap between the ranges does occur (146-152 and 130-153, respectively). However, the ranges of vertebrae number might show greater overlap between the two species once a larger sample of ***B. niedeni*** becomes available for study.

A comparison of phallus morphology among ***B. niedeni***, ***B. taitanus***, ***B. boulengeri***, and ***B. uluguruensis*** (Barbour and Loveridge) reveals common patterns but also what we interpret as specific differences (see Fig. 3). ***Boulengerula taitanus***, ***B. boulengeri***, and ***B. uluguruensis*** all have dorsolateral longitudinal ridges but differ with regards to the extent of their development. In addition, the four species have differently arranged phallus ornamentation. In ***B. boulengeri*** the longitudinal ridges are very prominently developed and each ridge terminates close to the base of the phallus in a simple, semidetached pointed tip. In ***B. uluguruensis*** and ***B. taitanus*** prominent horn-like tuberosities are present at the end

of each ridge and inclined against the longitudinal axis of the phallus. The horn-like tuberosities are smaller and only moderately developed and inclined in *B. uluguruensis*, but *B. taitanus* shows a more pronounced development and a slightly stronger inclination. *Boulengerula niedeni* differs from the other species in having no markedly developed longitudinal ridges. Instead, large, horn-like tuberosities are attached more apically to an otherwise almost smooth phallus. *Boulengerula niedeni* is further unique in that the horn-like tuberosities are in close proximity dorsally and possess elaborate ornamentation. The phallus morphology of *B. denhardti* and *B. fischeri*, both known from only the type specimen, and *B. changamwensis*, which is known from only a few specimens (Malonza & Müller 2004), is currently unknown. At present, the intra- and interspecific variation of phallodeal morphology is poorly understood (Gower & Wilkinson 2002), but the main differences we observed are constant and we interpret them as further evidence that *B. niedeni* is a distinct species. We note however, some variation in the degree of phallus ornamentation in *B. niedeni*, which might reflect seasonal variation.

Discussion

Wilkinson *et al.* (2003) analysed the relationships of African caecilians using mitochondrial DNA sequences. Their phylogeny confidently resolved their only two representatives of *Boulengerula*, *B. boulengeri* and *B. taitanus*, as sister taxa, although Wilkinson *et al.* (2003) pointed out the deep molecular divergence between the two. In their discussion of interrelationships within *Boulengerula*, Wilkinson *et al.* (2004) suggested that the synonymy of *Afrocaecilia* with *Boulengerula*, proposed by Nussbaum and Hinkel (1994), might have been premature. Against this backdrop it is intriguing to note an apparently more similar general phallus morphology with inclined dorsolateral tuberosities in *B. uluguruensis* and *B. taitanus* as compared to *B. boulengeri* (see Fig. 3). This common phallus morphology might be a putative synapomorphy of *Afrocaecilia sensu* Taylor (1968). However, *B. niedeni*, which appears most similar to *B. taitanus* based on external morphology, shows a strikingly different phallus morphology. It seems therefore as if phallus morphology might be a useful character in distinguishing species of *Boulengerula* although further research is necessary to investigate and compare the phallus morphology in other members of *Boulengerula*. The diagnostic value of the phallus has previously been suggested for caecilians (Gower & Wilkinson 2002, Kupfer & Himstedt 2002).

Taylor (1968) and Nussbaum and Hinkel (1994) used the presence of a diastema between vomerine and palatine teeth as a character to diagnose species of *Boulengerula* (*sensu* Nussbaum & Hinkel 1994). The few specimens examined for oral morphology in our comparatively small sample of *B. niedeni* show different states of this character, ranging from a very pronounced diastema as in *B. changamwensis* to no diastema as in *B. taitanus*. It appears therefore as if the presence of a diastema between vomerine and palatine teeth, at least in *B. niedeni*, is much more variable than previously thought. Tooth replacement has been reported in other caecilian species (Taylor 1968) and seems to be the most likely cause for the observed variation in the arrangement of vomeropalatine teeth in our series. Future studies on other *Boulengerula* should investigate the intraspecific variability of this character and our observation suggests that this character should be treated with caution when dealing with very small sample sizes.

Local farmers report *Boulengerula niedeni* to be more abundant during the wet seasons, something previously reported for other East African caecilians (e.g. Loveridge, 1935). All specimens collected during this study were from small-scale agricultural plots (shambas); natural forest has all but disappeared from this isolate (Wilder *et al.* 1998). Our personal experience and published work (e g. Glaser 1984, Measey 2004) indicate that shambas can be suitable habitats for some caecilian species, including members of the genus *Boulengerula*. However, understanding of what constitutes suitable habitat for caecilians is currently uncertain (Gower & Wilkinson 2005). For example, specimens of *B. taitanus* found in shambas were significantly smaller but more abundant than those inhabiting naturally forested areas of the Taita Hills (Measey 2004). The only known locality for this new species is Sagalla Hill (c. 2900 hectares above 1000m asl), a small mountainous area that is ecologically isolated within the arid Tsavo plain. The currently limited information about *B. niedeni* implies that it should be considered 'data deficient' (IUCN 2001). However, as all specimens were collected in an anthropogenically disturbed area on a small, isolated mountain, it is clear that detailed future investigations are needed.

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Appendix

Material Examined

Boulengerula niedeni.–Kenya: Coastal Province: Taita-Taveta District: Sagalla Hill: BMNH 2005.10–11; NMK A/4294; NMK A/4298/1–7.

Boulengerula taitanus.– Kenya: Coastal Province: Taita-Taveta District: Dawida: Wundanyi: NMK A/3110–2 (specimen field numbers: MW501–11, 514, 516–525, 528–41, 544–48, 814); Mt. Mbololo: MCZ 20001, MCZ 20002, MCZ 20004, MCZ 20005, MCZ 20007–12, MCZ 20014, MCZ 20015, MCZ 20017, MCZ 20018, MCZ 20021, MCZ 85094.

Institutional codes: BMNH: The Natural History Museum, London, UK; MCZ: Museum of Comparative Zoology, Harvard, USA; NMK: National Museums of Kenya, Nairobi, Kenya.

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Original article

Niche separation and comparative abundance of *Boulengerula boulengeri* and *Scolecomorphus vittatus* (Amphibia: Gymnophiona) in an East Usambara forest, Tanzania

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Abstract.—The ecology of the sympatric caecilians *Boulengerula boulengeri* and *Scolecomorphus vittatus* was studied in Nilo Forest Reserve in the East Usambara Mountains, Tanzania. Three sampling methods (timed digging, pitfall trapping and casual visual encounter surveys of the forest floor) yielded 85 *B. boulengeri*, found only by digging soil, and 23 *S. vittatus*, mostly collected above ground. The difference between these taxa in the proportions of captures above and below ground is statistically significant and is taken to indicate different ecologies. *B. boulengeri* is interpreted as predominantly a burrower in soil, and *S. vittatus* as an animal spending more time than *B. boulengeri* above ground. Niche separation appears to be correlated with some morphological differences. The vast majority of all vertebrate specimens dug from the top 300 mm of soil were *B. boulengeri*, and this species appears to be more abundant than *S. vittatus* in East Usambara forest soils. As an abundant endogeic animal, *B. boulengeri* may play an important role in the ecology of forest soils.

Key words—caecilians, Eastern Arc, ecology, Caeciliidae, Scolecomorphidae, soil

Caecilians are elongate, limbless, snake-like amphibians found mainly in the wet tropics. Little is known about caecilian ecology (Himstedt 2000). Most ecological information that has been published has been gleaned from examination of preserved specimens, and from piecemeal natural history observations made during the often opportunistic collection of caecilians. Recently, however, several largely field-based studies have made encouraging breakthroughs. These include quantitative estimates of abundance through surveys (Oommen *et al.* 2000; Measey *et al.* 2003a; Measey & Di-Bernardo 2003; Measey in press), testing and implementation of marking methods in recapture experiments (Measey *et al.* 2001, 2003b), investigations of diet and condition based on randomised sampling (Measey *et al.* 2004, Measey & Gower in press; Gaborieau & Measey 2004), growth (Kupfer *et al.* 2004a), and reproductive ecology (Kupfer *et al.* 2004b).

The majority of these studies have focussed upon autecology in agricultural or otherwise disturbed habitats. In this paper, we present a

quantitative study of two sympatric caecilian species from a forest reserve in Tanzania - the caeciliid *Boulengerula boulengeri* Tornier and the scolecomorphid *Scolecomorphus vittatus* (Boulenger). These species are endemic to Tanzania, and *B. boulengeri* is endemic to the East Usambara Mountains (Taylor 1968; Nussbaum & Hinkel 1994; Nussbaum 1985). They are the only caecilian species thus far reported from the East Usambaras. Here we compare their ecology using quantitative field collection data, and relate comparisons to what is known of their morphology.

The East Usambara Mountains of Tanzania are one of the component blocks of the Eastern Arc of East Africa, a region of very high endemicity and a global biodiversity hotspot (e.g., Loveridge 1942; Lovett & Wasser 1993; Myers *et al.* 2000). The study site, Nilo Forest Reserve (FR), is in the northwestern part of the East Usambaras. It lies on hilly ground (400 - 1506 m a.s.l) and comprises submontane and lowland forest, some of which is under cultivation and some cleared for human settlement. At 6,025 hectares, it is the second largest protected block of forest in the East Usambaras.

MATERIALS AND METHODS

This study was conducted as part of the East Usambara Biodiversity Surveys (EUBS), a collaboration between the East Usambara Conservation Area Management Programme (EUCAMP) and Frontier-Tanzania. The survey of Nilo FR was carried out between June 2000 and March 2001 (for details see Frontier-Tanzania 2002). This survey aimed to provide an inventory of selected plant, invertebrate and vertebrate taxa, and to quantify ecological parameters in the context of forest disturbance and regeneration. For the survey, Nilo FR was divided into a grid of 450 m (East - West) x 900 m (North - South) quadrats. The vast majority of these were surveyed in vegetation analyses, totalling 122 quadrats. All of the caecilian work reported here occurred in the forested areas within Nilo FR.

Three methods were used to collect caecilians - timed digging, pitfall trapping, and visual encounter. Timed digging took place within 38 of the 450 m x 900 m quadrats in the central part of Nilo FR. Selected quadrats were contiguous along the North-South axis of the grid, but spaced every other quadrat along the East-West axis. Each timed dig comprised two person hours (SPL and assistant concurrently for one hour each) and took place adjacent to 50 m x 20 m vegetation plots situated in the southwest corner of each quadrat. Hoes were used to search leaf litter and dead wood, and to dig over approximately the top 300 mm of the soil.

Ten pitfall trap sites were set up in Nilo FR, with their location chosen to lie outside the vegetation plots and to cover differing environmental conditions (vegetation, altitude, slope etc) within the reserve. Six of these trap sites lay within the central region of Nilo FR that were chosen for timed dig sampling, and four lay elsewhere in the reserve. Each pitfall trap site consisted of three unconnected lines of 20-litre plastic buckets (275 mm deep, 290 mm diameter at opening) sunk to ground level. Along each line, 11 buckets were spaced approximately 5 m apart, with a drift fence formed by a continuous plastic sheet (1 m wide, of which 0.5 to 0.75 m was held perpendicular to the ground) extending across the centre of the buckets. Trap sites were checked each morning and afternoon for 10 days.

Searching by visual encounter took place casually and irregularly, mostly during daylight, whenever fieldworkers moved about in Nilo FR. This form of collecting was not systematic or randomised, in that areas between field camps and sampling sites were walked much more frequently than within quadrats. Digging took place between June and December 2000

Table 1. Numbers of individuals of two species of caecilian collected by three different methods in surveys of Nilo Forest Reserve, Tanzania. Visual encounter represents animals casually found above ground. For reasons unknown, Frontier-Tanzania (2002) report three *Scolecomorphus vittatus* collected by digging and do not mention pitfall captures.

Species	Collection method			Totals
	Timed digging	Pitfall	Visual encounter	
Boulengerula boulengeri	85	0	0	85
Scolecomorphus vittatus	2	2	19	23
Totals	87	2	19	108

but pitfall trapping and visual encounter were spread throughout the duration of the Nilo FR biodiversity survey.

Collected caecilians were killed using the anaesthetic MS222, Sandoz, individually tagged, fixed in formalin, and stored in ethanol. The position and method of capture of each specimen was recorded. Material is being deposited in the collection of the Department of Zoology, The Natural History Museum, London. Capture data were subject to a χ^2 test of the null hypothesis that the proportions of above and below ground captures are not different for each species, or alternatively that the proportions of the two species are not different for above and for below ground captures. Only one species was caught by pitfall trapping and visual encounter (see below) so that the captures for these two methods can safely be pooled without having to assume that they are equivalent. The test is based on proportions and so does not require us to assume that each capture above ground is equivalent to each below ground. Statistical analyses were performed by hand and with GenStat (2000).

RESULTS

The numbers of *B. boulengeri* and *S. vittatus* collected by the three methods are presented in Table 1. No caecilians were observed above the soil (including in litter) during digging. In total 108 caecilians, 85 *B. boulengeri* and 23 *S. vittatus*, were collected. *Boulengerula boulengeri* were found only during timed digging in soil, while *S. vittatus* were found using all three methods. Abundance within the soil was much higher for *B. boulengeri* than for *S. vittatus* (1.12 versus 0.03 individuals per person hour of digging). Categorising the data into subterranean (timed digging) and surface (pitfall plus visual encounter) collections, the relative proportions of each species found above and below ground are significantly different (Likelihood $\chi^2 = 92.81$, df = 1, $P < 0.001$). Unsurprisingly, a Fisher exact test (sometimes recommended where expected values in contingency tables are low, as here) on the same data also yields a significant result ($P < 0.001$). The only *S. vittatus* captured by digging in soil were found in a single quadrat, which also yielded two *B. boulengeri*. *Scolecomorphus vittatus* collected by visual encounter were predominantly found after rain, mostly during daylight. Background demographic data for the two study species are lacking, but the frequency distributions of total length of individuals of the two species (Fig. 1) are not notably skewed, and are interpreted as being consistent with no obvious collecting bias toward especially small or large animals.

For our samples, mean total length is significantly greater for *S. vittatus* (237.6 versus 158.7 mm, *t*-test, $P < 0.001$), with the largest *S. vittatus* 83% longer than the largest *B. boulengeri*. Of the specimens in which sex could be determined, there were more females than

males for both *B. boulengeri* (41 : 31) and *S. vittatus* (10 : 9) but neither ratio is significantly different from unity (Likelihood $\chi^2 = 0.24$ and 0.82 for *B. boulengeri* and *S. vittatus* respectively, $P > 0.5$).

Some reptiles and other amphibians were also collected during searches for caecilians. Timed digging found one or two individuals each of the microhylid frog, *Probreviceps macrodactylus macrodactylus* (Nieden), and the skinks, *Leptosiaphos kilimensis* (Stejneger) and *Proscelotes eggeli* (Tornier). Thus, more than 90% of all vertebrate specimens found by digging were *B. boulengeri*. Many frogs (approximately ten species), lizards (four skink and one gecko species), snakes (two species) and small mammals were also collected in pitfall traps, but individuals were not counted for all taxa.

DISCUSSION

Of the three sampling techniques employed, only digging is likely to yield endogeic species, those that live and feed primarily within the soil (see also Measey *et al.* 2003b; Gower *et al.* 2004). Pitfall traps and visual encounter methods sample species that spend at least some time on the surface. We interpret differences in captures of *B. boulengeri* and *S. vittatus* when using different sampling techniques as indicative of a real difference in their ecologies. That *B. boulengeri* and *S. vittatus* occur in significantly different proportions above and below ground indicates a degree of niche separation across the sampled habitats. *Scolecomorphus vittatus* spend some time in soil and *B. boulengeri* rarely (though not in this study) are found in pitfall traps or on the surface, so the difference is not absolute.

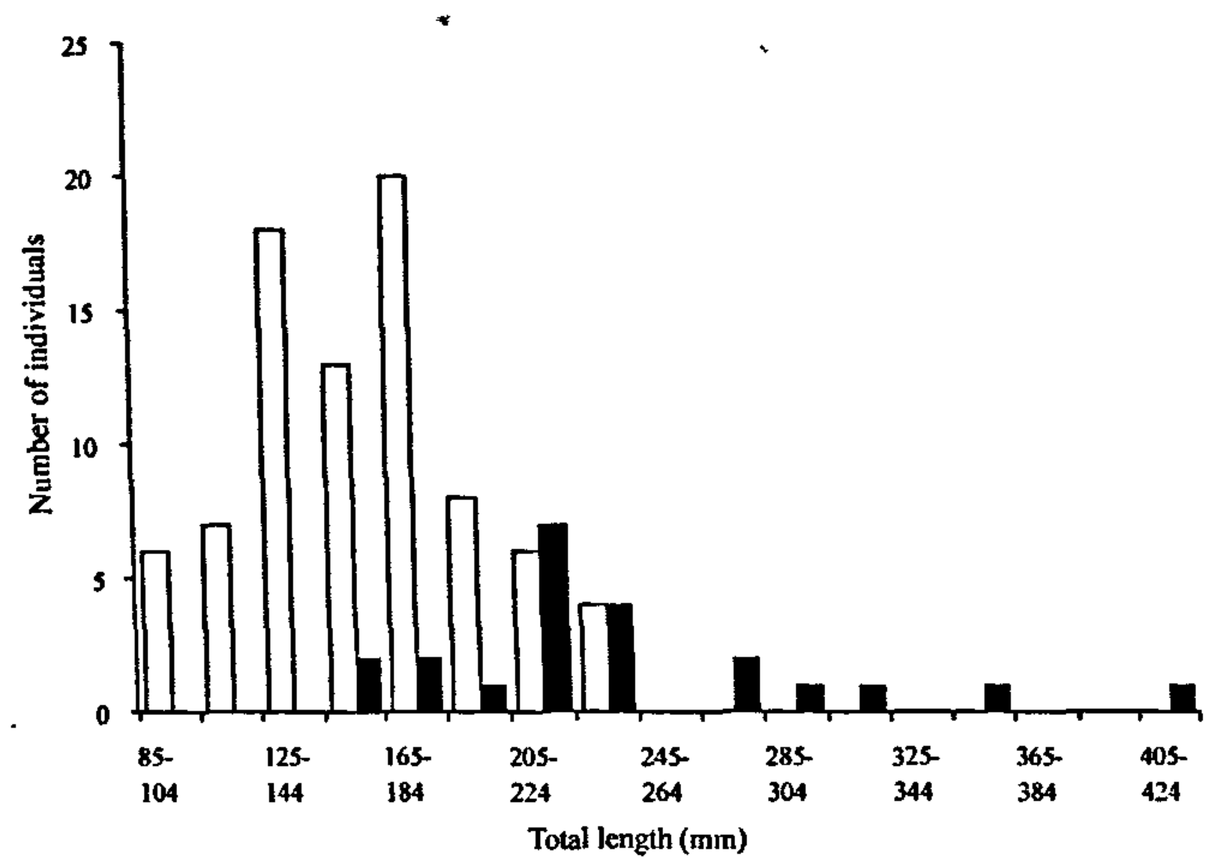

Figure 1. Frequency distributions of total length of complete caecilian specimens collected in Nilo FR, Tanzania. Open bars — *Boulengerula boulengeri* ($N = 82$); Black bars — *Scolecomorphus vittatus* ($N = 21$).

Table 2. Morphological differences between *Boulengerula boulengeri* and *Scolecomorphus vittatus*. Data from Taylor (1968), Wake (1985), Nussbaum and Hinkel (1994), Nussbaum (1985), Loader *et al.* (2003b), references cited therein, and personal observations. The *S. vittatus* sample collected from Nilo FR includes two apparent morphs. The taxonomic significance of these forms is under investigation, but the details do not affect this list of differences (except perhaps for maximum size) because most appear to hold at the generic level for *Scolecomorphus*. * G. J. Measey pers. comm.

Character	*Boulengerula boulengeri*	*Scolecomorphus vittatus*
Maximum length	< 280 mm*	> 400 mm
Body form	slender and subcylindrical	more tapered anteriorly
Head shape	subconical	slightly dorsoventrally compressed
Temporal region	strongly stegokrotaphic	zygokrotaphic
Nasal-premaxilla-septomaxilla	compound	unfused
Separate prefrontals	absent	present
Stapes	present	absent, and cheek less constrained
Eyes	covered by bone, strongly reduced	protrusible from cranium, less strongly reduced
Tentacles	short and globular	long and slender
Tentacle position	lateral, halfway along upper margin of mouth	underside of snout, level with or in front of margin of mouth
Tooth crowns	some bi-cusped	mono-cusped
Palatine teeth	substantial overlap with maxillary series	extend posteriorly from end of maxillary tooth row

Thus it is likely that there are opportunities for interspecific interactions, but our data suggest that intraspecific interactions are likely to dominate. With the exception of the aquatic and semiaquatic neotropical Typhlonectidae, adult caecilians are thought to be mostly terrestrial and soil dwelling. However, the literature suggests that some species differ in their soil-burrowing capability and propensity. Some species are dedicated soil dwellers (e.g., *Gegeneophis ramaswamii*, Measey *et al.* 2003b, c) while others (e.g., species of *Ichthyophis*, Ramaswamii 1941; Nussbaum & Gans, 1980) have been perceived to be relatively more frequently found in and under rotting vegetation. Through laboratory experiments, Ducey *et al.* (1993) demonstrated differing burrowing ability among some caecilian species. Arguments about burrowing ability have also been partly based on differences in morphology, with more loosely constructed crania and less cylindrical bodies thought to be associated with less adept burrowing (e.g., Nussbaum 1977, 1979).

Given that our data reveal real ecological differences, how do they match with what else is known of the biology of the study species? Although caecilians are often superficially considered to be rather morphologically uniform, *B. boulengeri* and *S. vittatus* are markedly different animals (see Table 2; Loader *et al.* 2003a). *Boulengerula* have bullet-shaped heads and near-cylindrical bodies, with relatively less mobile and more solid skulls. The skulls lack upper temporal fossae (stegokrotaphy), and they have markedly reduced eyes that are covered by bone. All these features might be expected to confer performance advantage in a predominantly burrowing, endogeic lifestyle.

Scolecomorphus also have the orbit covered by bone but the eye remains relatively well developed (Wake 1985). The eye migrates forward from the orbit with the tentacle during ontogeny. In adult *Scolecomorphus*, the eye lies in an open tentacular groove covered not by bone but only a relatively pigment-free and translucent patch of skin, and it can even be protruded with the tentacles (Taylor 1968; Nussbaum 1985; O'Reilly *et al.* 1996). The comparative morphology of these species is consistent with the visual system being more important for *S. vittatus* than for *B. boulengeri*, and thus with the former spending more time on or near the surface. *Scolecomorphus vittatus* have a less uniform body than *B. boulengeri*, with a more narrow anterior body and a highly zygokrotaphic skull, both of which we interpret as less well suited for burrowing in compact soil. The hypothesis that the two species have different burrowing abilities can be tested experimentally.

The two species also differ substantially in their sensory tentacles. In *B. boulengeri*, the tentacle lies in a lateral position close to the eye, where the head is almost as wide as the body. It is small and cannot be strongly protruded. Such an arrangement seems adequate for life in soil where the substrate is close to the sides of the head. The more protrusible tentacles of *S. vittatus* are much closer to the snout tip and more ventrally oriented when protruded, and we suggest this may provide a performance advantage in accessing sensory cues on the surface and in litter.

Other notable differences in the biology of these two species include maximum length, reproductive mode (Wilkinson & Nussbaum 1998; Loader *et al.* 2003b) and colour. These are less obviously related to propensity and/or ability to spend more or less time within soil, but are consistent with different autecologies. The probability that *B. boulengeri* and *S. vittatus* have different niches, combined with differences in their dentition and probable cranial mobility (Table 2), prompts the hypothesis that their diets also differ.

An alternative explanation for our data is that they are biased or otherwise atypical due to sampling error. For example, it might be hypothesised that *S. vittatus* are better able to escape collection by digging or that they are more patchily distributed and occur in areas of soil not sampled, or that *B. boulengeri* are less conspicuous when on the surface, avoid or escape from pitfalls, and/or only emerge at times when casual visual encounter was less likely (e.g., at night). However, biases would have to be strong to account for the magnitude of the differences observed. Additionally our interpretation is consistent with other (unpublished) field observations, including the extremely rare collection (< 10) of *B. boulengeri* in pitfall traps or on the surface in other Frontier Tanzania surveys in the East Usambaras over eight years.

Taken at face value, the collection data suggest that *B. boulengeri* is more abundant than *S. vittatus* in Nilo FR (at least within the top 300 mm of soil), with nearly four times as many specimens being collected during this study. This is with the strong caveat that we do not know the relative efficiencies of the different habitat-specific sampling regimes. That the greater abundance of *B. boulengeri* applies elsewhere receives support from additional fieldwork in Amani Nature Reserve forest in the southern part of the East Usambara. During twelve days (February-March, 2000) of dedicated caecilian sampling by digging soil and searching through leaf litter and dead wood, 124 *B. boulengeri* (approximately 1.5 per person hour of digging) and one *S. vittatus* (approximately 0.1 per hour) were found (DJG,SPL, MW unpublished data). In surveys conducted by digging randomised quadrats, Measey (in press) also found *B. boulengeri* to be much more abundant than *S. vittatus* in both forest and agricultural habitats in the southern part of the East Usambaras.

Oommen *et al.* (2000) and Measey *et al.* (2003a, b, c) commented on the high abundance of the caeciliid *Gegeneophis ramaswamii* in agricultural habitats in India, reported that they appeared to be the most abundant vertebrate in these habitats, and suggested they may have an impact on soil ecology through predation of invertebrate groups considered to be soil ecosystem engineers. In this study, *B. boulengeri* was the most abundant vertebrate dug from the surface 300 mm of the soil, so that we expect this species to have a significant role in forest soil ecology in the East Usambaras.

Standard methods for sampling terrestrial caecilians have yet to become established (Measey *et al.* 2003a, b) and this is usually attributed to the apparent rarity of these animals (e.g., Lips *et al.* 2001). However, caecilians can be abundant in tropical soils, and this makes ecological hypotheses amenable to testing in some situations. *Scolecomorphus* and *Boulengerula* are not sympatric across the whole of their ranges. For example, only species of *Boulengerula* are known from Kenya, and only *Scolecomorphus* are known from the Pare Mountains of Tanzania. This mixture of sympatry and allopatry might be exploited to investigate the degree to which ecological differences result from interspecific competition.

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Note added in proof

Since this paper was accepted, two publications have appeared that provide evidence that brevicipitines (Darst and Cannatella 2003; Van der Meijden et al. 2004) and *Hemisus* (Darst and Cannatella 2003) are more closely related to hyperoliids and arthroleptids than to non-brevicipitine microhylids. Each study included a single brevicipitine.

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Phylogenetic relationships of African microhylid frogs inferred from DNA sequences of mitochondrial 12S and 16S rRNA genes

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Abstract

The phylogenetic relationships of microhylid frogs are poorly understood. The first molecular phylogeny for continental African microhylids is presented, including representatives of all subfamilies, six of the eight genera, and the enigmatic hemisotid *Hemisus*. Mitochondrial 12S and 16S rRNA sequence data were analysed using parsimony, likelihood and Bayesian methods. Analyses of the data are consistent with the monophyly of all sampled subfamilies and genera. *Hemisus* does not nest within either brevicipitines or non-brevicipitines. It is possibly the sister group to brevicipitines, in which case brevicipitines might not be microhylids. *Phrynomantis* and *Hoplophryne* potentially group with non-African, non-brevicipitine microhylids, in agreement with recent morphological and molecular data. Within brevicipitines, *Breviceps* is recovered as the sister group to a clade of *Callulina*+ *Spelaeophryne* + *Probreviceps*. The relationships among the genera within this latter clade are unclear, being sensitive to the method of analysis. Optimal trees suggest the *Probreviceps macrodactylus* subspecies complex might be paraphyletic with respect to *P. uluguruensis*, corroborating preliminary morphological studies indicating that *P. m. rungwensis* may be a distinct species. *P. m. loveridgei* may be paraphyletic with respect to *P. m. macrodactylus*, though this is not strongly supported. Some biogeographic hypotheses are examined in light of these findings.

Keywords: Microhylidae; Brevicipitinae; Melanobatrachinae; Phrynomerinae; *Hemisus*; Africa, Eastern Arc

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Introduction

Microhylids are a diverse group of subterranean, terrestrial and arboreal frogs occurring in northern Australasia, South and Southeast Asia, sub-Saharan Africa, Madagascar, and North and South America. The approximately 350 nominate species are classified in 64 genera and 10 subfamilies. This is the largest number of genera found in any amphibian family, comprising some 15% of all frog genera (Frost 2002). The status, composition, inter- and intrarelationships of Microhylidae have not been studied in detail, and the family remains in general poorly understood. Indeed, even the monophyly of Microhylidae is far from established (see below). In association with their ecological diversity, microhylids display great morphological variation, particularly in their cranial and pectoral girdle structure (Parker 1934; Carvalho 1954; Blommers-Schlösser 1993; Wu 1994). The inadequate state of microhylid systematics partly stems from the lack of comparative morphological studies. Blair (1962) suggested the use of non-traditional character systems for clarifying evolutionary relationships in frogs. More specifically, Largen and Drewes (1989) suggested molecular data would be useful for resolving relationships among African microhylids.

The suprageneric taxonomy of Microhylidae has barely changed since Parker's (1934) milestone monograph but, given the generally inadequate state of current knowledge, this is unlikely to prove stable. Currently, the eight African (excluding Madagascar) genera are divided into three subfamilies (Frost 2002). The African Brevicipitinae consists of twenty species in five genera. Three of these genera (*Probreviceps* Parker, *Callulina* Nieden, *Balebreviceps* Largen & Drewes) are found in evergreen forest, whereas the remainder (*Breviceps* Merrem, *Spelaeophryne* Ahl) are known to also inhabit some drier habitats. Among the moist forest genera, *Probreviceps* is the most speciose (3 species) and, except for the Zimbabwean *P. rhodesianus* Poynton & Broadley, is found principally in the mountain forests of Tanzania (Howell 1993). *P. macrodactylus* (Nieden) is subdivided into three subspecies (Parker 1934): *P. macrodactylus macrodactylus* (Nieden) from the Usambara, *P. macrodactylus loveridgei* Parker from the Uluguru and Udzungwa, and *P. macrodactlyus rungwensis* Loveridge from Rungwe and the Udzungwa. The latter two subspecies are sympatric in the Udzungwa Mountains, suggesting that they may be separate species. *Callulina* is also found throughout the Eastern Arc Mountains, and is known from *C. kreffti* Nieden and a new species from the West Usambaras (de Sá, Loader and Channing, unpublished). *Balebreviceps* is monotypic, with *B. hillmani* Largen & Drewes known from the Bale Mountains, Ethiopia (Largen and Drewes 1989). The only species of *Spelaeophryne*, *S. methneri* Ahl, is found in both low and highland areas of southeastern Tanzania, and *Breviceps* (15 species) is confined to eastern and southern Africa, being "concentrated in South Africa" (Poynton 1964; see also Channing 2001; Minter 2003). The Indo-African Melanobatrachinae comprises four species: *Melanobatrachus indicus* Beddome (Western Ghats, India), *Hoplophryne rogersi* Barbour & Loveridge (East Usambara, Tanzania), *H. uluguruensis* Barbour & Loveridge (Uluguru and Udzungwa, Tanzania), and *Parhoplophryne usambaricus* Barbour & Loveridge (East Usambara, Tanzania). These species all appear to be strictly confined to forests. The subfamily Phrynomerinae comprises five species of *Phrynomantis* Peters that have a wide distribution across savanna and woodland habitats in sub-Saharan Africa.

Based on morphology and behaviour, Blommers-Schlösser (1993) argued that brevicipitines are not microhylids, but actually belong with the enigmatic African taxon *Hemisus* Günther in the Hemisotidae. Wu's (1994) phylogenetic analysis of morphology also found support for brevicipitines being more closely related to *Hemisus* than to non-brevicipitine microhylids. The currently more orthodox view that brevicipitines are microhylids and only distantly related to *Hemisus* was summarised by Ford and Cannatella (1993). Recent studies of larval morphology (Haas 2003) and DNA sequence data (Biju and Bossuyt 2003; Vences et al. 2003) have reinforced the view that *Hemisus* is only distantly related to a monophyletic Microhylidae, but none of these studies sampled any brevicipitine taxa.

The limited ability of most amphibians to disperse across biogeographical barriers (e.g. the sea or arid habitats) has led some workers (e.g. Savage 1973; Duellman and Trueb 1994; Bossuyt and Milinkovitch 2001) to argue that the distribution of amphibians reflects changes in geology and geography at various scales, such as continental drift and orogenesis. The current distribution of microhylids has been interpreted as reflecting the break-up of Gondwana (Savage 1973). At a finer scale, the high species diversity and strong patterns of endemism in amphibians (including microhylids) of the Eastern Arc are thought to be intimately related to more recent geographic events (Fjeldå and Lovett 1993; Howell 1993).

In this paper, we present the first phylogenetic analysis of mitochondrial DNA sequence data for African microhylids, sampling all subfamilies and six of the eight genera found in continental Africa. We focus especially on brevicipitines. *Hemisus* is also included, in order to explore the relationship of this genus with microhylids. The results of phylogenetic analyses are compared briefly with some existing biogeographic hypotheses.

