



Ancient forest fragmentation or recent radiation? Testing refugial speciation models in chameleons within an African biodiversity hotspot

Krystal A. Tolley^{1,2*}, Colin R. Tilbury², G. John Measey^{1,3},
Michele Menegon⁴, William R. Branch^{5,6} and Conrad A. Matthee²

¹Applied Biodiversity Research Division, South African National Biodiversity Institute, Claremont 7735, Cape Town, South Africa, ²Department of Botany and Zoology, University of Stellenbosch, Matieland 7602, South Africa, ³Department of Biodiversity and Conservation Biology, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa, ⁴Tropical Biodiversity Section, Museo Tridentino di Scienze Naturali, Via Calepina 14, I-38100 Trento, Italy, ⁵Bayworld, P.O. Box 13147, Humewood 6013, South Africa, ⁶Department of Zoology, P O Box 77000, Nelson Mandela Metropolitan University, Port Elizabeth 6031, South Africa

ABSTRACT

Aim East Africa is one of the most biologically diverse regions, especially in terms of endemism and species richness. Hypotheses put forward to explain this high diversity invoke a role for forest refugia through: (1) accumulation of new species due to radiation within refugial habitats, or (2) retention of older palaeoendemic species in stable refugia. We tested these alternative hypotheses using data for a diverse genus of East African forest chameleons, *Kinyongia*.

Location East Africa.

Methods We constructed a dated phylogeny for *Kinyongia* using one nuclear and two mitochondrial markers. We identified areas of high phylogenetic diversity (PD) and evolutionary diversity (ED), and mapped ancestral areas to ascertain whether lineage diversification could best be explained by vicariance or dispersal.

Results Vicariance best explains the present biogeographic patterns, with divergence between three major *Kinyongia* clades (Albertine Rift, southern Eastern Arc, northern Eastern Arc) in the early Miocene/Oligocene (> 20 Ma). Lineage diversification within these clades pre-dates the Pliocene (> 6 Ma). These dates are much older than the Plio-Pleistocene climatic shifts associated with cladogenesis in other East African taxa (e.g. birds), and instead point to a scenario whereby palaeoendemics are retained in refugia, rather than more recent radiations within refugia. Estimates of PD show that diversity was highest in the Uluguru, Nguru and East Usambara Mountains and several lineages (from Mount Kenya, South Pare and the Uluguru Mountains) stand out as being evolutionarily distinct as a result of isolation in forest refugia. PD was lower than expected by chance, suggesting that the phylogenetic signal is influenced by an unusually low number of extant lineages with long branch lengths, which is probably due to the retention of palaeoendemic lineages.

Main conclusions The biogeographic patterns associated with *Kinyongia* are the result of long evolutionary histories in isolation. The phylogeny is dominated by ancient lineages whose origins date back to the early Miocene/Oligocene as a result of continental wide forest fragmentation and contraction due to long term climatic changes in Africa. The maintenance of palaeoendemic lineages in refugia has contributed substantially to the remarkably high biodiversity of East Africa.

Keywords

Biodiversity hotspots, biogeography, Chamaeleonidae, climate shifts, East Africa, *Kinyongia*, lizards, phylogeny, refugia, reptiles.

*Correspondence: Krystal A. Tolley, Applied Biodiversity Research Division, South African National Biodiversity Institute, Claremont 7735, Cape Town, South Africa.
E-mail: k.tolley@sanbi.org.za

INTRODUCTION

Understanding historical biogeography and the processes that generate diversity is central to ensuring the protection of diversity. Such information is even more essential in the face of current rapid climate change, occurring currently with elevated losses of biodiversity (Parmesan, 2006; Chen *et al.*, 2009; Forister *et al.*, 2010), and substantial anthropogenic pressures that are already great enough to threaten unique floras and faunas (Brooks *et al.*, 2002; Driver *et al.*, 2005; Burgess *et al.*, 2007; Menegon *et al.*, 2008; Forister *et al.*, 2010; Sinervo *et al.*, 2010). Of particular interest are the tropics, where high species richness is accompanied by elevated levels of endemism and highly nested distribution patterns (*sensu* Burgess *et al.*, 2007; Mittelbach *et al.*, 2007; Fjelds  & Bowie, 2008). South and Central America provided early examples for investigation into origins of this elevated diversity (*sensu* Haffer, 1969), with a proposed refugial model invoking allopatric speciation through periodic habitat fragmentation brought on by Pleistocene glacial cycles (Haffer, 1997). Recent studies, however, show that the processes contributing to elevated Neotropical diversity are complex (Colinvaux *et al.*, 2000; Willis & Whittaker, 2000; Lessa *et al.*, 2003; Carnaval *et al.*, 2009), and a single explanatory model is not realistic (Rull, 2008).

Along these same lines, it has been proposed that excessive endemism in the African tropics is partly due to climatic stability through the Pleistocene, whereby the region was shielded from the large climatic fluctuations that caused repeated local and regional extinctions in higher latitudes (Fjelds  & Lovett, 1997; Hewitt, 2000). This climatic buffering may have permitted speciation through ecological diversification in areas that remained stable during periods when global/regional climate change were severe enough elsewhere to force major shifts in species distributions (Fjelds  & Lovett, 1997; Fjelds  *et al.*, 1997). Indeed, East Africa contains one of the highest known concentrations of endemic plants and vertebrates on Earth (Myers *et al.*, 2000; Lovett *et al.*, 2005), which is in part due to high species richness and endemism within Afro-montane forest patches throughout the Eastern Arc Mountains (EAM) and the Albertine Rift to the west (Burgess *et al.*, 2004, 2007). These montane forests are relicts of a once widespread pan-African forest, which became fragmented due to aridification starting in the early Oligocene, and have persisted through Pleistocene glaciation cycles, due to Indian Ocean circulation bringing reliable orographic rainfall (Lovett, 1993a,b; Wasser & Lovett, 1993; Jacobs, 2004). At present, they are confined to the summits and slopes of mountain blocks and volcanic cones (e.g. Mount Kilimanjaro) that are separated by vast expanses of savanna, resulting in what are essentially forest ‘islands’. In particular, the Eastern Arc Mountains, which are centred in the Afro-tropics, are considered to have been climatically stable in general (Lovett, 1993a; Mumbi *et al.*, 2008; Finch *et al.*, 2009), giving credence to hypotheses that link elevated diversity and environmental stability (Fjelds , 1994).

Although climatic stability of the Eastern Arc Mountains corresponds well with species richness for many taxa (Fjelds  & Lovett, 1997; Fjelds  *et al.*, 1997), the differing ages of topographical features which retain forests could also explain patterns of richness and diversity (Voelker *et al.*, 2010). East Africa has undergone orogeny through block faulting for several hundred million years, with the most recent uplift of the present-day Eastern Arc Mountains *c.* 7 Ma. In contrast, prominent volcanic features of the landscape are much younger, with the formation of major volcanoes such as Mount Kenya, Mount Kilimanjaro and Mount Meru all occurring between *c.* 1.5 and 2.5 Ma (Griffiths, 1993; Chorowicz, 2005; Le Gall *et al.*, 2008; Nonnotte *et al.*, 2008). Major volcanism continued in some areas through the Pleistocene to as recently as *c.* 150,000 years ago (Chorowicz, 2005; Nonnotte *et al.*, 2008). In the Albertine Rift, considerable volcanism and uplift also occurred in the Miocene and Plio-Pleistocene, with substantial activity persisting into the late Pleistocene, *c.* 40,000 years ago (Griffiths, 1993; Kampunzu *et al.*, 1998). Both lava and lahar flows had substantial impact on the region (Nonnotte *et al.*, 2008) and repeated eruptions would have devastated forested volcanic slopes and surrounds for hundreds of kilometres (Griffiths, 1993). Thus, patterns of diversity in forests on block faulted mountains (old) and volcanic slopes (young and volatile) might be viewed as a contrast between young and old forests due to geological history, rather than climatic stability.

Regardless, dated floral and faunal phylogenies have focused on palaeoclimate to construct hypotheses around events affecting biogeographical patterns on the African continent (Bowie *et al.*, 2004, 2006; Couvreur *et al.*, 2008). These in turn provide insight into competing, but not mutually exclusive hypotheses to explain these patterns. One such hypothesis is that high diversity in climatically stable refugia has been permitted through retention of ‘palaeoendemism’. This hypothesis predicts that there should be multiple ancient lineages in dated phylogenies (Wasser & Lovett, 1993; Burgess *et al.*, 1998). An alternative hypothesis is that high diversity in climatically stable refugia is generated through elevated speciation rates due to exploitation of novel niches (e.g. heterogeneous habitats, ecotones), resulting in an abundance of lineages with recent origins and parapatric distributions (*sensu* Haffer, 1969; Terborgh, 1992b).

