

Dispersal to or from an African biodiversity hotspot?

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Abstract

Biodiversity hotspots are centres of endemism and thus contain many range-restricted species. In addition, within these hotspots occur widespread species that might have originated within a hotspot before dispersing to neighbouring or distant regions. We test this hypothesis with a phylogeographic analysis of a miniature leaf litter frog, *Arthroleptis xenodactyloides*, that has a large distribution throughout the Eastern Arc biodiversity hotspot and other regions in East Africa. Maximum-likelihood and Bayesian estimates of the mitochondrial gene phylogeny are used as a proxy for understanding the evolutionary history of diversification and the historical relationships between populations. The north–south range of this species extends for approximately 1900 km; our sampling covers approximately 85% of this range. Using phylogenetic comparative methods, we estimate the region of origin and direction of dispersal within *A. xenodactyloides*. We compare contrasting hypotheses of latitudinal range expansion using bayes factors. The ancestral region of origin of *A. xenodactyloides* is reconstructed as having occurred within the Eastern Arc before dispersing southwards into the southern Rift Mountains, probably in the Pleistocene. The phylogeographic structure within this leaf litter frog is surprisingly similar to that of forest birds, revealing that similar geographic features might have had a driving role in diversification of these very dissimilar taxa. Latitudinal expansion occurred early in the evolutionary history of *A. xenodactyloides*, which may indicate that physiological adaptation facilitated its wide geographic distribution.

Keywords: Arthroleptidae, mitochondrial genetics, phylogenetic comparative methods, phylogeography, range expansion, speciation

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Introduction

Continental sub-Saharan Africa contains several biodiversity hotspots with high numbers of endemic and threatened species that, consequently, are conservation priorities (Myers *et al.* 2000; Burgess *et al.* 2004). Of these hotspots, the Eastern Arc Mountains and coastal forests of Tanzania and Kenya, hereafter referred to simply as the Eastern Arc, are of special note because they contain the highest known concentration of endemic plants and vertebrates in the entire world (Myers *et al.* 2000; Lovett *et al.* 2005; Burgess *et al.* 2007). Animal and plant species from the Eastern Arc continue to be described at a surprising pace, especially given that

most vertebrate discoveries include morphologically distinct and non-cryptic species (e.g. Channing & Stanley 2002; Davenport *et al.* 2006). To date, molecular phylogenetics has played only a limited role in the identification of new species or evolutionarily significant units within species in the Eastern Arc (e.g. Fjeldså *et al.* 2006; Loader *et al.* 2006). Identifying and defining these taxa using molecular methods, however, may prove pivotal for both discovering and interpreting broad-scale biogeographic patterns and then setting conservation priorities at the regional level within this biodiversity hotspot (e.g. Rissler *et al.* 2006).

The climate of the Eastern Arc is under the direct influence of the climatic regime of the Indian Ocean (Marchant *et al.* 2006) and is believed to have been relatively stable for the past 30 million years (Lovett *et al.* 2005). This stability may have had a causative role in the accumulation of species

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diversity, although the processes underlying this accumulation remain unclear (e.g. Lovett *et al.* 2005). In contrast, recent phylogenetic studies have emphasized the role of historical climatic variation in generating the diversity of the Eastern Arc (reviewed in Fjelds  & Bowie 2008). Cyclical climatic variation is generally held to be an important causative agent in speciation by isolating populations in refugia (e.g. Haffer 1969). However, the emphases on both climatic stability and variation are not necessarily contradictory because, in combination, these factors may be important components for accumulating high species diversity. Long-term climatic stability on a regional scale may be important for maintaining levels of species diversity, whereas cyclical climatic variation at local levels could lead to the generation of new species. As centres of endemism, areas of high species richness, such as the Eastern Arc, are generally easy to recognize, but further study is required to determine whether these centres of endemism are also centres of diversification.

Previous work indicates that the Eastern Arc is both a centre of endemism and a historically important centre of diversification (Jetz *et al.* 2004). This suggests that the combination of geological and climatic processes underlying species diversity in this hotspot have functioned as a 'speciation pump' (*sensu* Terborgh 1992; e.g. Haffer 1969). Using climatic data, Jetz *et al.* (2004) modelled species distributions in sub-Saharan Africa to analyze the determinants underlying patterns of endemism. These authors found that the Eastern Arc (among other regions) contains unusually high species richness, even if environmental differences between it and other regions are taken into account. A complementary approach is to use biogeographic and phylogeographic studies to understand temporal changes in distributions of clades or specific species. Of particular interest is the analysis of phylogeographic patterns of currently widespread species in order to determine whether these are immigrants from or emigrants to regions believed to be centres of diversification.

Phylogeographic analysis provides the means to evaluate competing hypotheses of modes of diversification across geographic space within a lineage (Knowles 2004). Regions of origin, barriers to, and directions of dispersal can be estimated, as can parameters that estimate, for example, the relative timing of dispersal. One interesting question concerning species diversity in biodiversity hotspots is whether this diversity originated within or outside of the hotspot. Within nearly any biodiversity hotspot, there are at least some species that occur both within the hotspot as well as in other neighbouring or even distant regions. Widespread species might have originated in biodiversity hotspots before dispersing or, conversely, originated in other regions before invading the hotspot. If biodiversity hotspots are demonstrated to be sources of both endemic and widespread species, this further supports arguments

that these regions should be conserved because they are important centres of diversification.