Material and methods

Samples

A total of 27 terminal taxa were used in this study (Table 1). Sequences for 23 terminal taxa were generated from newly collected material from Tanzania and Ivory Coast. These were supplemented by sequences for 4 species obtained from GenBank (Benson et al. 1998). Although microhylids are also distributed elsewhere in sub-Saharan Africa, collecting was concentrated in Tanzania because all but one genus (*Balebreviceps* from the Bale Mts, Ethiopia; Largen and Drewes 1989) of African microhylids occur there. All species known to occur in Tanzania are represented in this study by at least one specimen, except for *Parhoplophryne usambaricus* which is known from the single type specimen only (Barbour and Loveridge 1928). Beyond Tanzania, this study lacks intensive sampling of *Breviceps*, with only one of 15 species included. The sub-Saharan *Phrynomantis* is represented by two of the five known species. The only species of *Probreviceps* not included in this study is the Zimbabwean *P. rhodesianus*.

Four non-African microhylids were included, including representatives of at least two major lineages within the family, the exclusively Madagascan Scaphiophryninae (*Scaphiophryne* Boulenger) and the more cosmopolitan Microhylinae (*Microhyla* Tschudi, *Kaloula* Gray). All microhylid taxa for which 12S and 16S data are currently deposited in GenBank were included, with the exception of the Madagascan dyscophine *Dyscophus guineti* (Grandidier), for which the available data do not match the regions sequenced here and contain several ambiguities. In addition to microhylids, we included the East African *Hemisus marmoratus* Steindachner and West African *H. sudanensis* (Steindachner).

DNA extraction, amplification and sequencing

DNA was extracted from liver and/or thigh muscle preserved in aqueous 95% ethanol, and purified using phenol/chloroform extractions. The primers used in amplification and sequencing were 12Sa and 12Sb for the 12S rRNA gene (Kocher et al. 1989), and 16Sa and 16Sb for the 16S rRNA gene (Palumbi 1996). Successful polymerase chain reaction (PCR) gel bands were removed and purified. PCR products were sequenced using an ABI 377 automated sequencer (PE Biosystems, Warrington, UK), following the manufacturer's protocols. Each published sequence represents a consensus of both strands. GenBank accession numbers for sequences are given in Table 1.

Phylogenetic analysis

Sequences were aligned manually. Length differences were resolved by inserting alignment gaps, and positions that could not be aligned unambiguously were excluded. Parsimony and maximum likelihood (ML) analyses were performed with PAUP*4b6 (Swofford 1998); ML analyses used models recommended by Modeltest 3.04 (Posada and Crandall 1998), with empirical base frequencies. All analyses were heuristic, with 10 random addition sequence replicates and tree bisection recombination branch swapping. Zero length branches were suppressed. Bayesian analysis was performed using MrBayes (Huelsenbeck and Ronquist 2001) with a six substitution category model and empirical base frequencies. The Markov chain Monte Carlo search was run with four chains for 1,000,000 generations. The first 1000 generations were discarded as 'burn-in', and subsequent trees were sampled every 1000 generations.

Faith and Cranston's (1991) permutation tail probability (PTP) was determined with parsimony analyses of 99 randomisations of the data. Support for clades was measured with bootstrap proportions (Felsenstein 1985; 1000 pseudoreplicates), and decay indices (Bremer 1988) determined by enforcing converse topological constraints. The significance of length differences between most parsimonious and suboptimal trees found in constrained analyses was assessed using a non-parametric test (Templeton 1983). This test is only unbiased when comparing trees chosen a priori, i.e. not on the basis of their fit to the data. When trees are selected because of their maximal fit to the data, the tests are too liberal. Thus, we here accept the failure to reject the null hypothesis at face value, while rejection of the null hypothesis is interpreted more cautiously (see Wilkinson et al. 2003). Rate heterogeneity among taxa was investigated by performing relative rates tests using RRTree (Robinson-Rechavi and Huchon 2000).

We chose not to include a range of putative outgroups (e.g. ranids, hyperoliids, artholeptids, rhacophorids) for three main reasons. First, the monophyly of, and interrelationships among, many major groups of neobatrachian frogs are not well established (e.g. Ford and Cannatella 1993; Hay et al. 1995; Haas 2003) so that selection of specific outgroups would be somewhat arbitrary. Second, countering this by including a broad range of outgroups was resisted because, based on preliminary analyses, it increases ambiguity in the alignment and the potential for long-branch attraction. Third, previous studies (e.g. Hay et al. 1995; Wilkinson et al. 2003; Hertwig et al. 2004) suggest that 12S and 16S mitochondrial data alone are unlikely to provide a robust, well-resolved picture of higher relationships across such a wide range of amphibian families. Thus, we use unrooted trees to test previous hypotheses of

Table 1. Details of *Hemisus* and microhylid samples used in analyses

	Species	Voucher	Locality	GenBank accession no.
1	*Hemisus marmoratus* Steindachner	MW 1856	Sali FR, Mahenge Mts., Tanzania	AY531831, AY531854
2	*Hemisus sudanensis* (Steindachner)	MOR C00.1	Comoé National Park, Ivory Coast	AY531830, AY531853
3	*Phrynomantis microps* Peters	MOR C97.1	Comoé National Park, Ivory Coast	AY531832, AY531855
4	*Phrynomantis bifasciatus* (Smith)	MW 3842	Mkomazi Game Reserve, Tanzania	AY531833, AY531856
5	*Scaphiophryne brevis* (Boulenger)			AF 026357, AF 215384
6	*Scaphiophryne gottlebei* Busse & Böhme			AF 215144, AF 215385
7	*Hoplophryne uluguruensis* Barbour & Loveridge	KMH 22723	West Kilombero Scarp FR, Uzungwa Mts, Tanzania	AY531835, AY531858
8	*Hoplophryne rogersi* Barbour & Loveridge	KMH 23364	Nilo FR, East Usambara Mts. Tanzania	AY531834, AY531857
9	*Microhyla* cf. *ornata* (Duméril & Bibron)			AF 249003, AF 215371
10	*Kaloula taprobanica* Parker			AF 249004, AF 249057
11	*Breviceps mossambicus* Peters	MW 1826	Sali FR, Mahenge Mts., Tanzania	AY531836, AY531859
12	*Breviceps mossambicus* Peters	MW 1848	Sali FR, Mahenge Mts., Tanzania	AY531837, AY531860
13	*Spelaeophryne methneri* Ahl	KMH 21547	Uluguru Mountains, Milawilila FR, Tanzania	AY531838, AY531861
14	*Spelaeophryne methneri* Ahl	MW 1850	Sali FR, Mahenge Mts., Tanzania	AY531839, AY531862
15	*Callulina* n. sp.	MW 3215	Ambangula FR, West Usambara Mts, Tanzania	AY531841, AY531864
16	*Callulina* n. sp.	MW 1968	Mazumbai FR, West Usambara Mts, Tanzania	AY531840, AY531863
17	*Callulina kreffti* Nieden	KMH 23534	Nilo FR, East Usambara Mts., Tanzania	AY531842, AY531865
18	*Probreviceps m. rungwensis* Loveridge	KMH 19141	West Kilombero Scarp FR, Uzungwa Mts, Tanzania	AY531843, AY531866
19	*Probreviceps m. rungwensis* Loveridge	KMH 18974	Ndundulu FR, Uzungwa Mts., Tanzania	AY531844, AY531867
20	*Probreviceps uluguruensis* (Loveridge)	KMH 21570	Uluguru South FR, Uluguru Mts., Tanzania	AY531845, AY531868
21	*Probreviceps uluguruensis* (Loveridge)	KMH 21577	Uluguru South FR, Uluguru Mts., Tanzania	AY531846, AY531869
22	*Probreviceps m. loveridgei* Parker	KMH 21461	Mkungwe FR, Uluguru Mts., Tanzania	AY531847, AY531870
23	*Probreviceps m. loveridgei* Parker	KMH 21532	Kasanga FR, Uluguru, Tanzania	AY531848, AY531871
24	*Probreviceps m. loveridgei* Parker	KMH 22702	West Kilombero Scarp FR, Uzungwa Mts., Tanzania	AY531849, AY531872
25	*Probreviceps m. loveridgei* Parker	KMH 22067	West Kilombero Scarp FR, Uzungwa Mts., Tanzania	AY531850, AY531873
26	*Probreviceps m. macrodactylus* (Nieden)	KMH 16360	Amani NR, East Usambara Mts, Tanzania	AY531851, AY531874
27	*Probreviceps m. macrodactylus* (Nieden)	KMH 21399	Nilo FR, East Usambara Mts., Tanzania	AY531852, AY531875

Vouchers were identified through comparisons with published descriptions (Barbour and Loveridge 1928; Parker 1934; Laurent 1972; Poynton and Broadley 1985; Rödel 2000) and paratype material held in the Natural History Museum, London. Voucher specimens are stored in the Zoology department of the Natural History Museum, London (KMH and MW field series) and M.-O. Rödel's research collection (MOR) deposited in the Staatliches Museum für Naturkunde Stuttgart and the Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn. FR = Forest Reserve, NR = Nature Reserve, m = macrodactylus.

monophyly and sister-group relationships, and we explore the implications of alternative rootings.

Results

A total of 760 aligned sites were analysed, of which 479 were constant, 44 variable but parsimony uninformative, and 237 parsimony informative. The data have a parsimony PTP of 0.01, allowing rejection of the null hypothesis that they contain no more hierarchical structure than expected by chance alone. Relative rates tests indicated that *Spelaeophryne methneri*, *Hemisus marmoratus*, and *Breviceps mossambicus* evolved more rapidly than the other taxa ($p = 0.04$). There is no significant base composition bias for any taxon, whether or not uninformative sites are considered. Plots of transitions vs. transversions (not shown) suggest that saturation is not a problem with these data.

Parsimony analysis yielded three most parsimonious trees (MPTs), which differed only in the position of the two Uluguru samples of *Probreviceps macrodactylus loveridgei* (Fig. 1). The ML analysis used the GTR + I + G model (as recommended by both criteria used in Modeltest). The optimal ML tree (Fig. 2) is similar to the MPTs. Most relationships common to parsimony and ML trees are well supported as judged by bootstrap proportions and decay indices (Fig. 1). Bayesian posterior probabilities are high (>0.87), perhaps unreasonably so, for all splits in the optimal ML tree (Fig. 2), including for relationships not found in the MPTs. A minority of the investigated splits were not significantly better supported than alternatives, as judged by Templeton tests (Fig. 1).

Discussion

The unrooted optimal trees recovered by parsimony and ML (Figs. 1 and 2) are consistent with the monophyly of all previously recognised genera, subfamilies (except Microhylinae) and families, in that the trees can be rooted such that all these taxa are clades. The ML tree can be rooted such that Microhylinae (*Microhyla* + *Kaloula*) is a clade, but the corresponding split has a low posterior probability and is not recovered in the MPTs, which allow for this clade only as one of the possible resolutions of a polytomy. With the exception of the Brevicipitinae, bootstrap proportions for the splits corresponding to the other supraspecific taxa are high (>95%).

Higher relationships

Despite uncertainty over the position of the root, we are able to examine relationships among four main groups: Brevicipitinae (B), *Hemisus* (H), Scaphiophryninae (S), and the remaining, paraphyletic non-brevicipitine, non-scaphiophrynine microhylids (N). Ford and Cannatella (1993) defined Scoptanura as non-scaphiophrynine microhylids, including brevicipitines. Our optimal trees are inconsistent with the Scoptanura hypothesis (H, S (B, N)). Templeton tests ($p < 0.031$) do not require us to attribute the difference (16 steps) between our MPTs and the best trees consistent with Scoptanura monophyly to random sampling error. The same is also true ($p < 0.02$) for the alternative hypothesis (H, N (B, S)). Assuming that brevicipitines are monophyletic (see below) and that *Hemisus* is monophyletic, our data suggest that the Brevicipitinae is the sister group to *Hemisus*, to a clade containing all non-brevicipitine microhylids sampled here, or to a clade including both these groups.

Given that *Hemisus* is only distantly related to non-brevicipitine microhylids (Biju and Bossuyt 2003; Haas 2003; Vences et al. 2003), the implication is that if brevicipitines are the sister group to *Hemisus*, then they are not microhylids. Support for the resolution ((S, N)(H, B)) comes from Blommers-Schlösser's (1993) and Wu's (1994) phylogenetic analyses of morphology. These tentative insights point to a need for a major revision of microhylid classification. Additional taxon sampling and data from other (probably nuclear) genes and/or from more morphological systems will be needed to further resolve phylogenetic relationships before this can be undertaken with confidence.

Non-brevicipitine microhylids

The non-brevicipitine microhylids sampled here were recovered as a putative clade in all analyses. The bootstrap proportion, decay index, and posterior probability for this group are high, and Templeton tests ($p > 0.0339$) do not compel us to attribute this support to sampling error (Figs. 1 and 2). The position of *Hoplophryne* Barbour & Loveridge within a putative clade comprising a mixture of widely geographically distributed, non-brevicipitine microhylids is uncontroversial. The similar nesting of *Phrynomantis* is supported by detailed studies of morphology (Laurent 1941; Haas 2003). Noble (1931) placed *Phrynomantis* in its own subfamily, not closely allied to any other microhylids. Parker (1934) excluded *Phrynomantis* from Microhylidae based on the presence of intercalary cartilages, a character now known to be present in other microhylids as well (Wu 1994). Data from larval morphology strongly support the nesting of *Phrynomantis* within a clade of non-scaphiophrynine microhylids (Haas 2003).

Savage (1973) speculated that the three extant African microhylid subfamilies (Brevicipitinae, Melanobatrachinae, Phrynomerinae) diversified prior to Gondwana

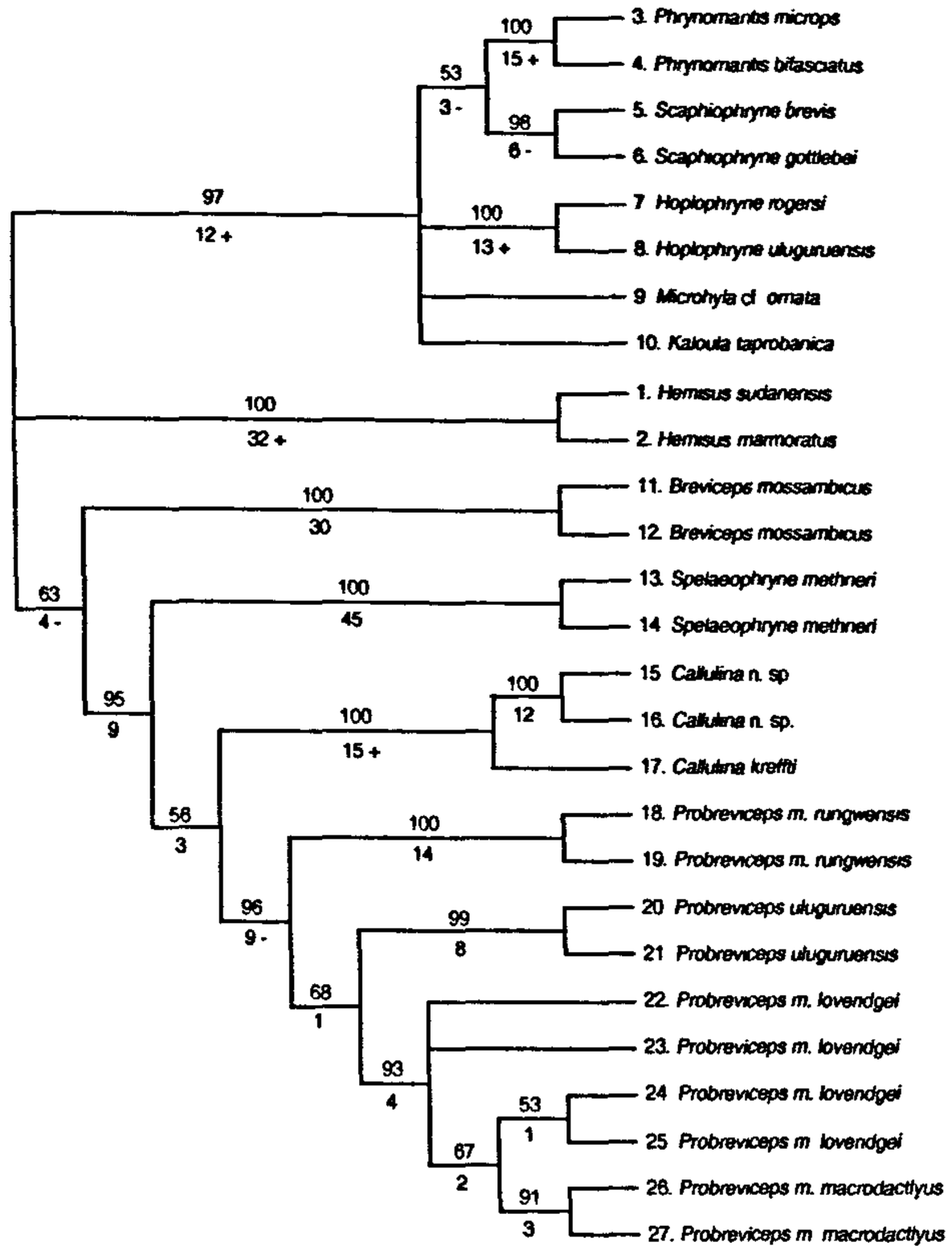

Fig. 1. Strict consensus of three unrooted most parsimonous trees (MPTs). Descriptive statistics (with all characters/without uninformative characters): tree length = 677/629 steps, CI = 0.5746/0 5421, RI = 0.7732/0.7732. Numbers above branches are bootstrap proportions. Numbers below internal branches are decay indices, symbols following the decay index values show the results of Templeton tests for 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 is indicated for previously hypothesised supraspecific taxa. m = *macrodactylus*.

fragmentation. In contrast, Duellman and Trueb (1994, p. 489) argued that a brevicipitine–phrynomerine lineage diversified only after Gondwana fragmentation. We reject Duellman and Trueb's hypothesis, because there is no rooting of our optimal trees in which *Phrynomantis* and brevicipitines form a clade. We are not compelled to attribute the difference (16 steps) between our MPTs and the best trees in which *Phrynomantis* and brevicipitines are a potential clade to sampling error (Templeton test, $p < 0.02$).

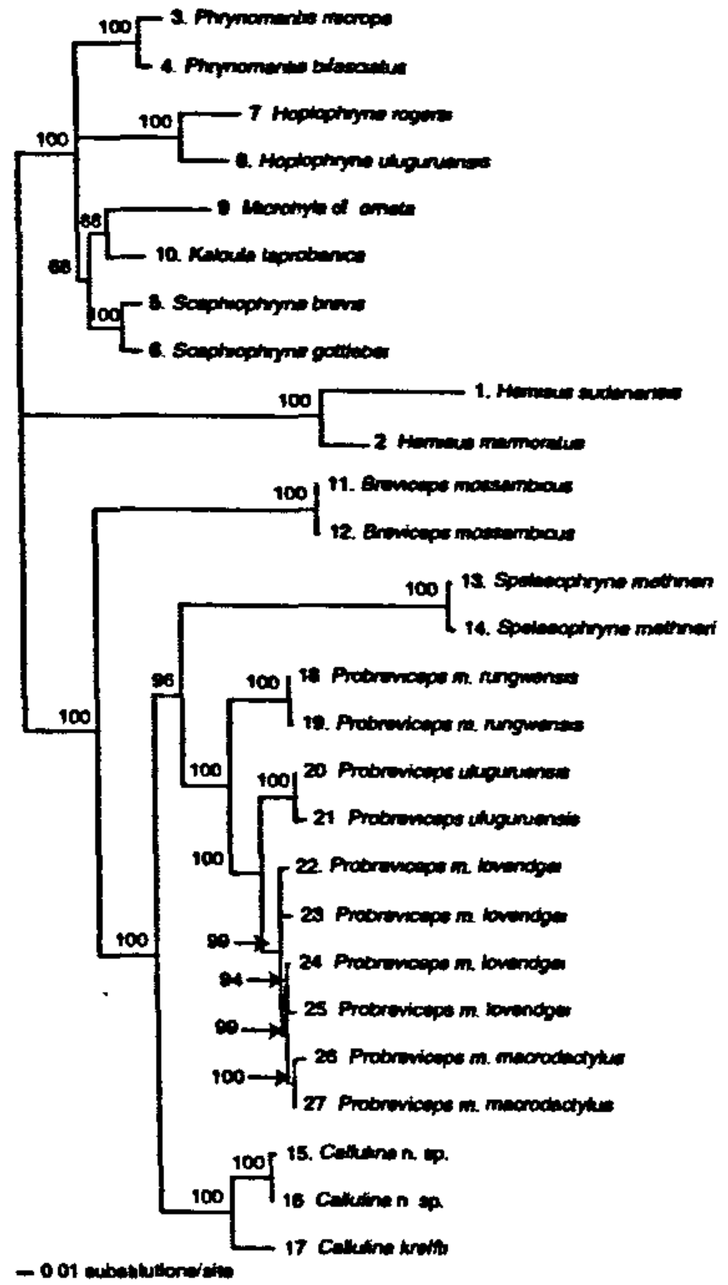

Fig. 2. Maximum likelihood phylogram (unrooted), showing branch lengths (−ln likelihood = 4275 79863, proportion of invariant sites = 0.3491, gamma shape parameter = 0.4952); support values above nodes are Bayesian posterior probabilities.

Brevicipitines

Whether trees are rooted with *Hemisus* or any of the non-brevicipitine microhylids sampled, the data presented in this paper support the monophyly of Brevicipitinae. Quantitative support for this node is not compelling (Figs. 1 and 2), although it is further corroborated by morphological evidence (Parker 1934; Blommers-Schlösser 1993; Wu 1994) and is accepted here. Parker (1934) commented on the special nature of the brevicipitine vomer (prevomer in Parker's usage) which is reduced posteriorly (post-choanally) but bearing a large anterior and medial expansion. Parker also noted other characters (e g. retention of a complete shoulder girdle) that readily distinguished brevicipitines from all other microhylids, but further work is required to determine derived and plesiomorphic conditions.

Phylogenetic relationships of the genera within Brevicipitinae have been briefly explored by Poynton (1964, 1999), Poynton and Pritchard (1976), Largen and Drewes (1989), and Wu (1994). As their genus names suggest, *Probreviceps* and *Breviceps* have been thought to be closely related, and Poynton (1999, p. 515) proposed that *Breviceps* "can be derived from sylvicolous East African *Probreviceps*". This was based on the observation of clinal variation in the lengths of limbs and digits along the continuous North to South distribution of the two genera (Poynton and Pritchard 1976). *Probreviceps* from Tanzania have the longest limbs and toes, followed by *P. rhodesianus* (further South, in Zimbabwe), then *Breviceps* (which occurs further southwards) with the shortest. In contrast, Wu (1994) hypothesised that *Callulina* and *Probreviceps* comprise a clade, with successive sister groups formed by a paraphyletic *Breviceps*, and *Spelaeophryne*. Focussing on pectoral girdle morphology, Largen and Drewes (1989) questioned the monophyly of *Probreviceps* + *Breviceps* by suggesting that *Probreviceps* is more closely related to *Balebreviceps* (not included in our analyses). Our analyses strongly exclude *Breviceps* from a clade comprising *Probreviceps*, *Callulina* and *Spelaeophryne*. Judged by the Templeton test ($p < 0.03$), it is unnecessary to attribute the difference (14 steps) between our MPTs and the best trees containing a *Probreviceps* + *Breviceps* clade to random sampling error. Despite this, the optimal trees recovered in our analyses (Figs. 1 and 2) do not preclude the possibility that *Breviceps* evolved from a *Probreviceps*-like ancestor, as in Poynton's hypothesis.

Bootstrap support for the *Spelaeophryne* + *Callulina* + *Probreviceps* clade, and for the monophyly of the constituent genera, is high in all analyses, although the best trees in which *Probreviceps* is constrained to be non-monophyletic do not have a significantly worse fit to the data (Fig. 1). The relationships among these three genera are not clearly resolved by our data, although no analyses recovered one of the three possible resolutions, i.e. the pairing of *Callulina* + *Spelaeophryne*. Currently, morphological data that might provide decisive support for one of the two competing hypotheses (in the optimal parsimony and ML trees) are lacking. The conflict and lack of resolution might be caused by heterogeneous rates of molecular evolution (i.e. *Spelaeophryne* relative to other brevicipitines), inadequate taxon sampling (*Balebreviceps hillmani*; additional species of *Breviceps*), or simply too few sequence data.

The referral of a new species to *Callulina* based on morphology (de Sá, Loader & Channing, unpublished) is strongly supported by our molecular analyses. The

status of the *Probreviceps macrodactylus* complex has not been investigated previously in a phylogenetic context. Our analyses suggest (Figs. 1 and 2) that *P. macrodactylus* is paraphyletic with respect to *P. uluguruensis*, but this is poorly supported as judged by the Templeton test ($p > 0.21$), bootstrap proportion and decay index values (Fig. 1). *Probreviceps macrodactylus rungwensis* can be distinguished from other *Probreviceps* by its large tympanum and notably pointed snout (J. C. Poynton, pers. comm.), and it perhaps represents a distinct species. We sampled *P. m. rungwensis* from the Udzungwa only, so that future sampling of this taxon from its type locality of Rungwe (part of the Southern Highlands rather than the Eastern Arc) is recommended, particularly in light of the apparently significant biogeographical barrier (the 'Makambo Gap', e.g. Keilland 1990; Lovett 1990; Gravlund 2002) between these regions. Limited morphological studies on *P. m. macrodactylus* and *P. m. loveridgei* (Parker 1934; Poynton, unpublished) and our molecular data suggest that there are very few differences between these subspecies, and the molecular data suggest that the latter may be paraphyletic with respect to the former (Figs. 1 and 2).

Tanzanian *Probreviceps* are confined to upland evergreen forest of the isolated constituent blocks of the Eastern Arc Mountains and Southern Highlands (e.g. Howell 1993). Taken at face value, the optimal phylogenies recovered in our analyses (Figs. 1 and 2) suggest that divergence of lineages giving rise to extant Udzungwa and Uluguru *Probreviceps* has occurred at least twice. The combined distributional and phylogenetic evidence does not fit with a simple, single vicariance/dispersal event, but is seemingly in accordance with the hypothesis that climatic fluctuations have repeatedly isolated (and reconnected) Eastern Arc montane forests over the last 2.8 Myr and driven speciation (e.g. see Roy 1997, and references therein). However, we stress that the relationships on which this is based are not well supported.

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Note added in proof

Since this paper was accepted, two publications have appeared that provide evidence that brevicipitines (Darst and Cannatella 2003; Van der Meijden et al. 2004) and *Hemisus* (Darst and Cannatella 2003) are more closely related to hyperoliids and arthroleptids than to non-brevicipitine microhylids. Each study included a single brevicipitine.

Darst, C.R., Cannatella, D.C., 2003. Novel relationships among hyloid frogs inferred from 12S and 16S mitochondrial DNA sequences. Mol Phyl. Evol. 31, 462–475.

Van der Meijden, A., Vences, M., Meyer, A , 2004. Novel phylogenetic relationships of the enigmatic brevicipitine and scaphiophrynine toads as revealed by sequences from the nuclear Rag-1 gene Proc. Roy. Soc. Lond B (Suppl.) 271, S378–S381.

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Five new species of *Nectophrynoides* Noble 1926 (Amphibia Anura Bufonidae) from the Eastern Arc Mountains, Tanzania

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Five new species of *Nectophrynoides* Noble 1926 are described from the forests of the Eastern Arc Mountains of Tanzania. Two of the new species were already recognised as such, but not formally described. Two more were recognised as undescribed in the collection of the Natural History Museum, London. The fifth was collected during a field survey of the Udzungwa Scarp Forest Reserve in early 2003. A description of the advertisement call and some ecological information are provided for the latter species. Little is known of the ecology and behaviour of any of the other new species. An updated key to the species of *Nectophrynoides* is given. The high species diversity and pattern of endemism of *Nectophrynoides* suggests the genus is closely associated with the geological and climatic history of the Eastern Arc Mountains. Given the limited distribution of the new species and continuing habitat loss in the Eastern Arc Mountains (a globally recognised biodiversity hotspot), a rapid conservation assessment of the status of these species is necessary.

KEY WORDS: Bufonidae, *Nectophrynoides*, Tanzania, Eastern Arc, distribution, biogeography, endemics, conservation, new species.

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INTRODUCTION

The first species of *Nectophrynoides* was described by ROUX (1906) as *Nectophryne tornieri*, but subsequent work by NOBLE (1926) described this species as forming the new East African genus *Nectophrynoides*. NOBLE (1926) also tentatively included *Pseudophryne vivipara* Tornier 1905 in the genus (now recognised as *N. viviparus*). Further additions to the genus included two species from Ethiopia, *N. osgoodi* (formerly *Bufo osgoodi* Loveridge 1932) and *N. malcolmi* (Grandison 1978). *Nectophrynoides* underwent further modifications by DUBOIS (1987), who restricted the genus to species only found in Tanzania, based on their mode of reproduction. The Ethiopian species were included in two new genera *Altiphrynoides* and *Spinophrynoides* (DUBOIS 1987). A further four species were described from Tanzania (PERRET 1971, 1972; CLARKE 1988; POYNTON et al. 1998), and eight species of *Nectophrynoides* are currently recognised (POYNTON 1998). The genus is mainly restricted to the forested areas of the Eastern Arc Mountains of Tanzania, with the exception of one species, *N. viviparus*, which also occurs on the Poroto and Rungwe Mountains in Tanzania. *Nectophrynoides* species seem to be forest associated (HOWELL 1993), occurring on submontane and montane forest patches along the Eastern Arc. However, they are absent in the Taita Hills, Pare Mountains, Nguu Mountains and Malundwe Hill, though these areas are poorly explored. The genus is considered an ancient Afrotemperate relict, as recognised by POYNTON et al. (1998) and POYNTON (2003).

Here we describe five new species of *Nectophrynoides* and highlight the conservation importance of the forest fauna inhabiting the Eastern Arc Mountains. Two of the new species were already identified as such but without formal description (POYNTON et al. 1998). Another two species were recently found in the herpetological collection of the Natural History Museum, London. The fifth was collected during a herpetological survey in the Udzungwa Scarp Forest Reserve in early 2003.

MATERIALS AND METHODS

The following measurements were taken to the nearest 0.1 mm with a calliper under a dissecting microscope: snout urostyle length (SUL), tibia length folded (TL), foot length from the proximal end of the metatarsal tubercle to tip of fourth toe (FL), metatarsus length (ML), forearm length (FOL), head width at the angle of the jaw (HW), head length from the angle of the jaw to tip of the snout (HL) and distance between nostrils (ND).

Following ELIAS & SHAPIRO (1957), the term coni is used to describe the very small conical warts, visible only under strong magnification, covering the entire surface of the skin. Specimens were dissected to ascertain sex, reproductive status, and to verify the absence of the tympanum and middle ear.

A Sharp minidisc recorder MD-MT 877H and a Sony C-76 directional microphone were used to record the male voice. The sound analysis was carried out on an Apple Macintosh G4 personal computer. Canary 1.2.4 (Cornell Bioacoustic Workstation) software was used for the measurement of temporal and spectral parameters of the call.

The signal was sampled at 44.100 Hz and 16 bit resolution. The call variables analysed were pulse duration, inter-pulse duration, fundamental frequency and dominant frequency. For each parameter the mean and standard deviation were calculated. Bioacoustic terminology follows LITTLEJOHN (2001). A Garmin e-map GPS was used to obtain the coordinates of specimens collected in the Udzungwa Mountains.

Abbreviations of institutions and field tags are as follows: BMNH (Natural History Museum, London, UK); MCZ (Museum of Comparative Zoology, Harvard, USA); MTSN (Museo Tridentino di Scienze Naturali, Trento, Italy); ZMUC (Zoological Museum, University of Copenhagen, Denmark); CAM (Charles Andekia Msuya field tags); KMH (Kim M. Howell field tags); MW (Mark Wilkinson field tags); WTS (William T. Stanley field tags).

The complete list of specimens examined is reported in Appendix 2.

Nectophrynoides vestergaardi n. sp. (Figs 1a, 2a, 3a)

Nectophrynoides sp. from W. Usambara (POYNTON et al. 1998).

Holotype. An adult female in the Natural History Museum, London, BMNH 1982.509 (KMH 1926), collected in 1981 in Shume Magamba Forest Reserve by Kim M. Howell and Simon Stuart.

Paratypes. BMNH1982.510, a female with embryos. BMNH1982.512, BMNH1982.516 and BMNH1982.517 are adult females with eggs at different stages of development. BMNH1982.511, BMNH1982.514 and BMNH1982.515 are males. BMNH1982.516 is a juvenile. All specimens were collected at the same locality, with the same collection data as the holotype.

Referred material. BMNH 1982.499 and BMNH 1982.500 from Shume Magamba Forest Reserve. MW 03211 from Ambangula Forest Reserve (cleared and stained), BMNH 1982.501 from Mazumbai Forest Reserve. ZMUC-R131228; ZMUC-R131229; ZMUC-R131230; ZMUC-R131231; ZMUC-R131233; ZMUC-R131234; ZMUC-R131235; ZMUC-R131236; ZMUC-R131240; ZMUC-R131241 from Mazumbai Forest Reserve. All sites are in the West Usambara Mountains.

Type locality. Shume Magamba Forest Reserve, at 1800 m above sea level, West Usambara Mountains, Tanga Region, north eastern Tanzania (4°66'S, 38°25'E).