While some authors support the latter model (*sensu* Terborgh, 1992b; Fjelds  & Lovett, 1997; Plana, 2004; Blackburn & Measey, 2009), multiple examples of both animals and plants are required to obtain a more comprehensive view of the most important events that shaped East African biota. A suite of recent studies in hyper-diverse Madagascar have given credence to a synergism of processes (for a review, see Vences *et al.*, 2009) leaving it unclear as to whether the search for a dominant factor is realistic. It might also transpire that particular hypotheses work better for specific taxa, with some taxa displaying the majority of their diversity as palaeoendemism while others show evidence of relatively rapid adaptation and speciation (Grenyer *et al.*, 2006; Tolley *et al.*, 2008).

Obtaining dated phylogenies for groups that are particularly diverse but also restricted to forested habitat is likely to produce the most useful data to test hypotheses of how refugia contribute to high endemism and diversity.

In this regard, forest-restricted chameleons are an excellent group with which to test the competing refugial models. In general, chameleons have strong associations with vegetation structure and have limited dispersal capability (Bickel & Losos, 2002; Tolley *et al.*, 2006, 2008, 2010; Measey *et al.*, 2009; Hopkins & Tolley, 2011), and in East Africa, a species-rich genus of chameleon (*Kinyongia*; Tilbury *et al.*, 2006) is strongly associated with forested habitat (Tilbury *et al.*, 2006; Mariaux *et al.*, 2008; Menegon *et al.*, 2009). Species of this genus occur in most of the major inselbergs (e.g. Taita Hills, Usambara Mountains), on volcano slopes (e.g. Mount Kilimanjaro, Mount Meru, Mount Kenya), and in mountain chains (e.g. Rwenzori Mountains and Virunga Mountains) that have been historically forested (but may presently suffer from land transformation). These chameleons do not occur in the surrounding savanna, although a few species can tolerate altered habitats in formerly forested areas (e.g. small subsistence gardens or 'shambas'). There are currently 17 described species in the genus, but other lineages/species may exist (Menegon *et al.*, 2009).

Using Bayesian phylogenetic dating methods, we tested the contrasting hypotheses by investigating the evolutionary relationships within the genus *Kinyongia* in the context of East African biogeography. We hypothesized that biogeographic patterns and evolutionary relationships correspond to the changes in climate that have driven forest distribution, but that this will be offset by the relative ages of the topographic

features where forests occur (i.e. old mountain blocks versus young volcanic features). A phylogeny dominated by palaeoendemics with non-overlapping distributions for sister taxa would indicate a good fit for the palaeoendemic model. Alternatively, the prevalence of recent radiations and parapatric distributions of closely related sister species (perhaps on the same mountain block) should indicate that chameleon diversity is dependent upon an elevated speciation rate model. Chameleons of this genus have not moved into the expanding savanna that replaced forest throughout the Miocene and Pliocene, and therefore are not expected to show extensive radiations that correspond with habitat changes, as is evident in other genera, e.g. *Bradypodion* (Tolley *et al.*, 2006, 2008). Thus, we expect the palaeoendemic refugial hypothesis to be more appropriate for this taxon, with its high dependence on forest habitat.

MATERIALS AND METHODS

Phylogenetic analyses

A phylogenetic analysis was performed on 53 individuals from the genus *Kinyongia* using Bayesian inference based on a 2139 bp dataset for two mitochondrial (ND2, 856 bp; 16S, 462 bp) and one nuclear (RAG-1, 821 bp) marker (Fig. 1; and see Appendix S1 in the Supporting Information). The dataset included multiple examples from 15 of the 17 described species [samples of two new species (Nečas, 2009; Nečas *et al.*, 2009) were unavailable: *Kinyongia asheorum* from Mount Nyiru and *Kinyongia vanheygeni* from the Poroto Mountains]. In addition, some taxa occur in more than one mountain block or

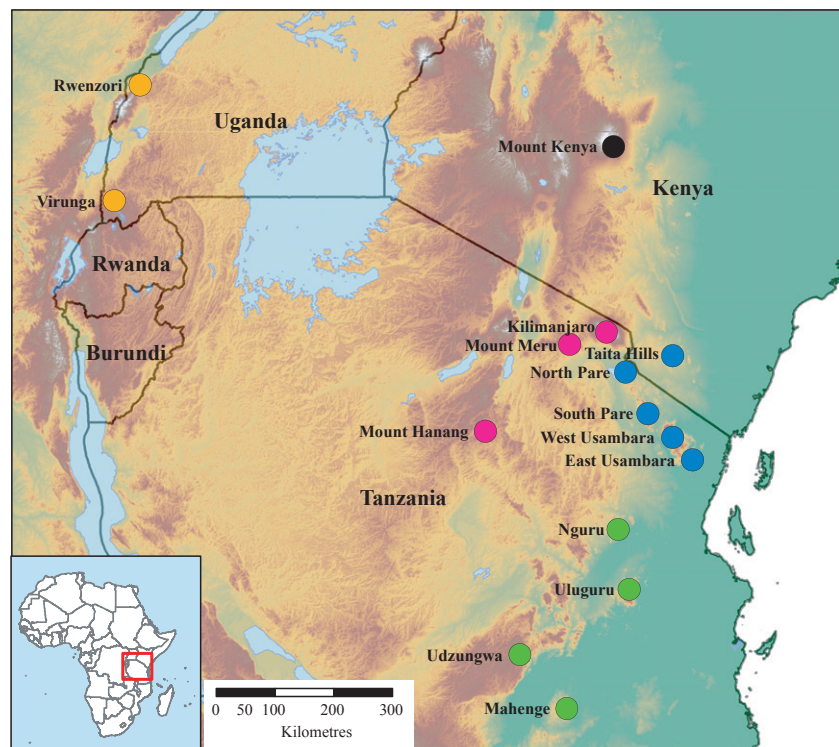


Figure 1 Map of East Africa showing major mountain blocks and volcanic features. Sites from which chameleons were sampled are indicated by dots colour coded to the main biogeographic regions (orange, Albertine Rift; black, Kenyan Highlands; blue, northern Eastern Arc; green, southern Eastern Arc; pink, volcanic mountain cones).

volcanic mountain, but our sampling included only one locality (we lacked *Kinyongia tavetana* from South Pare Mountains and *Kinyongia uthmoelleri* from Ngorongoro). In all purely molecular phylogenies to date, chameleon genera aside from *Brookesia* form a polytomy (Townsend & Larson, 2002; Matthee *et al.*, 2004; Tilbury *et al.*, 2006; Tilbury & Tolley, 2009b; Townsend *et al.*, 2011), so the choice of outgroup (*Bradypodion*) is not dependent upon an explicit sister species relationship between the ingroup and the outgroup taxa.

Sequences for 33 of these individuals are from earlier studies (Matthee *et al.*, 2004; Tilbury *et al.*, 2006; Menegon *et al.*, 2009; Tilbury & Tolley, 2009b) and are available in GenBank (Appendix S1). For the remaining samples, total genomic DNA was extracted from ethanol preserved (95%) tail tips, liver or muscle using the Qiagen DNA Easy extraction kit (Qiagen Ltd., Crawley, UK) following the standard protocol, or by salt extraction. Fragments were amplified in 25 μL reaction volumes containing 2 μL of the DNA extract (*c.* 25 ng μL^{-1}), 0.25 μM of each primer, 0.2 mM dNTPs, 2.5 mM MgCl_2 , 10 \times thermophilic buffer (50 mM KCl, 10 mM Tris-HCl, pH 9) and 0.25 U Super-Therm Taq DNA polymerase. The polymerase chain reaction (PCR) profile was 95 $^\circ\text{C}$ for 1 min, followed by 35 cycles of 35 s at 95 $^\circ\text{C}$, 30 s at 50–55 $^\circ\text{C}$ (primer dependent) and 1 min at 72 $^\circ\text{C}$, with a final extension at 72 $^\circ\text{C}$ for 30 s. The PCR product was electrophoresed on a 1% agarose gel containing GoldViewTM (Geneshun Biotech Ltd., Guangdong, China), and visualized by ultraviolet light. PCR products were sent to Macrogen (Seoul, Korea) for clean-up and sequencing. Sequences were checked and aligned with GENEIOUSPRO v. 4.8 (Drummond *et al.*, 2007). All new sequences have been deposited in the European Molecular Biology Laboratory (EMBL) (Appendix S1).

The phylogenetic analysis was implemented in MRBAYES v. 3.0b4 (Huelsenbeck & Ronquist, 2001). MODELTEST v. 3.06 (Posada & Crandall, 1998) was run initially, and indicated that the GTR+G+I model of DNA substitution was the best fit for the combined dataset and for mitochondrial markers, but a simpler model (HKY+I+G) was appropriate for the nuclear marker. Thus, MRBAYES was run specifying six rate categories ($nst = 6$) for the mitochondrial markers, and two rate categories for RAG-1 ($nst = 2$), plus invariable sites, and the alpha shape parameter for the gamma distribution to account for among-site rate heterogeneity. The dataset was constructed with seven partitions, which were allowed to estimate model parameters independently for each codon for ND2 and RAG-1, plus independently for 16S. The Markov chain Monte Carlo (MCMC) was run three times in parallel (each with four independent chains) for 10×10^6 generations with trees sampled every 1000 generations. To ensure that over-parameterization would not affect the tree topology or support values, an additional MCMC was run with only 3 partitions (one for each marker) using the same model priors as above. Burn-in was determined by examining stationarity of log likelihood tree scores, standard deviation of split frequencies, and an effective sample size (ESS) > 200 for all parameters.