Within East Africa, the frog genus *Arthroleptis* (Neobatrachia: Ranoidea: Arthroleptidae) provides an excellent system for studying the evolutionary history of species distributions. *Arthroleptis* species are terrestrial, leaf litter generalists (Blackburn & Moreau 2006) and all are believed to have direct development, i.e. they have lost the free-living, feeding tadpole stage (Blackburn 2008). There are many *Arthroleptis* species endemic to the Eastern Arc and the more southern mountains (Channing 2001; Channing & Howell 2006; Blackburn 2009). Some species, such as *A. francei*, *A. tanneri* and *A. troglodytes* are known from a single mountain or mountain block; in these examples, the Mulanje Massif, the West Usambara mountains and the Chimanimani mountains, respectively. In contrast, others have disjunct distributions throughout many of the mountains, including *A. affinis* (e.g. Poynton & Loader 2008), *A. reichei* (Channing & Howell 2006) and the miniature *A. xenodactyloides* (Channing 2001; Poynton 2003a; Channing & Howell 2006; Measey *et al.* 2007). Whereas many miniature *Arthroleptis* species are restricted to very small ranges, *A. xenodactyloides* has the largest geographic range of any *Arthroleptis* species occurring in the mountains of East Africa. At least two different *Arthroleptis* clades have diversified in the mountains of East Africa (Blackburn 2008), and it seems that there are possibly two centres of diversification, one in the northernmost mountains (endemics include *A. tanneri*, *A. xenodactylus* and two new species; Grandison 1983; Blackburn 2009) and one in the southernmost mountains (endemics include *A. francei* and *A. troglodytes*). Because the large geographic range of *A. xenodactyloides* encompasses both of these potential centres of diversification, the distribution of this species suggests that historical scenarios in which it originates in one region and then disperses to another are plausible, regardless of the place of origin and direction of dispersal. As such, *A. xenodactyloides* proves an ideal candidate for a phylogeographic study and for evaluating the 'speciation pump' model.

We estimated historical relationships within *A. xenodactyloides* using an intraspecific mitochondrial DNA phylogeny. Mitochondrial gene phylogenies are particularly useful for phylogeographic studies because they coalesce more quickly than nuclear genes and thus avoid the problems of reticulating phylogenies and the obfuscation of geographic movements that may have occurred over short periods of time. We then reconstructed the region of origin and direction of dispersal over the history of *A. xenodactyloides*. This allows for the evaluation of whether this species originated within or outside of the Eastern Arc. We employ a novel approach by using phylogenetic comparative methods to analyze geographic data, which are here represented by a single continuously varying character (decimal degrees latitude).

Materials and methods

Sampling

This study used a total of 25 specimens of *Arthroleptis xenodactyloides* from 11 localities that span approximately 85% of the north–south range of the species (Table 1; Fig. 1) and include all localities for which specimens were available. This species occurs in many East African mountain blocks and other low-lying areas, including Pemba Island just to the north of Zanzibar. The most northern locality, the Taita Hills in southern Kenya, represents both the northern extent of the Eastern Arc and the most northern extent of the geographic range of *A. xenodactyloides* (Channing & Howell 2006; Measey *et al.* 2007). The most southern locality, the Mulanje Massif in southern Malawi, is the most southerly locality for which tissues of *A. xenodactyloides* were available. However, the southernmost extent, and coincidentally the type locality, of this species, is the Chirinda Forest in the eastern highlands of Zimbabwe (Channing 2001; IUCN *et al.* 2006). Unfortunately, specimens with associated tissue samples from Zimbabwe and Mozambique were not available for analysis. Frost (2008)

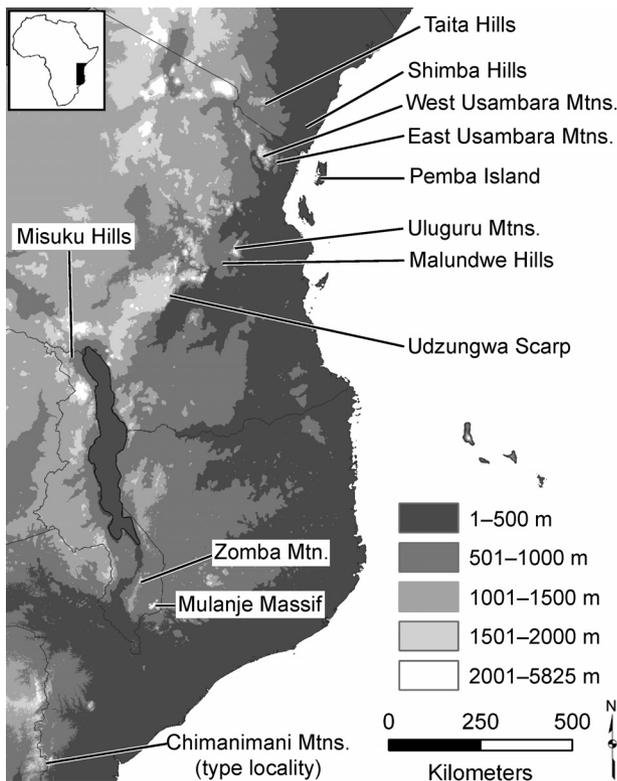


Fig. 1 Map of localities for *Arthroleptis xenodactyloides* specimens used in this analysis. Type locality in the Chimanimani Mountains of eastern Zimbabwe is also indicated. Inset at upper left indicates the region of Africa that is the focus of this study.

suggests that *A. xenodactyloides* also occurs in the eastern Democratic Republic of Congo, but we are unaware of any records of this species in this country. The north–south range of *A. xenodactyloides* covers approximately 1900 km, and the specimens in this analysis span nearly 1400 km of this, which we believe is sufficient for addressing basic questions regarding the history of this species. Using latitude as a proxy for biogeographic patterns is sufficient because the east–west distribution of *A. xenodactyloides* is narrow at most latitudes. To evaluate phylogeographic patterns, we utilize specimens that were obtained from throughout the Eastern Arc (Table 1; Fig. 1); data from one *A. xenodactyloides* specimen (GenBank DQ283431) come from a previous study (Frost *et al.* 2006). Institutional abbreviations follow Leviton *et al.* (1985).