Diagnosis. A medium sized *Nectophrynoides* with slender limbs. Tips of fingers and toes rounded to pointed, not expanded or truncated. Length of foot greater than or equal to length of tibia. Tympanum present and clearly visible. Fingers only webbed at base. Two phalanges of fifth toe free of main webbing, three and half of fourth toe free of main webbing on outer side and four free on inner side. Parotoid

glands present as a discrete raised elongated ridge from the angle formed by the squamosal and prootic to the scapular region.

In preserved specimens, dorsal ground colour light brown, darker on sides. A light maxillary patch usually present and may be conspicuous. Outer edge of parotoid glands darkened. A clearly raised glandular ridge on outer margin of eyelids is discernible. Almost always a fine dark mid dorsal vertebral line from snout to urostyle is present. The tibia/foot ratio in the type series varies between 0.89 to 1.00 (mean 0.93 ± 0.04) (see Table 1).

Comparison with other species in the genus. Resembling *N. tornieri* (Roux 1906) in size and body shape but easily distinguished from it by the rounded fingers and toes without truncated ends. The presence of an elongated and continuous parotoid gland from otic to scapular region, the larger size and the foot length equal or greater than tibia length, allows *N. vestergaardi* to be distinguished from *N. minutus* Perret 1972.

N. vestergaardi differs from *N. asperginis* Poynton et al. 1998, *N. laevis* n. sp. *N. pseudotornieri* n. sp. and *N. wendyae* Clarke 1988 by the presence of a tympanum. *N. cryptus* Perret 1971 and *N. frontierei* n. sp. sometimes possess a reduced,

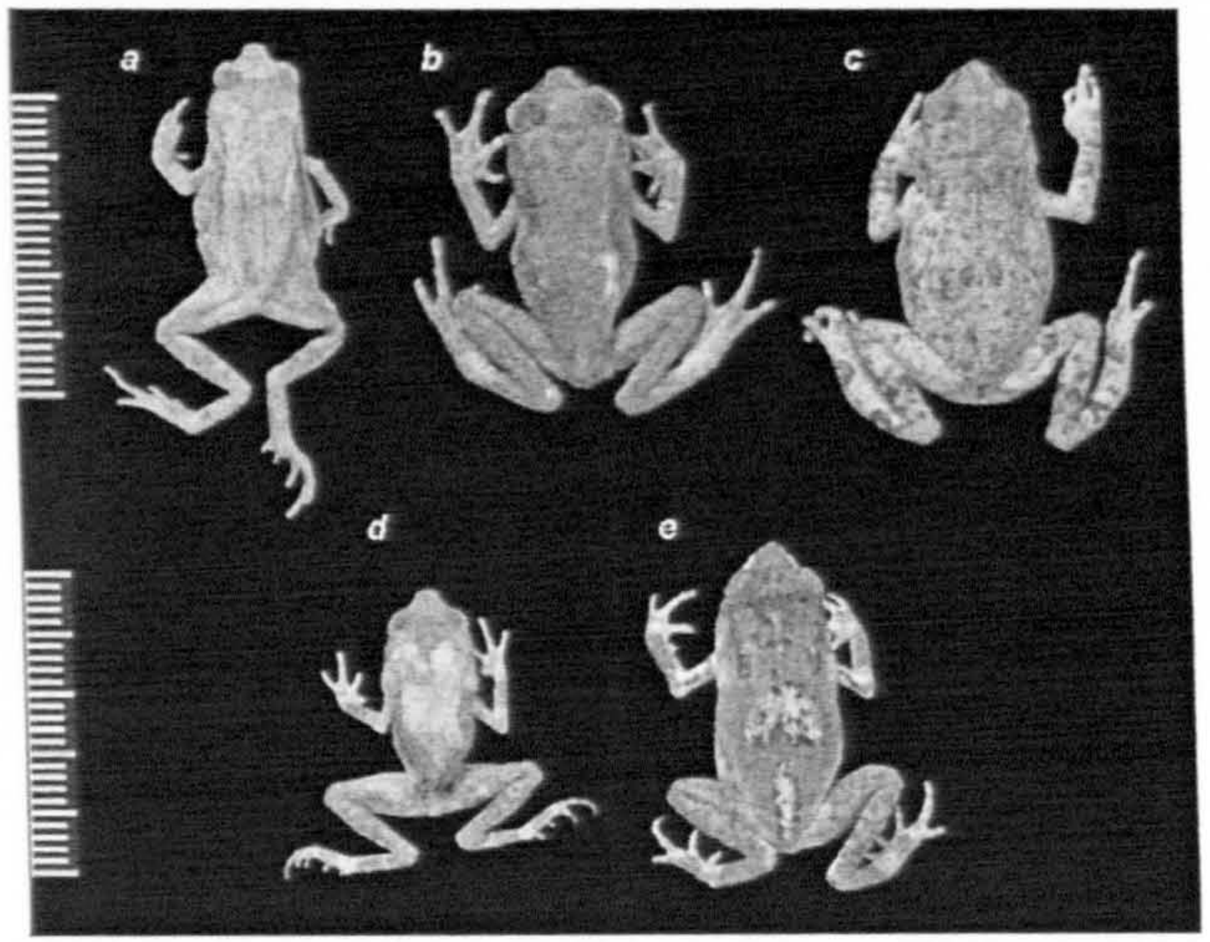

Fig. 1. — Dorsal aspects of the holotypes of (a) *N. vestergaardi*; (b) *N. pseudotornieri*; (c) *N. laevis*; (d) *N. frontierei* and (e) *N. poyntoni*. Scale bars = 25 mm.

weakly discernible tympanum, *N. vestergaardi* is distinguished from them by the presence of narrow and elongated parotoid glands (scapular in *N. cryptus*, absent in *N. frontierei*). Readily distinguished from *N. viviparus* (Tornier 1905) by the absence of massive glands on the limbs and by the smaller size. The species resembles *N. poyntoni* n. sp. in size, body and head shape, and in rounded tips of fingers and toes. However, it differs in both fore and hindlimb proportions (limbs are significantly shorter in *N. vestergaardi*, Table 1), in parotoid glands shape (forming a continuous ridge from otic to scapular region in *N. vestergaardi* whereas in *N. poyntoni* the parotoids are discontinuous) and in dorsal and lateral colour pattern (pale dorsum with darker sides and a thin vertebral line in *N. vestergaardi*).

Description. Holotype: BMNH 1982.509, an adult female containing embryos at about Gosner stage 44. Distance from tip of snout to urostyle 24.0 mm, width of

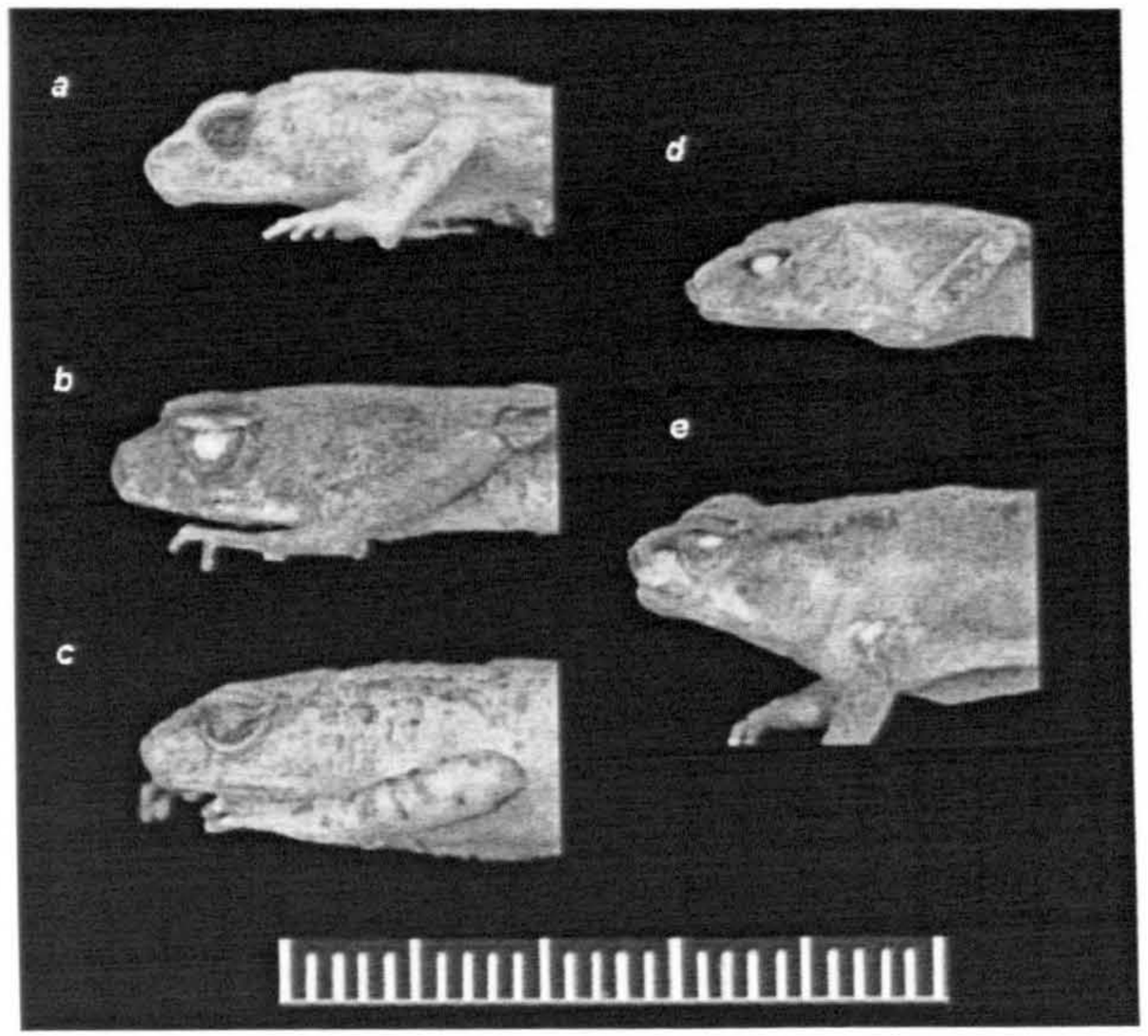

Fig. 2. — Aspects of the side of the head of the holotypes of (a) *N. vestergaardi*; (b) *N. pseudotornieri*; (c) *N. laevis*; (d) *N. frontierei* and (e) *N. poyntoni*. Scale bar = 25 mm.

head at jaw articulation 9.1 mm, length of tibia 9.9 mm, length of foot 10.4 mm. Tympanum and tympanic annulus easily discernible. Parotoid glands present as a discrete raised elongated mass from the angle formed by the squamosal and prootic to the scapular region.

Snout short, nostrils closer to the snout tip than eye, situated laterally, *canthus rostralis* slightly concave. Eye pupil horizontal, eyes prominent and visible ventrally. Trace of web on hands, two phalanges of fifth toe free of main webbing, three and half of fourth toe free on outer side, four free on inner side. Tips of fingers and toes rounded, not expanded or truncated. Two subequal metatarsal tubercles on feet. The body skin is completely covered by coni with scattered glandular humps surmounted by small clear spines on eyelids, tympanic area, body sides and limbs, but almost absent from body dorsum and head. The tibia/foot ratio in the holotype is 0.88 (Table 1).

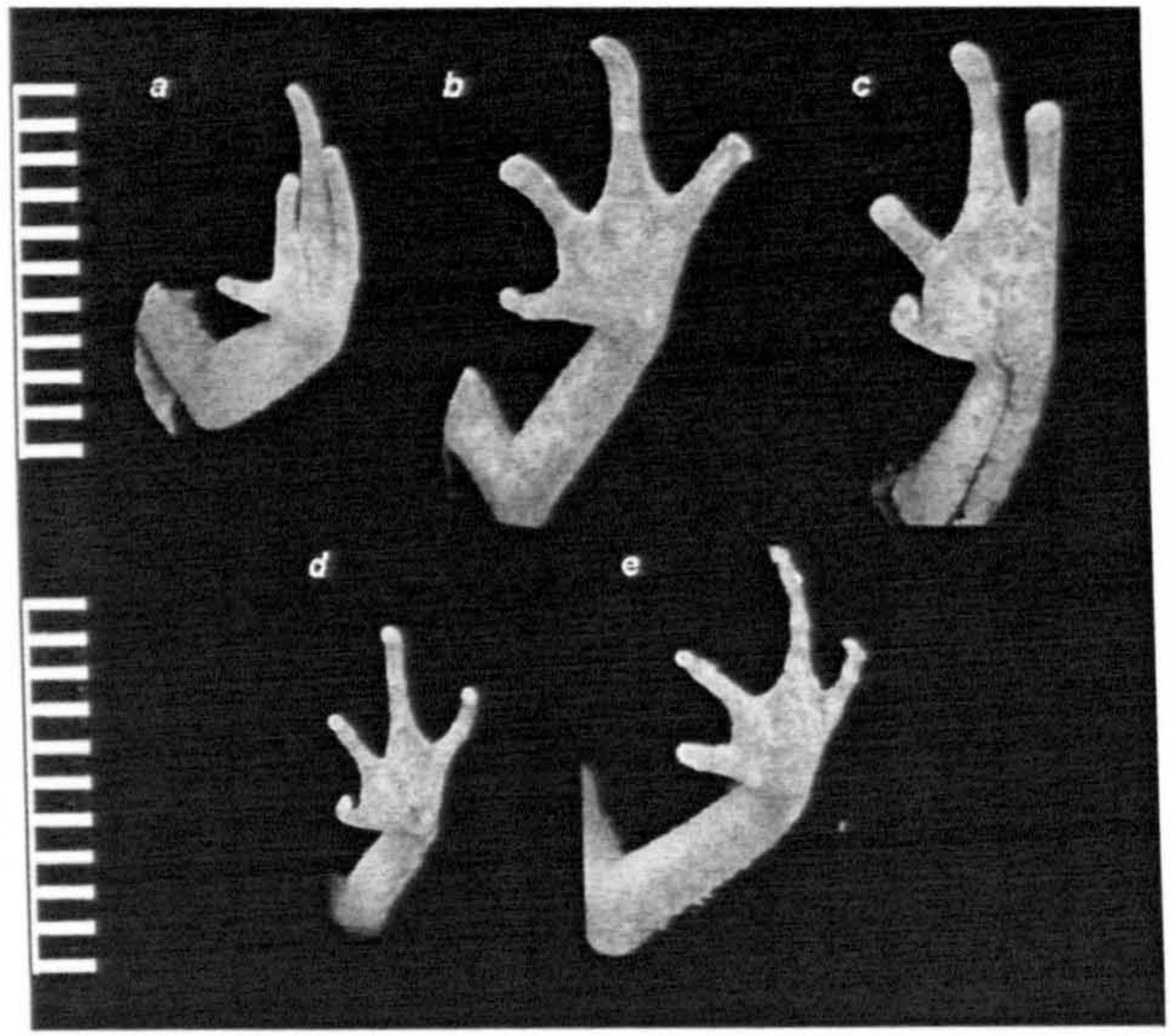

Fig. 3. — Aspects of the hand of the holotype of (a) *N. vestergaardi*; (b) *N. pseudotornieri*; (c) *N. laevis*; (d) *N. frontierei* and (e) *N. poyntoni*. Scale bars = 10 mm.

Colour pattern. In preservative the body appears always bicoloured, with sides darker than dorsum. The pale brown colour appearance is the result of a mixture of a very pale surface together with very small condensations of melanophores. A black thin vertebral stripe runs from the tip of the snout to urostyle. A light maxillary patch is present as a darker area on the ventral side of *canthus rostralis*, parotoid glands and on the tympanic region. The ventral region is a slightly translucent pale cream colour. The chin area is cream. There are a few sparse condensations of melanophores on limbs and dorsum.

Variation in the paratypes. There is little variation in the dorsal pattern. No evident differences between sexes, no evident nuptial pads or significant asperities on first and second fingers of males. The dorsal skin varies from smooth, as in BMNH 1982.510, to apparently rough and covered by conical glands, as in BMNH 1982.511.

A brown, interrupted stripe running from snout tip to the end of scapular parotoids is present. The contrast between the colour of the dorsum and the sides is more evident in: BMNH 1982.510, BMNH 1982.511, BMNH 1982.512, BMNH 1982.513 and BMNH 1982.515. In BMNH 1982.511, BMNH 1982.513 and BMNH 1982.515 a further darker area located in the inguinal area and in the inner part of the knee give a bicoloured appearance to the inner side of the thigh and tibia. The vertebral line is weakly discernible in BMNH 1982.511.

The tibia/foot ratio in the paratype series ranges from 0.88 to 1.0 (mean 0.93 ± 0.04) (see Table 1).

Advertisement call. Not recorded.

Sexual dimorphism. Except for the larger size of females, no apparent secondary sexual characters were observed.

Etymology. The species is named in honour of Martin Vestergaard, a Danish zoologist, who first recognised the population as being an undescribed species.

Habitat and life notes. The type series was collected in montane forest dominated by *Ocotea usambarensis* and *Podocarpus* sp. (S. STUART pers. comm.). The specimens from Mazumbai Forest Reserve were collected during the day at 1750 m in an upper montane forest, "at an altitude where the forest becomes lower and thinner as it gradually turns into ericaceous zone at Mount Sagara" (VESTERGAARD 1994). One specimen from Ambangula FR was found at 1230 m in submontane forest. Little is known of the breeding biology of this species, the presence of a small number (18) of large developed embryos in females of this species implies ovoviviparity.

Table 1.

Summary (mean ± standard deviation) of morphometric diagnostic ratios between *N. poyntoni* and *N. vestergaardi*.

Ratio	*N. poyntoni* (n = 7)	*N. vestergaardi* (n = 9)	Mann-Whitney test
SUL/TL	2.2452 ± 0 0835	2.5445 ± 0.1265	W = 28 0 *P* = 0.0010
SUL/FOL	3.4063 ± 0.2538	3.8557 ± 0.2671	W = 33.0 *P* = 0.0059
SUL/ML	3.2059 ± 0.2498	3.5879 ± 0.2829	W = 36.5 *P* = 0 01
TL/FL	1.1130 ± 0.0222	0.9348 ± 0.0422	W = 91.0 *P* = 0 0010

Nectophrynoides pseudotornieri n. sp. (Figs 1b, 2b, 3b)

Nectophrynoides sp. from North Uluguru (POYNTON et al. 1998).

Holotype. An adult male in the Natural History Museum, London, BMNH 2000.229 (KMH 21477), collected in the Uluguru North Forest Reserve at 1080 m in August 2000 by Nike Doggart.

Paratype. An adult female in the Natural History Museum, London, BMNH 2000.230 (CAM 716), collected in Uluguru North Forest Reserve in locality "camp 1" located 3 km West and 1.3 km North of Tegetero Village (06°55'45"S, 37°42'20"E) at about 1345 m, 30th July 1996 by Kim M. Howell and Charles A. Msuya.

Type locality. Uluguru North Forest Reserve, at 1080 m, Uluguru Mountains, Morogoro Region, eastern Tanzania (06°52'40"S, 37°55'00"E).

Diagnosis. A medium sized *Nectophrynoides* with slender limbs. The SUL/hindlimb ratio is 1.12. Tips of fingers expanded and slightly truncated, tips of toes rounded. Foot length greater than tibia length. Tympanum absent. Fingers widely webbed at base with a rib of webbing reaching the second tubercle of the third finger. Main webbing on feet reaching the second tubercle of third, fourth and fifth toe. Parotoid glands present in the scapular region as a slightly raised and weakly discernible mass, twice as long as wide, and smaller than eye length.

In preserved specimens, dorsal ground colour varies from dark brown to light brown with irregular darker areas.

The skin surface is homogeneously covered by coni, lacking pointed tubercles and recognisable superficial glands.

Comparison with other species in the genus. Resembling *N. tornieri* in body shape but substantially larger. Easily distinguished from *N. tornieri*, *N. poyntoni*, *N. vestergaardi*, *N. minutus* and *N. viviparus* by the absence of tympanum.

Differing from *N. wendyae*, *N. cryptus* and *N. frontierei* in having expanded tip of fingers (the two latter species sometimes also have a weakly discernible tympanum under the skin). *N. laevis* has shorter hindlimbs (SUL/hindlimb = 1.20 versus 1.12 in *N. pseudotornieri* holotype), lacks hand webbing and has clearly raised parotoid glands, longer than the horizontal diameter of the eye. *N. asperginis* has rounded finger tips, is smaller with dark dorsolateral bands, and has a more developed webbing.

Description. Holotype: BMNH 2000.229, an adult male. Distance from tip of snout to urostyle 25.0 mm, width of head at jaw articulation 9.4 mm, length of tibia 11.4 mm, length of foot 12.2 mm. Tympanum and tympanic annulus completely absent. Parotoid glands present on the scapular region as a slightly raised and weakly discernible mass.

Snout short, nostrils closer to the snout tip than eye, situated laterally, *canthus rostralis* slightly concave. Eye pupil horizontal, eyes prominent and visible ventrally. Fingers widely webbed at base with a rib of webbing reaching the second tubercle of the third finger. Three phalanges of fourth toe free of web on both inner and outer sides, two of the fifth toe. Tips of fingers expanded and slightly truncated. Two subequal metacarpal tubercles. The inner metatarsal tubercle is about twice as large as the outer one. The skin surface is homogeneously covered by coni, lacking pointed tubercles and recognisable superficial glands, except for the abdomen and the inner side of the thigh which are covered by pavement-like glands. The tibia/foot ratio in the holotype is 0.93.

Colours and markings (Fig. 1b). The body appears dark brown dorsally. The dorsum is finely marbled with different shades of brown. The sides are slightly darker than the dorsum with more contrasted and extensive marbling. Hands and feet are lighter in colour. The belly is creamy with sparse condensation of melanophores mainly on throat and limbs. In the living specimen the dorsum and head were light brown, iris was gold (collector's notes).

Variation in the paratype. BMNH 2000.230, an adult female. Distance from tip of snout to urostyle 29 mm, width of head at jaw articulation 10.1 mm, length of tibia 12.7 mm, length of foot 13.4 mm. The SUL/hindlimb = 1.11. The specimen is in a poor condition and is dehydrated. Differing from the holotype in having the parotoid glands almost invisible (probably due to poor preservation). The ground colour is pale yellowish brown covered by dense melanophores. The throat is yellowish in colour. Collector's report the coloration of the living specimens as "golden, tan back". The tibia/foot ratio in the paratype is 0.94 (Table 1).

Advertisement call. Not recorded.

Etymology. The Latin word *pseudo* means "false" or "seeming", indicating an overall similarity with *N. tornieri*.

Habitat and life notes. Rainforest mainly covers the Uluguru North Forest Reserve. Submontane forest occurs at 800-1500 m and montane patches at 1500-2000 m, with elfin forest occurring on ridges above 1900 m (LOVETT & POCS 1993, DOGGART et al. 2000). This species was collected in submontane forest, with a canopy height of 30-50 m. Nothing is known of the breeding biology of this species.

Nectophrynoides frontierei n. sp. (Figs 1d, 2d, 3d)

Holotype. An adult male in the collection of the Natural History Museum, London, BMNH 2000.231(KMH 16367), collected in the Amani-Sigi Forest, Amani Nature Reserve, East Usambara Mountains, at 920 m by members of Frontier-Tanzania on 21th February 1999.

Paratype. BMNH 2000.232 (KMH 16100). Sub-adult. Same locality as the holotype, collected 17th February 1999 by members of Frontier-Tanzania.

Type locality. Amani-Sigi Forest, Amani Nature Reserve, East Usambara Mountains, north eastern Tanzania (05°07'S, 38°39'E).

Diagnosis. A small *Nectophrynoides* with a maximum recorded length of 18.3 mm. Limbs slender, tips of fingers and toes rounded to pointed, not expanded or truncated. Head longer than wide. Tympanum and tympanic annulus reduced to absent. Columella not clearly visible. Eyes visible ventrally. Parotoid glands absent, skin of the parotoid region with a few small glands, which are rounded to oval in shape. A glandular ridge on outer edge of eyelids is present. Nostrils are closer to the tip of snout than to eye and are directed laterally. Length of foot smaller than length of tibia. Two metatarsal and one metacarpal tubercles are present. Webbing absent on hands and only found at the base of the toes. In preserved specimens, dorsal ground colour grey to light brown with light and dark marbling. Ground colour in life was brown with a faint hourglass pattern. Belly was grey with white speckling, the dorsal colouration of the head was light brown, white ventrally, and the same is true for the legs. The skin surface is smooth. A close inspection of the skin reveals that it is uniformly covered by very small coni. Pointed warts present on the tympanic region, head, eyelids and on the limbs, sparsely on body.

Comparison with other species in the genus. Resembling *N. minutus* in size and body shape. The tympanum, if present in *Nectophrynoides frontierei*, is weakly discernible, thus differing from *N. minutus*, *N. tornieri*, *N. poyntoni*, *N. vestergaardi* and *N. viviparus* which have a well developed and clearly visible tympanum. Differing from *N. cryptus* (which in some specimens has a reduced tympanum) and from *N. laevis* in lacking parotoid glands. In *N. pseudotornieri* the parotoid glands are weakly discernible but *N. frontierei* is readily distinguished from it by the rounded tip of the fingers and the smaller size. Differing from *N. asperginis* which has dark dorsolateral bands and more developed webbing.

Description. Holotype: BMNH 2000.231, male. Distance from tip of snout to urostyle 18.3, width of head at jaw articulation 6.9, length of tibia 9.0, length of foot 7.7.

A small *Nectophrynoides* with a moderately robust body. Long slender limbs. Head longer than wide. The tympanum is faintly discernible on both sides of the head. Reflection of the skin on the left side reveals a clear annulus and tympanum. A columella is not clearly visible. The auditory apparatus is evidently partially degenerate. A similar situation is known for a few specimens of *N. cryptus* as stated by PERRET (1972) "Le tympan (...) sous le tegument, il n'est généralement qu'ébauché, l'annulus est incomplet et la columelle absente" which was observed by the authors among the material examined (see Appendix 2). Horizontal pupil. Eye visible ventrally. Sides of the head vertical. *Canthus rostralis* slightly concave, with the snout extending beyond the upper lip. Parotoid glands absent, skin of the parotoid region with a few small, separated, rounded to oval glands. A glandular ridge on outer edge of eyelids is present. Nostrils are closer to the tip of snout than to eye, below the level of the *canthus rostralis* and directed laterally. Webbing on hands absent and only found at the base of the toes. Fingers with rounded to pointed tip, not expanded or truncated. One metacarpal tubercle and two sub-equal metatarsal tubercles are present. The tibia/foot ratio is 1.16 in the holotype.

Colours and markings. The dorsal ground colour is a uniform light brown, with lighter areas corresponding to layers of skin that were probably being shed. The skin is uniformly covered by melanophores, except for the belly and the inferior part of the limbs, where they are less numerous. Light blotches are present on the urostyle region and on the inner side of the thigh. The belly and throat are pale grey with sparse melanophores. Ground colour in life was brown with a faint hourglass pattern. The belly was grey with white speckling. Head and limbs were light brown above and white below. Iris in life was golden.

Variation in the paratype. BMNH 2000.232. Little variation in morphology is evident.

The weak development of the auditory apparatus is consistent with the degenerate condition of the holotype, but this could also be a consequence of the juvenile stage of the specimen. The tympanic area is barely discernible on the left side of the head, but absent on the right. Removal of skin on the left side reveals a barely detectable annulus and tympanum, but no visible columella. It should be noted that this condition was never observed in juvenile specimens of *N. tornieri*, which is sympatric with *N. frontierei*. Pointed warts are more numerous and also present on the surface of the dorsum. The ground colour is marbled in different shades of brown and grey, and the sides are lighter than the dorsum. A light rhombic pattern is present in the middle of the dorsum of the head, in line with the tympanic region. A pale weak vertebral stripe is present on the sacrum. In life, the paratype had a dark brown dorsum, with a grey-white speckled belly. The head and legs were dark brown with a white-grey throat. The iris was golden.

Advertisement call. Not recorded.

Etymology. The species *Nectophrynoides frontierei* is dedicated to the organisation Frontier-Tanzania, whose members collected the type series of this species and have also made significant contributions to understanding the biological diversity of Tanzania.

Habitat and life notes. The holotype was collected in a Sherman trap at an altitude of 920 m, 10 m from a dry riverbed. The paratype was collected in a pitfall trap at an altitude of 950 m, about 20 m from a stream. Both were found in closed submontane moist forest (collector's notes). Nothing is known of the breeding biology of this species.

Nectophrynoides laevis n. sp. (Figs 1c, 2c, 3c)

Holotype. An adult male in the Natural History Museum, London, BMNH 2000.233 (FJM 559), collected in Uluguru South Forest Reserve by Felix Mkonyi on 4th April 2002.

Type locality. Uluguru South Forest Reserve, Uluguru Mountains, Morogoro Region, eastern Tanzania (7°01'-7°12'S, 37°36'-37°45'E).

Diagnosis. A medium sized *Nectophrynoides*, with relatively short limbs. The SUL/hindlimb ratio is 1.20. Tips of fingers rounded and expanded. Length of foot

greater than length of tibia. Tympanum and tympanic annulus absent. No webbing on hands. Feet with main webbing reaching the median subarticular tubercle of fourth toe. Parotoid glands present in the scapular region as a raised and easily discernible structure. The parotoid gland is twice as long as wide, and longer than the eye.

The dorsal ground colour is pale grey with scattered and irregular dark brown to black blotches. A thin, interrupted vertebral line is present. Belly whitish with a thin dark line running from the chin to the vent and on the inner side of upper limbs, absent from hands and feet.

Comparison with other species in the genus. Resembling a small *N. viviparus* in body shape but lacking tympanum, massive parotoids and glands on limbs. It is easily distinguished from *N. poyntoni*, *N. vestergaardi*, *N. minutus* and *N. tornieri* by the absence of a tympanum.

Differing from *N. wendyae*, *N. frontierei* and *N. cryptus* in having expanded tips of fingers (the latter two species occasionally have a weakly discernible tympanum, outlined under the skin). *N. laevis* can be distinguished from *N. pseudotornieri* by its shorter hindlimbs (SUL/hindlimb * 1.20 versus 1.12 in *N. pseudotornieri* holotype), the absence of hand webbing, and clearly raised parotoid glands which are longer than the horizontal diameter of the eye. *N. asperginis* differs from *N. laevis* in having rounded finger tips, absence of parotoids, and by being smaller with dark dorsolateral bands.

Description. Holotype: BMNH 2000.233, male. Distance from tip of snout to urostyle 24.8 mm, width of head at jaw articulation 8.7 mm, length of tibia 10.2 mm, length of foot 12.0 mm. Tympanum and tympanic annulus absent. Parotoid glands present in the scapular region as a raised and easily discernible mass, more than twice as long as wide, and longer than horizontal diameter of the eye.

Snout short, nostrils situated laterally, much closer to the tip of the snout than to the eye, *canthus rostralis* concave. Eyes barely prominent, though still visible ventrally. Pupil is horizontal. A raised glandular ridge on the edge of eyelids is present. No webbing on the hands, three phalanges free of main web on both sides of fourth toe as well as two and half on the inner side and two on the outer side of the third toe. Tips of fingers expanded, the longer one slightly truncated. Two white metatarsal tubercles on feet, the inner greater than the outer. The skin surface appears smooth, uniformly covered by coni, lacking pointed tubercles and recognisable superficial glands. The tibia/foot ratio in the type specimen is 0.85 (Table 1).

Colours and markings. In the preserved specimen, dorsal ground colour is pale grey covered by dense melanophores, with scattered and irregular dark brown to black blotches. A thin interrupted vertebral line is present. The belly is whitish with a thin dark line running from the chin to the vent and on the inner side of the limbs, although absent from the surface of the hands and feet.

Etymology. The skin surface of the holotype of this species appears to be very smooth. The Latin word for smooth is "laevis".

Advertisement call. Not recorded.

Habitat and life notes. Uluguru South Forest Reserve is covered by moist forest, surrounding the upland grassland, swamps and forest patches of the Lukwangule plateau (LOVETT & POCS 1993). No data are available on the habitat of the site where the specimen was collected. Nothing is known about the breeding biology of this species.

Remarks. At present the holotype is the only known representative of this species. The morphological variation in this species is therefore completely unknown and deserves further research. The ecology and breeding behaviour is also unknown and it is vital that further research addresses these topics in order to evaluate the conservation status of this species.

Nectophrynoides poyntoni n. sp. (Figs 1e, 2e, 3e, 4)

Holotype. An adult male in the collection of the Museo Tridentino di Scienze Naturali, Trento, Italy, MTSN 5077, collected between the 2nd and the 6th of January 2003 in the Mkalazi Valley by Michele Menegon and Sebastiano Salvidio.

Paratypes. MTSN 5074, MTSN 5075, BMNH 2000.234 (ex MTSN 5078) and MTSN 5080 are adult males. MTSN 5076 and BMNH 2000.235 (ex MTSN 5079) are adult females with immature ovaries. All paratypes have the same locality and collectors' data as the holotype. BNHM 2000.236 (ex MTSN 5078) has been cleared and stained.

Type locality. Mkalazi Valley, at about 1200 m, Udzungwa Scarp Forest Reserve, Udzungwa Mountains, Iringa Region, south eastern Tanzania (08°23'44.9"S, 35°58'55.4"E).