Log-likelihood tree scores for the MCMC reached a plateau in < 20,000 generations for all runs, but the average standard deviation of the split frequencies did not stabilize until 5×10^6 generations. Thus, the first 5000 trees were considered burn-in and were removed from the analysis. The MCMC was run separately for the nuclear and the mitochondrial datasets using the same conditions as above, to ensure the two types of markers did not produce conflicting topologies. Nodes with posterior probability ≥ 0.95 were considered supported. Finally, a maximum likelihood (ML) search was run in GARLI v. 1.0 (Zwickl, 2006), using the GTR+I+G model indicated by MODELTEST for the combined dataset, with all parameters estimated, and a random starting tree. This analysis was run three times to ensure that independent ML searches produced the same topologies. Nodes with a bootstrap value of $\geq 75\%$ were considered supported in this analysis.

A separate phylogenetic analysis was run to estimate divergence times using an uncorrelated log normal relaxed molecular clock in BEAST v. 1.4.8 (Drummond & Rambaut, 2007). Input xml files were generated in BEAUTY v. 1.4.8 (Drummond & Rambaut, 2007) and edited to allow the parameters to be estimated separately for each data partition (one for each gene), using a Yule model of speciation, a random starting tree, and the GTR+I+G model of sequence evolution. The MCMC was run twice, for 20 million generations each, and a 10% burn-in, with runs combined using LOGCOMBINER v. 1.4.8 (Drummond & Rambaut, 2007). There are no primary calibration points within *Kinyongia*, so the BEAST analysis included two individuals from each *Kinyongia* lineage, plus 2–3 representatives from most other genera of chameleons in order to accommodate two fossil calibration points. The outgroup consisted of three species from the chameleon genus *Brookesia* (*Brookesia thieli*, *Brookesia brygooi* and *Brookesia peyrierasi*) as this genus has been shown to be a sister clade to all the other genera (Townsend & Larson, 2002; Townsend *et al.*, 2011). The BEAST analysis was run using several different scenarios for priors on calibration points (Appendix S2). Two calibration priors were always included for genera in which fossils have been dated: *Chamaeleo andrusovi*, *c.* 18 Ma (Čerňanský, 2010), and *Bradypodion* sp., *c.* 5.2 Ma (P. Haarhoff, West Coast Fossil Park, pers. comm., 2007), providing lower age limits for the genera. Assignment of priors was guided by previous studies, which suggest that most chameleon genera probably diverged no earlier than 35 Ma (Matthee *et al.*, 2004), and that *Bradypodion* is *c.* 15 million years old (Tolley *et al.*, 2008). The choice of priors for the root node was guided by information from molecular dating (Matthee *et al.*, 2004; Townsend *et al.*, 2011), based on the split between *Brookesia* and the other genera of chameleons between *c.* 45 and 75 Ma. Finally, the taxonomic scope of the dating analysis was widened and BEAST was run using outgroup taxa from the family Agamidae (Appendix S2). For each run, the effective sample size for all parameters was checked using TRACER v. 1.4.1 (Rambaut & Drummond, 2007).

Biogeographic analyses

To investigate historical biogeography and generate a hypothesis of dispersion over the landscape for the genus *Kinyongia*, ancestral character state reconstruction for biogeographic areas based on areas of avian endemism (Fjelds  & Bowie, 2008) was carried out in MESQUITE v. 2.74 (Maddison & Maddison, 2006). For each taxon, the present area of occurrence was coded as belonging to one of five biogeographic areas: southern Eastern Arc, northern Eastern Arc, volcanoes, Albertine Rift and the Kenyan Highlands (Fig. 1). Character history (i.e. areas) was traced on the ultrametric BEAST tree using the likelihood reconstruction in MESQUITE and the Markov *k*-state 1 parameter mode (all states equally probable). Character states were considered unequivocal when proportional likelihoods were ≥ 0.95 .

An index of evolutionary distinctiveness (ED) was investigated (Isaac *et al.*, 2007) using the Tuatara v. 1.01 package for MESQUITE (Maddison & Mooers, 2007). This analysis uses total branch lengths from tip to root of terminal taxa weighted by the number of descendant taxa to generate an index of distinctiveness for each taxon. Essentially, taxa that occur on long branches will receive higher values of ED than those on short branches, given the same number of daughter lineages in a clade. This method also returns high scores for young taxa in cases where long branches lead to the split between sister species (Isaac *et al.*, 2007). The analysis was conducted using the ultrametric tree produced by BEAST, but did not include an evaluation of taxa outside the *Kinyongia* clade. Two other measures of conservation importance were also estimated, the equal splits distinctiveness measure (EDc) (Redding & Mooers, 2006) and Vane-Wright *et al.*'s (1991) measure of taxonomic distinctness. EDc is similar to ED, whereby terminal branch length of a taxon is added to the length of all ancestral branches, which are equally weighted according to the number of splits in that clade (Redding & Mooers, 2006). Vane-Wright *et al.*'s (1991) taxonomic distinctness measure takes into account the number of branches leading to each terminal, from tip to root.

Faith's phylogenetic diversity (PD; Faith, 1992) was estimated for each mountain block and for each of the five biogeographic areas. PD differs from other metrics such as ED, because it is a sum of the phylogenetic diversity of the species occurring in a geographic area, rather than an index of the distinctiveness of a single lineage. The ultrametric dated BEAST tree was used in conjunction with a presence/absence matrix coded for the taxa occurring in each mountain block. PD (using the dated phylogeny, PD is given as 'evolutionary age') was estimated in R 2.5.1 (R Development Core Team, 2005) using the APE package (Paradis *et al.*, 2004) and a script written by R. Grenyer (University of Oxford, pers. comm.). To determine whether PD in a given mountain block was different than expected by chance, 10,000 randomizations of PD were performed using the R script (e.g. Forest *et al.*, 2007) to obtain a null model for comparison to the observed PD.

RESULTS

Phylogenetic analyses

The phylogeny (Fig. 2) showed three well-supported clades that correspond to the geographic regions Albertine Rift/Kenyan Highlands (AR/KH), northern Eastern Arc (NEA), southern Eastern Arc (SEA). Two reversals of this pattern are present: one NEA species (*Kinyongia tenuis*, East Usambara Mountains) is within the SEA clade, and one SEA species (*Kinyongia fischeri*, Nguru Mountains) is within the NEA clade. Most lineages in the phylogeny are found only in a single mountain block, with two exceptions (*K. tavetana*: North Pare Mountains, Mount Kilimanjaro and Mount Meru and *K. uthmoelleri*: South Pare Mountains, Mount Hanang). Levels of sequence divergence between terminal lineages (Appendix S3) are higher than those found at the species level in other chameleon genera (Tolley *et al.*, 2004, 2006; Tilbury *et al.*, 2006; Tilbury & Tolley, 2009a,b).

The effects of different dating scenarios on the divergence dates were minimal (Appendix S2), with 95% highest posterior density (HPD) overlapping between scenarios. Only the run using the agamids as the outgroup taxa showed older dates across the phylogeny, but all 95% HPD intervals for this run were markedly larger than in the other runs. Overall, the dating analysis suggests that the origin of the genus occurred > 30 Ma (Fig. 3; Appendix S2). The three main clades within *Kinyongia* are also ancient, with origins roughly 25–30 Ma. Most diversification within clades is dated to the mid-Miocene, although a few extant lineages are more ancient and date to the early Miocene (e.g. *K. uthmoelleri*, *Kinyongia excubitor*, *Kinyongia uluguruensis*). Colonization of extant species from the North (*K. tavetana*) and South Pare (*K. uthmoelleri*) Mountains to the forested slopes of the three volcanoes (Mount Kilimanjaro/Meru and Hanang, respectively) occurred more recently (5–6 Ma) but pre-dates the formation of the volcanoes themselves (Griffiths, 1993; Le Gall *et al.*, 2008). Relatively few lineages can be dated to the Pliocene, and none to the Pleistocene.

Biogeographic analyses

The likelihood optimization of ancestral areas show the highest probability of origin in the southern Eastern Arc (Fig. 4), but the proportional likelihood is low (0.48), and the result should be considered equivocal. The distribution over the present biogeographic regions appears to be rooted deep in the past, with little subsequent interchange between areas. There are a few exceptions (Fig. 4), with (1) the movement of species into the forested slopes of newly formed volcanoes (*K. tavetana* and *K. uthmoelleri*), (2) the Miocene interchange at the contact zone between the northern and southern Eastern Arc (East Usambara: *K. tenuis*, and Nguru: *K. fischeri*), and (3) the early divergence of chameleons from the Albertine Rift (*Kinyongia adolffriderici*, *Kinyongia carpenteri*, *Kinyongia xenorhina*) and the Kenyan Highlands (Fig. 3).