In addition, data from specimens of four closely related *Arthroleptis* species – *A. schubotzi*, *A. xenodactylus*, and two new species (*A. sp. nov.* A and *A. sp. nov.* B; Blackburn 2009) – provided phylogenetic context for the origin and dispersal of *A. xenodactyloides* (Blackburn 2008, 2009). Based on a recently generated and more extensive *Arthroleptis* phylogeny (Blackburn 2008), two distantly related *Arthroleptis* species (*A. stenodactylus* and *A. wahlbergii*) were used as outgroups in the phylogenetic analysis. All data from species other than *A. xenodactyloides*, as well as several *A. xenodactyloides* specimens, were generated during the course of other, related research (Blackburn 2008, 2009).

DNA extraction, amplification and sequencing

Genomic DNA was extracted from tissue samples using a Qiagen DNeasy Tissue Kit (Cat. No. 69506). Phylogenetic relationships were estimated through analysis of DNA sequences of the mitochondrial 12S and 16S ribosomal RNA (rRNA) genes and the intervening transfer RNA (tRNA) for Valine; approximately 1900 base pairs (bp) were amplified using the primers pairs (i.e. 12L1 and 16sh; 12sm and 16sa; 16sc and 16sd) of Darst & Cannatella (2004). Standard reaction mixtures and thermal cycle profiles were utilized for both polymerase chain reaction (PCR) and sequencing reactions and follow Blackburn (2008). Sequencing reactions were purified using the Performa DTR V3 96-well plates from Edge BioSystems (Cat. No. 63877) and sequenced using cycle sequencing on an ABI3730xl Genetic Analyzer.

Because PCR products were amplified with varying success, the full-length sequence could not be obtained for several specimens. In two cases (*A. xenodactyloides*: MCZ A-138390; *A. sp. nov.* A: MCZ A-138394), two non-overlapping fragments were obtained. For three other specimens, only one fragment could be amplified with lengths ranging between 700 and 1200 bp (*A. xenodactyloides*: MCZ A-138383, A-138386, A-138452). While data for these terminal taxa is obviously incomplete, taxa with missing data can still add

Table 1 Specimens included in analysis

Species	Locality	Coordinates	Catalog No.	GenBank No.	Source
<i>Arthroleptis</i> sp. nov. A	Nguru Mountain, Tanzania	6°03'09"S, 037°32'26"E	MCZ A-138393	FJ188698	Blackburn, 2009
"	"	"	MCZ A-138394	FJ188699–70	Blackburn, 2009
<i>A.</i> sp. nov. B	Baga II Forest Reserve, West Usambara Mountains, Tanzania	04°48'S, 038°27'E	CAS 168829	FJ151064	Blackburn, 2008
"	Mazumbai Forest Reserve, West Usambara Mountains, Tanzania	04°49'45"S, 038°30'46"E	MCZ A-138384	FJ151064	Blackburn, 2009
<i>A.</i> cf. sp. nov. B	Chome Forest Reserve, South Pare Mountains, Tanzania	04°17'S, 037°55'40"E	FMNH 251864	FJ188696	Blackburn, 2009
<i>A. schubotzi</i>	Bwindi Impenetrable National Park, Uganda	00°59'34"S, 29°36'57"E	CAS 201752	FJ151061	Blackburn, 2008
"	"	"	CAS 201753	FJ151116	Blackburn, 2008
<i>A. xenodactyloides</i>	Tegetero Village, Uluguru Mountains, Tanzania	6°56'30"S, 37°43'10"E		DQ283431	Frost <i>et al.</i> (2006)
"	Amani, East Usambara Mountains, Tanzania	05°05'S, 038°36'E	CAS 168608	FJ151063	Blackburn, 2008
"	Malundwe Hills, Tanzania	07°23'34"S, 037°18'15"E	FMNH 272057		This study
"	Ruo River Gorge, Mulanje Massif, Malawi	15°56'S, 035°37'E	MCZ A-137002	FJ151096	Blackburn, 2008
"	"	"	MCZ A-137003	FJ151097	Blackburn, 2008
"	Mughese Forest Reserve, Misuku Mountains, Malawi	09°39'29"S, 033°32'27"E	MCZ A-137056	FJ151102	Blackburn, 2008
"	"	"	MCZ A-137057	FJ151103	Blackburn, 2008
"	Ngezi-Vumawimbi Nature Forest Reserve, Pemba Island, Tanzania	04°55'11"S, 039°41'28"E	MCZ A-138383		This study
"	Ambangulu Tea Estate, West Usambara Mountains, Tanzania	05°04'03"S, 038°25'13"E	MCZ A-138385		This study
"	"	"	MCZ A-138386		This study
"	Amani Nature Reserve, East Usambara Mountains, Tanzania	05°05'37"S, 038°36'00"E	MCZ A-138390		This study
"	"	"	MCZ A-138391		This study
"	Ngezi-Vumawimbi Nature Forest Reserve, Pemba Island, Tanzania	04°55'11"S, 039°41'28"E	MCZ A-138452		This study
"	"	"	MCZ A-138453		This study
"	"	"	MCZ A-138454		This study
"	Mkaja, Udzungwa Scarp Forest Reserve, Tanzania	08°20'31"S, 035°58'02"E	MTSN 5094		This study
"	"	"	MTSN 5095		This study
"	Chawia, Taita Hills, Kenya	03°25'S, 038°20'E	NMK A/4537/26		This study
"	Fururu Forest, Taita Hills, Kenya	"	NMK A/4538/28		This study
"	Mbololo Forest, Taita Hills, Kenya	"	NMK A4540/15		This study
"	Ngangao, Taita Hills, Kenya	"	NMK A/4542/17		This study
"	Ronge Forest, Taita Hills, Kenya	"	NMK A/4543/10		This study
"	Sagalla Forest, Taita Hills, Kenya	"	NMK A/4544/15		This study
"	Makadara Forest, Shimba Hills, Kenya	04°15'S, 039°25'E	NMK A/4653/1		This study
"	Zomba Plateau, Southern Region, Malawi	15°20'S, 35°18'E	TMP 84805	FJ151080	Blackburn, 2008
<i>A. xenodactylus</i>	Amani Nature Reserve, East Usambara Mountains, Tanzania	05°05'37"S, 038°36'00"E	MCZ A-138404	FJ151156	Blackburn, 2008
"	"	"	MCZ A-138405	FJ151157	Blackburn, 2008
"	Nguru Mountain, Tanzania	6°03'09"S, 037°32'26"E	MCZ A-138400		This study
"	"	"	MCZ A-138402		This study
"	"	"	MCZ A-138403		This study
<i>A. stenodactylus</i>	Amani, East Usambara Mountains, Tanzania	6°56'30"S, 37°43'10"E	CAS 168455	FJ151054	Blackburn, 2008
<i>A. variabilis</i>	Etome, Petit Mount Cameroon, Cameroon	6°56'30"S, 37°43'10"E	MCZ A-136744	FJ151083	Blackburn, 2008