Diagnosis. A medium sized *Nectophrynoides*, with slender limbs and tips of fingers and toes rounded, not expanded or truncated. Tympanum present. Foot shorter than tibia. No webbing on hands. Webbing present on fourth and fifth toes. The parotoid gland is subdivided into an anterior and posterior part, running from the posterior edge of the eye to the scapular region. The anterior part of the parotoid is formed by a row of small glands, barely larger than those of the dorsal surface, while the posterior half is large and bean-shaped, twice as long as wide, starting behind the optical ramus of the squamosal and ending at the scapular region. Dorsal ground colour brown to light brown, two black stripes from the otic to the scapular region give a bicoloured appearance to the scapular part of the parotoid glands. The dorsum also often has scattered and variable dark blotches.

The tibia/foot ratio in the type series ranges from 1.07 to 1.13 (mean 1.11 ± 0.02) (Table 1).

The advertisement call is composed of a group of trains of 6 to 8 pulses. The train duration is about 1 sec. The pulse duration is about 60 msec with an inter-pulse duration of about 80 msec. Each pulse has a very stable dominant frequency of about 2.9 kHz (mean 2.89 ± 0.03) with a second harmonic emphasized at about 8.7 kHz.

Comparison with other species in the genus. *Nectophrynoides poyntoni* resembles *N. tornieri* in body size and shape but it is easily distinguished by its rounded fingers and toes (always expanded and truncated in *N. tornieri*), and by the characteristics of its advertisement call (Fig. 5). Easily distinguished from *N. viviparus* by the lack of massive glands on limbs and the smaller size.

The presence of a clearly raised bicoloured parotoid gland in the scapular region and the bigger body size allow *N. poyntoni* to be distinguished from *N. minutus* and from *N. frontierei*, which have parotoid glands reduced to just a few small conical glands.

The presence of a clear tympanum differentiates this species from *N. asperginis*, *N. laevis*, *N. pseudotornieri*, *N. wendyae* and *N. cryptus*. The last species occasionally has a depression marking the tympanic area or a reduced tympanum outlined under the skin, but is easily distinguished from *N. poyntoni* in having a longer foot than tibia. *N. poyntoni* resembles *N. vestergaardi* in size, body and head shape and in having rounded tips of fingers and toes, but it differs morphometrically from it in both hind and forelimb proportions (Table 1), in the shape of the parotoid glands and also in the dorsal pattern.

Description. Holotype: MTSN 5077, male. Distance from tip of snout to urostyle 24 mm, width of head at jaw articulation 8.5 mm, length of tibia 10.4 mm, length of foot 9.3 mm. Tympanum and tympanic annulus present, the anterior parotoids are formed by a row of smaller glands aligned antero-posteriorly. The posterior parotoid is located in the scapular region, is twice as long as wide, and as long as the horizontal diameter of the eye (Fig. 2e). Snout short, nostrils closer to the snout tip than eye, situated laterally, *canthus rostralis* slightly concave. Eyes prominent and visible ventrally. Pupils are horizontal. No webbing on the hands. Webbing present on fourth and fifth toes. Tips of fingers and toes rounded, not

Fig. 4. — *N. poyntoni* (holotype MTSN 5077) in life.

expanded or truncated. Two subequal white metatarsal tubercles on feet. The body skin is completely covered with small pointed coni, with scattered glandular humps surmounted by small clear spines on dorsum, sides, eyelids and limbs.

The tibia/foot ratio in the holotype is 1.10 (Table 1).

Colours and markings. Ground colour apparently brown, but close inspection shows the colour to be formed of darker brown melanophores with dense patches of dark brown. A black stripe runs from the tip of the snout to the end of the parotoid glands, darkening the outer edge of these glands. The sides of the head are pale beige, as well as the upper part of the arms. The dorsum has a light brown mid dorsal stripe with a black border. The stripe is interrupted in the mid dorsal area by a beige inverted 'v' -shaped broad band, which has a black border. Several dorsal glands are marked by condensations of melanophores, often present on the margins of the pale pink areas, forming interrupted stripes (Fig. 3e). Ventral surface is grey, with a sparse number of melanophores.

Variation in the paratypes. The clear dorsal markings described in the holotype are the most extensive among the whole type series. Variation is evident in the dorsal pattern. A dark, sometimes interrupted stripe runs from snout tip to the end of the scapular parotoids. The dorsal pattern varies from being almost completely

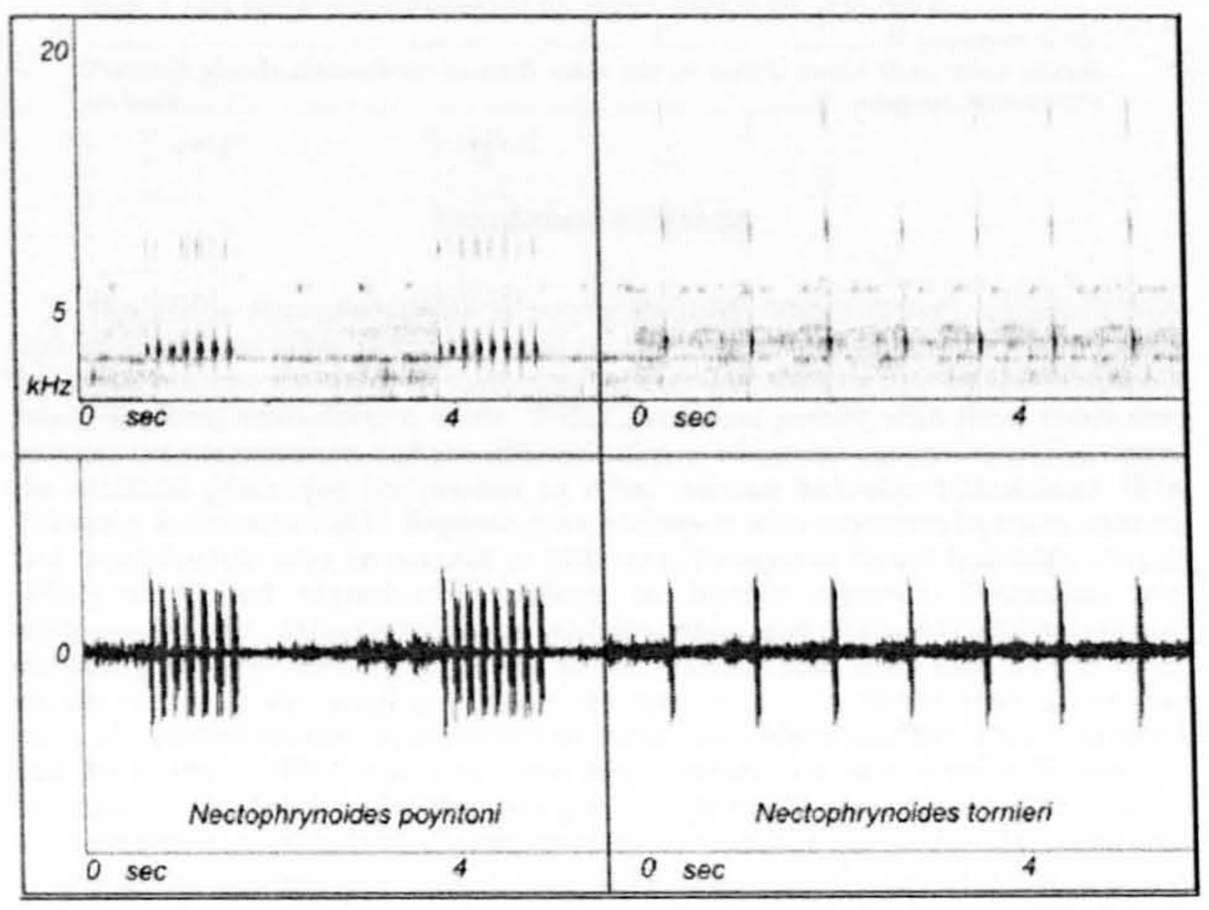

Fig. 5. — The pulse trains arrangement of the advertisement call of *N. poyntoni* compared with that of the syntopic *N. tornieri*.

brown with weak condensation of melanophores (in MTSN 5076) to specimens with dorsum, head and limbs covered with several scattered irregular black blotches (in MTSN 5075 and in BMNH 2000.234). MTSN 5080 has a dark, thin, interrupted vertebral line.

Sexual dimorphism. No apparent secondary sexual characters were observed; no nuptial pads or significant asperities on first and second finger of males. The two dissected females had eight (BMNH 2000.235) and 10 (MTSN 5076) large yolky eggs.

Advertisement call. The analysis was carried out on a sequence of 21 pulse trains obtained from one captive male. Many other males were heard calling in the forest. The male advertisement call is monophasic and high pitched, consisting of a sequence of similar pulse trains (Fig. 5). The pulse duration is about 60 msec, with an inter-pulse interval of about 80 msec. The pulse train duration is about 1 sec with an interval of about 2.5-3.5 sec. The call has a very stable dominant frequency of 2.9 kHz (mean 2.89 ± 0.03) with the second harmonic emphasized at 8.7 kHz (Fig. 4). The high pitched call of *N. poyntoni* may be adaptive; higher frequencies of the call differ from the low frequencies produced by the noise of turbulent water (Hödl & Amézquita 2001). The first pulse always had the highest intensity maxima (mean – 37.04 ± 2.8 dB of the first vs – 41.2 ± 2.1 dB of the second), followed by a series of pulses of lower intensity.

The call of the new species is highly distinctive and can be easily distinguished in the field from the call of *Nectophrynoides tornieri* with which it is syntopic. The advertisement call of the latter species, recorded in the same place under the same conditions, was characterised by a sequence of single pulses with a dominant frequency at about 3.30 kHz (mean 3.29 ± 0.01) (Fig. 5).

Etymology. The species is named in honour of Prof. John Poynton, who has greatly improved the understanding of the amphibians of Tanzania.

Habitat and life notes. Specimens of *N. poyntoni* were found in the Udzungwa Scarp Forest Reserve at about 1200 m. The site is characterised by a submontane closed moist forest of the Eastern Arc type (Shangali et al. 1998), with a canopy of 30 to 40 m and emergents exceeding 50 m.

Toads were active during the first part of the night and were found on leaves 60 -160 cm above ground level. Some specimens were found during the day in their hiding places, under fallen trees and coarse wood debris. The species was rather common along the Mkalazi stream but much less numerous than *N. tornieri*.

Calls were emitted from the late afternoon, but mainly after sunset, from trees and bushes, never far from streams. The presence of a small number of large yolky eggs suggests that this species is ovoviviparous (see *N. vestergaardi* for similar comments on reproductive biology).

REVISED KEY TO THE SPECIES OF *NECTOPHRYNOIDES*

1	Ventral interfemoral glands made conspicuous by darkened skin .... *N. wendyae* Clarke 1988	
—	No conspicuous ventral interfemoral pigmentation.....	2

2	Tympanum not or weakly visible (completely absent or degenerated)	3
—	Tympanum visible (tympanic membrane, annulus and columella present at close inspection)	7
3	Tip of fingers expanded	4
—	Tip of fingers not expanded	5
4	Hindlimb short (SUL/hindlimb > 1.15), no hand webbing, parotoid gland larger than eye diameter ... *N. laevis* n. sp.	
—	Hindlimbs long (SUL/hindlimb < 1.15), webbing present on hands, parotoid gland smaller than eye diameter ... *N. pseudotornieri* n. sp.	
5	Parotoid gland present, if only weakly ... *N. cryptus* Perret 1971	
—	Parotoid glands absent	6
6	Dark dorsolateral band, webbing reaching middle tubercle of fourth toe and tubercle of second finger, foot longer than tibia ... *N. asperginis* Poynton, Howell, Clarke & Lovett 1998	
—	No dark dorsolateral band, no webbing on the hand, length of foot shorter than tibia ... *N. frontierei* n. sp.	
7	Adults without massive glands on limbs	8
—	Adults with massive glands on limbs ... *N. viviparus* (Tornier 1905)	
8	Tips of fingers rounded to pointed, not or only slightly expanded	9
—	Tips of fingers widely expanded, ends truncate ... *N. tornieri* (Roux 1906)	
9	Parotoid glands continuous from ear region to scapular region, sides darker than dorsum, foot not shorter than tibia ... *N. vestergaardi* n. sp.	
—	Parotoid glands discontinuous, foot shorter than tibia	10
10	Easily discernible scapular parotoid glands much larger than other glands on back, a dark stripe running from the tip of the snout to the scapular region ... *N. poyntoni* n. sp.	
—	Parotoid glands discontinuous, each mass not or barely larger than other glands on back ... *N. minutus* Perret 1972	

TAXONOMIC REMARKS

The genus *Nectophrynoides* is poorly defined. NOBLE (1926) separated *Nectophrynoides* from other bufonids based on the presence of an omosternum, simple T- shaped but not flattened or spatulated terminal phalanges and by the viviparous [ovoviviparous] reproductive mode. Today, problems persist with these characters because the omosternum and the different degree of lateral expansion of the tips of the terminal phalanges are present in other African bufonids (GRANDISON 1978, CHANNING & STANLEY 2003). Reproductive biology is also unknown in many species, and ovoviviparity may be present in different lineages of forest bufonids. DUBOIS (1987) also used reproductive biology to further separate Tanzanian *Nectophrynoides* from Ethiopian species (*Altiphrynoides* and *Spinophrynoides*), without discussing the reproductive modes of all the relevant taxa from East Africa. Based on our current understanding of the characters defined by NOBLE (1926), the diagnosis of *Nectophrynoides* with respect to other bufonids is unclear. This is exemplified by CLARKE (1988) who was uncertain whether his new species *N. wendyae* belonged to *Nectophrynoides* or a new genus. This doubt appears to be the result of a number of poorly defined characters used to diagnose the genus. Re-assessment of this group is urgently required, especially in light of the descriptions provided here and other those of newly described species (POYNTON 1998, CHANNING & STANLEY 2003). Detailed morphological and behavioural work is necessary to define

characters that diagnose the genus, clearly separating *Nectophrynoides* from all other bufonids. Once this is accomplished, it should be possible to ascertain the relationships within the East African bufonids. Molecular data would also be a suitable tool to further assess the phylogenetic relationships of *Nectophrynoides* and their close relatives.

DISTRIBUTION

A map of the known distribution of species in the genus *Nectophrynoides* and closely related taxa is given in Fig. 6 (based on POYNTON 1998; see also AKKER & HIGHSTEAD 1992, POYNTON 2003, LOADER et al. 2004). Only a preliminary interpretation of the distribution is made because there are certain to be biases in sampling intensity between mountain blocks (EMMRICH 1994, BURGESS et al. 1998). Given the current distribution of *Nectophrynoides*, it appears that the forest patches of major importance in terms of numbers of species found in Eastern Arc, are the Udzungwa and Uluguru Mountains. The former harbour five species, including three strictly endemic species (i.e. *N. asperginis*, *N. poyntoni* and *N. wendyae*) confined to the Udzungwa Scarp Forest Reserve in the south-eastern part of the massif. These three species seem to be locally allopatric, while *N. tornieri* is sympatric with at least two of them, i.e. *N. asperginis* and *N. poyntoni* (POYNTON et al. 1998 and this study). The importance of the Udzungwa forest as a major biogeographic relict has been previously shown by POYNTON (2003) and FJELDSÅ & LOVETT (1997). Six species are found in the remnant forest patches on the Uluguru Mountains, three of which are strictly endemic. The Ulugurus are an area of high biodiversity and are considered one of the most important areas for conservation in the Eastern Arc (BURGESS et al. 1998, 2002).

The East and West Usambara Mountains are inhabited by two strictly endemic species (*N. vestergaardi* and *N. frontierei*, respectively). The Ukaguru Mountains are of interest, based on the occurrence at least one endemic bufonid genus (*Churamiti* and the undescribed *Nectophrynoides*-like genus, POYNTON et al. 1998) and given preliminary evidence of a rich endemic amphibian fauna (CHANNING & STANLEY 2003). The occurrence of endemic genera, new species, and the absence of a number of widespread Eastern Arc taxa suggest a relatively long period of isolation in this area. Clearly further research on the distribution of amphibians in the Ukaguru Mountains and throughout the Eastern Arc is needed to clarify these preliminary observations.

The forests of the Eastern Arc are thought to be ancient, with forest persisting throughout severe climatic fluctuations and fragmentation (LOVETT & POCS 1993). This environmental stability coupled with isolation of the mountains is believed to have had an important influence on the evolution of forest species (HOFFMAN 1993, LOVETT & POCS 1993, FJELDSÅ & LOVETT 1997, ROY 1997, GRAVLUND 2002). High levels of endemism of species are claimed as evidence of this history (BURGESS et al. 1998). The genus *Nectophrynoides* is endemic to the Eastern Arc Mountains, with the exception of *N. viviparus* which also occurs in the Southern Highlands. According to HOWELL (1993) and POYNTON (2000, 2003), the genus is cool-adapted, forest associated and not known to occur below 400 m (POYNTON et al. 1998). Because of these ecological limitations, the ability of *Nectophrynoides* to disperse across the

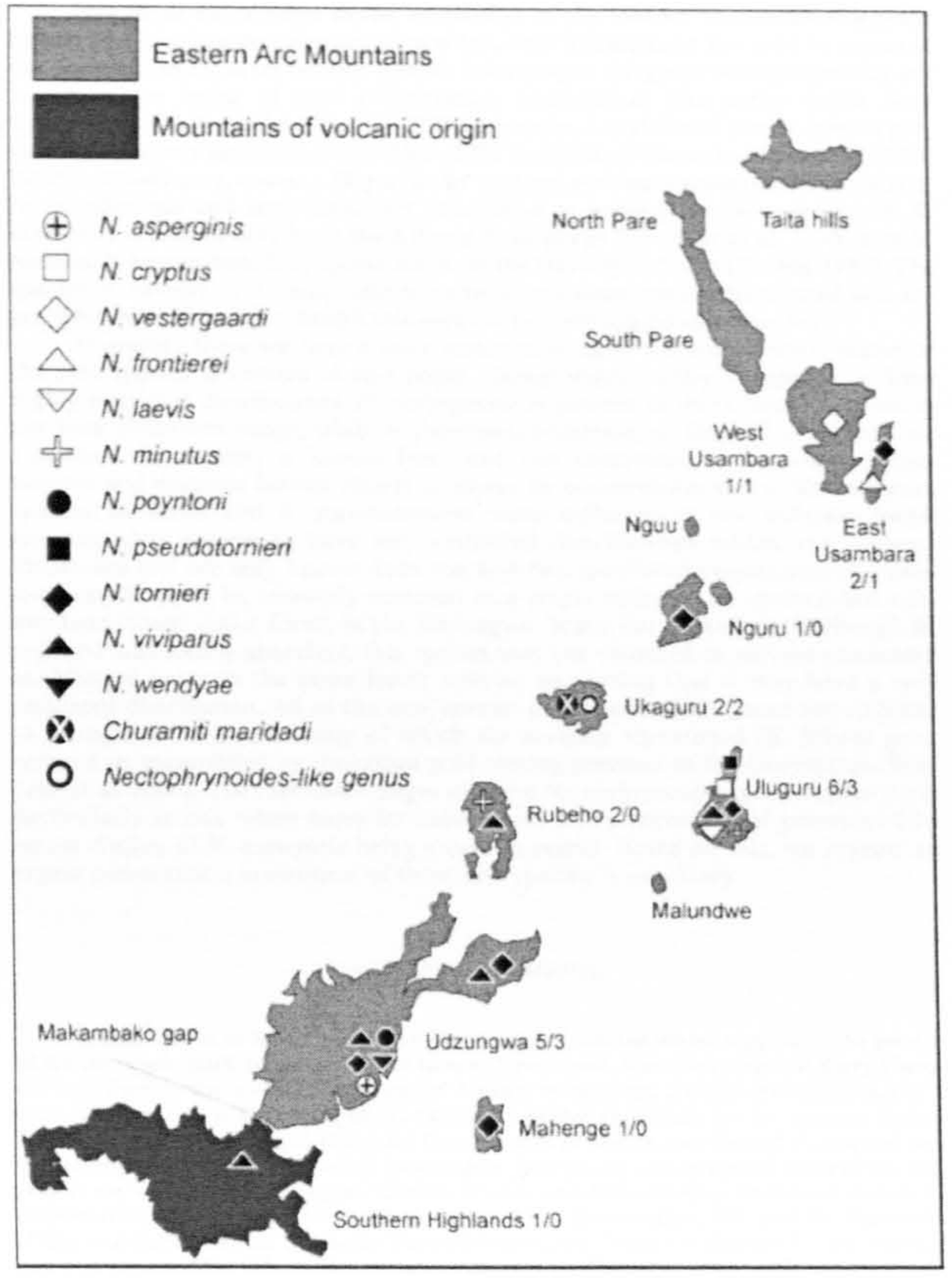

Fig. 6. — Map of the updated distribution of the forest dependent bufonid species included in the genus *Nectophrynoides*, *Churamiti* and in the undescribed *Nectophrynoides*-like genus recorded for the Ukaguru Mountains (Poynton 1998). The numbers following the name of the mountain represents the total number of species/strictly endemic species (see also Table 2).

Table 2.

Summary of the distribution of the species belonging to the genus *Nectophrynoides* and the closely related bufonids *Churamiti maridadi* Channing & Stanley 2002 and a *Nectophrynoides*-like undescribed genus.

Mountain block	Species present	No. species present/ strictly endemic
Taita Hills	—	—
Pare	—	—
West Usambara	*N. vestergaardi*	1/1
East Usambara	*N. tornieri, N. frontierei*	2/1
Nguu	—	—
Nguru	*N. tornieri*	1/0
Ukaguru	*Churamiti maridadi*, *Nectophrynoides*-like undescribed genus	2/2
Uluguru	*N. tornieri, N. cryptus, N. minutus, N. viviparus, N. pseudotornieri, N. laevis*	6/3
Malundwe	—	—
Rubeho	*N. minutus, N. viviparus*	2/0
Mahenge	*N. tornieri*	1/0
Udzungwa	*N. tornieri, N. asperginis, N. viviparus, N. wendyae, N. poyntoni*	5/3
Southern Highlands	*N. viviparus*	1/0

dry habitats found between mountains in the Eastern Arc would appear to be limited. Consequently, as has been speculated in other forest amphibian species (Howell 1993), the distribution of *Nectophrynoides* is thought to reflect both the long history of the forests and periods of isolation of the fragmented mountain blocks. The description of five new species with restricted distributions appears to support the idea that forest amphibians have been influenced by a long history of fragmentation and isolation in the Eastern Arc (Howell 1993, Burgess et al. 1998). The use of dwarf forest bufonids as indicators for reconstruction of the biogeography of the Eastern Arc appears to merit of further study.

CONSERVATION

The high biodiversity of the Eastern Arc Mountains is now well established (Mittermeier et al. 1999, Myers et al. 2000, Newmark 2002). In quantitative analyses (Mittermeier et al. 1999) comparing 24 leading global biodiversity hotspots, the Eastern Arc and Coastal forests in Tanzania and Kenya ranked among the top eight "hottest" hotspots. Although the Eastern Arc is not the most speciose of hotspots, the number of species per km^2 is high. Although large patches of undisturbed montane forests are still present, the lowland and submontane layers of the forest have suffered extensive losses and fragmentation due to human disturbance (Newmark 2002). Conservation of these forests in light of the increasing levels of deforestation has been singled out as a priority (Newmark 2002).

This paper contributes to the knowledge of the eastern Tanzanian mountain forests by providing descriptions of new taxa and highlighting the need to preserve the habitats these species occupy. Species belonging to the genus *Nectophrynoides* are recognised as being of high conservation importance. The entire genus *Nectophrynoides* has been placed in the CITES Appendix I, and five of the six species previously described are listed in the IUCN 2002 Red List of Threatened Species (CEPF 2002; S. STUART pers. comm.). Of particular concern are two species that have restricted distributions and accordingly are considered as being critically endangered. *N. asperginis* is known only from the Kihansi Falls gorge (POYNTON et al. 1998) and *N. wendyae* is known from four specimens from the Udzungwa Scarp (CLARKE 1988). The species *N. tornieri, N. cryptus,* and *N. minutus* are more widely distributed and are probably more abundant, though this needs to be investigated quantitatively.

At present there we have a poor understanding of the conservation status of the new species described in this paper, though significantly all appear to have highly restricted distributions. *N. vestergaardi* is present in three forest reserves in the West Usambara range, while *N. frontierei*, occurring on the well explored East Usambara Mountains, is known from just two specimens from Amani Nature Reserve and deserves further efforts to assess its conservation status. The Uluguru species, *N. laevis* and *N. pseudotornieri*, were collected in two different forest reserves; they appear to have very restricted distributions within the Uluguru Mountains and are only known from one and two specimens respectively. *N. poyntoni* was found to be relatively common in a single valley in an undisturbed submontane closed moist forest in the Udzungwa Scarp Forest Reserve. Although *N. poyntoni* was locally abundant, this species was not collected in surveys conducted in different areas in the same forest reserve, suggesting that it may have a very restricted distribution. All of the new species described in this paper are endemic to a single forest patch, some of which are severely threatened (S. STUART pers. comm.), as exemplified by the recent gold mining pressure in the Usambaras (DOGGART et al. 2004). The restricted ranges of some *Nectophrynoides* species make them particularly at risk when faced by habitat loss and environmental pressures (the recent decline of *N. asperginis* being a case in point). Based on this, we suggest an urgent conservation assessment of these new species is necessary.

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APPENDIX 1

Summary of morphological measurements of the specimens used for statistical analysis comparing *N. poyntoni* and *N. vestergaardi*.

Species	Museum number	Sex	SUL	HW	HL	ND	TL	FOL	ML	FL
N. poyntoni	MTSN 5074	m	20.2	7.3	7.2	1.5	9.1	5.8	6.1	8.0
N. poyntoni	MTSN 5075	m	19.1	8.0	7.8	1.7	9.2	6.6	6.5	8.1
N. poyntoni	MTSN 5076	f	23.9	8.5	8.6	1.9	10.4	7.0	7.0	9.4
N. poyntoni	MTSN 5077	m	24.0	8.5	8.4	2.0	10.4	6.9	7.2	9.3
N. poyntoni	BMNH 2000.234	m	22.0	8.9	8.3	1.9	9.5	6.4	6.5	8.5
N. poyntoni	BMNH 2000.235	f	20.2	6.7	7.2	1.7	8.9	5.4	7.3	8.3
N. poyntoni	MTSN 5080	m	21.4	8.3	7.7	1.9	9.6	6.3	6.5	8.7
N. vestergaardi	BMNH 1982-499	f	25.7	9.2	9.0	2.0	10.8	7.1	7.9	11.4
N. vestergaardi	BMNH 1982-501	m	21.8	8.2	8.2	1.8	9.2	5.5	5.9	9.2
N. vestergaardi	BMNH 1982-513	f	21.8	8.3	8.4	1.8	8.4	5.9	6.3	9.3
N. vestergaardi	BMNH 1982-510	f	21.4	7.9	7.4	1.7	7.7	5.0	5.1	8.2
N. vestergaardi	BMNH 1982-509	f	24.0	8.9	9.1	2.2	9.5	6.7	7.2	10.7
N. vestergaardi	BMNH 1982-514	m	19.0	8.0	7.5	1.7	7.2	5.1	5.6	8.1
N. vestergaardi	BMNH 1982-515	m	18.7	7.1	7.2	1.8	7.5	5.1	5.1	8 1
N. vestergaardi	BMNH 1982-512	f	20.9	7.6	7.7	1.7	8.2	4.9	5.6	8.9
N. vestergaardi	BMNH 1982-511	m	20.3	7.5	8.0	1.6	7.9	5.2	5.7	7.9

For abbreviations see Materials and methods.

APPENDIX 2

Specimens examined (collection abbreviations are as indicated in Materials and methods).

Nectophrynoides asperginis Poynton, Howell, Clarke & Lovett 1998
BMNH 1998-136 (holotype), BMNH 1998-137, BMNH 1998-138, BMNH 1998-140, BMNH 1998-141, BMNH 1998-142, BMNH 1998-143, BMNH 1998-145 (paratypes) from Kihansi River Gorge, Udzungwa Mountains.

Nectophrynoides cryptus Perret 1971
BMNH 1972-1289, BMNH 1972-1290, BMNH 1972-1292, BMNH 1972-1293, BMNH 1972-1289 from Vituri, Uluguru Mountains (paratypes), BMNH 1992-273 Bondwa peak, Uluguru Mountains. MCZ A-12470, MCZ A-12471, MCZ A-84830 from Nyingwa, Uluguru Mountains.

Nectophrynoides minutus Perret 1972
MCZ12460, MCZ12464, MCZ12465 from Bagilo, Uluguru Mountains (paratypes). CAM 701, CAM 769, CAM 770 from Uluguru North Forest Reserve, Uluguru Mountains, KMH 21465, KMH 21468, KMH 21471, KMH 21472, KMH 21474, KMH 21479, from Uluguru Mountains, KMH 25059, KMH 25495 from Ukwiva, Rubeho Mountains, KMH 25523 from Mangalisa, Rubeho Mountains.

Nectophrynoides tornieri (Roux 1906)
BMNH 1947-2-19-44 (paratype), BMNH 1974-446, BMNH 1974-456, BMNH 1974-457, BMNH 1974-459, BMNH 1974-471, BMNH 1974-472, BMNH 1974-473, BMNH 1974-474, BMNH 1974-475, BMNH 1974-477, BMNH 1974-479, BMNH 1974-485 from Amani, East Usambara.

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A New Species of *Callulina* (Anura: Microhylidae) from the West Usambara Mountains, Tanzania

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ABSTRACT.—**The description of the species *Callulina kreffti* was based on specimens collected in the East Usambara Mountains of Tanzania. Successive collecting has shown this species to be widely distributed through the Eastern Arc Mountains. Advertisement calls from populations in the type locality of *Callulina kreffti* were compared with calls from populations in the West Usambara Mountains. Analysis of the calls suggested that these two populations of *Callulina* represent two separate taxa. Subsequent morphological and molecular investigations indicated that these two populations are distinct. Herein, we describe a new *Callulina* species on the basis of call, morphology and molecular sequences.**

The monotypic genus *Callulina* was described by Nieden (1910) to accommodate a brevicipitine microhylid, *Callulina kreffti*, which resembled *Probreviceps* and *Breviceps* but differed from these species with the following characters: the fingers and toes distinctly broadened, inner and outer metatarsal tubercles in contact, and diapophyses of sacral vertebra expanded. A further nine new species (five *Breviceps*, three *Probreviceps*, and one new monotypic genus *Spelaeophryne*) all belonging to the Brevicipitinae were described between Nieden's (1910) description of *Callulina* and Parker's (1934) monograph of the Microhylidae. Parker's monograph (1934:180) distinguished *Callulina* and *Spelaeophryne* from *Probreviceps* and *Breviceps* by the former genera having a double condylar articulation between the coccyx (= urostyle) and the sacral vertebra, whereas the latter two genera exhibit a fused condition. Furthermore, Parker (1934) differentiated *Callulina* from *Spelaeophryne* based on the shape of the terminal phalanges, T-shaped in *Callulina* and simple-shaped in *Spelaeophryne*. However, no other brevicipitine apart from *Callulina* has T-shaped phalanges.

Callulina is known from the Usambara, Uluguru, Nguru, Udzungwa, and Pare Mountains (Frost, 2002), which all form part of the Eastern Arc Mountains of Tanzania. The type locality of *C. kreffti* is Amani, Tanga, Tanzania (ZMB 21778); this locality is within the East Usambara.

During an amphibian survey in Tanzania 1999–2000 we realized that the *Callulina* from the West Usambara could be distinguished from the type species in the East Usambara by their advertisement call. Subsequent analyses of external morphology and DNA sequence data support the recognition of these two disjunct populations as distinct taxa. We describe the population from the West Usambara Mountains as a new species.

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MATERIALS AND METHODS

Material was collected from the Mazumbai Forest Reserve in the West Usambara Mountains, Tanzania (Fig. 1). Male specimens were collected while calling from bushes and trees after heavy rains. Calls were recorded using a Sony TCD-5M and directional microphone, or a Marantz model PMD-430 stereo cassette tape recorder and a KE66 Sennheiser directional microphone. Air temperature was recorded using an electronic thermometer. Calling males were collected and deposited as voucher specimens at the USNM. Specimens were fixed in 10% formalin (commercial grade) and subsequently stored in 70% ethanol; sample tissues of muscle and liver were removed and preserved in 95% ethanol. Specimens were cleared and double-stained for bone and cartilage using a modified Dingerkus and Uhler's (1977) technique. Specimens examined are deposited at USNM, BMNH, and MCZ (Leviton et al., 1985).

Standard measurements were taken to the nearest 0.1 mm using digital calipers: SUL (snout–urostyle length), TL (tibiofibula length), ED (horizontal eye diameter), TD (horizontal tympanum diameter), ETD (eye–tympanum distance), ND (nostril diameter), NED (nostril–eye distance), HW (head width at level of jaw articulation), LF3 (length of Finger 3 measured

FIG. 1. Map showing the position of the West Usambara Mountains relative to the other highlands of Tanzania. Map courtesy of D. Moyer.

from the distal edge of the basal subarticular tubercle), LT4 (length of Toe 4 measured from the proximal edge of the basal subarticular tubercle), TSL (length of tarsus), HL (humerus length), NLD (nostril–lip distance), WDF3 (width of disc of Finger 3), WDTF3 (width of Finger 3 at level of distal subarticle tubercle), IOD (interorbital distance). Summary statistics (mean ± SD) are provided in Table 1 for 49 specimens of *C. kreffti* (including the holotype) and for 19 specimens of the new species. Specimens examined and locality data are provided in Appendix 1. The calls were analyzed using the software package CANARY 1.2.4 (R. A. Charif, S. Mitchell, and C. W. Clark, Cornell Laboratory of Ornithology, Ithaca, NY, 1995).