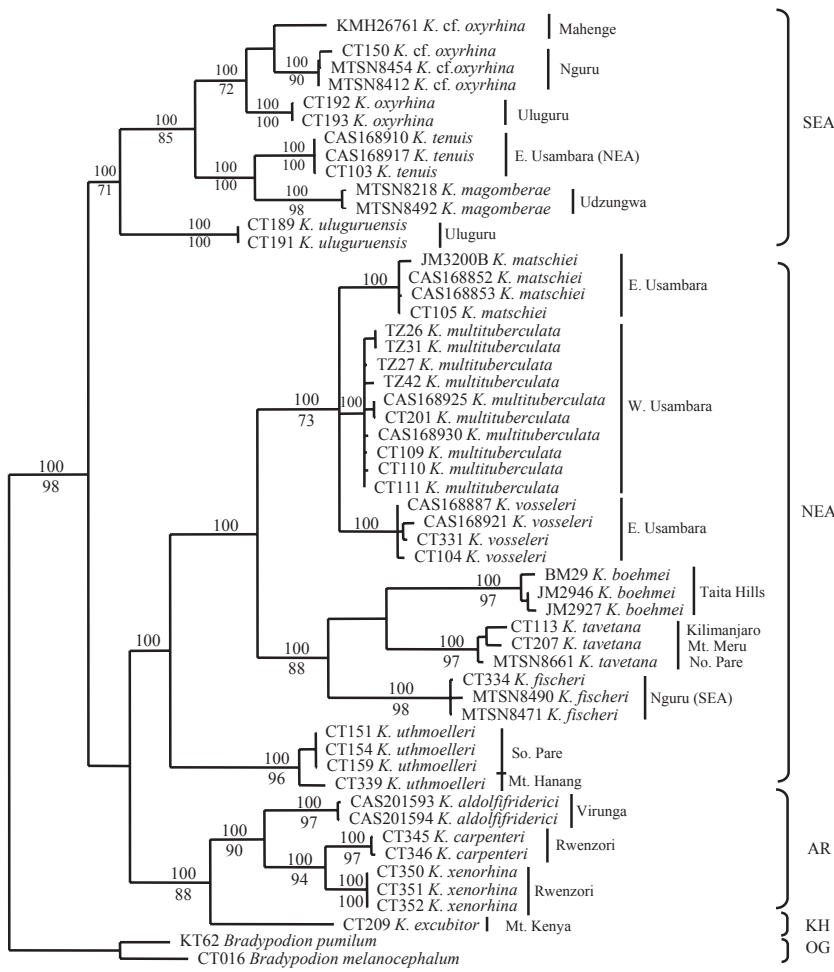


Figure 2 Bayesian consensus phylogram for *Kinyongia*. Node support is shown by posterior probabilities above each branch, and maximum likelihood (ML) bootstrap values below. Geographic areas are indicated: SEA, southern Eastern Arc; NEA, northern Eastern Arc; AR, Albertine Rift; KH, Kenyan Highlands. OG, outgroup.

Measures of diversity showed similar patterns to each other. The analysis of evolutionary diversity (ED) suggests that exceptionally distinct lineages occur in the Kenyan Highlands (*K. excubitor*), the South Pare Mountains (*K. uthmoelleri*) and the Uluguru Mountains (*K. uluguruensis*). The ED values for these three species are outside the 95% confidence interval of the mean for ED. Clade-weighted evolutionary distinctiveness (EDc) showed one addition to the pattern: *Kinyongia oxyrhina* from the Uluguru Mountains also lies outside the 95% confidence interval for the mean of EDc. Vane-Wright *et al.*'s distinctness measure appears to be the least sensitive to the identification of distinctive lineages, with only two taxa, *K. excubitor* and *K. uluguruensis*, falling outside the 95% confidence intervals for that metric.

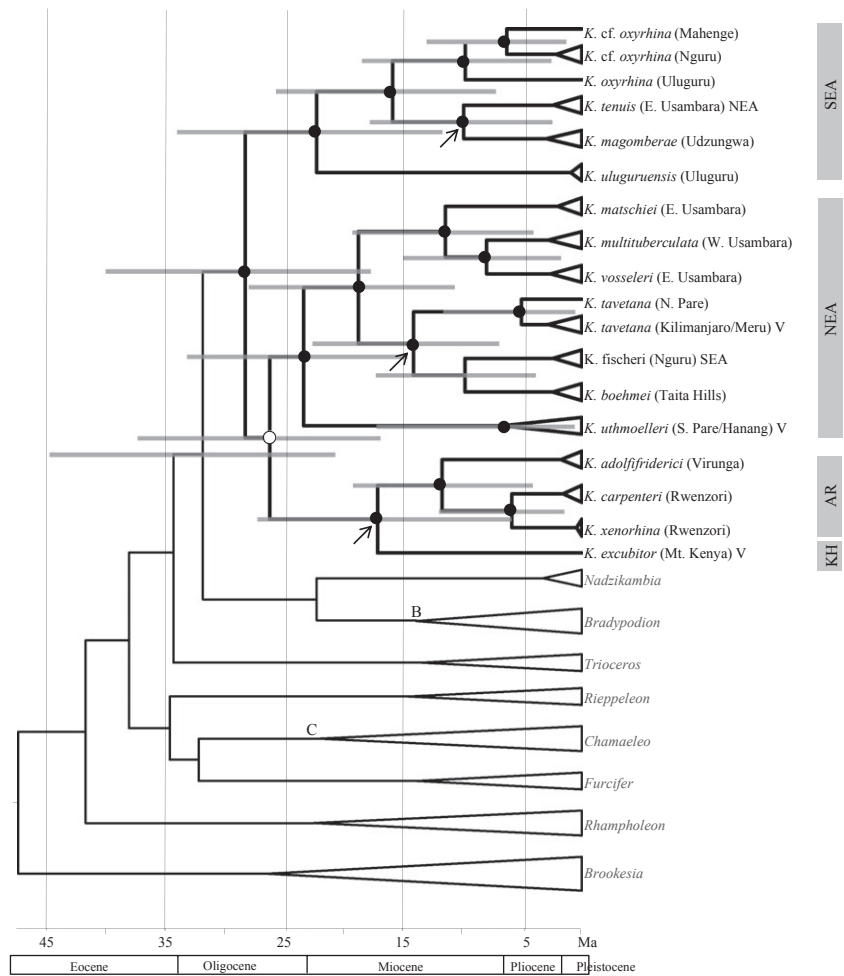
Phylogenetic diversity (PD) was highest in the mountain blocks closest to the coastal plains (Fig. 5), especially East Usambara, Nguru and Uluguru. In these three mountain blocks, the species richness (two species each) is not remarkable (Fig. 5) suggesting that higher PD is not due to a greater number of species. Randomizations indicated that PD was actually lower than expected by chance in all of these mountains, and in several others (East and West Usambara, Nguru, Udzungwa, Uluguru, Taita Hills, and the Albertine Rift: $P < 0.05$, two-tailed comparison of observed PD to

randomized distribution). PD was not different than expected in the remaining mountains (Kenyan Highlands, Mount Kilimanjaro, Mount Meru, Mount Hanang, North and South Pare, and Mahenge), nor was it lower for the volcanic mountains, than for the mountain blocks. When considering the biogeographic regions, PD was nearly double in the Eastern Arc compared with the other areas (volcanoes, Albertine Rift, Kenyan Highlands). Randomizations, however, indicate that PD was lower than expected for all areas (10,000 randomizations, two-tailed comparison to random values), suggesting that taken as a whole, the phylogeny is over-dispersed.

DISCUSSION

The genus *Kinyongia* is dominated by ancient lineages, most dating to the mid- and late Miocene, with a notable lack of evidence for the Plio-Pleistocene radiation that characterizes many East African taxa (Terborgh, 1992a,b; Fjeldså & Lovett, 1997; Bowie *et al.*, 2004, 2006; Plana, 2004; Blackburn & Measey, 2009). The genus is characterized by the retention of palaeoendemics in stable refugia, and has not undergone recent radiations into novel heterogeneous habitats. In most clades, sister species diverged in the mid-late Miocene and the lack of co-occurring sister species on mountain blocks

Figure 3 Bayesian chronogram of phylogenetic relationships inferred for Chamaeloniidae using BEAST run *i* (see Appendix S2). Fossil calibration points are labelled with C (*Chamaeleo andrusovi*) and B (*Bradypodion* sp.). Node support with posterior probabilities ≥ 0.95 and maximum likelihood bootstrap $\geq 75\%$ are indicated with a black circle, with nodes supported only by posterior probabilities ≥ 0.95 indicated with an open circle. Genera outside *Kinyongia* (grey text) are included for primary fossil calibration points. Shaded bars represent 95% highest probability densities of divergence times within *Kinyongia*. Tips are collapsed for each species and are labelled according to taxon and the mountain isolate on which each species occurs. NEA, northern Eastern Arc Mountains; SEA, southern Eastern Arc Mountains; AR, Albertine Rift; KH, Kenyan Highlands. Isolated volcanic cones are indicated by V. Arrows point to the Miocene interchange at the contact zone between the northern and southern Eastern Arc and the early spread of chameleons into the Albertine Rift the Kenyan Highlands.



supports a model of divergence in allopatry. While some species do co-occur on a mountain block, they are either distantly related (e.g. *K. oxyrhina* and *K. uluguruensis*) or a sister taxon relationship is not supported (e.g. *Kinyongia matschiei* and *Kinyongia vosseleri*). Although sister taxa are distributed on the Rwenzori Massif (*K. carpenteri* and *K. xenorhina*), it is unknown to what extent their distributions overlap (Tilbury, 2010). Overall, lineage diversification reflects vicariance through isolation in refugia, rendering the elevated speciation rate model inappropriate for *Kinyongia*. Although this model is well supported for numerous other taxa to date, it probably does not explain the exceptional levels of diversity and endemism for all taxa throughout East Africa.