substantial information to a phylogenetic analysis (Wiens 2006; Wiens & Moen 2008) and these taxa were thus included in the analysis.

Phylogenetic analysis

DNA sequences of unequal length were aligned in ClustalX version 1.83.1 using default parameters. Non-overlapping DNA fragments from a single specimen were aligned separately and then merged in MacClade version 4.06 to form a single terminal taxon for analysis. The alignment was then checked by eye, and minor manual adjustments were made so as to minimize the change across sites. The alignment was subsequently trimmed such that nearly all taxa were complete for both the 5' and 3' sites (alignment available from DCB upon request). The resulting 1886 bp alignment corresponds to positions 2546–4450 of the *Xenopus laevis* mitochondrial genome (GenBank NC-001573).

A maximum-likelihood (ML) estimate of phylogeny was generated by analyzing the aligned sequence data in GARLI version 0.95 (Zwickl 2006), using a random starting tree and a GTR + I + Γ model of sequence evolution with all parameters estimated. ML analysis was repeated 10 times to insure that GARLI searches were not stuck at local optima; the topology and $-\ln L$ were nearly identical among replicates. The analysis was terminated one million generations after the last topological improvement. Support for the ML phylogeny was estimated using non-parametric bootstrapping in GARLI. One thousand bootstrap replicates were performed using the same model of sequence evolution with each search terminated after 10 000 generations after the last topological improvement. Branches present in $\geq 70\%$ of the bootstrap trees were considered well supported following Hillis & Bull (1993).

A Bayesian estimate of phylogeny was obtained using MrBayes version 3.1.1 and a GTR + I + Γ model of sequence evolution. Bayesian analysis was run for five million generations, sampled every 1000 generations, using four chains, a temperature of 0.2, and default priors. The first one million generations were discarded as burn-in. The phylogeny and posterior probabilities were then estimated from the remaining 4000 trees. Following Wilcox *et al.* (2002), topologies with posterior probabilities ≥ 0.95 were considered well supported.

Genetic distances were calculated in MEGA version 4.0.1 (Tamura *et al.* 2007) using the maximum composite likelihood (MCL). The MCL calculations used data for both transitions and transversions and assumed a heterogeneous pattern of sequence evolution with a Γ distribution of among-site rate variation (Γ parameter = 1.0). Gaps and missing data were deleted from the analysis and standard errors were calculated with 500 bootstrap replicates.

Phylogeographic analysis

To estimate the phylogeography and reconstruct the history of latitudinal change within *A. xenodactyloides*, we utilized tools from phylogenetic comparative methods rather than standard biogeographic methods. When considering the geographic units for analysis, it was not clear whether certain mountains should be considered as single or several units (e.g. the West and East Usambaras, or the several blocks of the Taita Hills), or if, instead, larger groups of mountains should be used (e.g. Northern Eastern Arc, southern and central Eastern Arc, and Malawi Rift; i.e. Bowie *et al.* 2006). Because defining geographic regions is difficult, analyses such as dispersal-vicariance analysis as implemented in DIVA version 1.1 (Ronquist 1997) or lagrange (Ree & Smith 2008) are inappropriate. Rather than determining patterns of relationships between particular mountain blocks, we were interested in broader questions including the approximate region of origin (within or outside the Eastern Arc) and the diversification across geographic space. Because the geographic range of *A. xenodactyloides* is oriented roughly north–south, with approximately 30% of the northern range occupying the Eastern Arc, we used ancestral state reconstruction of latitude and phylogenetic comparative methods to estimate the region of origin, direction of subsequent range expansion, and relative timing of north–south dispersal.