Tissue samples were obtained from the specimens listed in Appendix 1. DNA extraction followed Hillis et al. (1996). Two segments of the mitochondrial genome were amplified using the polymerase chain reaction (PCR). A segment of the 12s r RNA of ~350 base pairs and a segment of the 16s r RNA of ~500 bp were amplified. Primers used were: 12Sa (5′-AAACTGGGATTAGATACCCCACTAT-3′), 12Sb (5′-GAGGGTGACGGGCGGTGTGT-3′), 16SaR (5′-CGCCTGTTTACCAAAAACAT-3′), and 16Sd (5′-CTCCGGTCTGAACTCAGATCACGTAG-3′). Double-stranded (DS) PCR amplifications were performed in a final volume of 50 µl containing 0.4 µl of each primer, 1.0 µl of each dNTP, 3.0 µl of 25 mM MgCl, and 1.25 units? of *Taq* (*Thermus aquaticus*) DNA polymerase; the reaction was overlaid with 50 µl of mineral oil. PCR conditions were as follows: 94°C for 60 sec, 57°C for 60 sec, and 72°C for 60 sec, with 25 cycles for the 12S amplification and 30 cycles for the 16S amplification. Purification of DS amplified product was done using Wizard® PCR Preps Kit (Promega). Of the purified DS fragment, 0.5 µl were mixed with 1.5 µl of a single IRD-labeled primer, 7.2 µl of Sequencing Buffer, 1 µl of Sequitherm Excel™ II (Epicentre Technologies Co.) DNA polymerase, and 6.8 µl of dH_20. Subsequently, 4.0 µl of this mix was added to each of four tubes containing 2 µl of each nucleotide, respectively. PCR conditions were as follows (30 cycles): 92°C for 30 sec, 55°C for 30 sec, and 70°C for 30 sec. Single-stranded (SS) segments were amplified and

TABLE 1. Comparison of morphometrics in *Callulina* (in millimeters), abbreviations given in Materials and Methods.

	Callulina kreffti (N = 47)				*C. kisiwamisitu* n. sp. (N = 19)			
Measures	Min.	Max.	Med.	SD	Min.	Max.	Med.	SD
SVL	13.4	38.0	24.2	5.73	21.1	41.4	30.01	5.83
TL	4.7	12.8	8.55	1.91	6.8	15.2	10.62	2.26
TD	0.6	1.8	1.2	0.22	0.7	1.8	1.23	0.32
ETD	0.6	2	1.05	0.30	1	2.4	1.46	0.37
ED	1.6	3.8	2.8	0.43	2.4	4.4	3.15	0.55
ND	1.1	2.7	1.8	0.36	1.4	2.8	1.97	0.39
NED	1.4	3.2	2.1	0.37	1.7	3.1	2.38	0.38
HW	4	10.5	6.3	1.44	5	11.5	7.56	1.84
LF3	1.6	5	3.2	0.70	2.5	6	4.18	0.84
LT4	2.3	7.4	4.75	1.00	4	8.6	5.65	1.26
TSL	3.2	9.6	5.95	1.34	4.8	11.2	7.75	1.71
HL	3.9	11.7	7.2	1.62	5.4	13.4	9.04	2.13
NLD	0.7	1.6	1.1	0.22	0.9	1.6	1.21	0.19
WDF3	0.7	1.9	1.2	0.29	0.7	1.5	1.04	0.22
WDTF3	0.5	1.2	0.8	0.18	0.7	1.3	0.94	0.18
IOD	3.3	7.5	5	0.89	3.8	7.3	5.41	0.99
WDF3/WDTF3	0.58	0.78			0.83	1.00		

FIG. 2. Photograph of *Callulina kisiwamistu* in life.

infrared labeled fragments were sequenced in a LI-COR 4200 IR DNA Sequencer on 6% acrylamide gels. Phylogenetic analyses were performed using PAUP* (Swofford, 1998).

Callulina kisiwamsitu sp. n.
Figure 2

Holotype.—USNM 556132(originally field number RdS 930), adult male, collected by R.O. de Sá and A. Channing on 14 March 2000, at Mazumbai, West Usambaras Mts. (04°48'46.5"S, 38°30'12"E), Tanzania.

Paratypes.—USNM 556133, adult male, same data as holotype; USNM 556134 adult female, USNM 556139 adult male, collected by R. O. de Sá and A. Channing on 15 March 2000, at Mazumbai, West Usambara Mtns.; MCZ A-13632, and MCZ A-13633, adult females, collected by A. Loveridge on 24 December 1926, at Phillipshof, Usambara Mtns, Tanga Territory (these specimens were originally identified as *C. kreffti*); BMNH 1982.592, immature juvenile, collected by Kim Howell on 13 February 1981, at Shume-Mugambo FR, West Usambara Mtns.; BM 2002-45, adult male, collected by Simon Loader on 28 October 2001, at Mazumbai FR, West Usambara Mtns.; BMNH 2002-46, adult female with eggs, collected by Simon Loader on 28 October 2001, at Mazumbi FR, West Usambara Mtns.

Referred Specimens.—USNM 556135, USNM 556136-138, 556141, USNM 556140, BMNH 1982.591, BMNH 1986.595-96, BMNH 1986.597, BM 2002-47 (specifically not designated as types; see Appendix 1 for details).

Diagnosis.—The new species is assigned to the genus *Callulina* based on the following characteristics: (1) triangular-shaped terminal digits (simple, not expanded, in *Balebreviceps*, *Breviceps*, *Probreviceps*, and *Spelaeophyrne*); (2) expanded terminal phalanges (simple in *Spelaeophyrne*, *Probreviceps*, *Breviceps*, and *Balebreviceps*); and (3) a double condylar articulation between the urostyle and the sacral vertebra (fused in *Balebreviceps*, *Breviceps*, and *Probreviceps*).

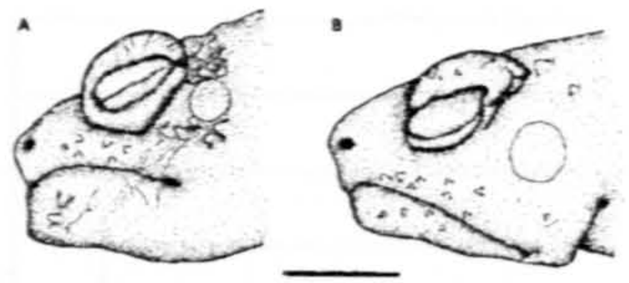

FIG. 3. Lateral view of the head region of (A) *Callulina kreffti* (MCZ A-13625) and (B) *Callulina kisiwamsitu* (USNM 556134). Bar = 5 mm.

The new species is overall morphologically similar to *C. kreffti* (Table 1). However, the two taxa are distinguished by the following characters: (1) *Callulina kisiwamsitu* has a rounded canthus rostralis and a distinctly truncated snout (the latter is less pronounced in *C. kreffti*, Fig. 3); (2) In *C. kisiwamsitu*, there is no contact between inner and outer tubercles on either the hand and the foot (tubercles very close or in contact in *C. kreffti*, Figs. 4 and 5); (3) Inner metatarsal tubercle larger than outer metatarsal tubercle in *C. kisiwamsitu* (metatarsals tubercles are about equal in size in *C. kreffti*, Fig. 5); (4) Dorsal and lateral body surfaces of *C. kisiwamsitu* have uniformly small warts (*C. kreffti* have large, broad-based warts as well as small warts); (5) The ratio between the widths of Finger 3 at the level of the distal subarticle tubercle relative to the width of its toe tip is always more than three-fourths in *C. kisiwamsitu* (three-fourths or less in *C. kreffti*, see Table 1); (6) Cleared-and-stained specimens show Y-shaped expanded terminal phalanges in *C. kisiwamsitu* (T-shape in *C. kreffti*, Fig. 6); (7) In *C. kisiwamsitu*, the distance between the tympanum and the posterior corner of the eye is equal to or greater than the tympanum diameter (Fig. 3; usually less than the tympanum diameter in *C. kreffti*, in a few cases it is slightly larger); and (8)

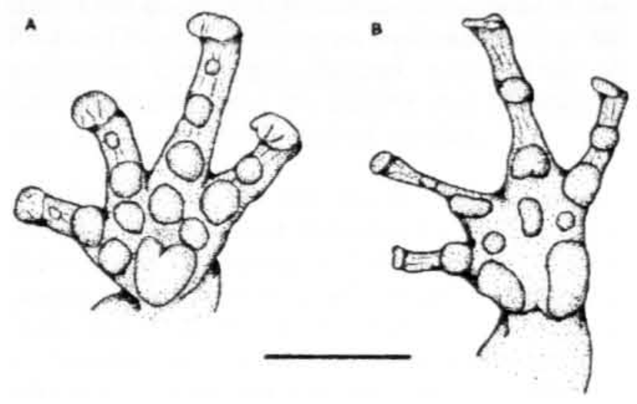

FIG. 4. Ventral view of right hand of (A) *Callulina kreffti* (MCZ A-13625) and (B) *Callulina kisiwamsitu* (USNM 556134). Bar = 5 mm.

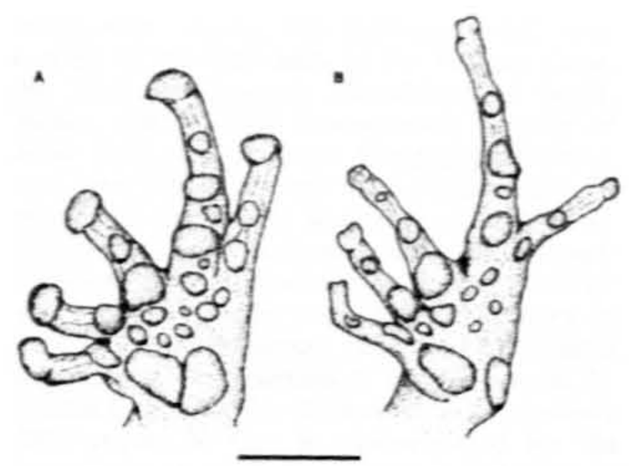

FIG. 5. Ventral view of right foot of (A) *Callulina kreffti* (MCZ A-13625) and (B) *Callulina kisiwamsitu* (USNM 556134). Bar = 5 mm.

Peak dominant frequency of advertisement calls of *C. kisiwamsitu* is always below 2 Khz, and peak dominant frequency in *C. kreffti* is always above 2 Khz, usually around 2.5–2.6 KHz (Fig. 7).

Description of the Holotype.—Body stout; head small, as wide as the body; head width about equal to head length; snout truncate in lateral view (Fig. 3); snout tip extending slightly anteriorly to the jaws; lower and upper jaw with small warts, less dense on upper jaw; canthus rostralis rounded; loreal region sloping at a shallow angle, without warts; nostril openings rounded, directed laterally, nearer to the tip of snout than the eye. Tongue rounded. Tympanum slightly ovoid, distinct, defined by smooth, light colored skin, with warts around edge of the disc. Warts on dorsal surface of head small, rounded. Pupil horizontal. Forelimbs slender, short, covered with small warts, more densely concentrated on the ventral surfaces. Hind limbs stout, also covered with small warts, concentrated ventrally. Digits of hands and feet moderately long, relatively slender; tip of digits truncate, slightly triangularly expanded, more pronounced on hand than on foot. A white spot defines the dorsal junction between the penultimate and ultimate phalanges in hands and feet; this spot is

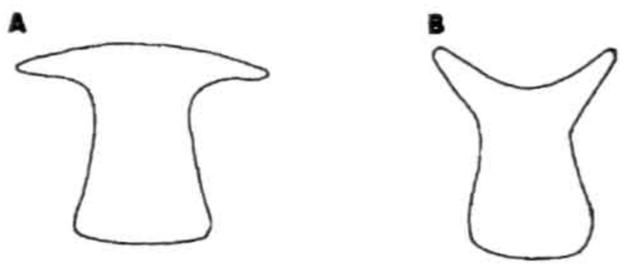

FIG. 6. Distal phalanges of (A) *Callulina kreffti* (MCZ A-105871) and (B) *Callulina kisiwamsitu* (USNM 556141).

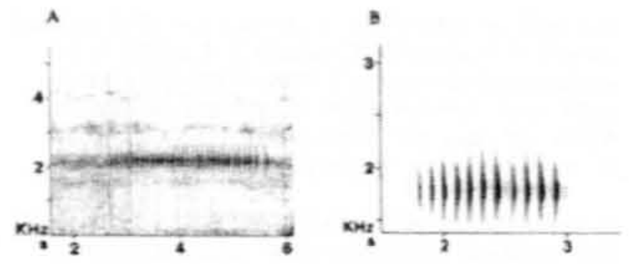

FIG. 7. Calls of (A) *Callulina kisiwamsitu* and (B) *Callulina kreffti*.

a fold of skin. Subarticular tubercles are distinct and rounded. Palmar tubercles are distinct and not contacting; inner and outer metatarsal tubercles distinct, not contacting each other, inner tubercle larger. Dorsal and ventral surfaces of body glandular, with rounded shallow warts; laterally the warts are slightly larger and white.

Measurements.—SUL = 30.4; TL = 10.5; ED = 3.3; TD = 1.1; ETD = 1.3; ND = 1.8; NED =2.3; HW = 7.0; LF3 = 4.1; LT4 = 5.3, TSL = 7.8; HL = 8.7; NLD =1.1; WDF3 = 1.1; WDTF3 = 1.0; IOD = 5.4.

Coloration.—In life, the holotype was brown with irregular dark brown marbling and a thin cream middorsal line; warts on the lateral surfaces of body were clearly white. Ventral surface was cream with brown marbling on edges. In preservative, the overall coloration is similar to that in life; however the warts on side of body are less pronounced, both relative to their color and their size.

Variation.—Females are larger than males, but otherwise all specimens examined are morphologically very similar to the holotype. However, in some individuals, the first and second toes are subequal in size instead of having a smaller first toe. Tympanum diameter is always at least equal to the distance from the tympanum to posterior edge of the eye; however, this distance may be larger than the tympanum diameter itself. Tympanum is partially hidden in some individuals. Warts on the edge of the eyelid tend to be more pronounced in larger specimens; however, this is difficult to detect in old museum specimens. Coloration is mainly uniform, however some specimens may also have three to four dark bands across the back. The middorsal stripe may be poorly defined. The extent of dark marbling varies slightly on the ventral regions.

Advertisement Call.—Calls of *C. kisiwamsitu* were recorded between 12 and 16 March 2000, at Mazumbai Reserve, Tanzania, by RdS and AC, between 2000 and 2300 h, with air temperatures ranging from 17–20°C. Call rate was determined for a three-minute recording period of individual USNM 556136 recorded on 15 March 2000, 2300 h, air temperature 20°C. Other call characteristics are based on analyses of 59 calls (six individuals).

The call is a long trill (Fig. 7), with average 13.3 notes per call (range 8–18), average call duration is 126 msec. There is an average of 5.44 pulses per note. The intensity of the dominant frequency averages 1.84 KHz; sometimes a second and third harmonic are present at about 3 and 5.5 KHz.

Phylogenetic Analyses.—Alignment of nine DNA-sequences resulted in a matrix of 760 unambiguously aligned characters, of which 541 were constant and 219 variable; of which 136 were informative under parsimony (only 1 gap present, alternative coding of this gap made no difference in resulting trees). Two non-brevicipitine microhylids were used as outgroups, *Hoplophryne* and *Phrynomantis*. An exhaustive search option using parsimony yielded five best trees (377 steps), a strict consensus tree is shown in Figure 8. Maximum Likelihood analyses (heuristic search using 10 random addition sequence replicates and TBR swapping method under a GTR + G model as suggested by Modeltest 3.04 [Posada and Crandall, 1998]), also agrees with the MP analysis in that *Callulina* forms a clade. Support for clades was measured with bootstrap proportions (Felsenstein, 1985; 1000 pseudoreplicates) and decay indices (Bremer, 1988) determined by enforcing a converse topological constraint. In summary, the tree demonstrates that *C. kisiwamsitu* forms a clade with *C. kreffti*, and furthermore that the two samples of *C. kisiwamsitu* form a well-supported clade (bootstrap proportions of 100% and a decay index 10). For further details of analyses, see Loader et al. (2004).

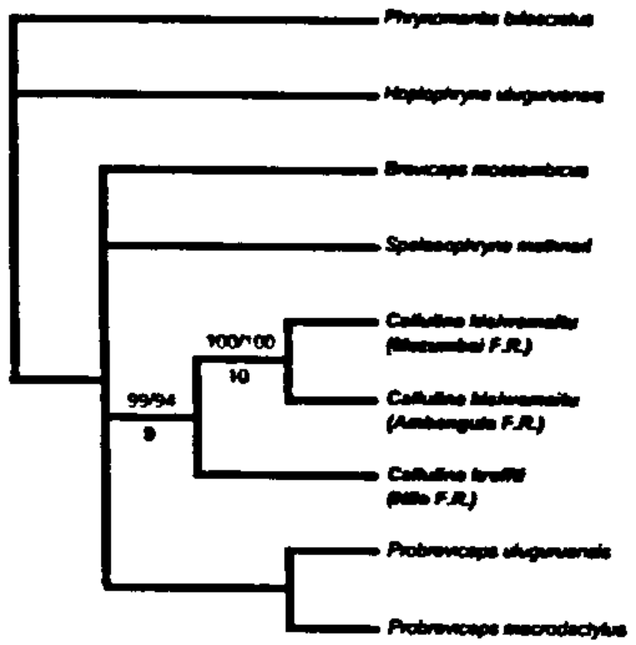

FIG. 8. Strict consensus tree of 760 base pairs of 12S and 16S mtDNA sequence data, using MP. Bootstrap proportions for both MP and ML analyses are shown above branches and decay index values are below.

Etymology.—The specific name derives from the Swahili *kisiwa* (island) and *msitu* (forest) and refers to the habitat of this species that is now just a remnant forest that once covered the West Usambara Mountains. The word is a noun in apposition.

Natural History.—Observations of calling behavior were noted. As the rainy season starts, males climb into low bushes and other vegetation where they call. It was often observed that calling males were positioned vertically on small trunks, from 0.5–2 m off the ground, and initially were mistaken as notches in the trunks. Sometimes they were also found calling at the junction of branches. Gut contents of one specimen consisted of relatively large arthropods (Hemiptera, Orthoptera, Diplopoda), and nematodes; parasitic nematodes were found in the lungs. Nothing else is known of the natural history of this species.

Distribution.—The new species is presently known from remnant forest patches on the West Usambara Mountains: Mazumbai FR, Ambangula FR, Shume-Mugambo FR, and Philipshof.

Remarks.—Molecular analyses of sequence data demonstrate that the two populations of the new species sampled are sister taxa to *C. kreffti*. Furthermore, the two samples of *C. kisiwamsitu* in the West Usambara Mountains (Ambangula FR and Mazumbai FR) are geographically more distant from each other (31.13 km apart), than samples from Mazumbai are to *C. kreffti* from Nilo, East Usambara (19.73 km apart). This suggests that the phylogenetic relationships do not appear to be the result of clinal variation among separated populations but perhaps could be indicative of the populations of the East and West Usambara mountains being specifically distinct. This assessment is consistent with the known differences in the amphibian assemblages of the East and West Usambara Mountains (Howell, 1993). The recognition of another distinct species in this area is therefore not unsurprising.

Overall the current morphological, behavioral (calls) and molecular data support the recognition of the disjunct populations of *Callulina* in the East and West Usambaras as separate species. We anticipate that other disjunct populations of *Callulina* throughout the Eastern Arc Mountains may also prove to be distinct species.

Acknowledgments.—We thank the Tanzanian Wildlife Division and Tanzania Commission for Science and Technology (COSTECH) for research permit 9-55-NA-99-40 to AC, 99-287-NA-99-66 to RdS, and RCA 2001-272 to SPL. J. Hanken and J. Rosado are thanked for permission to loan specimens from the Harvard MCZ collection. R. Günther allowed SPL access to herpetological collection at the Museum für Naturkunde, Berlin. J. Channing was a helpful and gracious companion during the fieldwork. AC was funded by the University of the Western Cape, the National Research Foundation of South Africa, the Wildlife Conservation Society of New York, the National Geographic Society, and the Royal Society of London. SPL is supported by NERC studentship NER/S/A/2000/03366 and a Systematic Association Grant. National Science Foundation Award 9815787 also provided partial support for this work to RdS. D. Bygott is thanked for sharing his Swahili expertise in the selection of species names. H. Müller is thanked for his translation of Nieden's 1910 paper. N. Cox is acknowledged for the information provided on geographical data in the Eastern Arc Mountains. K. Howell, J. Poynton, D. Gower, M. Wilkinson, and B. Clarke are thanked for advice and practical support.

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APPENDIX 1

Material Examined.—Callulina kisiwamsitu: BM 1982.591-2, Shume-Mugambo FR, 7000 ft. 4°40'S, 38°15'E. collected on 13 February 1981 by Kim Howell; BM 2002-45-46, Mazumbi FR, 4°48'45 4"S, 38°30'12.9"E, collected by Simon Loader and Jean Mariaux on 28 October 2001; BM 2002-47, Ambangula FR, 5°03'97 0"S, 38°24'63.0"E, collected by Simon Loader, Wilirk Ngalason and David Gower on 15 May 2002; USNM 556132-141, Tanga, Lushoto, Mazumbi FR, 4°48'45.4"S, 38°30'12.9"E, collected by Rafael O. de Sá and Allan Channing on 12–16 March 2000; MCZ A12632-33 Philipshof, West Usambara Mountains; *Callulina kreffti*: ZMB 21777–78 and 23341 Amani, East Usambara Mountains; MCZ A 13623–27, Amani, East Usambara Mountains; MCZ A 13628–29, Kizeru (Nilo FR), East Usambara Mountains; MCZ A 25490–93, Magrotto Hill, East Usambara Mountains; MCZ A 105871 and MCZ 107068, Magrotto Hill, East Usambara Mountains; USNM 200072, Magrotto Hill, East Usambara Mountains and USNM 226754 Amani, East Usambara Mountains; BM 2000-185, 187–188, Mtai FR, East Usambara Mountains; BM 2000-186, Kwamgumi FR, East Usambara Mountains, collected by Frontier; BM2000-189, 193–194, Segoma FR, East Usambara Mountains, collected by Frontier; BM 2000-190–192, 195–196, Amani, East Usambara Mountains, collected by Frontier.

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The caecilian amphibian *Scolecomorphus kirkii* Boulenger as prey of the burrowing asp *Atractaspis aterrima* Günther: trophic relationships of fossorial vertebrates

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Abstract

A report is given of an adult caecilian, *Scolecomorphus kirkii*, found in the gut of a specimen of the snake *Atractaspis aterrima* from the Udzungwa Mountains, Tanzania. Both predator and prey are largely fossorial in soil, and their ecology is poorly known, such that this is the first reported predator of any scolecomorphid caecilian. The caecilian was ingested head first and much of the flesh from the anterior of the specimen had been digested. The prey/predator mass ratio is 0.48. This value is substantially higher than reported for *A. aterrima* from West Africa, and refutes the notion that this species feeds only on small prey. Most reported predators of caecilians are snakes, and a brief review is presented.

Key words: Africa, Atractaspididae, diet, Gymnophiona, soil, Tanzania

Résumé

Rapport est donné du cas d'un cécilien adulte, *Scolecomorphus kirkii*, trouvé dans l'intestin d'un serpent *Atractaspis aterrima*, dans les Monts Udzungwa, en Tanzanie. Le prédateur et la proie sont en grande partie fouisseurs et on connaît mal leur écologie, à tel point que ceci est le premier cas rapporté de prédateur d'un cécilien scolécomorphe. Le cécilien a été absorbé la tête la première et une grande partie de la chair de l'avant de l'animal avait été digérée. Le rapport de masse proie/prédateur est de 0,48. Ce chiffre est nettement plus élevé que celui rapporté pour *A. aterrima* en Afrique de l'Ouest et contredit l'idée que cette espèce se nourrit de petites proies. La plupart des prédateurs des céciliens rapportés sont des serpents et on en présente une brève révision.

Introduction

Burrowing asps of the genus *Atractaspis* are venomous, fossorial snakes inhabiting rainforest, woodland, savanna, and semi-desert in mostly tropical Africa and the Arabian peninsula (e.g. Kochva, 2002). Despite their injurious (although rarely fatal, e.g. Spawls & Branch, 1995) potential for humans, very little is known about the natural history of the genus, perhaps because their fossorial lifestyle makes them relatively elusive (e.g. Akani *et al.*, 2001). Data on the diet of *Atractaspis* is scant. For example, Cansdale (1961: 68) wrote that 'It is hard to speak about the feeding habits of snakes which are so poorly known, but their diet is known to include skinks, Worm snakes, small rodents and shrews.' Diet and feeding in *Atractaspis* is of particular interest because of the uncertain phylogenetic position of the Atractaspididae, and an unusual dentition that is highly reduced apart from very long maxillary fangs (e.g. Reinhardt, 1843). This reduced dentition prevents the 'pterygoidwalk' mode of swallowing used by many other higher snakes. Instead, a mechanism convergent with that seen in alethinophidian snakes has evolved in *Atractaspis*. This is perhaps an adaptation to their fossorial habit (Deufel & Cundall, 2003).

Caecilian amphibians (order Gymnophiona) are an inadequately understood and relatively understudied component of tropical vertebrate faunas. As with *Atractaspis*, caecilians are fossorial, elongate and limbless, and their predator–prey relationships are inadequately known. There are few reports of predators of caecilians, and

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apparently none for the endemic African family Scolecomorphidae. Here we add to the meagre knowledge of diet in atractaspidid snakes and the predators of caecilians with a brief report of the discovery of a scolecomorphid caecilian in the gut of an *Atractaspis*.

Material, methods and results

The snake specimen is catalogued in the Zoological Museum of the University of Copenhagen, Denmark (ZMUC) as R68320. ZMUC R68320 was collected during 1997–1998 by residents of the Masisiwe area in the Udzungwa Mountains, Tanzania (08° 17′S, 35°54′E, *c.* 1840 m a.s.l.). Using the key of Broadley & Howell (1991), it is identified as *Atractaspis aterrima*. This identification is based on the single subcaudals and entire anal shield, the short frontal (almost as broad as long), the high number of ventrals (274), and the number of mid-body scale rows (23), a combination of features that is diagnostic for specimens of *A. aterrima* from the Eastern Arc region of East Africa. This species is further distributed westward from Uganda to Senegal. The total length (TL) of ZMUC R68320 is 522 mm, and the mass of the preserved specimen minus gut contents is 27.5 g. Other than the caecilian described below, the only other contents in the gut was a short (36 mm, preserved mass 0.5 g), autotomized section of the tail of an unidentified lizard. The snake is a female. It is in a fairly good condition, although it was beaten during capture so that its skin is ruptured in three places and the vertebral column is broken in one place.

The caecilian (ZMUC R0277, Fig. 1) removed from the gut of ZMUC R68320 is a mature female, based on examination of its gonads. It has a TL of 356 mm and preserved mass of 13.2 g. Identification of species of *Scolecomorphus* is largely determined by colour pattern (Nussbaum, 1985), and ZMUC R0277 is identified as *Scolecomorphus kirkii*. In addition to its colour pattern, the numbers of annuli (147) and vertebrae (158) are within the known range for females of this species (140–152 and 150–165, respectively; n = 23, Nussbaum, 1985).

Given its orientation in the snake's gut, the caecilian is interpreted as having been ingested head first. Its anterior end is heavily digested with most of the skin and other, outer soft tissue of the cranium and mandibles having been removed so that many of the bony elements are exposed. The eyes remain in place in the tentacular grooves. The skin is missing from the anterior of the body, up to the level of about the 24th annulus. Much of the flesh is also missing from this region, including the more superficial 'external muscular sheath' (Nussbaum & Naylor, 1982). Deeper muscle remains attached to the vertebrae so that only a very small amount of bone is directly visible. A similar pattern of loss of flesh from the head was reported by Barbour & Loveridge (1928) for an individual of the Tanzanian caeciliid *Boulengerula boulengeri* extracted from the gut of the elapid snake *Elapsoidea nigra*. The amount of flesh remaining intact on the anterior of ZMUC R0277 increases steadily up to the 24th annulus. From the 24th to about the 60th annulus, the body and skin are in good condition. From about the 60–117th annulus, the body wall is notably flaccid and there are substantial patches where the outer layer of skin is missing. This might be attributable to imperfect preservation, to the fact that the snake was damaged, or perhaps it instead indicates that the caecilian was bitten and possibly envenomated here by the *A. aterrima*. The remaining length of the specimen, from about the 118th annulus to the posterior terminus, is in good condition with no indication of any external damage. X-ray examination revealed no damage to any of the bones of the cranial, mandibular, or axial skeleton.

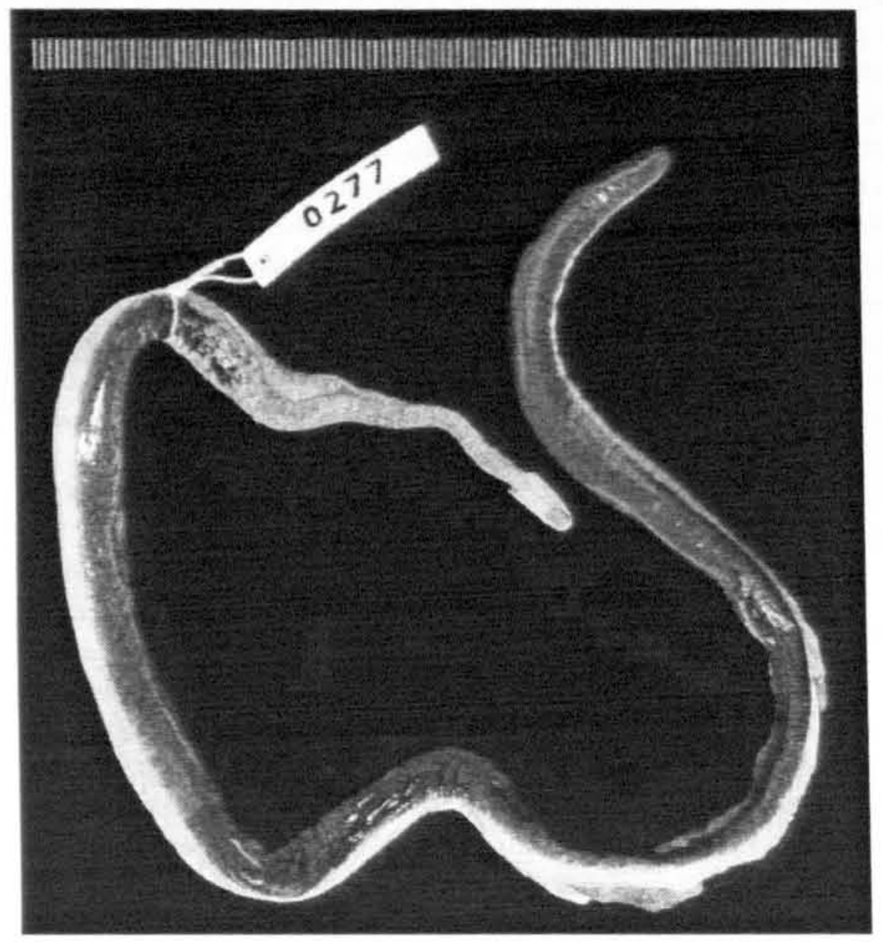

Fig 1 Dorsal view of a partially digested caecilian amphibian, *Scolecomorphus kirkii*, removed from the gut of a specimen of the atractaspidid snake *Atractaspis aterrima* from Tanzania. Scale bar in millimetres.

Discussion

Spawls *et al.* (2002): 439) stated that 'virtually nothing is known' of the natural history of *A. aterrima*, and this is, to the best of our knowledge, the first report of this genus feeding on caecilians. That *Atractaspis* feed on other highly fossorial, elongate lower vertebrates, at least occasionally, is demonstrated by reports of scolecophidian snakes (Barbour & Loveridge, 1928: 137; Loveridge, 1933: 279) and an amphisbaenian squamate (Loveridge, 1951: 202) in the gut of specimens from Uganda and Tanzania. Akani *et al.* (2001) reported that the gut and faeces contents of three individuals of *A. aterrima* from Nigeria were snakes (including the colubrid *Natriciteres* sp.) and forest skinks (*Panaspis* sp.). For at least four sympatric species of *Atractaspis*, Akani *et al.* reported an overall statistically significant positive relationship between predator size and prey size, and that 'although the sample size was small', the slopes of this relationship were significantly different among the species studied, with *A. aterrima* taking the proportionately smallest prey.

The predator mass : prey mass values given by Akani *et al.* (2001) were largely for fresh specimens, and the predator mass values included gut contents (L. Luiselli, pers. comm.). Assessing an accurate comparable ratio in the present case is problematic because mass was only measured for the preserved predator and prey, and both values will undoubtedly be lower than they were in life – the snake has a midventral incision along much of its length, and the caecilian is partly digested. Additionally, this study is based on a minimal sample, and the following, limited interpretation must be treated with care.