The equivocal result for the ancestral area optimization coupled with the short branch lengths near the root of the phylogeny points to a widespread distribution for the genus during the Oligocene, with the break-up of pan-African forest fragmenting the genus into three main clades at the start of the Miocene. In addition, the origin of the genus pre-dates the present Eastern Arc formation. Thus, vicariance associated with pan-African forest contractions seems to be the best explanation for both regional and local biogeographic patterns (rather than colonization from one of the regions).

Historical biogeography

The divergence time estimated for the origin of *Kinyongia* is ancient, within the Oligocene, c. 32 Ma (Fig. 3). The overall timing of this early divergence corresponds to cool conditions beginning in the late Eocene (Zachos *et al.*, 2001) which initiated fragmentation of the pan-African forest (Wasser & Lovett, 1993; Jacobs *et al.*, 1999; Couvreur *et al.*, 2008). Given that these chameleons are strongly linked with forest habitat and have not diversified into new habitats (i.e. savanna and grasslands), it is perhaps inevitable that their biogeographic patterns mirror that of African forests, which showed major diversification pulses at c. 33, 17, 8 and 5 Ma (Couvreur *et al.*, 2008).

The presence of palaeoendemics is ubiquitous within the phylogeny, given the presence of multiple taxa with long branches and the lack of closely related sister taxa. As the pan-African forests disappeared (Wasser & Lovett, 1993) and major tracts of forests were turned over to open habitats (Cerling *et al.*, 1997; Jacobs *et al.*, 1999; Jacobs, 2004), *Kinyongia* would have been reduced to a few isolated lineages persisting in montane forest patches, a scenario not unique within chameleons. Forest-restricted species of the dwarf chameleons (genus *Bradypodion*) are on long branches in the phylogeny (with no

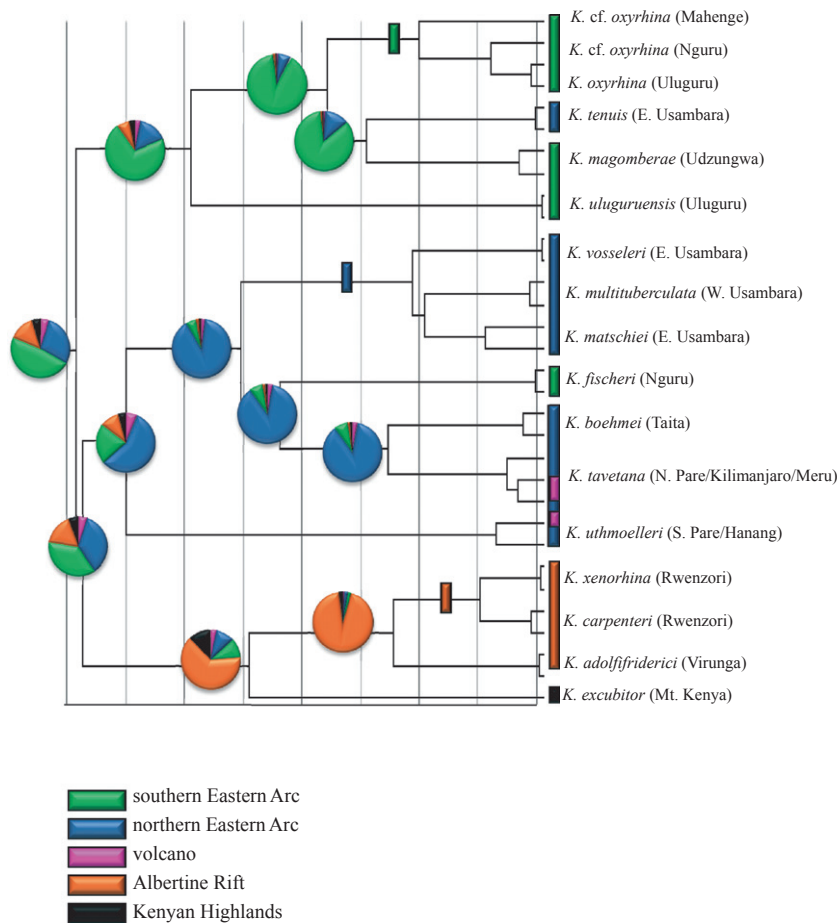


Figure 4 Likelihood optimization probabilities for the ancestral areas represented by pie charts at each node in the *Kinyongia* phylogeny. Terminal taxa are colour coded according to the area in which they occur. Unequivocal states are indicated along the branches by a colour-coded bar.

closely related sister taxa) as a result of extinction filtering through a reduction in the extent and connectivity of southern African forests in the Miocene (Tolley *et al.*, 2008). However, unlike *Kinyongia*, some clades of *Bradypodion* have successfully radiated into adjacent open habitats (Tolley *et al.*, 2008), possibly suggesting that both a palaeoendemic and an elevated speciation rate model applies to that genus. Although these models were not explicitly tested, forest-restricted and open-habitat-adapted sister species also occur in the chameleon genus *Rieppeleon* (Matthee *et al.*, 2004). Overall, these findings suggest that chameleons do not conform to any one model of speciation. Multiple speciation modes would suggest that chameleons per se, are able to take advantage of adjacent novel habitats leading to parapatric (or sympatric) speciation, but that this has not occurred in *Kinyongia* despite prolonged stability of habitats and available alternative niches. Indeed, patterns of speciation in East African vertebrates can often be explained by vicariance at some level (Matthee *et al.*, 2004; Bowie *et al.*, 2006; Blackburn & Measey, 2009; Taylor *et al.*, 2009; Lawson, 2010) through isolation in refugia, rather than parapatric speciation (Voelker *et al.*, 2010).

In the present study, the incidence of major episodes of Miocene lineage diversification suggests that forest re-expansion and connectivity during climatic optima (Zachos *et al.*, 2001; Couvreur *et al.*, 2008) influenced diversification. Multi-

ple reconnections of forests (Couvreur *et al.*, 2008) are reflected in the *Kinyongia* phylogeny by the mid-late Miocene divergence of *K. tenuis* (distributed in the northern Eastern Arc) from members of the SEA clade, and the mid-late Miocene divergence of *K. fischeri* (distributed in the southern Eastern Arc) from members of the NEA clade (Fig. 3). Connections between the Albertine Rift and the Kenyan Highlands are considerably older (Fig. 3), and support hypothesized ancient connectivity between those areas (e.g. Lovett, 1993a; Fjeldså & Lovett, 1997). Although there is a possibility of recent corridors linking the Albertine Rift with the western Kenyan Highlands, this does not appear to have influenced the current biogeographic patterns in that area for *Kinyongia*, as it may have for other taxa (Bowie *et al.*, 2005; Schick *et al.*, 2005). Since the mid-late Miocene, geomorphological changes in the Kenyan Highlands brought aridification by halting the deep westward penetration of Indian Ocean rainfall, and by altering drainage patterns that had previously promoted wet corridors (Lovett, 1993a). These events correspond well with the divergence time estimates for *K. excubitor* (Kenyan Highlands) from lineages in the Albertine Rift (Fig. 3). The Highland lineage appears to have remained isolated from the Albertine lineages since that time, but also to have remained isolated from the Eastern Arc lineages due to increasing arid conditions and the establishment of a dry