All coordinate data were converted to decimal degrees. As all localities are south of the equator, interpretation of these data is straightforward: low values correspond to more northerly localities whereas high values correspond to more southerly localities. For those specimens for which coordinate data was not recorded, data were estimated using gazetteers (US National Geospatial-Intelligence Agency 2008). In all phylogeographic analyses, so as to not inadvertently bias reconstructions at basal nodes in the phylogeny, data for the two outgroup species was excluded. For these analyses, the ML estimate of phylogeny was used, and polytomies were resolved using TreeEdit version 1.0a10 (Rambaut & Charleston 2002). Ancestral states of latitudinal position were reconstructed using the Phylogenetic Generalized Least Squares (PGLS) Model as implemented in Compare version 4.6 (Martins 2004); the linear model with default parameter values was used. We report the adjusted standard error for PGLS estimates as suggested by Rohlf (2001). In addition, we utilized the parameter h^2 estimated in the Phylogenetic Mixed Model (PMM) in Compare to determine whether latitudinal patterns could be explained simply by phylogeny (Housworth *et al.* 2004). If $h^2 = 1$, then the results are identical to the independent contrasts of Felsenstein (1985) and all latitudinal patterns are adequately explained by the phylogeny. In contrast, if $h^2 = 0$, then phylogeny does not explain the latitudinal patterns. In other words, the estimation of h^2 can be interpreted as a measure

of the relative degree that long-term ($h^2 = 1$) or short-term ($h^2 = 0$) temporal changes have been important in the establishment of the current latitudinal distribution. If $h^2 = 0$, then reconstructing latitude at internal nodes is relatively meaningless because there has been substantial latitudinal movement throughout the phylogeny.

We were interested to further determine the relative timing of dispersal within *A. xenodactyloides*. To this end, we used Continuous, as implemented in BayesTraits (Pagel & Meade 2008), to estimate the parameter δ (a scale of overall path length), which is a measure of whether character change, in this case latitudinal spread, occurred early or late in the phylogeny. Post burn-in Bayesian trees were analyzed in a Markov chain Monte Carlo analysis using default parameter values. Two sets of analyses were performed. In the first analyses, δ was estimated based on the data, while in the second analyses the value of δ was set to one to test a gradualist hypothesis of latitudinal change. If $\delta = 1$, then a pattern of gradual latitudinal spread across the phylogeny cannot be rejected. If $\delta < 1$, shorter paths contribute more to trait evolution and thus we interpret that latitudinal spread was concentrated early in the history of *A. xenodactyloides*. If $\delta > 1$, longer paths contribute more, and thus latitudinal spread probably occurred more recently. For both sets of analyses, we conducted analysis using both a random walk and a directional model of change. The marginal likelihoods of each model were compared using bayes factors to evaluate whether temporal change in latitude occurred early ($\delta < 1$) or late ($\delta > 1$) in the phylogeny and whether a hypothesis of gradual change ($\delta = 1$) could be rejected; a bayes factor value of 10 or greater was considered strong evidence in favour of the better model.

Results

Phylogeny of Arthroleptis xenodactyloides

In general, phylogenetic relationships between species and between populations within *Arthroleptis xenodactyloides* were well resolved (Fig. 2). Interspecific phylogenetic relationships were similar to those found by Blackburn (2008, 2009) and received high support from both ML non-parametric bootstrapping and Bayesian posterior probabilities. The topology of phylogenetic relationships was identical when the analysis was limited to only those characters represented for all terminal taxa (data not shown). The monophyly of all ingroup species was also highly supported.

Populations of *A. xenodactyloides* were resolved as belonging to one of two reciprocally monophyletic clades (Fig. 2). Support for each of these clades was weak, but a pattern emerges nonetheless. The first clade contained only populations from the northernmost part of the Eastern Arc, specifically the East and West Usambara mountains of northeastern Tanzania and the Shimba and Taita Hills of

southeastern Kenya. Within this first clade, there was high support that the specimen from the Shimba Hills is more closely related to the specimens from the East and West Usambaras than to those from the Taita Hills. The second clade contained species distributed from Pemba Island and within the Eastern Arc to the mountains of southern Malawi. Within this second clade, there was high support that the specimens from the Uluguru Mountains and Malundwe Hills are sister taxa and that those specimens from Pemba Island are sister to a more exclusive clade containing specimens from southern Eastern Arc and Malawian mountains. Whereas there was generally good phylogenetic resolution between populations, there was little to no resolution between specimens from the same or nearby localities. This indicates that while there was substantial divergence between populations there was little within populations. One exception occurred in the clade containing populations from the Udzungwa Scarp, Misuku Hills, Zomba Plateau and Mulaje Massif. While there was strong support that these populations form a clade, resolution between populations was not possible.

Relative to the closely related *A. xenodactylus*, which is endemic to the northern part of the Eastern Arc, there was substantial genetic divergence within *A. xenodactyloides*. There was little sequence divergence among the specimens of *A. xenodactylus* from Nguru and the East Usambara mountains (0.2%, ± 0.2), which are separated by approximately 160 km. In contrast, within *A. xenodactyloides*, there was much greater divergence when taken as a whole (6.6%, ± 1.3) or when considering the northern (2.3%, ± 0.8) and southern (4.2%, ± 1.1) clades separately. Specimens from the Taita Hills are essentially genetically identical (0.0%, ± 0.0) whereas other localities such as Pemba Island (0.8%, ± 0.4) or the East and West Usambaras (0.3%, ± 0.2) exhibit some genetic diversity. Surprisingly, the genetic diversity within Pemba Island was as great as that from the clade comprising specimens obtained from the Udzungwa Scarp and far south to the Mulanje Massif in southern Malawi (0.8%, ± 0.4). The genetic divergence between the specimen from the Uluguru mountains and nearby Malundwe Hills was seemingly very high (2.0%, ± 0.8), although, as the sequence data from the Uluguru specimen was collected by other authors (Frost *et al.* 2006), we cannot rule out the possibility that errors in base-calls have artificially inflated this pairwise divergence.