Prey/predator mass ratio (MR: equivalent to the weight ratio WR of Greene, 1983) have been calculated from the preserved masses. The greatest WR value for *A. aterrima* recorded by Akani *et al.* from their Nigerian sample was about 0.06 (Akani *et al.*'s Fig. 1). In the present Tanzanian case, MR = 0.32 (0.48 if predator mass is recorded minus gut contents), which is almost an order of magnitude greater, and about one and a half times the value of the greatest MR value for any of the species of *Atractaspis* reported by Akani *et al.* (2001) (Fig. 1). The MR value reported here for the Tanzanian specimens is, of course, associated with measurement error caveats, but it might be taken to indicate that *A. aterrima* does not necessarily tend 'to prey upon very small prey' (Akani *et al.*, 2001: 92).

Even including gut contents and allowing for a 10% decrease in mass upon preservation in alcohol (as reported for lizards by Colbert, 1967), the mass (*c.* 70 to >118 g) of three Nigerian specimens of *A. aterrima* given by Akani *et al.* (2001) (Fig. 1) is substantially greater than the mass of the Tanzanian specimen under consideration here, despite the latter being longer. This is also true for seven West African *A. aterrima* in the collections of The Natural History Museum, London (TL 279–645 mm; preserved mass 5.2–48.2 g). For ZMUC Tanzanian specimens of *A. aterrima*, the largest male has a TL of 660 mm and a preserved mass of 71 g (n = 3), and the largest female a TL of 670 mm and of mass 53 g (n = 3). The two heaviest *A. aterrima* reported by Akani *et al.* were females that were gravid, but their *c.* 70 g specimen was not (L. Luiselli, pers. comm.), and the apparent discrepancy between the two studies is not fully explained by reduction in mass caused by preservation. The relationship between mass and length in fresh and preserved *Atractaspis* requires further investigation.

If there is truly a great variation in relative prey size within *A. aterrima*, then this might be attributable to a

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large number of factors, potentially including geography and the presence of sympatric species of *Atractaspis*. Simpler hypotheses are also worth considering, and it might be noted that 'feeding both on small and large prey is common for most snakes' (Marques, 1996). Furthermore, it is known that some snakes can change their diet under various circumstances (e.g. de Queiroz *et al.*, 2001), so that the proportionately very small size of the prey taken by the two largest *A. aterrima* reported by Akani *et al.* (2001), might conceivably be related to their gravidity. Clearly, more data are needed for a satisfactory assessment of diet in *Atractaspis*.

As far as we are aware, this is the first report of any predator of the African endemic Scolecomorphidae. In life, *S. kirkii* have a striking colour pattern comprising a very dark dorsum and a strongly contrasting (Fig. 1), pinkish venter. Nussbaum (1998: 54) has suggested that the bright colouration of some caecilians might be aposematic, associated with possible skin toxins. It might be speculated that the striking colour pattern of *S. kirkii* is, at least in part, an adaptation to avoid predation, but *A. aterrima* do not seem to be deterred by this and/or any toxins that this caecilian might produce.

Species of *Scolecomorphus* are known only from Malawi and Tanzania in East Africa, where they inhabit rainforest of the highlands, including the Udzungwa, Uluguru, Usambara and Pare Mountains of the Eastern Arc (Nussbaum, 1985). Caecilians other than *Scolecomorphus* are known from some of the western part of the range of *A. aterrima*, but apparently the only report of their predators is of the fossorial, venomous atractaspidid *Polemon acanthias* preying on the caecilid *Geotrypetes seraphini* in Ghana (Cole, 1967).

As with many aspects of the biology of caecilians, knowledge of their predators is inadequate. Taylor (1968: 393) suggested that for terrestrial caecilians 'doubtless snakes and carnivorous birds are the most active predators'. For snakes, this is supported in that they are the taxa in the vast majority of literature reports of caecilian predators. These reports include preying on the caecilid *Caecilia intermedia* and an unspecified caecilian by the fossorial/cryptic, nonvenomous colubrid *Ninia atrata* and the venomous elapid *Micrurus ancoralis ancoralis*, respectively, in Colombia (Boulenger, 1913); the semi-aquatic typhlonectid *Chthonerpeton indistinctum* by the aquatic, diurnal aglyphous colubrid *Sordellina punctata* in Brazil (Procter, 1923); several individuals of the caecilid *B. boulengeri* by the mostly nocturnal, fossorial/cryptic and venomous elapid *E. nigra* in Tanzania (Barbour & Loveridge, 1928: 182; Jakobsen, 1997: 65); the caeciliid *Siphonops annulatus* by the largely nocturnal, venomous colubrid *Clelia clelia* (Sawaya, 1937) and *Siphonops* sp. by the nocturnal, venomous elapid *Micrurus corallinus* (Marques & Sazima, 1997) and the venomous *M. decoratus* (Marques, 2002) in Brazil; the caeciliid *Caecilia gracilis* by the fossorial, nonvenomous aniliid *Anilius scytale* in Surinam (Taylor, 1968: 34; Nussbaum & Hoogmoed, 1979); the caeciliid *Gymnopis multiplicata* by the venomous elapid *Micrurus multifasciatus hertwigi* (Burger, 1997) in Costa Rica; the caeciliids *Microcaecilia* sp. and *Oscaecilia* sp. by the venomous elapid *Micrurus lemniscatus* in Brazilian Amazonia (Martins & Oliveira, 1998); the ichthyophiid *Ichthyophis* sp. by the nocturnal, venomous elapid *Bungarus candidus* (Grossmann & Schäfer, 2000) and the fossorial, nonvenomous cylindrophiid *Cylindrophis ruffus* in Thailand (Greene, 1983; Kupfer *et al.*, 2003). Neotropical coral snakes of the venomous elapid genus *Micrurus* are clearly the most commonly reported snakes preying on caecilians. Rose (1996: 60) reports that among nine species of *Micrurus* known to prey on caecilians, *M. m. mipartitus* from Panama and Colombia, and *M. bocourti* from Ecuador and Peru, are 'known to feed only on caecilians'. Marques & Sazima (1997) found that although caecilians are a significant prey item of species of *Micrurus*, their most common prey are amphisbaenian fossorial reptiles.

Some, but not all, of the records of snakes preying on caecilians report the headfirst ingestion of prey. Marques & Sazima (1997) suggest that instances of tail-first ingestion of fossorial vertebrates by elapid coral snakes are associated with underground feeding. Where reported snake predators are not fossorial or semi-fossorial, they are often nocturnal and/or the caecilian has been found being ingested while moving on the surface (e.g. Burger, 1997; Grossmann & Schafer, 2000; Kupfer *et al.*, 2003). However, the sample size is currently very small and more data are required before any firm general conclusions can be drawn.

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Herpetofauna of Mahenge Mountain, Tanzania: a window on African biogeography

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Mahenge Mountain is part of the Eastern Arc Mountains in Tanzania. It is bordered by the Kilombero flood plain to the west and forms a continuous mountain block with the Mbarika to the south, lying close to the Udzungwa and Southern Highland Mountain blocks. The amphibian fauna of the forests of Mahenge Mountain has not been investigated until recent fieldwork carried out in the Sali Forest Reserve that has revealed a diverse forest fauna. In this study new records are reported, details of habitats are given and a checklist of the amphibians and reptiles of Mahenge Mountain are provided. In addition, biogeographical analyses were carried out to investigate patterns of spatial turnover in species. This was achieved by calculating the standard similarity index between the amphibian assemblages of the highland Mahenge Mountains (>850 m) and the lowland Kilombero floodplain (<400 m). The results show significant differences (62%) between the two areas, reflecting large altitudinal turnover in species between the widespread lowland fauna and the montane fauna. This is further emphasized by a comparison of transects in lowland Mozambique and highland Zimbabwe, which has a comparable similarity index measure of 54%, impressive when comparing the difference in distance and altitude between the two transects. The reasons for this marked lowland–highland differentiation in Tanzania are poorly understood. Concern is raised for the preservation of this highland endemic fauna in the light of continuing habitat destruction.

Key words:

INTRODUCTION

Mahenge Mountain is part of a chain of East African mountain blocks, the Eastern Arc (Fig. 1). The Arc is notable for a high degree of plant and animal diversity (Clarke 1988; Howell 1993; Lovett 1993; Burgess *et al.* 1998; Newmark 2002); it is one of the world's biodiversity hotspots (Myers 2000). Yet many habitats still remain poorly sampled, hampering an understanding of the biogeography and evolution of the area (Schiøtz 1981; Burgess 1998). This paper reviews what is known of the Mahenge Mountain herpetofauna, and reports new records from a small patch of forest that still survives, the Sali Forest Reserve at 08°54–57'S, 36°37–41'E. The Mahenge fauna is compared with the fauna of the Kilombero floodplain, at the foot of Mahenge Mountain, to highlight features of biogeographical and conservation importance.

Mahenge Mountain forms the tip of a spur, the Mbarika Mountains, which are attached to the great mountain complex situated north of Lake Nyassa. The spur diverges from the northern Udzungwa range of this complex, separated by a broad floodplain of the Kilombero River. This plain, whose amphibian fauna was discussed by Hinde *et al.* (2001), lies at an altitude of about 300–400 m a.s.l. The Mbarika mountain chain borders this plain on the east; Mahenge Mountain, the highest peak in the range, reaches a height of 1400 m at 08°51'S, 36°41'E (Muhulu Forest Reserve).

Botanically the area is 'hardly explored [and] in urgent need of biological inventory' (Rodgers 1993: 330). During the 1960s it was a base of A. Rees, who was then in the Tanzanian Game Division, and who made an important collection of amphibians which was included in more general papers by Poynton (1977, 1991, 2003). Virtually no habitat data were provided by Rees (1964). In October 2001, S.P.L. and J.M. made a collection in the Sali Forest Reserve, concentrating on amphibians and chamaeleons. No other reptiles were collected apart from a few specimens taken opportunistically.

The forest reserve is 1890 hectares in extent, with altitudes ranging from 1050–1300 m. It contains submontane forest and montane grassland, but it has been subject to cultivation, pit sawing, and extensive damage (Lovett & Pocs 1993). Night and day collections were carried out over a three-day intensive survey period during which there were no rains. Drift fences were constructed in the forest to collect leaf-litter amphibians. Digging and searching through leaf litter and forest was also carried out. Specimens were euthanased using MS222 or ether, and preserved in buffered formalin. Tissue samples for DNA analysis taken for all specimens and stored in ethanol. In addition, all specimens were searched for external and internal parasites. These were removed and preserved separately for subsequent studies.

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Fig. 1. Mountains of Eastern Arc and outlying mountain blocks (modified from Wasser & Lovett 1993).

LIST OF THE MAHENGE HERPETOFAUNA

Data are provided below for material recently collected, and new to the Mahenge herpetofauna. Comments on the ecology and distribution of these taxa are also provided. Note however that habitat data are lacking for specimens collected by Rees. Taxa found during this study that were already known from the area are only mentioned in the checklist.

Material has been deposited in the Natural History Museum London (field tag prefix MW) and the Museum d'Histoire Naturelle, Geneve (field tag prefix TZ, museum accession numbers (where available) with prefix MHNG).

CLASS AMPHIBIA

ORDER GYMNOPHIONA

Family Scolecomorphidae

Genus *Scolecomorphus*

Scolecomorphus kirkii Boulenger, 1883

Habitat. Found on the edge of Sali Forest Reserve at 900 m, in a banana plantation.

Previous records. Malawi: Thyolo/Cholo mountains, Zomba Plateau, Shire Highlands and Namadzi. Tanzania: Southern Highlands, Udzungwa, Uluguru, Nguru and Rubeho moun-

tains (Poynton & Broadley 1985; Nussbaum 1985; Emmrich 1994). MW 01842, MW 01846.

Remarks. In local ki-pogoro language referred to as 'Namtokosa', which is a reference to its snake-like movement.

ORDER ANURA

Family Bufonidae

Genus *Nectophrynoides*

Nectophrynoides tornieri (Roux, 1906)

Habitat. Found in a gully/swamp near Gongo Peak, Sali Forest Reserve, at 1000 m in undisturbed submontane forest. Usually found in submontane/montane forest but also found in disturbed habitats, often in banana leaf axils.

Previous records. East Usambara, Magrotto Hill, Nguru, Uluguru, Ukaguru and Udzungwa mountains (Poynton, unpubl.). MW 01823, MW 01824.

Family Ranidae

Genus *Arthroleptides*

Arthroleptides yakusini Channing, Moyer & Howell, 2002

Habitat. Found clinging to rock face on the River/Stream 'Gongo' at 960 m in Sali Forest Reserve. Surrounded by pristine submontane forest, with relatively few disturbed patches. Associated with undisturbed forest and streams.

Previous records. Referable to a new species found in the Uluguru and Udzungwa mountains (Channing *et al.* 2002). MW 01844, MW 01852, MW 01854, TZ 360 (MHNG 2620.086), TZ362 (MHNG 2620.088).

Family Hyperoliidae

Genus *Leptopelis*

Leptopelis vermiculatus (Boulenger, 1897)

Habitat. Found in a gully/swamp near to Gongo Peak in Sali Forest Reserve at 1000 m in pristine submontane forest. Associated with submontane/montane forest.

Previous records. East and West Usambara, Ukaguru, Udzungwa, Uluguru and Rungwe mountains (Schiøtz 1999; pers. comm.). MW 01830, MW 01832, TZ 363 (MHNG 2620.094).

Genus *Afrixalus*

Afrixalus fornasini (Bianconi, 1849)

Habitat. Found on the edge of Sali Forest Reserve at 900 m in a banana plantation. Associated with 'farmbush' fauna.

Previous records. Widely distributed in Tanzania; in southeastern Tanzania found at Kilwa, Kisaye, Kitaya, Kugota, Liwale, Tunduru (Poynton 1991). Outside Tanzania found in lowlands from south of Durban (KwaZulu-Natal) to Kenya (Poynton & Broadley 1987). MW 01828, TZ 355 (MHNG 2620.072), TZ 359 (MHNG 2620.076).

Afrixalus uluguruensis (Barbour & Loveridge, 1928)

Habitat. Found in a gully/swamp close to Gongo Peak at 1000 m in pristine submontane forest. Associated with evergreen forest, and edges of forest. Often found in the leaf axils of banana trees.

Previous records. East and West Usambara, Udzungwa and Uluguru mountains (Schiøtz 1999; pers. comm.). MW 01834, MW 01836, MW 01838, MW 01840, TZ 364 (MHNG 2620.077), TZ 366 (MHNG 2620.079).

CLASS REPTILIA

ORDER SQUAMATA

SUBORDER SAURIA

Family Gekkonidae

Genus *Cnemaspis*

Cnemaspis africana (Werner, 1895)

Habitat. Found lying on a leaf at night, about 0.5 m off the ground in submontane forest, around 900 m. Often found in woodland and hill forest, from sea level to about 2000 m; thought to be forest dependent (Sprawls *et al.* 2002).

Previous records. Eastern Arc Mountains, and Shimba Hills, Kenya, and Mt Kilimanjaro. TZ351 (MHNG 2641.084); TZ 352 (MHNG 2641.085).

Family Chamaeleonidae

Genus *Chamaeleo*

Chamaeleo melleri (Gray, 1864)

Habitat. Found close to forest in dry woodland. Usually found in wooded savanna and woodland at low altitude, sea level to 1200 m (Sprawls *et al.* 2002).

Previous records. Tanzania, northern Mozambique and Malawi. TZ353 (MHNG 2620 063)

Chamaeleo dilepis Leach, 1819

Habitat. Found on edge of Sali Forest Reserve, associated with coastal forest, woodland, thicket and both moist and dry savanna, from sea level to about 1500 m (Sprawls *et al* 2002).

Previous records. Widely distributed in Tanzania,

found also in Rwanda, Burundi, Kenya, Zaire and South Africa,. TZ 354 (MHNG 2620.049), TZ 378 (MHNG 2620.051), TZ 379 (MHNG 2620.052).

Rhampholeon brevicaudatus (Matschie, 1892)

Habitat. Found in submontane forest around 850–900 m in Sali Forest Reserve. Found elsewhere in evergreen forest and coastal thicket from sea level to 1300 m (Sprawls *et al.* 2002).

Previous records. Eastern Arc Mountains, Shimba Hills, southeastern Kenya, Rondo plateau and Masasi (Sprawls *et al.* 2002) TZ 346, TZ 350, TZ 374.

Rhampholeon cf. *moyeri*

Habitat. Found in submontane forest around 850–900 m in Sali Forest Reserve. Found in submontane evergreen forests.

Previous records. Udzungwa Scarp Forest, 1200–2000 m (Menegon, *et al.* 2002) TZ 343, TZ 345.

SUBORDER SERPENTES

Family Typhlopidae

Genus *Rhinotyphlops*

Rhinotyphlops mucruso (Peters, 1854)

Habitat. Found alive on Mahenge-Ifikara Road (8°28'0.3"S, 36°41'20.7"E, 333 m). Inhabits miombo woodland and coastal mosaic vegetation.

Previous records. Coastal Tanzania and some inland localities: Lake Victoria, Shinyanga Region, Tabora, Singida, Iringa, Rovuma and Rukwa regions. Coastal Kenya, Mozambique, Malawi, Zambia, Botswana and Namibia (Sprawls *et al.* 2002). TZ 377 (MHNG 2635.070).

REVISED CHECKLIST OF AMPHIBIANS AND REPTILES FROM MAHENGE MOUNTAIN

ORDER GYMNOPHIONA

Family Scolecomorphidae
[1,2]*Scolecomorphus kirkii*

ORDER ANURA

Family Bufonidae
Bufo gutturalis
Bufo maculatus
Stephopaedes loveridgei
[1]*Nectophrynoides tornieri*

Family Microhylidae
Breviceps mossambicus
Phrynomerus bifasciatus bifasciatus
Spelaeophryne methneri

Family Hemisotidae
[2]*Hemisus marmoratum*

Family Arthroleptidae
Arthroleptis stenodactylus
Arthroleptis xenodactyloides

Family Ranidae
[1]*Arthroleptides yakusini*
Pyxicephalus edulis
Rana angolensis
Ptychadena anchietae
Phrynobatrachus acridoides
Phrynobatrachus natalensis

Family Rhacophoridae
Chiromantis xerampelina

Family Hyperoliidae
Leptopelis flavomaculatus
[1]*Leptopelis vermiculatus*
Kassina senegalensis
Afrixalus brachycnemis
[1]*Afrixalus fornasini*
[1]*Afrixalus uluguruensis*
Hyperolius tuberilinguis
Hyperolius puncticulatus
Hyperolius mitchelli
Hyperolius nasutus

SUBORDER SAURIA

Family Gekkonidae
[1]*Cnemaspis africana*

Family Chamaeleonidae
[1]*Chamaeleo melleri*
[1]*Chamaeleo dilepis*
[1]*Rhampholeon brevicaudatus*
[1]*Rhampholeon* cf. *moyeri*

SUBORDER SERPENTES

Family Typhlopidae
[1]*Rhinotyphlops mucrosa*

Family Colubridae
[4]*Dasypeltis medici*

[1]Additions to previous lists made by this study
[2]Species in bold collected in Sali Forest Reserve during this study.
[3]Additions made by Hinde et al. (2001) to previous lists.
[4]D.G. Broadley (pers. comm.)

DISCUSSION

The Mahenge fauna described here was collected above an altitude of 850 m. A comparison with the amphibian fauna of the Kilombero Valley, below an altitude of 400 m, shows a difference in species composition. The following species recorded from

this lowland area, based on data gained from the Rees and the Frontier-Tanzania collections in Institution. City (unpubl.). Specific determination is still in doubt in some cases.

Afrixalus fornasini, *Afrixalus* sp. A. *Afrixalus sp.* Poynton & Broadley (1987), *Arthroleptis stenodactylus, Arthroleptis xenodactyloides, Bufo gutturalis, B. maculatus, B. reesi, Chiromantis xerampelina, Hemisus marmoratus, Hildebrandtia ornata, Hylarana galamensis, Hyperolius mitchelli, H. tuberilinguis, Hyperolius reesi, H. viridiflavus, Hyperolius* sp., *Kassina senegalensis, Leptopelis argenteus, L. flavomaculatus, Phrynobatrachus acridoides, P. natalensis, P. mababiensis, Phrynobatrachus* sp, *Phrynomantis bifasciatus, Ptychadena anchietae, P. mascareniensis, P. mossambica, Pyxicephalus edulis, Rana angolensis, Speleophryne methneri, Stephopaedes loveridgei, Xenopus muelleri.*

Of these 33 species, 19 are recorded also from the Mahenge uplands. A comparison of the Kilombero and Mahenge amphibian faunas can be made using a standard similarity index:

$$\frac{2 \times \text{number of taxa common to both areas}}{\text{sum of totals of taxa both areas}}$$

expressed as a percentage (Poynton & Boycott 1996). A value of 62% is obtained. It is noteworthy that a comparison of the fauna of the eastern Zimbabwe highlands and the Mozambique coast gave a value of 54% (Poynton & Broadley 1991). In view of the marked environmental contrast between the Zimbabwean and Mozambican localities, as well as the distance between them, the degree of species spatial turnover between the Kilombero Valley and Mahenge Mountain – a mere 450 m in altitude and less than 100 km – is impressive.

Nevertheless, this result is consistent with the finding of greater taxonomic differentiation between highland and lowland amphibian faunas in Tanzania compared with areas to the south (Poynton 2003). A reason for this may be found in the greater taxonomic richness in Tanzania, but the fundamental reason for the marked lowland–highland differentiation in the African amphibian fauna is poorly understood (Poynton 1998, 2000). The environmental variable that seems most broadly associated with the differentiation is temperature, but exactly how this impinges on the life of amphibians has not yet been clarified (Poynton 1998). In the lowland-to-highland transition of the Kilombero-Mahenge area, the differentiation is so focused that it suggests that this area is a particularly fruitful zone for close biogeographical study.

No endemics have been found in the Mahenge amphibian fauna; the highland element in this fauna consists of species that are widespread on the southern Tanzanian mountain ranges, if not also on the more northern Uluguru and Usambara ranges. The Mbarika range has evidently linked the Mahenge area, with the main montane mass in southern Tanzania, despite a gap at the head of the Kilombero Valley, but it is not known whether faunal links still exist, because the range has not been surveyed. Collections of butterflies (Keilland 1990) in the forest patches of the Mbarika range, suggest that there are likely to be faunal links between amphibians in these areas. Butterfly species with an affinity to the montane mass of the Udzungwa and Southern Highlands were collected, as well as new 'races', suggesting a substantial period of isolation in the Mbarika range (Keilland 1990). There must, however, be concern about the future prospects of the highland fauna in the face of continuing habitat destruction. The area, as noted earlier, has been subjected to damaging exploitation (Lovett & Pocs 1993). The study of Hinde *et al.* (2001) showed that in the valley, the response of the amphibian fauna to disturbance is complex, as it is elsewhere in Africa (Poynton 1998). The Kilombero Valley and adjoining Mahenge highland seem to be promising zones for further investigation along the lines of Hinde *et al.* (2001). In light of the characteristic montane grassland fauna of the Udzungwa mountains (Poynton 2003), a survey of the grassland areas as well as montane forest in Mahenge is necessary.

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Original article

Phylogenetic relationships of African caecilians (Amphibia: Gymnophiona): insights from mitochondrial rRNA gene sequences

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Abstract.—Africa (excluding the Seychelles) has a diverse caecilian fauna, including the endemic family Scolecomorphidae and six endemic genera of the more cosmopolitan Caeciliidae. Previous molecular phylogenetic studies have not included any caecilians from the African mainland. Partial 12S and 16S mitochondrial gene sequences were obtained for two species of the endemic African Scolecomorphidae and five species and four genera of African caeciliids, aligned against previously reported sequences for 16 caecilian species, and analysed using parsimony, maximum likelihood, Bayesian and distance methods. Results are in agreement with traditional taxonomy in providing support for the monophyly of the African caeciliid genera *Boulengerula* and *Schistometopum*, and for the Scolecomorphidae. They disagree in indicating that the Caeciliidae is paraphyletic with respect to the Scolecomorphidae. Although more data from morphology and/or molecules will be required to resolve details of the interrelationships of the African caecilian genera, the data provide strong support for at least two origins of caecilians in which the eye is reduced and covered with bone, and do not support the hypotheses that the caecilian assemblages of Africa, and of East and of West Africa are monophyletic.

Key words—Amphibians, biogeography, evolution, eyes, phylogeny, vertebrates, viviparity

Caecilians (Gymnophiona) are one of the three extant orders of amphibians. The caecilian fauna of Africa, taken as excluding the Seychelles, includes the endemic family Scolecomorphidae (six species in two genera) and six genera and 16 species of the more cosmopolitan Caeciliidae, which also has representatives in the Seychelles, India, and Central and South America. African caecilians make up approximately 13% and 25% of the recognised caecilian species and genera respectively, and thus constitute a substantial proportion of known gymnophionan diversity. Previous molecular phylogenetic analyses (Hedges *et al.* 1993; Gower *et al.* 2002; Wilkinson *et al.* 2002) have included, at most, only a single African caecilian, the insular caeciliid *Schistometopum thomense*. Apart from an unconfirmed report from Central Africa (Nussbaum & Pfrender 1998), this caeciliid is known only from Sao Thome in the Gulf of Guinea. Thus we have no molecular phylogenetic insight into the relationships of any mainland African caecilians. Of the six currently recognised caecilian families (Nussbaum & Wilkinson 1989) only the Scolecomorphidae remains unstudied with regards to molecular data.

Table 1. Voucher specimens deposited in the collections of the Department of Zoology, Natural History Museum, London (BMNH) and the National Museums of Kenya, Nairobi (NMK).

Taxon	Voucher	Provenance
Boulengerula boulengeri	BMNH 2002.95	East Africa, Tanzania, East Usambara, Amani
Boulengerula taitanus	NMK A/3112*	East Africa, Kenya, Taita, Wundanyi
Geotrypetes seraphini	BMNH 2002.96	West Africa, Cameroon (pet trade)
Herpele squalostoma	BMNH 2002.97	West Africa, Cameroon (pet trade)
Schistometopum gregorii	BMNH 2002.98	East Africa, Tanzania, Bagamoyo
Scolecomorphus uluguruensis	BMNH 2002.99	East Africa, Tanzania, Uluguru, Uluguru North
Scolecomorphus vittatus	BMNH 2002.100	East Africa, Tanzania, East Usambara, Amani

*Field tag MW 512

Building on the foundations provided by Hedges *et al.* (1993), Wilkinson *et al.* (2002) used partial 12S and 16S SSU mt DNA sequence data to provide well supported resolution of the phylogenetic relationships of representatives of the three families of caecilians present in India, and suggested that expanding the sampling of African caecilians was a priority for caecilian molecular phylogenetics.

Here we report new 12S and 16S SSU rDNA partial sequences for seven species of African caecilians, including the first sequences for representatives of the Scolecomorphidae, and the first sequences for any East African caeciliid. At the generic level, our sampling of African caecilians is incomplete only in the omission of the monotypic caeciliids *Sylvacaecilia* from Ethiopia and *Idiocranium* from Cameroon, and of the West African scolecomorphid *Crotaphatrema* (three species). The new sequences increase the diversity of caecilians for which these comparative mitochondrial sequence data are available, from 16 to 23 of the approximately 160 currently recognised caecilian species, and from 11 to 15 of the 33 genera. The new sequences allow the first molecular tests of the monophyly of *Scolecomorphus* and the Scolecomorphidae, of *Boulengerula* and of *Schistometopum*, and investigation of the relationships of the caeciliid assemblages of East and West Africa to each other and a range of non-African caecilians.

MATERIALS AND METHODS

Details of voucher specimens are presented in Table 1. Sequencing methods are as given in Wilkinson *et al.* (2002). Sequences have been deposited in GenBank (Benson *et al.* 1998) with accession numbers A7450612 - A7450625.

The newly determined sequences were added to an alignment of concatenated partial 12S and 16S caecilian sequences (Wilkinson *et al.* 2002) and the alignment adjusted manually. Regions in which positional homology could not be assessed with confidence due to length variation were excluded, except where length variation was concentrated in a minority of taxa. In the latter case, regions of uncertainty in the alignment were represented by replacing the sequence data for the minority of taxa with missing entries. This increases the available data for the remaining majority of taxa and reduces the amount of useful information that is discarded. Following Wilkinson *et al.* (2002), the sequence of the rhinatrematid caecilian *Epicrionops marmoratus* was designated as a single outgroup and used to root trees.

Parsimony, maximum likelihood (ML) and distance analyses were performed with PAUP* 4.0b10 (Swofford 1998). LogDet and Maximum Likelihood distance (MLD) analyses used the minimum evolution objective function. ML and MLD analyses used models

of evolution selected by Modeltest (Posada & Crandall 1998) and the corresponding estimated proportion of invariant sites was used in the LogDet analyses. Alignment gaps were treated as missing data. Tree searches were heuristic with 100 (parsimony and distance analyses) or 10 (ML) random addition sequences and TBR branch swapping. A Bayesian analysis was performed using MrBayes 2.01 (Huelsenbeck & Ronquist 2001) using a general time reversible (GTR) model, with rate variation across sites modelled with a discrete gamma distribution (G) and proportion of invariant sites (I). The Metropolis coupled, Markov chain Monte Carlo analysis was run with four chains for 1,000.000 generations. Trees were sampled every 1000 generations, with the first 1000 generations discarded as "burn in".

A parsimony PTP test (Faith & Cranston 1991) was used to test the null hypothesis that the alignment has no more hierarchical structure than expected by chance alone (99 random permutations). Support for clades was measured with bootstrap proportions (Felsenstein 1985) (100 pseudoreplicates) and Bayesian posterior probabilities. Leaf stabilities based on the bootstrap difference measure (Thorley & Wilkinson 1999) were determined using RadCon (Thorley & Page 2000) from sets of bootstrap trees. For these measures, trees were treated as unrooted to allow the stability of the rooting on *Epicrionops marmoratus* to be assessed (Wilkinson *et al.* 2002). Relative rates tests were performed using the program RRTree (Robinson *et al.* 1998). Suboptimal ML trees, conforming to various *a priori* hypotheses, were found through searches enforcing user-defined topological constraints.

Differences between optimal and suboptimal ML trees were assessed using the Kishino-Hasegawa (KH) test (Kishino & Hasegawa 1989) using RELL with 1000 bootstrap replicates. The KH test is biased when, as here, the trees are not selected *a priori* because we are more likely to wrongly reject the null hypothesis than we would like at our selected Type I error rate, with the strength of this liberal bias unknown. Thus, whereas failing the test does allow us to accept the null hypothesis, passing the test does not fully justify rejecting the null hypothesis (Goldman *et al.* 2000). Here a significant result is taken to support the tentative rejection of the null hypothesis, and we used the conservative two-tailed version of the KH test to compensate to some uncertain extent for the liberal bias due to inappropriate tree selection. Although the Shimodaira-Hasegawa test (Shimodaira & Hasegawa 1999) is unbiased, it requires that all plausible trees are included. The identification of this set is problematic for trees with more than only a few taxa, and we have not used this test here.

RESULTS

All PCR amplifications from genomic DNA yielded products of the expected size, which, on sequencing, contained negligible levels of site ambiguity. Some taxa are relatively unstable in the phylogenetic analyses (see below) but there is no obvious reason to suspect that any of the data could have been derived from nuclear copies of mitochondrial sequences.

After incorporation of the new sequences and the exclusion of regions that could not be aligned with confidence, the alignment comprised 914 sites. Of these, 448 were invariant and 123 were parsimony uninformative, leaving 343 parsimony informative sites. There is no significant variation in base composition across the alignment as a whole (χ^2 tests for homogeneity, $P = 0.858$, d.f. = 66). In contrast, extensive and significant ($P = 0.001$) variation in base composition is evident in the variable sites. Remarkably, if sequences are ranked by their combined GC content, the eight African taxa have the eight highest GC contents (Table 2).

Table 2. Base composition and leaf stabilities. Sequences are ranked according to the proportion of guanine and cytosine in the variable sites included in the alignment (GC). Leaf stabilities are from parsimony (MP) and distance (MLD) bootstrap analyses.

	GC	MP	MLD
Epicrionops marmoratus	0.3231	0.6915	0.7597
Ichthyophis tricolor	0.3297	0.7386	0.8122
Ichthyophis bannanicus	0.3414	0.7386	0.8122
Uraeotyphlus sp.	0.3480	0.738	0.8122
Typhlonectes natans	0.3489	0.6271	0.7444
Caecilia sp.	0.3575	0.6273	0.7458
Siphonops annulatus	0.3616	0.5604	0.6171
Hypogeophis rostratus	0.3742	0.7608	0.8322
Praslinia cooperi	0.3767	0.8048	0.8762
Grandisonia alternans	0.3995	0.7672	0.8396
Average	0.4005	0.6949	0.78
Grandisonia brevis	0.4062	0.7778	0.8482
Grandisonia larvata	0.4093	0.7643	0.839
Grandisonia seychellensis	0.4100	0.7644	0.8339
Gegeneophis ramaswamii	0.4150	0.7604	0.8352
Dermophis mexicanus	0.4158	0.7051	0.8297
Schistometopum thomense	0.4260	0.7166	0.8301
Schistometopum gregorii	0.4318	0.7166	0.8301
Boulengerula boulengeri	0.4427	0.6558	0.7648
Boulengerula taitanus	0.4439	0.654	0.7642
Herpele squalostoma	0.4538	0.6284	0.7351
Geotrypetes seraphini	0.4560	0.5476	0.6042
Scolecomorphus vittatus	0.4593	0.6182	0.6869
Scolecomorphus uluguruensis	0.4820	0.6182	0.6869

Transition - transversion ratios, based on uncorrected pairwise differences, range from 0.79 to 4.0 (Fig. 1). The many very low ratios occuring in taxa with high total pairwise differences suggest that saturation and/or lineage specific relative rate variation may be a problem in this data set. The four lowest transition - transversion ratios, and nine of the 18 ratios that are less than one, involve the African caciliid *Geotrypetes seraphini* (Fig. 1), suggesting that saturation or lineage specific rate variation could be a particular problem in accurately placing this taxon. Relative rates tests revealed no significant differences in evolutionary rates in any taxa, with the exceptions that *G seraphini* and *Typhlonectes natans* were both significantly faster than *Ichthyophis tricolor* ($P = 0.045$ and $P = 0.033$, respectively). The data have a parsimony PTP of 0.01, allowing rejection of the null hypothesis that they contain no more structure than expected by chance alone. Using the likelihood ratio test and the Akaike information criterion, Modeltest selected TrN (Tamura & Nei 1993) + I + G and GTR (Rodriguez *et al.* 1990) + I + G models, respectively. We used the simpler TrN + I + G model, and this yielded a single ML tree (Fig. 2).