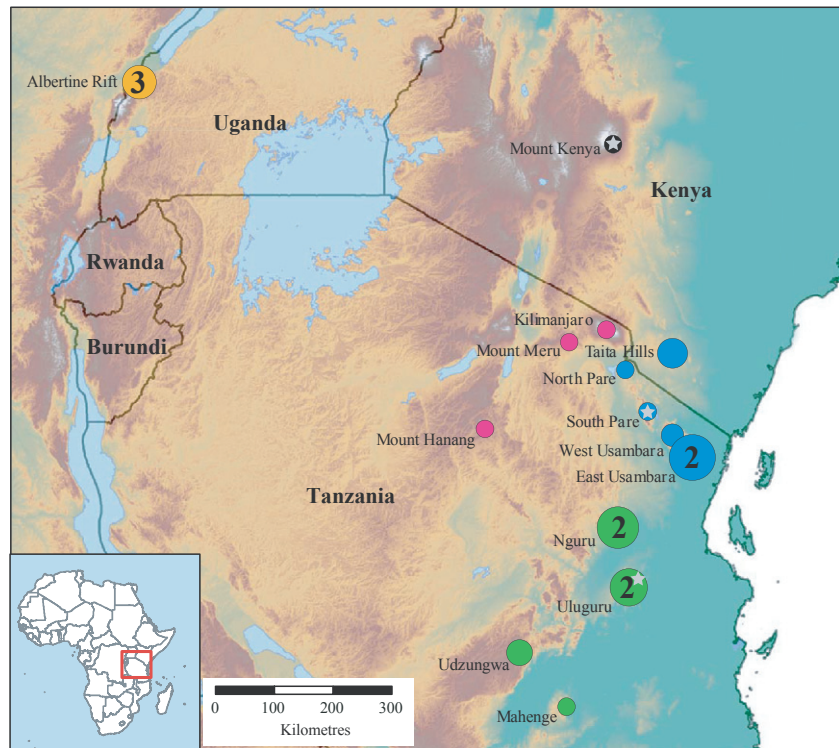


Figure 5 Phylogenetic diversity (PD) indicated by the relative size of the circles for each of the mountains from which *Kinyongia* were sampled. For mountains with more than one species, the number of species is indicated within the circles. Stars indicate the mountains where evolutionary diversity (ED) is highest.

corridor between the highlands and the northernmost Eastern Arc Mountains (Lovett, 1993a).

The late Miocene was a period of renewed aridification and increase of open habitats, due to changes in global circulation and tectonic events leading to continental uplift that altered rainfall patterns (Axelrod & Raven, 1978; Jacobs *et al.*, 1999; Zachos *et al.*, 2001; Jacobs, 2004; Sepulchre *et al.*, 2006). The spread of open environments and reduction of forest is reflected in the phylogenetic tree by a prolonged period with very little lineage diversification. The most recent diversification events appear to be associated within single taxa that occur in both mountain blocks and on volcanic slopes (e.g. *K. tavetana*, *K. uthmoelleri*), with estimated timing of diversification in the Pliocene. These divergence times pre-date the emergence of Mount Kilimanjaro, Mount Meru and Hanang (Griffiths, 1993; Schuller, 1997) suggesting diversification did not occur *in situ*. It is likely that forested patches were periodically widespread in the Pliocene (Plana, 2004), but that the most recent contractions have left remnant isolated populations in relict forests on any elevated land mass high enough to attract substantial orographic rainfall.

Patterns of diversity

While wholesale climate changes since the Eocene have profoundly changed the African landscape from moist tropical forest to savanna, many forest fragments persist in East Africa due to orographic rain as a result of Indian Ocean circulation (Lovett, 1993a,b; Sepulchre *et al.*, 2006). Although the East African landscape has undergone much uplift and subsequent

erosion, the present Eastern Arc Mountains were formed c. 7 Ma by block faulting associated with the East African Rift System (Griffiths, 1993). The resulting montane forests were probably stable enough throughout the Pliocene to allow populations of chameleons to persist despite an overall transition from forest to savanna across the larger landscape.

Mountain blocks closest to the coastal plains (East Usambara, Nguru and Uluguru) are likely to have received regular rainfall throughout the history of *Kinyongia*, and are the same mountains for which phylogenetic diversity (PD) is highest, due to the presence of palaeoendemics. This could suggest that these forests have survived in some form since these palaeoendemics shared a common ancestor, resulting in a phylogeny that is characterized by lineage extinction rather than radiation (or possibly, that many species have yet to be discovered). The other measures of diversity – evolutionary distinctiveness (ED), clade-weighted evolutionary distinctiveness (EDc) and Vane-Wright *et al.*'s (1991) measure of taxonomic distinctness – are basically reflective of lineages with the longest branch lengths (*K. excubitor*: Mount Kenya; *K. uthmoelleri*: South Pare; *K. uluguruensis* and *K. oxyrhina*: Uluguru Mountains), that is, those with exceptionally distinct evolutionary histories. Although these species are each evolutionary distinctive, the mountain blocks they inhabit are not necessarily exceptional in this respect. Only the Uluguru Mountains have both high PD and two species with high evolutionary distinctiveness. Unfortunately, we cannot conclude that this mountain block is the most diverse overall, as two species were missing from this analysis (*K. asheorum* from Mount Nyiru and *K. vanheygeni* from the Poroto Mountains),

and other new species may be unrepresented. Nonetheless, at this stage it appears that the Uluguru Mountains stand out as being diverse for *Kinyongia* as well as for other chameleons (Matthee *et al.*, 2004).

CONCLUSIONS

The biogeographic patterns associated with *Kinyongia* are the result of long evolutionary histories in isolation, resulting in a genus that is characterized by palaeoendemics. Recent radiation into newer, open habitats has not occurred in this genus. Phylogenetic diversity is lower than expected by chance, which suggests there are fewer lineages than expected, pointing to extinction filtering (and/or undiscovered taxa). Thus, the overall pattern of diversity is dominated by the presence of palaeoendemics, with mountain blocks which contain several of these palaeoendemics having the highest phylogenetic and evolutionary diversity (e.g. Uluguru Mountains).

The hypothesis that stable climates and heterogeneous habitats have provided refugia that elevate speciation rates, while relevant for some taxonomic groups, is not supported for this genus of forest-dependent chameleons. Sister taxa are not parapatric on the same mountain blocks, nor is there a preponderance of recent radiations and young lineages as would be expected under such a scenario. By explicitly testing these alternative hypotheses using a dated phylogeny for a forest-restricted group, we show a lack of congruence with the best-studied taxa (e.g. birds), and thus a single explanatory model for the high diversity and endemism of East Africa is not apparent. This may well apply for other mega-diverse regions (e.g. Grenyer *et al.*, 2006; Rull, 2008; Vences *et al.*, 2009). We conclude that efforts towards synthetic biogeographic hypotheses must be based on diverse and multiple taxa.

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REFERENCES

Axelrod, D.I. & Raven, P.H. (1978) Late Cretaceous and Tertiary vegetation history of Africa. *Biogeography and ecology of*

southern Africa (ed. by M.J.A. Werger), pp. 77–130. Junk, The Hague.

- Bickel, R. & Losos, J.B. (2002) Patterns of morphological variation and correlates of habitat use in chameleons. *Biological Journal of the Linnean Society*, **76**, 91–103.
- Blackburn, D.C. & Measey, G.J. (2009) Dispersal to or from an African biodiversity hotspot? *Molecular Ecology*, **18**, 1904–1915.
- Bowie, R.C.K., Fjelds , J., Hackett, S.J. & Crowe, T.M. (2004) Molecular evolution in space and through time: mtDNA phylogeography of the olive sunbird (*Nectarinia olivacea/obscura*) throughout continental Africa. *Molecular Phylogenetics and Evolution*, **33**, 56–74.
- Bowie, R.C.K., Voelker, G., Fjelds , J., Lens, L., Hackett, S.J. & Crowe, T.M. (2005) Systematics of the olive thrush *Turdus olivaceus* species complex with reference to the taxonomic status of the endangered Taita thrush *T. helleri*. *Journal of Avian Biology*, **36**, 391–404.
- Bowie, R.C.K., Fjelds , J., Hackett, S.J., Bates, J.M. & Crowe, T.M. (2006) Coalescent models reveal the relative roles of ancestral polymorphism, vicariance, and dispersal in shaping phylogeographical structure of an African montane forest robin. *Molecular Phylogenetics and Evolution*, **38**, 171–188.
- Brooks, T.M., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Rylands, A.B., Konstant, W.R., Flick, P., Pilgrim, J., Oldfield, S., Magin, G. & Hilton-Taylor, C. (2002) Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology*, **16**, 909–923.
- Burgess, N.D., Clarke, G.P. & Rodgers, W.A. (1998) Coastal forests of eastern Africa: status, endemism patterns and their potential causes. *Biological Journal of the Linnean Society*, **64**, 337–367.
- Burgess, N.D., Lovett, J.C., Rodgers, A., Kilahama, F.B., Nasheda, E., Davenport, T.R.B. & Butynski, T.M. (2004) Eastern Arc Mountains and southern rift. *Hotspots revisited: Earth's biologically richest and most endangered ecoregions* (ed. by R.A. Mittermeier, P. Robles-Gil, M. Hoffmann, J.D. Pilgrim, T.M. Brooks, C.G. Mittermeier, J.L. Lamoreux and G.A.B. da Fonseca), pp. 245–255. Cemex, Mexico.
- Burgess, N.D., Butynski, T.M., Cordeiro, N.J., Doggart, N.H., Fjelds , J., Howell, K.M., Kilahama, F.B., Loader, S.P., Lovett, J.C., Mbilinyi, B., Menegon, M., Moyer, D.C., Nasheda, E., Perkin, A., Rovero, F., Stanley, W.T. & Stuart, S.N. (2007) The biological importance of the Eastern Arc Mountains of Tanzania and Kenya. *Biological Conservation*, **134**, 209–231.
- Carnaval, A.C., Hickerson, M.J., Haddad, C.L.F.B., Rodrigues, M.T. & Moritz, C. (2009) Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. *Science*, **323**, 785–789.
- Cerling, T.E., Harris, J.M., MacFadden, B.J., Leakey, M.G., Quade, J., Eisenmann, V. & Ehleringer, J.R. (1997) Global vegetation change through the Miocene/Pliocene boundary. *Nature*, **389**, 153–158.