Phylogeography

The reconstructed latitude at the base of *A. xenodactyloides* phylogeny is -5.63 (in decimal degrees; approximately $5^{\circ}37'48''S$), which is within the Eastern Arc (Fig. 3). While the standard error associated with this estimate is large (Table 2), the estimate, the nesting of the specimens from the most southern localities being well within the phylogeny

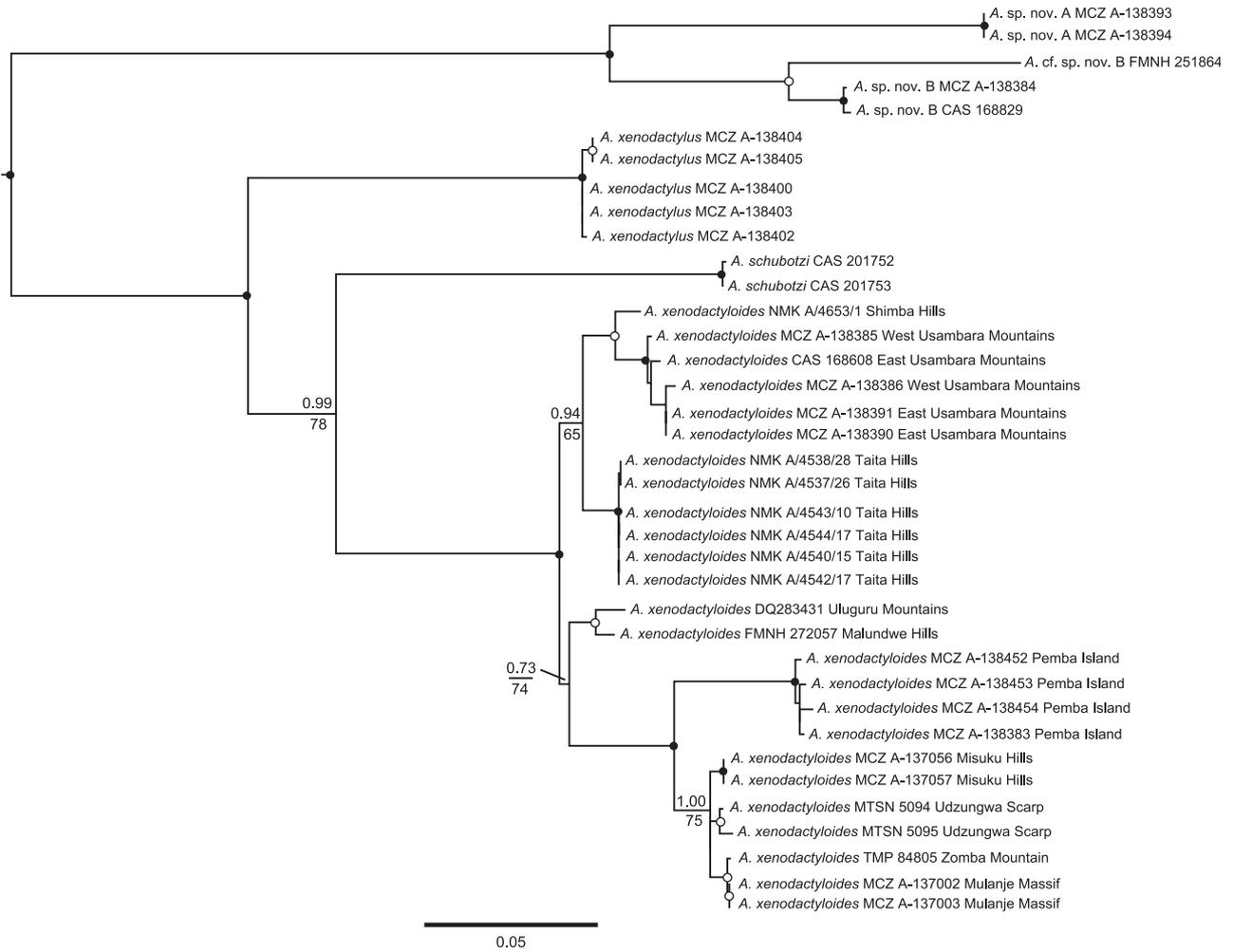


Fig. 2 Maximum-likelihood phylogram estimated from mitochondrial DNA sequences depicting relationships among populations of *Arthroleptis xenodactyloides* and related species from the Eastern Arc. Nodes with circles indicate Bayesian posterior probabilities of 1.00; solid black circles indicate non-parametric bootstrap proportions of 100%, whereas white circles indicate $\geq 94\%$ support. For selected nodes with lower support values, numbers above branches are Bayesian posterior probabilities and below branches are non-parametric bootstrap proportions. The outgroup species, *A. stenodactylus* and *A. variabilis*, are not shown. Scale bar, 0.05 substitutions/site.

of *A. xenodactyloides*, and the fact that closely related *Arthroleptis* species are endemic to the northern Eastern Arc, all point to an origin within the Eastern Arc. *Arthroleptis xenodactyloides* dispersed to more southern latitudes at the base of the clade containing specimens ranging from the Uluguru mountains, Pemba Island and the Malawian mountains. The origin and initial diversification of lineages within *A. xenodactyloides* thus occurred within the Eastern Arc with subsequent dispersal to the Malawian mountains and, presumably, those farther south in Zimbabwe. The PMM estimated that $h^2 = 1$ (variance = 70.38; log likelihood = -13.66), which indicates that the phylogeny sufficiently explains the pattern of latitudinal diversity. Thus, reconstructing ancestral states is meaningful and there is no need to invoke short-term temporal processes to explain the latitudinal spread of *A. xenodactyloides*.