Relationships among the non-African taxa are mostly those found in previous analyses, with minor differences in the relationships among the Seychellean caeciliids excluding *Praslinia*, and of the Neotropical caeciliid *Siphonops annulatus*, both of which were relatively unstable in previous analyses (Wilkinson *et al.* 2002). Thus there is an Indo-Seychellean caeciliid clade (*Gegeneophis, Prasilina, Grandisonia, Hypogeophis*), that is more closely related to a *Dermophis-Schistometopum* clade than to most other caecilians, there is a *Typhlonectes-Caecilia* clade, and the Uraeotyphlidae and Ichthyophiidae are each other's closest relatives and sister to all other caecilians except the rhinatrematid *Epicrionops*. These core relationships were also recovered in parsimony, distance and Bayesian analyses (trees not shown).

Indications of the support for the relationships recovered in the ML tree are given by Bayesian posterior probabilities and the bootstrap proportions from MLD and parsimony analyses (Fig. 2), as well as from the stability of relationships across the different methods of analysis. Each congeneric pair of African caecilians (*Boulengerula, Scolecomorphus* and *Schistometopum*) are recovered as each others' closest relatives in all analyses and with high support, consistent with current taxonomy. Similarly, the *Dermophis-Schistometopum* pairing appears well supported.

Other interrelationships of African caecilians are generally less well supported. All African caecilians fall within Nussbaum's (1991) informal 'higher' caecilians, a group comprising the Caeciliidae, Typhlonectidae and Scolecomorphidae (united by branch H, Fig. 2). Support for this group is not very strong ($P = 0.67$, BP = 60 - 68) but it is recovered in all optimal trees, is independently supported by morphological phylogenetic analyses (Nussbaum 1979; Duellman & Trueb 1986; Hillis 1991; Wilkinson & Nussbaum 1996; Wilkinson 1997) and is accepted here.

The basal splits within the higher caecilians place the African caeciliids *Boulengerula* and *Herpele squalostoma* together (branch X), and these and the scolecomorphids as successive sister groups of the remaining higher caecilians (branches Y and Z). Each of these relationships has unimpressive bootstrap support and posterior probabilities. Despite lacking strong quantitative support, branch Y is recovered in the optimal trees from each of the analyses employing different methods, and branch X is contradicted only in two of five most parsimonious trees.

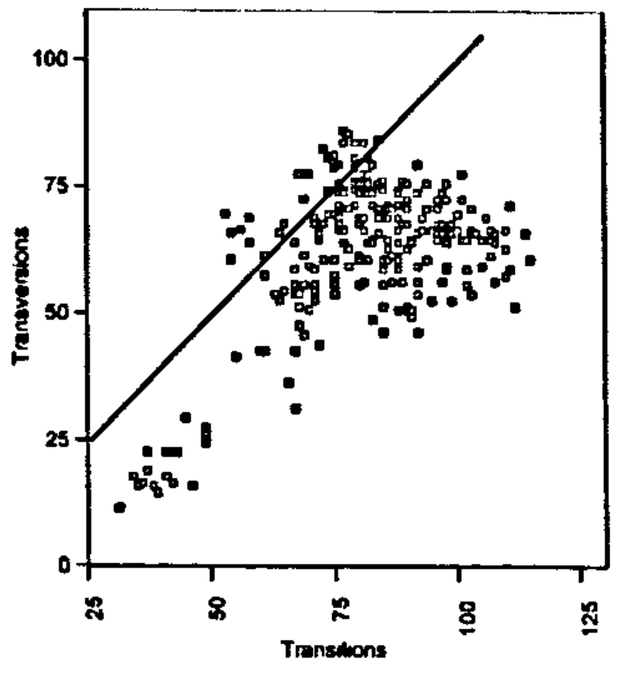

Figure 1. Scatter plot of pairwise uncorrected estimates of transitions and transversions. The straight line indicates a ratio of 1·1, with points above the line representing particularly low transition - transversion ratios. Shaded points are pairwise estimates of transition transversion ratios of less than one that involve *Geotrypetes seraphini*.

The remaining African caeciliid, *G. seraphini*, is nested within a cosmopolitan group of caeciliids (branch C, Fig. 2) that has low bootstrap support and is not recovered in all optimal trees, but which has a surprisingly high posterior probability (0.93). These relationships must be considered speculative and they are accepted only tentatively.

The precise relationships of *G. seraphini* differ greatly in the optimal trees recovered by the different analyses. It is recovered as sister to the *Dermophis-Schistometopum* clade (ML, MLD, Bayesian), sister to the Indo-Seychellean caeciliids (LogDet) or to *Scolecomorphus* (parsimony) and never with strong support. Apart from its tentative inclusion in clade C, all that can be confidently inferred about the relationships of *Geotrypetes* is that it lies outside the *Dermophis-Schistometopum* clade and the Indo-Seychellean clade. Leaf stabilities (Table 2) calculated from parsimony and MLD bootstrap analyses agreed in the rank order, and both identified *G. seraphini* as the least stable taxon. Leaf stabilities for African taxa except *Schistometopum* are lower than average, indicating that their positions are among the relatively least well supported. Leaf stability of *Epicrionops* is close to the average, indicating no special instability in the root.

Constrained analyses produced a number of suboptimal ML trees consistent with various hypotheses of taxonomic, biogeographic and biological interest that were tested against the unconstrained ML tree using the KH test (Table 3). Despite apparent strong support for the monophyly of *Boulengerula*, the best tree in which *Boulengerula* is not monophyletic does

Table 3. Kishino-Hasegawa tests comparing the fit of the data to the unconstrained ML tree (Fig. 2) and to a range of suboptimal trees, each constrained to make a particular set of taxa monophyletic or not monophyletic. D = difference in log likelihood between optimal and suboptimal trees; *P* = probability under the null hypothesis that the differences in fit are no greater than expected from random sampling error (none).

Suboptimal hypothesis	D	*P*
Boulengerula is not monophyletic	9 026	0.086
Schistometopum is not monophyletic	25 229	0.040
Scolecomorphus is not monophyletic	77 902	<0 001
African caecilians are monophyletic	38 623	0.002
African caeciliids (*Boulengerula* + *Geotrypetes* + *Herpele* + *Schistometopum*) are monophyletic	37 431	0 006
East African caeciliids (*Boulengerula* + *Schistometopum gregorii*) are monophyletic	68 212	<0.001
West African caeciliids (*Herpele* + *S. thomense*) are monophyletic	102 00	<0 001
Caecilians with rudimentary eyes (*Boulengerula* + *Herpele* + *Gegeneophis*) are monophyletic	31 596	0 005
Viviparous caecilians (*Typhlonectes* + *Dermophis* + *Geotrypetes* + *S. thomense* + *Scolecomorphus*) are monophyletic	17 896	0 111

not provide a significantly worse fit to the data. In contrast, trees in which the other individual African genera are not monophyletic have a significantly worse fit to the data, as do trees in which African caecilians, African caeciliids, and East and West African caeciliids are monophyletic. Optimal trees in which caecilians with rudimentary eyes are monophyletic can also be tentatively rejected. In contrast, the data do not allow rejection of the hypothesis that viviparity arose only once within caecilians.

DISCUSSION

Although greatly increasing the taxonomic sampling of African caecilians, our analyses offer incomplete and mostly tentative insights into their phylogenetic relationships. The molecular data support traditional taxonomy in being consistent with the monophyly of the three African genera, *Boulengerula*, *Schistometopum* and *Scolecomorphus* (and of the Scolecomorphidae). *Scolecomorphus* and *Schistometopum* are also characterised by unique morphological synapomorphies (e.g., Nussbaum 1985; Nussbaum & Pfrender 1998; Wake 1998; Gower & Wilkinson 2002; Loader *et al.* 2003). Nussbaum (1985: 47) reported that "Studies in progress indicate that *Herpele* and *Idiocranium* are distinctive western forms with no close relationship to other African caecilians" whereas our data provide tentative support for the pairing of *Herpele* with *Boulengerula*.

The data also support, albeit weakly, Nussbaum's (1991) 'higher' caecilian clade, as does morphology (Wilkinson & Nussbaum 1996; Wilkinson 1997). The results suggest that the Caeciliidae is paraphyletic, not only with respect to the Typhlonectidae (e.g., Hedges *et al.* 1993), but also with respect to the Scolecomorphidae, emphasising the need for more comprehensive taxonomic revision. Needless to say, any future revision intended to remove this paraphyly will require greater sampling of caeciliid taxa.

The parallel disjunct distributions of *Schistometopum gregorii* and *Schistometopum thomense*, and of *Scolecomorphus* and *Crotaphatrema* in East and West Africa has been noted previously (e.g., Nussbaum 1985; Nussbaum & Pfrender 1998). The tentative hypothesis that the East African *Boulengerula* and West African *Herpele* are sister taxa, adds a third potential component to this biogeographic parallelism. It is not clear whether the absence of caecilians from Central Africa is real or reflects lack of sampling (Nussbaum & Hinkel 1994), and thus whether the biogeographic pattern is real or apparent. However, this study demonstrates that the caecilian and caeciliid faunas of Africa, and those of East Africa and of West Africa are not monophyletic.

Schistometopum nests within a diverse group of caeciliids as the sister group of *Dermophis*, with which it was considered congeneric until Parker (1941). In contrast, the Scolecomorphidae and *Herpele-Boulengerula* clades appear to represent relatively deep branches in the higher caecilian clade, with a deep split between the species of *Boulengerula* as judged by branch lengths. If the split between *Schistometopum* and *Dermophis* corresponds to the vicariant separation of Africa from the Neotropics, then this suggests that the origins of some of the current diversity of African caecilians may predate the break-up of Gondwana.

Boulengerula taitanus was transferred to *Afrocaecilia* by Taylor (1968) but *Afrocaecilia* was subsequently synonymised with *Boulengerula* by Nussbaum & Hinkel (1994) based on phylogenetic analysis of morphological data. Wilkinson *et al.* (in press) argued that phylogenetic signal from the morphological data is weak and that the synonymy might have been premature. The deep divergence of *B. taitanus* and *B. boulengeri* indicated by the molecular data further suggests that a more detailed assessment of the taxonomy of these East African caeciliids is warranted.

Several caecilian genera have closed orbits, and in all of these except the scolecomorphids, the eyes are rudimentary (Wake 1985). It has been argued that rudimentation of the visual system has occurred independently multiple times within caecilians, and that characters of the visual system erroneously group rudimentary-eyed taxa in some morphological phylogenetic analyses (Wilkinson 1997). O'Keefe & Wagner

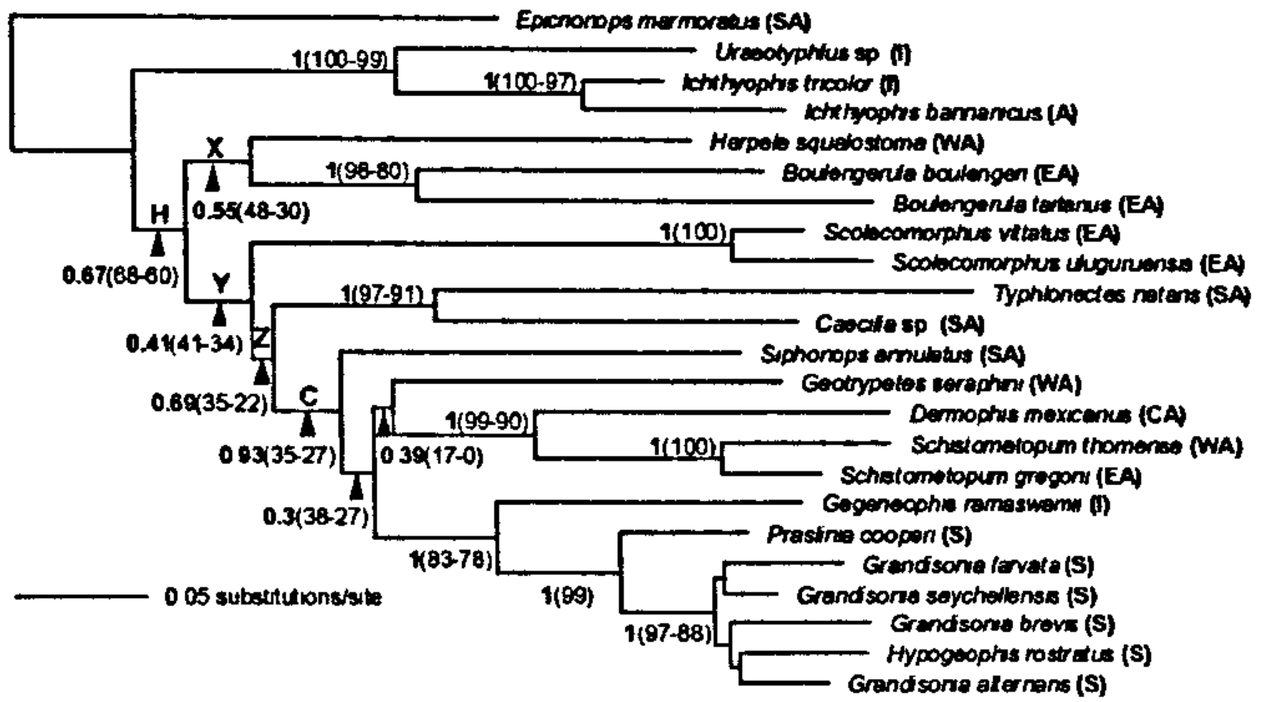

Figure 2 Single maximum likelihood tree (LnL = 7832 49). The chosen model of evolution (TrN + I + G) employed a symmetric rate matrix with AG and CT substitutions set at 3 1738 and 8 9975 respectively, and all other substitution types set at unity; base frequencies estimated at 0 4247, 0 2144, 0 1279 and 0 2330 for A, C, G and T respectively, a four category discrete approximation of a gamma distribution ($\alpha = 0\ 57$), and the proportion of invariant sites set at 0 276 Numbers in bold are Bayesian posterior probabilities Numbers in parenthesis are bootstrap proportions from MLD and (where different) parsimony analyses Letters in bold after taxon names indicate geographic provenance (A = South East Asia, CA = Central America, EA = East Africa, I = India, SA = South America, S = Seychelles, WA = West Africa) Other bold letters indicate internal branches discussed in the text.

(2001) further rejected the hypothesis that these characters are evolving independently. Our analysis provides the first support from molecular data for the parallel rudimentation of visual systems in caecilians, with this occurring independently in at least two lineages - *Boulengerula* (+ *Herpele*) and *Gegeneophis*.

Our analyses also suggest that viviparity has evolved at least three times, but using the KH test we are unable to reject trees in which all viviparous caecilians are a monophyletic group. The trees for this test were obtained using a backbone constraint that excluded *Schistometopum gregorii* and *Herpele squalostoma* because the reproductive modes of these species are uncertain (Wilkinson & Nussbaum 1998). Natives informed Loveridge (1936) that *S. gregorii* laid eggs in water, but we concur with Nussbaum & Pfrender (1998) that this is unlikely. The placement of *S. gregorii* and *H. squalostoma* in the optimal trees suggests that they are viviparous and oviparous respectively, predictions that can be tested empirically.

Two caeciliids, the West African *Geotrypetes seraphini* and the South American *Siphonops annulatus* are particularly unstable. Their phylogenetic placement is sensitive to method of analysis, is never well supported, and they have the lowest leaf stabilities. Little can be said of their relationships, other than that they lie somewhere within the higher caecilian clade, probably not basally, and that they lie outside the well-supported groupings of the scolecomorphids, *Typhlonectes* + *Caecilia*, *Dermophis* + *Schistometopum* or the Indo-Seychellean clade.

Although partial sequences of mitochondrial 16S and 12S have provided important insights into the phylogeny of caecilians (Hedges *et al.* 1993; Gower *et al.* 2002; Wilkinson *et al.* 2002), they are not adequate for resolving the relationships of African caecilians. In addition, there are strong base compositional biases and some very low transition - transversion ratios that suggest saturation and/or lineage specific rate variation may hinder accurate phylogenetic inference from these data. These potential problems seem particularly to affect comparisons involving the African caecilian sequences, all of which have below average leaf stabilities (Table 2). African sequences appear to provide disproportionately low estimates of transition - transversion ratios and this is particularly true of *Geotrypetes seraphini*. It is also remarkable that, when taxa are ranked by their GC content, the African caecilians exclusively occupy the highest ranks (Table 2). We would not have predicted any simple correlation between geography and base composition, are unaware of any comparable patterns in the literature, and consider its existence intriguing and worthy of further study.

Given that our alignment includes only 16 of 33 genera and 23 of c.160 caecilian species, partial 16S and 12S data should continue to be useful for placing many of the currently unsampled taxa. However, better resolution of the relationships of the African caecilians is unlikely to be achieved purely through the benefits of denser taxonomic sampling and will probably require data from more genes and morphology.

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- Decreases in profits are reported to have heavily impacted collective money circulation and village tax revenues.

Non-financial impacts

- Following the timber trade harvest and export bans, there has been increased rainfall and available water as a result of regeneration of the forest. During the intense logging of the 1970 and 1980s, the montane forests were severely degraded, impacting on local climatic conditions. Whereas previously there had been three agricultural seasons coinciding with rainfall patterns, this had been reduced to two and the timing and duration of the rains had altered. In recent years, villagers noted that the agricultural seasons were gradually returning to normal and so food production had increased.
- Other positive, non-financial impacts include community organisations, greater conservation education and awareness on farming techniques, tree planting and energy-saving stoves; improved road conditions due to fewer heavy timber lorries; and benefits associated from the ANR including village income from tourism revenues and improved communications.
- On the other hand, reduced employment, market access and, business opportunities have reduced individual and collective incomes with knock-on effects on their and earnings have all resulted from changes in trade and access regulations, which in turn has had knock-on effects on ability to pay for other services and to provide important livelihood components, for example, purchasing medicines, paying school fees and purchasing land and housing materials.
- Trade-offs exist for many livelihood impacts. Whilst reduced forest loss has benefited agricultural output, villagers also noted that linked to the regeneration of the forest was an increase in wildlife and a subsequent increase in problem (crop raiding) animals – particularly baboons. Similarly while villagers complain that they can no longer access or afford timber for construction, they also note that as a result they have learned to build better, superior houses from bricks.

Experience from the East Usambara Mountains in Tanzania clearly shows a mixture of positive and negative monetary and non-monetary impacts resulting from changes to wildlife access and trade regulations at local, national and international levels. It is also evident that regulatory measures have varying impacts on different sectors of society due to the different roles in the wildlife trade played by the rich and poor, women, men, elders and youth. Men are most affected by regulations on the timber trade and youth most affected by regulations on the trade in wild animals. According to local perceptions, wildlife access and harvest regulations have had a greater overall impact than national and international trade controls. Further, evidence suggests that some trade regulations have actually led to significant positive impacts on local livelihoods, whilst subsequent wildlife access regulations have caused the most negative impacts. Overall, wildlife access and trade restrictions in the East Usambara Mountains have had a significant financial effect on local people. Market demand, access, local control and business acumen were major factors influencing rural peoples' susceptibility to wildlife access and trade regulation.

The study also showed how wildlife access and trade regulations not only cause varying degrees of livelihood impacts, but also produce both short-term and long-term effects. In some cases, there are short-term and long-term trade-offs in the effects of trade regulation. Thus, a wildlife regulation may result in a direct, immediate livelihood impact (positive or negative), which is felt for a relatively long time. Alternatively, the impact may be only temporary, or perhaps felt some time after the regulation is introduced. These temporal aspects are discussed in more depth in Miombo (No. 26).

By better understanding not only the values and importance of natural resources to rural communities, but also the associated short- and long-term conservation and livelihood impacts of interventions, decision-makers will be better placed to achieve synergy between conservation and development goals.

CAECILIANS: MYSTERIOUS AMPHIBIANS OF THE EASTERN ARC MOUNTAINS

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The Eastern Arc (EA) Mountains are home to many fascinating animals, a proportion of which are restricted to small patches of forest in single mountain blocks. Many of these spatially restricted species are rarely seen or collected. Consequently, little is known of their biology. Nevertheless, it is unusual to misidentify the major group to which any given animal encountered in the field belongs. This is especially true for vertebrates, so that a frog is clearly identifiable as a frog, a bird is a bird etc. However, members of one major group of amphibians are often mistaken as belonging to other vertebrate or even invertebrate groups. These are the caecilian amphibians (Order Gymnophiona). So scant is the knowledge of these creatures that they must be the most poorly known group of tetrapods (the group comprising all amphibians, reptiles, birds and mammals). This is true for all aspects of their biology, including their systematics, i.e. their classification and evolutionary relationships.

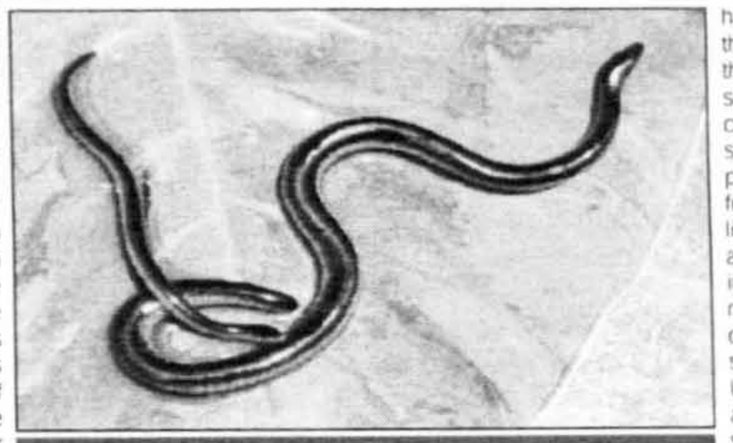

Photo 1. The caecilian, *Scolecomorphus vittatus*

Caecilians are poorly understood for a number of reasons, but the main factors are probably their cryptic habits, tropical distribution, and lack of dedicated study. Although one of the six recognised families (the South American Typhlonectidae) includes aquatic species, most caecilians are denizens of the soil, spending 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 very rarely seen in the wild. Caecilian morphology is well adapted to life underground. They have a robust skull that they push through the soil, and many have their eyes greatly reduced, sometimes to just a small group of cells concealed beneath bone and skin. In such forms, the eyes probably have a limited visual function, perhaps only detecting light and dark. The main sensory apparatus used by caecilians instead appears to be a pair of retractable, probably chemosensory tentacles on the snout that are unique to the group. Unlike other amphibians, caecilians are entirely limbless with elongate snake or worm like bodies. Their resemblance to worms is further enhanced by their moist, externally scaleless amphibian skin and its subdivision into conspicuous rings or annuli.

Bright colours are of no use underground or in communicating with poorly sighted kin. However, some caecilian species are surprisingly brightly coloured, a striking example being *Scolecomorphus vittatus* (Photo 1) from the East Usambara Mountains. This species has a dark dorsum combined with a vivid pink belly. Such contrasting colours are typically used as warnings to predators. The skin secretions of caecilians are almost certainly toxic as they are in other amphibians. Caecilians are carnivorous, with the terrestrial forms probably feeding mostly on earthworms, termites and other invertebrates that live within the soil. Dietary preferences and the impact of caecilians on soil ecosystems are particularly poorly understood and understudied.

continued page 4

Caecilians are often regarded as a small, conservative, and even 'primitive' group. However, despite there being only about 160 currently recognised species worldwide, these have diverse morphologies and natural histories. The smallest species reach little over 11cm (*Idiocranium russeli* from Cameroon and *Grandisonia brevis* from the Seychelles), but the largest species reach lengths of over 150cm (*Caecilia thompsoni* from South America). Numbers of vertebrae in the backbone vary from about 70 to over 300. Perhaps the most divergent caecilian discovered thus far is the South American *Atretochoana eiselti* which, at 80 cm, is by far the largest tetrapod to completely lack lungs, and rely entirely on cutaneous gas exchange.

Caecilians are also particularly diverse in their reproductive modes. Some have the typical amphibian life history of aquatic larvae that metamorphose into terrestrial adults, though the eggs are fertilised internally and are laid in terrestrial burrows rather than in water so that hatchling larvae must make their way to nearby water courses. Others develop directly within their terrestrial eggs and completely bypass the larval stage. Some species do not lay eggs but are instead viviparous.

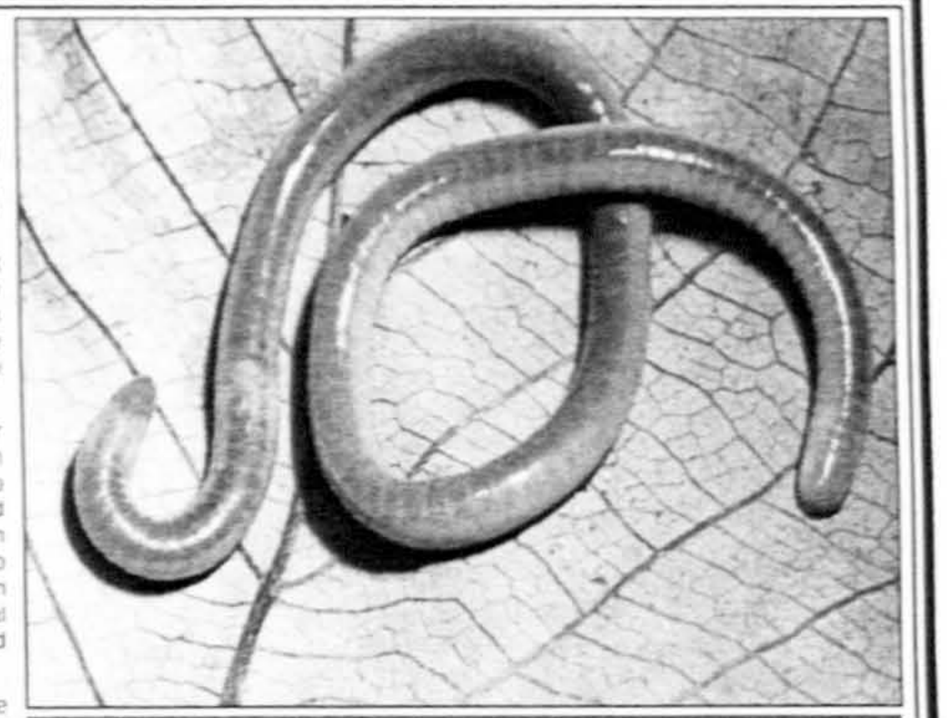

Photo 2. The caecilian Boulengerula boulengeri from the East Usambara Mountains. Photograph kindly provided by James Vonesh.

Local names for caecilians often reflect the misconception that they are snakes or earthworms. For example, peoples of the Mahenge Mountains of Tanzania refer to the caecilian *Scolecomorphus kirkii* as 'namtakosa', which means a snake. Unlike earthworms, caecilians are vertebrates with an internal skeleton and toothed jaws. They differ from reptiles, including snakes, in having eggs that lack the complex of amniotic membranes. Another obvious difference is the skin, dry and scaly in reptiles and moist, externally scaleless and highly glandular in caecilians. This was clearly recognised by the pioneer of East African amphibian studies, Sir Arthur Loveridge. His 1944 book 'A Mountain Safari' makes clear that caecilians "may be distinguished from snakes at a glance by the absence of a scaly covering, though some have scales embedded in the skin". Another trait, contributing to the view that caecilians form a natural group with frogs and salamanders, comprising what are recognised today as the three extant amphibian orders, is the adult dentition in which tooth crowns are attached to and sit upon distinct pedicels.

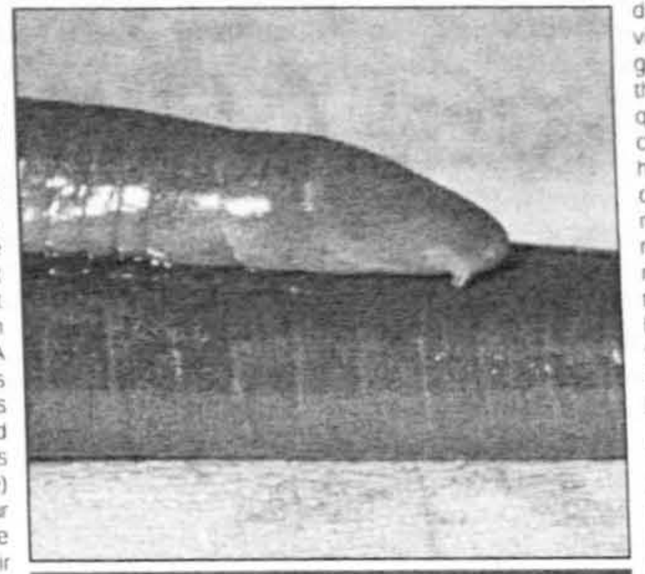

Photo 3. A species of the scolecomorphid from the Pare Mountains, note the protruding tentacle near the front of the snout.

Caecilians are distributed throughout much of the wet tropics, occuring in South America, Africa, the Seychelles, the Indian subcontinent and parts of SE Asia. They are absent (or at least unknown!) from Madagascar and Australasia. A centre of African caecilian diversity is the Eastern Arc (EA), which harbours six of the nineteen species, and representatives of both families (Caeciliidae and Scolecomorphidae) known from the continent. Our ongoing research on caecilians of the EA is focused primarily on their systematics. As a result of our work we anticipate a better understanding of the diversity of EA caecilians, their distribution and evolutionary relationships, along with better tools for reliable identification of caecilian species. We are also tying to understand the historical events that have produced the current geographical distributions of species. Some new species will be described, and we expect to have preliminary results on their ecology and natural history. Understanding the systematics of caecilians is a necessary prerequisite for the interpretation of all other aspects of caecilian biology, from physiology to ecology. Historically, caecilians have been neglected and understudied, but recent work has begun to uncover the fascinating biological diversity of these amazing creatures. We hope our work will continue to add to this growing knowledge.

Caecilians occur on all the mountains of the EA, where they can be found in moist soils both in forest and agricultural land. Most EA mountain blocks harbour species of both the genera found in the region, the caeciliid *Boulengerula* (Photo 2) and the scolecomorphid *Scolecomorphus* (Photo 1 & 3). These are very different animals.

Boulengerula are slender, with short globular tentacles and eyes very reduced and not visible externally. Species of *Scolecomorphus* are usually more robust with larger maximum sizes (up to 46 cm, in contrast to up to 36 cm in *Boulengerula*), and eyes that are visible when they protrude with closely associated, long tentacles. Species of *Boulengerula* lay eggs which develop directly, while *Scolecomorphus* are viviparous. The systematics of these genera have been recently reviewed, but there still remain several outstanding questions. Previous sampling of EA caecilians was very patchy. Recent work has partly remedied this, and we are optimistic that a combination of morphological and DNA sequence data for representatives of populations from each mountain block will allow us to further refine the taxonomy and provide robust hypotheses of the interrelationships of the species within each genus. This in turn will allow biogeographical hypotheses to be tested.

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A remarkable young *Scolecomorphus vittatus* (Amphibia: Gymnophiona: Scolecomorphidae) from the North Pare Mountains, Tanzania

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Abstract

A description is given of the external morphology of a single young caecilian (gymnophionan) amphibian from the North Pare Mountains, Tanzania, referable to *Scolecomorphus vittatus* (Boulenger, 1895). This is the smallest known free-living *Scolecomorphus*, and it is characterized by many remarkable features previously unrecorded for any life-history stage of any caecilian. The cheeks have conspicuous, posteroventrally divergent paraoral processes that border a concavity on the ventral surface of the snout. The mandibles are very flexible about their articulation with the cranium, and they have a broader curvature than the upper jaw. The dentition is heterogeneous, with adult-like monocusped teeth in single rows, but also some supernumerary teeth, some of which are bicornute. The posterior parts of the paraoral processes bear a small number of monocuspid teeth that lie outside, and project away from, the mouth. The nuchal region of the body bears a distinctive concavity on the underside of the throat, bordered by longitudinal ridges that terminate in fleshy nipples. All of these features are unknown in adult *Scolecomorphus*, and many are unique for caecilians, and they suggest a highly distinctive life-history stage. The discovery and description of this specimen adds substantially to the currently meagre information on the life history of scolecomorphids and of the diversity of caecilian reproductive biology. Two modes of viviparity in caecilians are identified, with *S. vittatus* resembling the caecilid *Geotrypetes* in giving birth to small altricial young that seem to require extended post-parturition parental care.