- Čerňanský, A. (2010) A revision of chamaeleonids from the Lower Miocene of the Czech Republic with description of a new species of *Chamaeleo* (Squamata, Chamaeleonidae). *Geobios*, **43**, 605–613.
- Chen, I.C., Shiu, H.-J., Benedick, S., Holloway, J.D., Chey, V.K., Barlow, H.S., Hill, J.K. & Thomas, C.D. (2009) Elevation increases in moth assemblages over 42 years on a tropical mountain. *Proceedings of the National Academy of Sciences USA*, **106**, 1479–1483.
- Chorowicz, J. (2005) The East African rift system. *Journal of African Earth Sciences*, **43**, 379–410.
- Colinvaux, P.A., De Oliveira, P.E. & Bush, M.B. (2000) Amazonian and neotropical plant communities on glacial time-scales: the failure of the aridity and refuge hypotheses. *Quaternary Science Reviews*, **19**, 141–169.
- Couvreur, T.L.P., Chatrou, L.W., Sosef, M.S.M. & Richardson, J.E. (2008) Molecular phylogenetics reveal multiple Tertiary vicariance origins of the African rain forest trees. *BMC Biology*, **6**, 54.
- Driver, A., Maze, K., Rouget, M., Lombard, A.T., Nel, J., Turpie, J.K., Cowling, R.M., Desmet, P., Goodman, P., Harris, J., Jonas, Z., Reyers, B., Sink, K. & Strauss, T. (2005) *National spatial biodiversity assessment 2004: priorities for biodiversity conservation in South Africa*. South African National Biodiversity Institute, Pretoria.
- Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.
- Drummond, A.J., Ashton, B., Cheung, M., Heled, J., Kearse, M., Moir, R., Stones-Havas, S., Thierer, T. & Wilson, A. (2007) *Geneious v4.8*. Biomatters Ltd., Auckland, New Zealand. Available at: <http://www.geneious.com>.
- Faith, D.P. (1992) Conservation evaluation and phylogenetic diversity. *Biological Conservation*, **61**, 1–10.
- Finch, J., Leng, M.J. & Marchant, R. (2009) Late Quaternary vegetation dynamics in a biodiversity hotspot, the Uluguru Mountains of Tanzania. *Quaternary Research*, **72**, 111–122.
- Fjelds , J. (1994) Geographical patterns of relict and young species of birds in Africa and South America and implications for conservation priorities. *Biodiversity and Conservation*, **3**, 107–126.
- Fjelds , J. & Bowie, R.C.K. (2008) New perspectives on the origin and diversification of Africa's forest avifauna. *African Journal of Ecology*, **46**, 235–247.
- Fjelds , J. & Lovett, J.C. (1997) Geographical patterns of old and young species in African forest biota: the significance of specific montane areas as evolutionary centres. *Biodiversity and Conservation*, **6**, 322–346.
- Fjelds , J., Ehrlich, D., Lambin, E. & Prins, E. (1997) Are biodiversity 'hotspots' correlated with current ecoclimatic stability? A pilot study using the NOAA-AVHR remote sensing data. *Biodiversity and Conservation*, **6**, 401–422.
- Forest, F., Grenyer, R., Rouget, M., Davies, T.J., Cowling, R.M., Faith, D.P., Balmford, A., Manning, J.C., Procheş, Ş., van der Bank, M., Reeves, G., Hedderson, T.A.J. & Salvoilainen, V. (2007) Preserving the evolutionary potential of floras in biodiversity hotspots. *Nature*, **445**, 757–760.
- Forister, M.L., McCall, A.C., Sanders, N.J., Fordyce, J.A., Thorne, J.H., O'Brien, J., Waetjen, D.P. & Shapiro, A.M. (2010) Compounded effects of climate change and habitat alteration shift patterns of butterfly diversity. *Proceedings of the National Academy of Sciences USA*, **107**, 2088–2092.
- Grenyer, R., Orme, C.D.L., Jackson, S.F., Thomas, G.H., Davies, R.G., Davies, T.J., Jones, K.E., Olson, V.A., Ridgely, R.S., Rasmussen, P.C., Ding, T.-S., Bennett, P.M., Blackburn, T.M., Gaston, K.J., Gittleman, J.L. & Owens, I.P.F. (2006) Global distribution and conservation of rare and threatened vertebrates. *Nature*, **444**, 93–96.
- Griffiths, C.J. (1993) The geological evolution of East Africa. *Biogeography and ecology of the rain forests of eastern Africa* (ed. by J.C. Lovett and S.K. Wasser), pp. 9–21. Cambridge University Press, Cambridge.
- Haffer, J. (1969) Speciation in Amazonian forest birds. *Science*, **165**, 131–137.
- Haffer, J. (1997) Alternative models of vertebrate speciation in Amazonia: an overview. *Biodiversity and Conservation*, **6**, 451–476.
- Hewitt, G. (2000) The genetic legacy of Quaternary ice ages. *Nature*, **405**, 907–913.
- Hopkins, K.P. & Tolley, K.A. (2011) Morphological variation in the Cape Dwarf Chameleon (*Bradypodion pumilum*) as a consequence of spatially explicit habitat structure. *Biological Journal of the Linnean Society*, **102**, 878–888.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.
- Isaac, N.J.B., Turvey, S.T., Collen, B., Waterman, C. & Baillie, J.E.M. (2007) Mammals on the EDGE: conservation priorities based on threat and phylogeny. *PLoS ONE*, **3**, 296.
- Jacobs, B.F. (2004) Palaeobotanical studies from tropical Africa: relevance to the evolution of forest, woodland and savannah biomes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **359**, 1573–1583.
- Jacobs, B.F., Kingston, J.D. & Jacobs, L.L. (1999) The origin of grass-dominated ecosystems. *Annals of the Missouri Botanical Garden*, **86**, 590–643.
- Kampunzu, A.B., Bonhomme, M.G. & Kanika, M. (1998) Geochronology of volcanic rocks and evolution of the Cenozoic western branch of the East African rift system. *Journal of African Earth Sciences*, **26**, 441–461.
- Lawson, L.P. (2010) The discordance of diversification: evolution in the tropical-montane frogs of the Eastern Arc Mountains of Tanzania. *Molecular Ecology*, **19**, 4046–4060.
- Le Gall, B., Nonnotte, P., Rolet, J., Benoit, M., Guillou, H., Mousseau-Nonnotte, M., Albaric, J. & Deverchère, J. (2008) Rift propagation at craton margin. Distribution of faulting and volcanism in the North Tanzanian divergence (East Africa) during Neogene times. *Tectonophysics*, **448**, 1–19.
- Lessa, E.P., Cook, J.A. & Patton, J.L. (2003) Genetic footprints of demographic expansion in North America, but not Amazonia, during the late Quaternary. *Proceedings of the National Academy of Sciences USA*, **100**, 10331–10334.