Table 2 Ancestral state estimation, in decimal degrees latitude, for selected nodes (numbers correspond to Fig. 3). PGLS, Phylogenetic Generalized Least Squares

Node	PGLS estimate	Standard error
I	-4.95	4.22
II	-4.87	5.65
III	-4.51	5.58
IV	-5.63	5.34
V	-4.73	5.15
VI	-6.06	6.02
VII	-8.55	4.70
VIII	-10.47	6.46

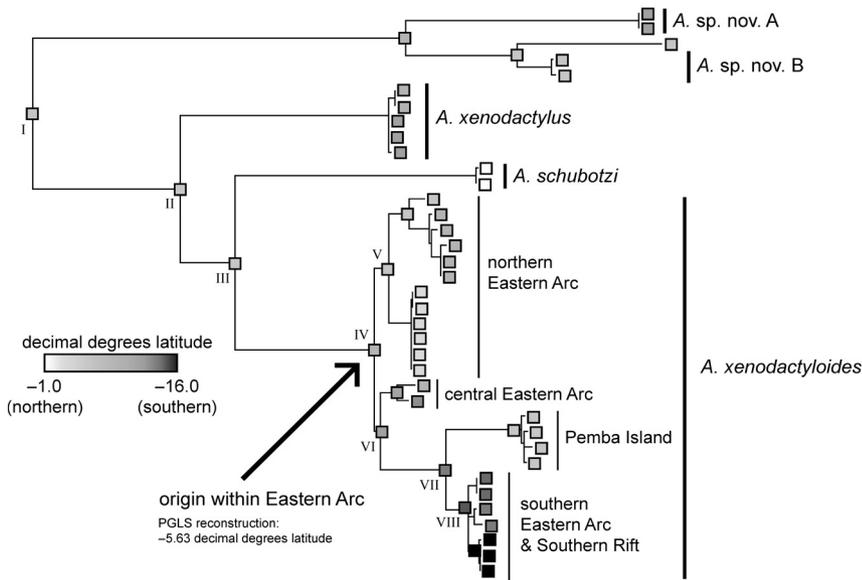


Fig. 3 Reconstruction of latitudinal change over the maximum-likelihood phylogeny of *Arthroleptis xenodactyloides* and its relatives. Ancestral states at internal nodes were reconstructed using the Phylogenetic Generalized Least Squares (PGLS) model. Values reconstructed at internal nodes and observed in terminal taxa are indicated in a greyscale gradation; white indicates decimal degrees latitude values of $-1.0 \leq x < -2.0$ whereas black indicates $-15.0 \leq x < -16.0$. PGLS reconstructions for nodes with roman numerals are provided in Table 2. The outgroup species, *A. stenodactylus* and *A. variabilis*, are not shown.

Table 3 Harmonic means of marginal likelihoods and values of δ for different models of temporal patterns of latitudinal change

Model of temporal change	Range of δ	Mean of δ	St. Dev. of δ	Marginal likelihood
δ (estimated; random walk)	0.001–0.671	0.175	0.098	–38.3439
δ (estimated; directional)	0.001–0.593	0.108	0.096	–38.5890
δ (gradualism; random walk)	1.000	N/A	N/A	–54.5838
δ (gradualism; directional)	1.000	N/A	N/A	–56.2871

When analyzed using either a random walk or directional model of character change in Continuous, the model in which $\delta = 1$ was rejected in favour of the model in which δ was estimated (Tables 3 and 4). Regardless of whether δ was estimated or set equal to 1, there was no evidence that a model of directional character change is better than one in which it is a random walk (Tables 3 and 4). These results imply that short path lengths in the phylogeny (i.e. those early in the phylogeny) contribute disproportionately to explaining trait variation; in this case, latitudinal spread. Furthermore, the results were very similar if only *A. xenodactyloides* was included in the analysis rather than analyzing all of the terminal taxa together (data not shown).

Discussion

Our analysis indicates that *Arthroleptis xenodactyloides*, a widespread leaf litter frog, originated in the Eastern Arc. Within these mountains, there is also a diversity of endemic *Arthroleptis* species with restricted geographic ranges. This biodiversity hotspot is thus a centre of diversification giving rise to species with both small and large geographic distributions. Further phylogeographic study is needed to determine whether this is also true of other widespread

Table 4 Hypothesis tests of patterns of temporal change in latitude using bayes factors. Asterisk indicates cases in which model with larger marginal likelihood is strongly rejected

Hypothesis	Bayes factor
Directional vs. Random walk	δ estimated 0.2451 $\delta = 1$ 1.7032
δ estimated vs. $\delta = 1$	Directional 17.6981* Random walk 16.2399*

frog genera in East Africa. Recently, Dillon & Fjeldså (2005) found that, by taking into account phylogenetic lineages within species, complexity is added to larger-scale biogeographic patterns. For the Eastern Arc in particular, Dillon & Fjeldså (2005) found that there is an even greater concentration of endemism at small spatial scales when intraspecific phylogenies are taken into account. This is in accordance with recent research demonstrating that comparative phylogeography can reveal important regional centres of diversification that may be obscured by species-level analyses (Rissler *et al.* 2006). In the case of *Arthroleptis*, the mountains of the Eastern Arc are an important centre

of both speciation and intraspecific diversification. This highlights this biodiversity hotspot as an ideal testing ground for determining the role of historical processes and geographic features in diversification at various time scales.

Phylogeography provides insight into the history of places and faunas (Avice 2000). Within the Eastern Arc, the many amphibians with disjunct distributions, including many cryptic taxa, suggests that amphibian phylogeography may provide practical insight into the historical patterns of geographic change within this biodiversity hotspot. The large number of recently described Eastern Arc amphibians is remarkable (e.g. Channing & Stanley 2002; Poynton 2003b; de Sá *et al.* 2004; Menegon *et al.* 2004, 2007; Müller *et al.* 2005; Loader *et al.* 2006; Pickersgill 2007) and contributes to making this one of the hotspots of global amphibian diversity (Stuart *et al.* 2004). *Arthroleptis* is one such genus that warrants further biogeographic and phylogeographic study. The taxonomic confusion surrounding several Eastern Arc *Arthroleptis* species (e.g. Poynton & Loader 2008) suggests substantial intraspecific and even interspecific cryptic diversity. New species have been recently described (Poynton 2003b; Blackburn 2009; Poynton *et al.* 2008) and there are at least several undescribed species (i.e. Blackburn, unpublished results). These are common leaf litter frogs, and samples are available from many localities within the Eastern Arc. As this genus includes both widespread and very localized species, further study of *Arthroleptis* biogeography may be particularly useful for understanding the history of the Eastern Arc.