Key words: viviparity, reproduction, Africa, caecilians, morphology, *Scolecomorphus vittatus*

INTRODUCTION

Caecilian amphibians have a variety of reproductive modes, including the classical biphasic amphibian life cycle of oviparity with an aquatic larval stage, as well as oviparity with direct development and viviparity (Wake, 1977*a*). Our understanding of the evolution of this diversity is hampered by a lack of information on the reproduction and development of most of the *c.* 160 recognized species of caecilians (Wilkinson & Nussbaum, 1998). The Scolecomorphidae is one of six currently recognized families of caecilians (Duellman & Trueb, 1986; Nussbaum & Wilkinson, 1989; Wilkinson & Nussbaum, 1999) and includes the genera *Scolecomorphus* (three species) and *Crotaphatrema* (three species), from East and West Africa, respectively (Nussbaum, 1985; Lawson, 2000). Scolecomorphids have several distinctive morphological synapomorphies, including the absence of stapes and the presence of protrusible eyes that migrate from the orbit during development in association with the tentacle (Taylor, 1968; Nussbaum, 1985; O'Reilly, Nussbaum & Boone, 1996). Barbour & Loveridge (1928) reported discovering small embryos in a female *Scolecomorphus uluguruensis*. Subsequently, Parker & Dunn (1964) provided a brief description of the dentition of *S. uluguruensis* embryos as part of their survey of tooth structure and function in the early development of viviparous caecilians, and Taylor (1968: fig. 360) figured one such embryo. As far as we are aware, there have been no other reports of early life-history stages in any species of *Scolecomorphus*. The life history of *Crotaphatrema* is even less well known, though Nussbaum (1985) suggested that this genus may be oviparous, based on his observation of large ova.

In 1973, CAM collected a juvenile caecilian from the herpetologically poorly known North Pare Mountains of Northern Tanzania, that is now part of the collections of the Natural History Museum, London (BMNH 1986.609). This specimen is interesting because it is the smallest, and presumably the youngest, known free-living *Scolecomorphus* and because it has distinctive features that are unknown in the adults or young of any other caecilian. Thus this specimen contributes to the meagre knowledge of the reproductive biology of *Scolecomorphus* and to knowledge of the morphological diversity of caecilians. In this paper, a description of the distinctive features of this specimen is provided and its significance discussed.

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DESCRIPTIVE ACCOUNT

The specimen, BMNH 1986.609, was collected by Charles A. Msuya on 26 December 1973 in Kifula Village, Ugweno, Pare District, Tanzania. It was dug from rich, moist soil in a mixed banana and coffee plantation. Its morphology and provenance supports a referral to *Scolecomorphus vittatus* (Boulenger, 1895) (see Discussion). BMNH 1986.609 has a total length of 72 mm, which is smaller than any previously reported specimens of *Scolecomorphus* other than intrauterine embryos (Barbour & Loveridge, 1928; Parker & Dunn, 1964; Nussbaum, 1985). Some meristic and additional morphometric data for BMNH 1986.609 are given in Table 1, along with comparative data for the otherwise smallest specimen known from the same locality, a 158 mm female in the collections of the California Academy of Sciences (CAS 159952). Comparative photographs of the two specimens are given in Figs 1 & 2. BMNH 1986.609 differs strikingly from CAS 159952 and from all other adult *S. vittatus* in several features.

Table 1. Morphometric and meristic data for the two specimens of *Scolecomorphus vittatus* shown in Figs 1 & 2. BMNH 1986 609 is the smallest known free-living specimen, while CAS 159952 is the smallest known adult individual from the same locality in the North Pare Mountains, Tanzania. All morphometric data given in mm

	BMNH 1986 609	CAS 159952
No. of primary annuli	121	125
Total length	72	157
Width at mid-body	2.8	5.6
Snout tip to first nuchal groove	4.8	5.6
Snout tip to second nuchal groove	6.0	7.3
Snout tip to third nuchal groove	7.2	10.1
Lower jaw tip to jaw angle	2.2	3.1
Snout tip to jaw angle	3.9	4.4
Head width at jaw angle	2.7	3.1
Head with at occiput	3 0	3.8
Internarial distance	1 1	1.3
Intertentacular distance	2.1	1.6
Eye spot to tentacle	0.4	Eye not visible
Eye spot to naris	1 3	Eye not visible
Tentacle to naris	0.8	0.7
Length of terminal shield	2.2	3.6

In adult scolecomorphids, as in many caecilians, the snout projects anteriorly beyond the margin of the mouth, forming a bluntly rounded subconical rostrum. In contrast, the rostrum of BMNH 1986.609 in lateral view is more wedge-shaped with a more pointed snout tip. On its ventral surface, the rostrum is distinctly concave transversely and its lateral margins form ventrolaterally directed ridges, referred to here as rostral ridges, the apices of which provide a sharper demarcation between the ventral and dorsal surfaces of the rostrum than is seen in other caecilians. More typically, the ventral surface of the caecilian rostrum is convex and without sharp differentiation between the ventral and dorsal surfaces. In adult *Scolecomorphus*, the tentacular apertures are on the ventral surface of the rostrum just anterior to the mouth. In BMNH 1986.609 they are also just anterior to the mouth but are positioned more laterally, in depressions on the lateral surfaces of the rostral ridges, and are barely visible in ventral view (Fig. 3). Compared to CAS 159952, a specimen more than twice its total length, the more laterally placed tentacular apertures of BMNH 1986.609 are also more widely separated (Table 1). The subdermal eyes are more clearly visible in BMNH 1986.609 than in CAS 159952. In BMNH 1986 609 they are close to the tentacular aperture (Table 1) and anterior to the assumed position of the orbit.

The entire mouth and the cheeks (i.e. the sides of the head adjacent to the upper margin of the mouth) of BMNH 1986.609 are highly unusual. In CAS 159952, as is typical of adult caecilians, there is a close fit between the upper and lower jaws when the mouth is closed, and the cheeks do not extend laterally much beyond the lateral margins of the lower jaw and the corresponding lateral margins of the mouth on the upper jaws. In BMNH 1986.609, the cheeks have pronounced posteroventrolateral projections of highly distinctive thickenings, referred to here as paraoral processes (Fig. 3). These do not extend posteriorly to the level of the jaw angle, but instead they diverge from, and partly overlap laterally, a thin web of tissue that forms the upper margin of the corner of the mouth. There is a minor asymmetry in this region in BMNH 1986.609, with the web of tissue on the right side seemingly slightly damaged. The rostral ridges extend onto the paraoral processes, which extends the ventral rostral concavity posterolaterally. The lateral expansion of the paraoral processes, and the cleft between their posteromedial edges and the skin of the corner of the mouth, gives the head anterior to the corner of the mouth a distinctive arrow-shape in ventral or dorsal view (Fig. 3).

In preservation, the lower jaw of BMNH 1986 609 is angled anteroventrally so that the mouth is open. It is very flexible about its articulation with the upper jaw and can be opened more widely than in adult preserved specimens. It is very short and gives the impression of lacking a close fit with the upper jaws when the mouth is closed. However, the mouth can be closed by simultaneously elevating the

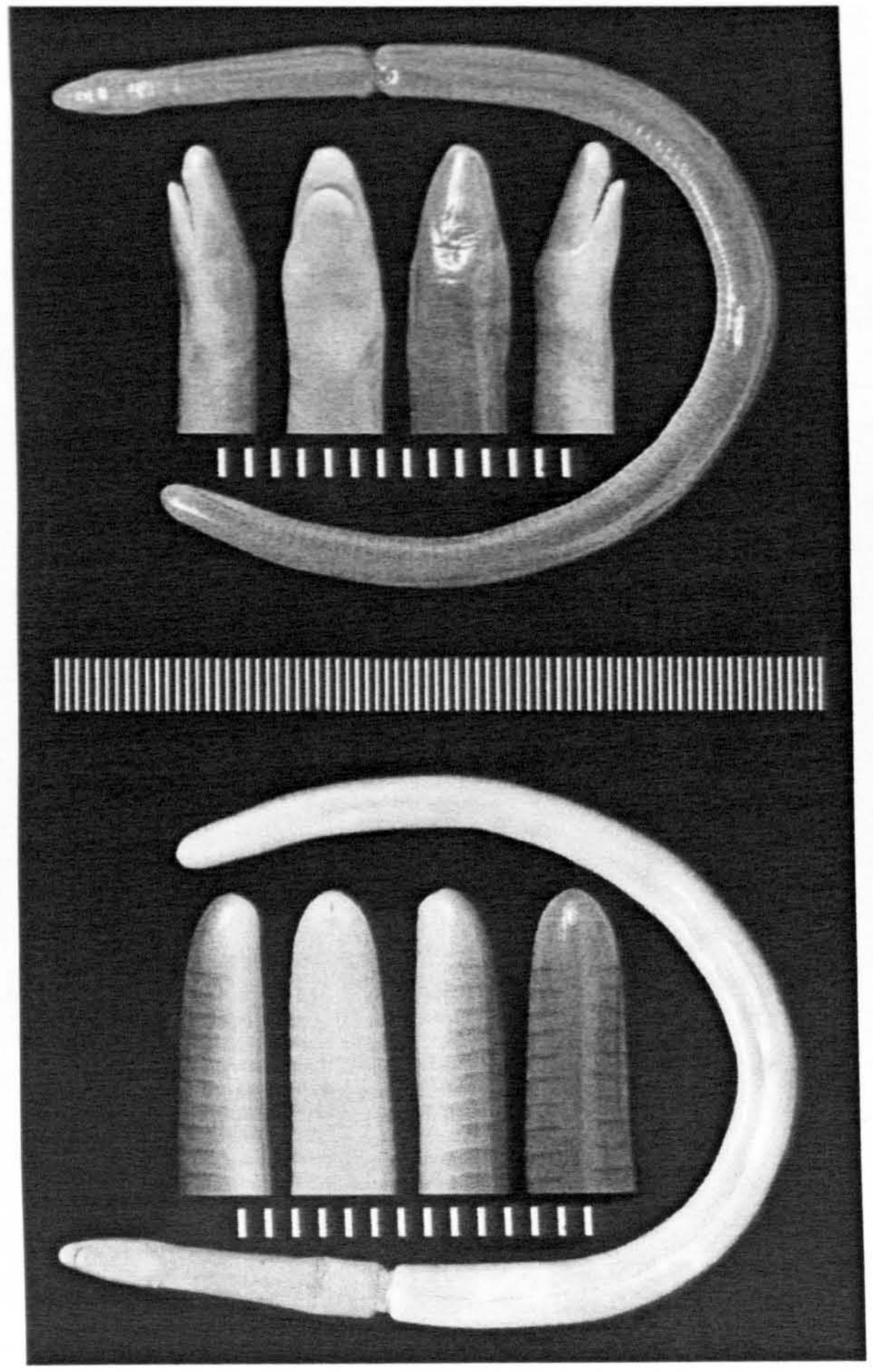

Fig. 1. *Scolecomorphus vittatus* CAS 159952. Whole specimen in dorsal and ventral views, with detail of head and vent region. Scale bars in mm.

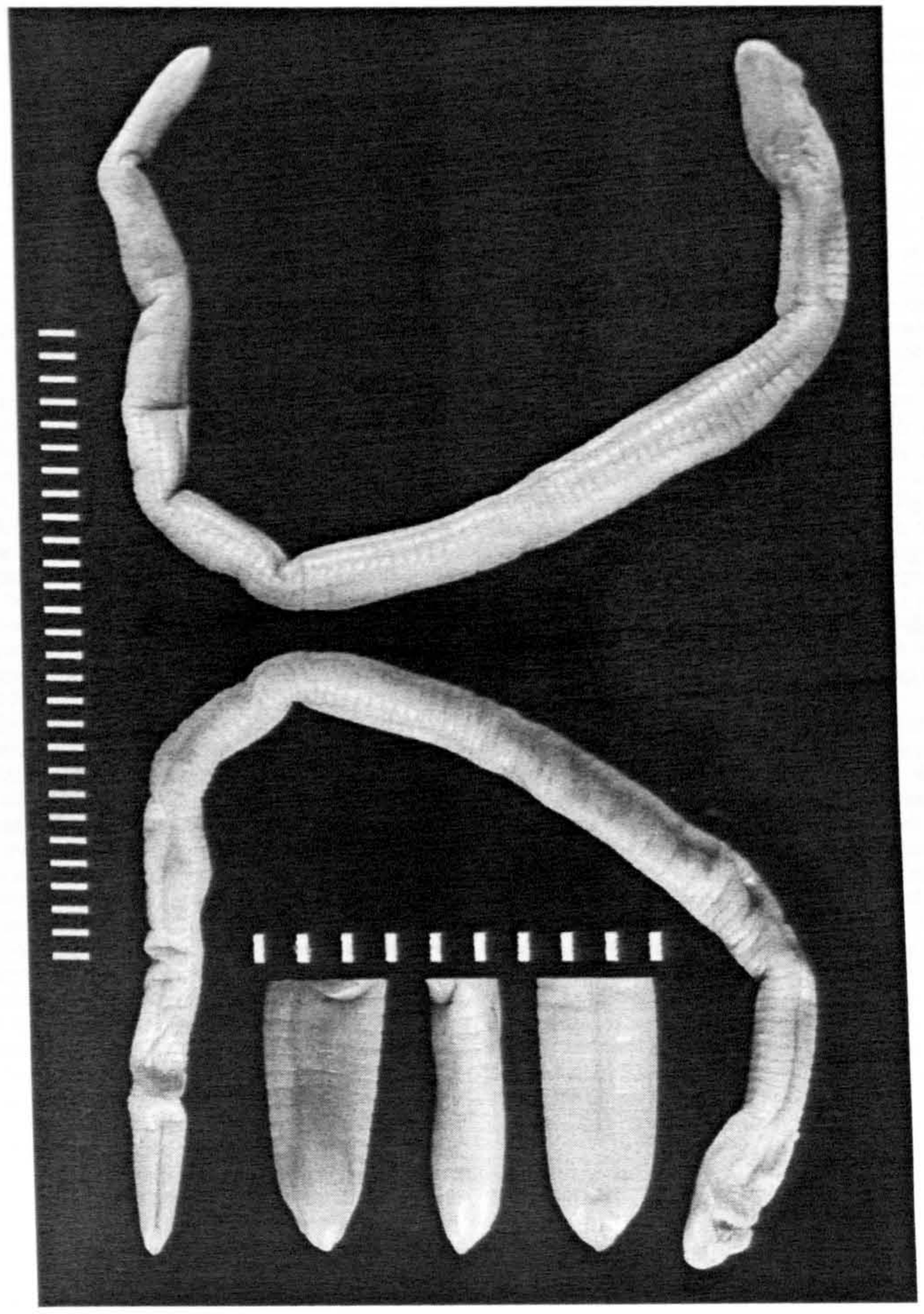

Fig. 2. *Scolecomorphus vittatus* BMNH 1986.609. Whole specimen in dorsal and ventral views, with detail of vent region. Scale bars in mm.

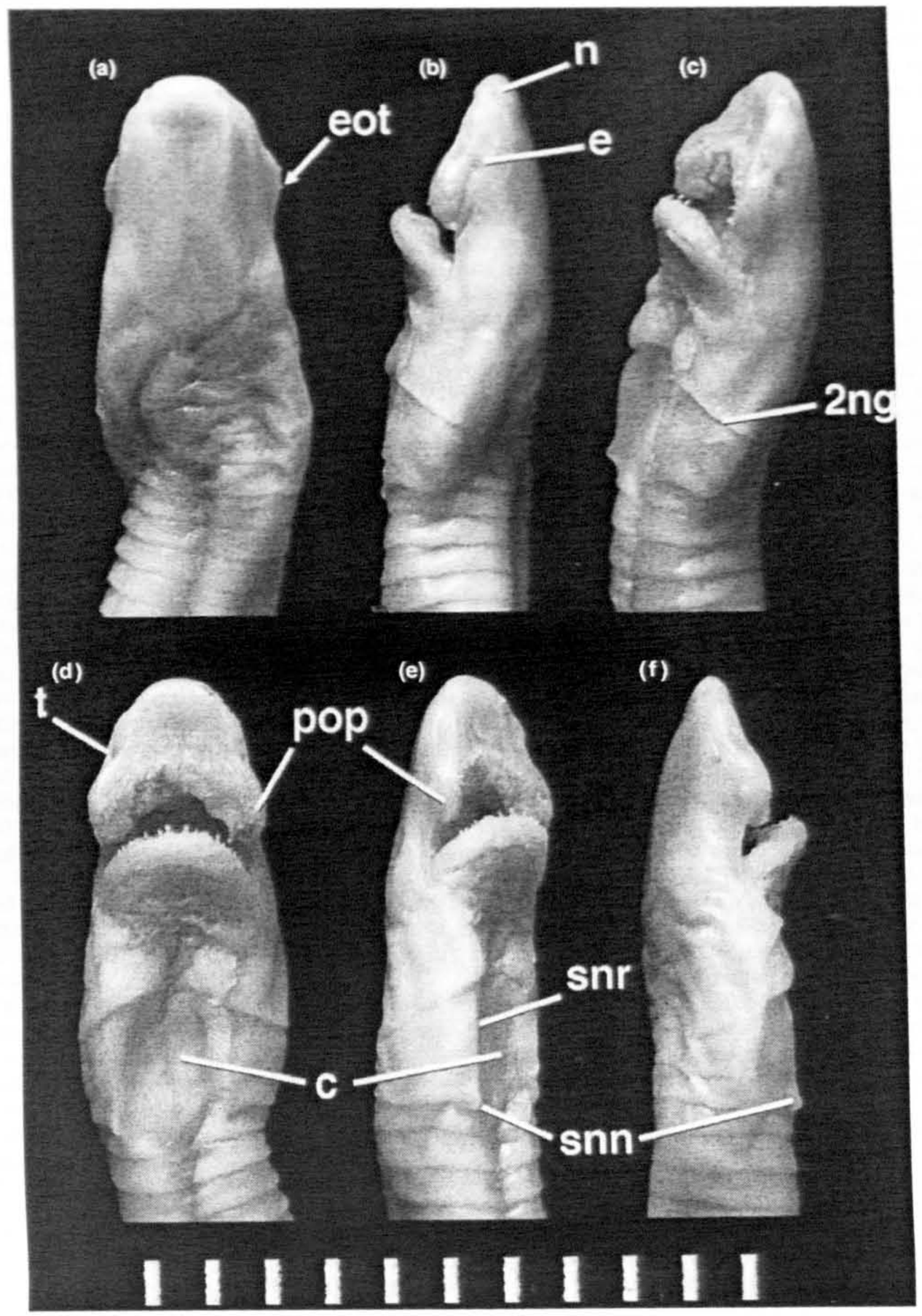

Fig. 3. *Scolecomorphus vittatus* BMNH 1986.609. Head and anterior of body in: (a) dorsal; (b) left lateral; (c) left ventrolateral; (d) ventral; (e) right ventrolateral; (f) right lateral views. c, subnuchal concavity; e, eye; eot, position of extraoral teeth on outer aspect of paraoral process; n, external naris; pop, paraoral process; snn, subnuchal nipple; snr, subnuchal ridge; t, tentacular fossa; 2ng, second nuchal groove. Scale bar in mm.

lower jaw and pushing downwards and backwards on the snout. This seems to flex the cranium. It also widens the angle between the paraoral processes.

Adult scolecomorphids have pointed and recurved monocusped teeth in three series, the dentary series in the lower jaw and the premaxillary–maxillary and prevomeropalatine series in the upper jaw. Nussbaum (1985) reported that the numbers of these teeth in *S. vittatus* vary between 17–31, 14–25 and 9–21, respectively, and tooth counts for CAS 159952 fall within these ranges (Table 1). The teeth of BMNH 1986.609 and their arrangement differ in several respects from those of adult scolecomorphids. They are mostly very small, which makes precise observations of number, morphology and disposition difficult without further preparation. They are also irregular in these respects, making the interpretation of pattern details subjective. Our description is one possible interpretation of these details.

The anterior margins of the upper jaws of BMNH 1986.609 bear a series of premaxillary–maxillary (PMM) teeth. This series is irregular as is the anterior margin of the upper jaws, and there is some damage to the adjacent gingivae, especially on the left side. The series comprises 19 teeth, 10 on the right and nine on the left, that extend from the midline onto the paraoral processes. The teeth are monocusped and recurved, as in adults. There are two similar but smaller teeth just anterior to the two most anteromedial members of the PMM series. These can be considered supernumerary and distinct from the PMM series *per se*. In addition, there are two small teeth that seem to have bicornute crowns. These also lie outside the arc of the main PMM series and can also be interpreted as supernumerary, although they are in line with gaps in the PMM series (between the third and fourth, and between the fourth and fifth posteriormost teeth of the left and right sides, respectively) and might be displaced members of that series. On each side there is an additional patch of four pointed monocusped teeth lying on the outer (lateral and dorsolateral) surface of the paraoral processes posteriorly. These teeth lie some distance outside of the mouth, beyond the rostral ridges. These extraoral teeth point upwards and outwards, although the two most dorsal members of each patch are barely erupted through the skin. We are unable to determine whether any teeth corresponding to the prevomeropalatine series of adults are present.

On the lower jaw of BMNH 1986.609 there are at least two rows of teeth in at least some places. All are monocusped and somewhat recurved. Of these, a major row comprises 23 teeth, 11 on the left and 12 on the right, that extend from the mandibular symphysis to near to the corner of the mouth. The most anteromedial teeth of this row are distinctly larger than the others and they differ from those of adult *Scolecomorphus* in having a relatively broad subterminal tooth crown (broader than the base) with an apical point. More posterolateral teeth are smaller and have a more narrow crown similar to the adult condition. An additional short tooth row comprising about six teeth on the left and three on the right lies labial to the anteromedial teeth of the main row. These teeth are very small, occupy the anterior margin of the mouth and are directed more anterodorsally. A single additional large tooth lies lingual to the large anteromedial teeth of the main series on the right side.

BMNH 1986.609 also has an unusual nuchal region. In adult caecilians, the ventral surface of the nuchal region, referred to here as the throat, shows little differentiation from the adjacent anterior body region. In adult *Scolecomorphus*, the main distinguishing features are the absence of annular grooves and the presence of a weak and narrow mid-ventral longitudinal nuchal groove (Fig. 1). In BMNH 1986.609, in contrast, there is a distinctive concavity on the throat that occupies most of the ventral surface of the second nuchal collar and has straight-sided lateral margins that are demarcated by longitudinal subnuchal ridges. The concavity extends onto the first nuchal collar anteriorly, but is less well indicated and seemingly less regular there. Posteriorly, it is continuous with a narrow but pronounced mid-ventral groove that extends across approximately the first 20 annuli (and reappears intermittently along the length of the body). The right subnuchal ridge terminates at the posterior margin of the second nuchal collar in a distinctive fleshy posteroventrally projecting nipple (Fig. 3). It seems that an antimeric nipple was present on the left sub-nuchal ridge also, but this region is damaged and most of the nipple is missing. The second nuchal groove of BMNH 1986.609 is much more pronounced than in adult *Scolecomorphus*. Unlike the adult condition, in which the groove is more or less transverse (Fig. 2), in BMNH 1986.609 it extends obliquely across the lateral nuchal region (Fig. 3).

X-ray examination reveals BMNH 1986.609 to be mostly poorly ossified. Only the otic capsules, the margins of the foramen magnum and the atlas, the floor of the braincase (os basale and parasphenoid) and to a lesser degree the next three anteriormost vertebrae and the posteriormost (pseudangular) parts of the lower jaw including the retroarticular processes, appear relatively well-ossified. An additional 20–25 anterior vertebrae that become progressively less distinct can be seen. Vertebrae cannot be distinguished further posterior and are presumed to be cartilaginous. No ossification is apparent within the paraoral processes. X-rays revealed the presence of some radio-opaque material in the hindgut. Dissection revealed that this included soil and mineral particles. The hindgut also contained a loosely aggregated flaky white substance but no recognizable remains of prey items.

DISCUSSION

The morphology of BMNH 1986.609 supports a referral to the genus *Scolecomorphus* rather than to any other East African caecilian genus. It differs from *Schistometopum* and *Sylvacaecilia* in lacking secondary annuli and in possessing a terminal shield, and from *Schistometopum, Sylvacaecilia* and *Boulengerula* in possessing a longitudinal rather than a transverse vent. The specimen can be fairly confidently referred to

Scolecomorphus vittatus. Nussbaum's (1985) key to the species of *Scolecomorphus* is based entirely on coloration. Although the colour is weakly indicated in BMNH 1986.609, either because it was poorly developed and/or because it has faded, a dark dorsal band is faintly indicated which is consistent with that reported for *S. vittatus*. In addition, the number of annuli of BMNH 1986.609 is 121, which lies outside the reported ranges (Nussbaum, 1985) for *S. kirkii* (130–152) and *S. uluguruensis* (124–149) but within the known range for *S. vittatus* (120–148). Provenance also supports the referral to *S. vittatus*. Although the caecilian fauna of the North Pare mountains is poorly known, the only caecilian species reported from this region is *S. vittatus*, and adult material from the same locality was assigned to this species by Howell & Msuya (1980) and Nussbaum (1985).

Judged against current knowledge of the morphology of caecilians, BMNH 1986.609 is highly unusual. In particular, the shape of the head is dominated by unique dentigerous paraoral processes which form lateral expansions of the upper jaws. Comparable features are unknown in any life-history stage of any caecilian species. It unlikely that these bilaterally symmetrical features are artefacts or otherwise anomalous, and they are interpreted as evidence of a novel cranial morphology and functional morphology of the mouth in early life-history stages of at least *S. vittatus*. The deep mid-ventral groove along the anterior trunk and sporadically elsewhere is probably an artefact of preservation. Dehydration in preservative can affect the shape of external surfaces in caecilians. The concavity on the nuchal region does not seem to be artefactual, but this needs to be tested by examination of additional material. The hypothesis that nuchal features are artefacts does not explain the symmetry of the subnuchal ridges or their termination at the posterior margin of the second nuchal collar in fleshy nipples. Whether or not the nuchal concavity is functionally associated with the rostral concavity and/or with the mouth, the co-occurrence of these features suggests the existence of a highly distinctive life-history stage in *Scolecomorphus*.

Little is known of the life history of *Scolecomorphus*. Based on Barbour & Loveridge's (1928) finding of embryos of *Scolecomorphus uluguruensis*, it has been assumed that all *Scolecomorphus* are viviparous (Nussbaum, 1979; Duellman & Trueb, 1986; Wilkinson & Nussbaum, 1998). Foetuses of viviparous caecilians have distinctive dentitions with multiple rows of small teeth, often with distinct cusp forms that are believed to be used in intra-oviductal feeding (Parker, 1956; Wake, 1977*b*). With respect to *S. uluguruensis*, Parker & Dunn (1964) briefly reported that: 'Thirty-seven of the fetuses, measuring from 24 to 36 mm, have been examined. All are in approximately the same stage of development, which would indicate a brief mating season, and all were still coiled and possessed triradiate, plumose external gills. There are no signs of erupted teeth in specimens of less than 30 mm, which retain some yolk, but older individuals of 30–36 mm have two staggered rows along the premaxillary-maxillary arch and a similar row along the outer aspect of the lower jaw. The individual teeth are not of the adult bicuspid [sic] type, but in a free-living specimen of 85 mm these are present in single series along the dentaries, premaxillae, maxillae, vomers, and palatines'. Unfortunately, Parker & Dunn also reported that this material is now lost and they did not describe the form of the embryonic teeth in any detail.

At 72 mm total length, BMNH 1986.609 is the smallest free-living *Scolecomorphus* known and presumably the youngest. It is poorly ossified and is probably a newborn. While it is thought unlikely that some demineralization has occurred in preservative, this cannot be ruled out, and should be checked when more material is available. The specimen has no scar tissue indicating the site of the previous attachment of gills. The second nuchal groove of adult *Scolecomorphus* occupies the position in which gills might be expected in *Scolecomorphus* embryos, and the more oblique form of this groove in BMNH 1986.609 may betray the previous presence of gills. Other than Parker & Dunn (1964), reported total length ranges (Barbour & Loveridge, 1928; Nussbaum, 1985) for the three currently recognized species are 163–463 mm for *S. kirkii*, 140–360 mm for *S. uluguruensis* and 140–376 mm for *S. vittatus*. Assuming that the life history of *S. vittatus* is similar to that of *S. uluguruensis*, and that Parker & Dunn (1964) did not overlook similar features in the 85 mm juvenile that they examined, then the peculiar head morphology seen in BMNH 1986.609 does not seem to persist in young *Scolecomorphus* for very long after parturition. Logically, these features are either characteristic of a post-parturition life-history stage or they are present also in (late) foetuses. The presence in BMNH 1986.609 of marginal tooth rows in which large adult-type teeth are accompanied by smaller teeth with supernumerary elements and occasional bicornute crowns, as well as the rather broad and short lower jaws, is significant. These are associated with foetuses of newborns of other viviparous caecilians (Wake, 1977*b*) and it is considered probable that the paraoral processes of BMNH 1986.609 are also characteristic of *Scolecomorphus* foetuses rather than being elaborated for a short post-natal period. Whether the nuchal concavity and extra oral dentition are also present in foetuses and whether all the unusual features of BMNH 1986.609 are functional in foetuses and/or in newborns is unclear (see later).

In most caecilians, the cheek region proximal to the corner of the mouth is supported chiefly by a bony quadrato-squamosal arch. In zygokrotaphic caecilians, this arch forms the lateral margin of the upper temporal fenestra (Nussbaum, 1977). Typically, the stapes articulates with the quadrate and connects the arch to the braincase posteriorly. Given only superficial morphological information on a single specimen, we are wary of drawing functional conclusions *per se*. However, based on the apparent absence of any ossification within the paraoral processes of BMNH 1986.609, we speculate that the paraoral processes of young *Scolecomorphus* are soft-tissue structures that are not supported by any lateral expansions of the quadrato-squamosal arch. In the typhlonectid *Atretochoana eiselti*, an unusual lateral expansion of the cheeks, associated with an enlarged gape, is correlated with a loss of the articulation between the quadrate and the stapes and the consequent freeing of the cheek from the posterior of the braincase (Wilkinson & Nussbaum, 1997). Scolecomorphids are unusual among caecilians in lacking a stapes, and thus in having a potentially less constrained cheek region, and the possibility of a relationship between foetal adaptation and adult morphology here is intriguing. The extreme flexibility of the lower jaw suggests a highly kinetic skull but the mobility of the expanded cheeks is not clear from BMNH 1986.609, and a better understanding of its novel cranial morphology requires more detailed anatomical study of additional material.

Some caecilians have a distinctive, deciduous 'foetal' dentition that sometimes extends outside the mouth, onto the external surface of the lower jaw (Parker, 1956; Wake, 1980; Wilkinson, 1991). In contrast, the extraoral teeth of BMNH 1986.609 are not of the 'foetal' type, and are on the upper jaw, on the dorsolateral surfaces of the paraoral processes. These are unreported in any life-history stage of any caecilian species and are highly unusual. Extraoral teeth have been reported in some fish, where they have been ascribed a hydrodynamic function (Sire, 2001). There may be several possible functions for these teeth in foetal or neonatal caecilians (involving for example communication and feeding), but we are confident only that they lack any hydrodynamic function in these soil-dwelling animals.

A further set of peculiar features of BMNH 1986.609 are the concavities and associated ridges and fleshy nipples on the snout and throat. These features contribute to an overall impression of an animal that is adapted to feeding on or clinging to a surface. Foetal caecilians are believed to feed *in utero* upon secretions and cells of the hypertrophied maternal oviduct lining, and the young of some species may feed upon maternal skin secretions (O'Reilly, Fenolio *et al.*, 1998; M. Wilkinson, R. A. Nussbaum & C. Jared, pers. obs., as reported in Pennisi, 1999). The concavities of BMNH 1986.609 could conceivably facilitate association with and/or feeding from either a flexible oviduct lining or the firmer and more concave external surface of the mother. The presence of environmental debris (mineral and soil particles) in the hindgut is evidence that BMNH 1986.609 had ingested material subsequent to its birth, suggesting that this life-history stage is a feeding stage and is not simply quiescent.

There seem to be at least two modes of viviparity in caecilians. Mode I caecilians (typhlonectids, the Central American caeciliids *Gymnopis* and *Dermophis*, and the West African caeciliid *Schistometopum thomense*) give birth to well-developed and well-ossified, precocial young that appear immediately capable of an independent existence. Some traces of characteristic foetal features (e.g. of the dentition) may be present in newborns (Taylor, 1955; Parker & Dunn, 1964; Wake, 1977*b*) but they mostly resemble adults. Mode II caecilians (the West African caeciliid *Geotrypetes*) give birth to smaller and more altricial young that seem to require extended post-parturition parental care (Wake, 1977*b*; O'Reilly, Fenolio *et al.*, 1998). Mode II newborns resemble younger mode I foetuses, and differ substantially from adult caecilians, for example in their dentition, in lacking pigment, and in being weakly ossified. BMNH 1986.609 is also generally weakly ossified and on that limited basis, *Scolecomorphus* seems to be most similar to mode II viviparous caecilians.

Throughout our discussion we have assumed that the unusual features of BMNH 1986.609 are typical of a life-history stage that is common to all *Scolecomorphus* species, and that we can meaningfully discuss the life history of *Scolecomorphus*. This assumption needs to be tested through the collection of additional material of all species of *Scolecomorphus*, encompassing a better range of life-history stages than are currently known for any species. If our assumption is correct, then paraoral processes, extraoral teeth, rostral and throat concavities, ridges and nipples, all of which are obviously derived within the Gymnophiona, will provide further compelling evidence for the monophyly of *Scolecomorphus*. If our assumption is incorrect, they should provide useful evidence of phylogenetic relationships within this fascinating genus.

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