- Lovett, J.C. (1993a) Climatic history and forest distribution in eastern Africa. *Biogeography and ecology of the rain forests of eastern Africa* (ed. by J.C. Lovett and S.K. Wasser), pp. 23–29. Cambridge University Press, Cambridge.
- Lovett, J.C. (1993b) Eastern Arc moist forest flora. *Biogeography and ecology of the rain forests of eastern Africa* (ed. by J.C. Lovett and S.K. Wasser), pp. 33–55. Cambridge University Press, Cambridge.
- Lovett, J.C., Marchant, R., Taplin, J. & Küper, W. (2005) The oldest rainforests in Africa: stability or resilience for survival and diversity? *Phylogeny and conservation* (ed. by A. Purvis, J.L. Gittleman and T.M. Brooks), pp. 198–229. Cambridge University Press, Cambridge.
- Maddison, W.P. & Maddison, D.R. (2006) *Mesquite: a modular system for evolutionary analysis. Version 2.73*. Available at: <http://mesquiteproject.org>.
- Maddison, W.P. & Moors, A.Ø. (2007) *Tuatara: conservation priority in a phylogenetic context*. Available at: <http://mesquiteproject.org/packages/tuatara>.
- Mariaux, J., Lutzmann, N. & Stipala, J. (2008) The two-horned chameleons of East Africa. *Zoological Journal of the Linnean Society*, **152**, 367–391.
- Matthee, C.A., Tilbury, C.R. & Townsend, T. (2004) A phylogenetic review of the African leaf chameleons: genus *Rhampholeon* (Chamaeleonidae): the role of vicariance and climate change in speciation. *Proceedings of the Royal Society B: Biological Sciences*, **271**, 1967–1976.
- Measey, G.J., Hopkins, K. & Tolley, K.A. (2009) Morphology, ornaments and performance in two chameleon ecomorphs: is the casque bigger than the bite? *Zoology*, **112**, 217–226.
- Menegon, M., Dognart, N. & Owen, N. (2008) The Nguru Mountains of Tanzania, an outstanding hotspot of herpetofaunal diversity. *Acta Herpetologica*, **3**, 107–127.
- Menegon, M., Tolley, K.A., Jones, T., Rovero, F., Marshall, A.R. & Tilbury, C.R. (2009) A new species of chameleon (Sauria: Chamaeleonidae: *Kinyongia*) from the Magombera Forest and the Udzungwa Mountains National Park, Tanzania. *African Journal of Herpetology*, **58**, 59–70.
- Mittelbach, G.G., Schemske, D.W., Cornell, H.V. *et al.* (2007) Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecology Letters*, **10**, 315–331.
- Mumbi, C.T., Marchant, R., Hooghiemstra, H. & Wooller, M.J. (2008) Late Quaternary vegetation reconstruction from the Eastern Arc Mountains, Tanzania. *Quaternary Research*, **69**, 326–341.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.
- Nečas, P. (2009) Das Poroto-Einhornchamäleon: *Kinyongia vanheygeni* n. sp. (Reptilia: Sauria: Chamaeleonidae), eine neue Art aus den Poroto-Bergen, Süd-Tansania. *Sauria*, **31**, 41–48.
- Nečas, P., Sindaco, R., Kořený, L., Kopečná, J., Malonza, P.K. & Modrý, D. (2009) *Kinyongia asheorum* sp. n., a new montane chameleon from the Nyiro range, northern Kenya (Squamata: Chamaeleonidae). *Zootaxa*, **2028**, 41–50.
- Nonnotte, P., Guillou, H., Le Gall, B., Benoit, M., Cotten, J. & Scaillet, S. (2008) New K–Ar age determinations of Kilimanjaro volcano in the north Tanzanian diverging rift, East Africa. *Journal of Volcanology and Geothermal Research*, **173**, 99–112.
- Paradis, E., Claude, J. & Strimmer, K. (2004) APE: analyses of the phylogenetics and evolution in R language. *Bioinformatics*, **20**, 289–290.
- Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology and Systematics*, **37**, 637–669.
- Plana, V. (2004) Mechanisms and tempo of evolution in the African Guineo-Congolian rainforest. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **359**, 1585–1594.
- Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.
- R Development Core Team (2005) *R: a language and environment for statistical computing, reference index version 2.5.1*. R Foundation for Statistical Computing, Vienna, Austria. Available at: <http://www.r-project.org>.
- Rambaut, A. & Drummond, A.J. (2007) *Tracer, version 1.4*. Available at: <http://beast.bio.ed.ac.uk/Tracer>.
- Redding, D.W. & Moors, A.Ø. (2006) Incorporating evolutionary measures into conservation prioritization. *Conservation Biology*, **20**, 1670–1678.
- Rull, V. (2008) Speciation timing and neotropical biodiversity: the Tertiary–Quaternary debate in the light of molecular phylogenetic evidence. *Molecular Ecology*, **17**, 2722–2729.
- Schick, S., Veith, M. & Lötters, S. (2005) Distribution patterns of amphibians from the Kakamega Forest, Kenya. *African Journal of Herpetology*, **54**, 185–190.
- Schulter, T. (1997) *Geology of East Africa*. Borntraeger, Berlin.
- Sepulchre, P., Ramstein, G., Fluteau, F., Schuster, M., Tiercelin, J.J. & Brunet, M. (2006) Tectonic uplift and eastern Africa aridification. *Science*, **313**, 1419–1423.
- Sinervo, B., Méndez-de-la-Cruz, F., Miles, D.B. *et al.* (2010) Erosion of lizard diversity by climate change and altered thermal niches. *Science*, **328**, 894–899.
- Taylor, P.J., Maree, S., van Sandwyk, J., Kerbis Peterhans, J.C., Stanley, W.T., Verheyen, E., Kaliba, P., Verheyen, W., Kaleme, P. & Bennett, N.C. (2009) Speciation mirrors geomorphology and palaeoclimatic history in African laminate-toothed rats (Muridae: Otomyini) of the *Otomys denti* and *Otomys lacustris* species-complexes in the ‘montane circle’ of East Africa. *Biological Journal of the Linnean Society*, **96**, 913–941.
- Terborgh, J. (1992a) *Diversity and the tropical rain forest*. Freeman, New York.
- Terborgh, J. (1992b) Maintenance of diversity in tropical forests. *Biotropica*, **24**, 283–292.
- Tilbury, C.R. (2010) *Chameleons of Africa: an atlas, including the chameleons of Europe, the Middle East and Asia*. Edition Chimaira, Frankfurt am Main.
- Tilbury, C.R. & Tolley, K.A. (2009a) A new species of dwarf chameleon (Sauria: Chamaeleonidae) from KwaZulu-Natal,

- South Africa with notes on recent climatic shifts and their influence on speciation in the genus. *Zootaxa*, **2226**, 43–57.
- Tilbury, C.R. & Tolley, K.A. (2009b) A re-appraisal of the systematics of the African genus *Chamaeleo* (Reptilia: Chamaeleonidae). *Zootaxa*, **2079**, 57–68.
- Tilbury, C.R., Tolley, K.A. & Branch, W.R. (2006) A review of the systematics of the genus *Bradypodion* (Sauria: Chamaeleonidae), with the description of two new genera. *Zootaxa*, **1363**, 23–38.
- Tolley, K.A., Tilbury, C.R., Branch, W.R. & Matthee, C.A. (2004) Phylogenetics of the southern African dwarf chameleons, *Bradypodion* (Squamata: Chamaeleonidae). *Molecular Phylogenetics and Evolution*, **30**, 354–365.
- Tolley, K.A., Burger, M., Turner, A.A. & Matthee, C.A. (2006) Biogeographic patterns and phylogeography of dwarf chameleons (*Bradypodion*) in an African biodiversity hotspot. *Molecular Ecology*, **15**, 781–793.
- Tolley, K.A., Chase, B.M. & Forest, F. (2008) Speciation and radiations track climate transitions since the Miocene climatic optimum: a case study of southern African chameleons. *Journal of Biogeography*, **35**, 1402–1414.
- Tolley, K.A., Raw, R.N.V., Altwegg, R. & Measey, G.J. (2010) Chameleons on the move: survival and movement of the Cape dwarf chameleon, *Bradypodion pumilum*, within a fragmented urban habitat. *African Zoology*, **45**, 99–106.
- Townsend, T. & Larson, A. (2002) Molecular phylogenetics and mitochondrial genomic evolution in the Chamaeleonidae (Reptilia, Squamata). *Molecular Phylogenetics and Evolution*, **23**, 22–36.
- Townsend, T.M., Tolley, K.A., Glaw, F., Böhme, W. & Vences, M. (2011) Eastward from Africa: palaeocurrent-mediated chameleon dispersal to the Seychelles islands. *Biology Letters*, **7**, 225–228.
- Vane-Wright, R.I., Humphries, C.J. & Williams, P.H. (1991) What to protect?—systematics and the agony of choice. *Biological Conservation*, **55**, 235–254.
- Vences, M., Wollenberg, K.C., Vieites, D.R. & Lees, D.C. (2009) Madagascar as a model region of species diversification. *Trends in Ecology and Evolution*, **24**, 456–465.
- Voelker, G., Outlaw, R.K. & Bowie, R.C.K. (2010) Pliocene forest dynamics as a primary driver of African bird speciation. *Global Ecology and Biogeography*, **19**, 111–121.
- Wasser, S.K. & Lovett, J.C. (1993) Introduction to the biogeography and ecology of the rain forests of eastern Africa. *Biogeography and ecology of the rain forests of eastern Africa* (ed. by J.C. Lovett and S.K. Wasser), pp. 3–7. Cambridge University Press, Cambridge.
- Willis, K.J. & Whittaker, R.J. (2000) The refugial debate. *Science*, **287**, 1406–1407.
- Zachos, J.C., Pagani, M., Sloan, L., Thomas, E. & Billups, K. (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science*, **292**, 686–693.
- Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. PhD Thesis, University of Texas, Austin, TX.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Details for the individuals used in the phylogenetic analyses for *Kinyongia*.

Appendix S2 Inferred dates for *Kinyongia* lineages under different dating scenarios.

Appendix S3 Sequence divergence estimates between lineages of *Kinyongia*.

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BIOSKETCH

Krystal Tolley leads the Molecular Ecology and Evolution Program at the South African National Biodiversity Institute in Cape Town. Her interests include historical biogeography and speciation processes in southern African reptiles, with particular emphasis on chameleons.

Author contributions: K.A.T., C.A.M., C.R.T. and W.R.B. conceived the original project; G.J.M., M.M. and C.R.T. conducted fieldwork; K.A.T. analysed the data and led the writing; all co-authors contributed to interpretation and writing.

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