A recent comparative study of East Africa's bird fauna revealed that, like *A. xenodactyloides*, certain widespread species, such as the Olive Sunbird (*Nectarinia olivacea*), may have originated within, and then dispersed from, the Eastern Arc Mountains (Fjeldså & Bowie 2008). Appropriate phylogeographic evidence for evaluating the 'speciation pump' hypothesis (Terborgh 1992), including the relative importance of the Eastern Arc in the origin of East African biodiversity, is currently available for very few species. However, taken together, the data available lend support to the idea that the geological and climatic processes characterizing the history of the Eastern Arc have functioned as a 'speciation pump.' We infer that this globally important biodiversity hotspot has been a centre of diversification for both range-restricted and regionally widespread species.

Utilizing comparative methods in phylogeography

Phylogenetic comparative methods may prove useful for studies of geographic patterns, especially in cases in which it is difficult to delimit geographic ranges of terminal taxa. This and two other recent studies have employed comparative methods that estimate parameters concerning temporal patterns of character change (Jones *et al.* 2005; Wollenberg

et al. 2008). In our study, the analysis of a single continuous variable is generally adequate for capturing large-scale geographic patterns because the range of *A. xenodactyloides* is distributed roughly north-south. With more complex ranges and more complicated histories of geographic dispersal (e.g. island archipelagos or continental areas characterized by high elevation 'insular' montane habitats), more sophisticated approaches will be necessary (see, for example, Lemmon & Lemmon 2008).

Comparative phylogeography in the Eastern Arc

Previous phylogeographic study of animals in the Eastern Arc have found similar patterns (summarized by Kahindo *et al.* 2007 and Fjeldså & Bowie 2008). In several avian clades, there is substantial divergence between – as well as reciprocal monophyly in mitochondrial gene lineages of – populations found in the northernmost mountains (Usambaras, Taita, Pare) and those just to the south (Uluguru, Ukaguru, Nguru, Malundwe). This pattern was found in akalats (Beresford *et al.* 2004), double-collared sunbirds (Bowie *et al.* 2004b), the green barbet (*Stactolaema olivacea*; Fjeldså & Bowie 2008), the streaky canary (*Serinus striolatus*; Fjeldså & Bowie 2008), and olive thrushes (Bowie *et al.* 2005), all of which are found in montane forests, although the latter has a broader distribution including woodlands and more disturbed habitats. While the pattern may be more complicated in montane greenbuls (Roy 1997; Roy *et al.* 1998, cited in Kahindo *et al.* 2007) and olive sunbirds (Bowie *et al.* 2004a), it is clear that lineages restricted to the central and southern Eastern Arc mountains are distinct and divergent from those restricted to the northernmost mountains.

The precise cause underlying these phylogeographic patterns – for animals as dissimilar as birds and frogs – in the Eastern Arc remains obscure. We speculate that one possibility is that riparian habitats, which in the past consisted of forest at lower elevations, formed corridors for dispersal. More extensive forest cover along rivers would allow forest-restricted animals to move between drainage basins in different mountains. With increasing aridity, montane forests would recede to higher elevations, thus eliminating, or at least significantly inhibiting, dispersal between basins. This may explain disjunct geographic distributions in which a given species presently occurs in forests on several mountains, but not in the intervening lowlands; examples in *Arthroleptis* include *A. affinis* and *A. xenodactylus*.

The divergence of populations found in the northernmost mountains from those of the mountains immediately to the south suggests a significant barrier for dispersal. This barrier is a broad, flat and relatively low-lying plain that corresponds roughly to the administrative Handeni District of Tanga Region and consists of a patchwork of

savannah and woodlands. Importantly, the Pangani River, which lies very close to the western side of the Pare and Usambara mountains, is the only major waterway in this plain. If, in the past, more extensive forests characterized riparian habitat at lower elevations, it seems likely that this large plain would still have been a barrier to dispersal as it has only one river. This leads us to propose two different hypotheses, which are not mutually exclusive, for gene flow between animal populations in the northernmost Eastern Arc mountains and those to the south. The first hypothesis is that dispersal occurred presumably during long, cool periods of climatic stability that facilitated the spread of forests across this broad plain. The second hypothesis is that forest-restricted animals dispersed to, and then spread within, coastal forests via forested riparian habitats and then gradually retreated to higher elevations in other basins as the forests receded with increasing aridification. These corridors might have existed simultaneously or, possibly, sequentially with riparian corridors still providing routes for dispersal when the plain was no longer forested. The great variation in divergence times estimated between bird populations in the Usambara Mountains and those to the south (Fjeldså & Bowie 2008) does not negate these scenarios. However, it does indicate that, over evolutionary time scales, cyclical climatic change likely generates many opportunities for gene flow between these mountains and that it is difficult to apply a simple model of one historical 'event' that resulted in the vicariance observed between populations of many species.

Estimating the timing of origin within and dispersal from the Eastern Arc

The timing of divergence between populations and palaeoclimatic data may serve to eventually test these contrasting hypotheses. However, there is currently a limited understanding of the temporal patterns of diversification among Eastern Arc organisms and of the long-term palaeoecology of these mountains (Lovett *et al.* 2005). Based on pairwise sequence divergences, Kahindo *et al.* (2007) suggested that most diversification within many Eastern Arc bird species occurred within the past three million years. Divergence between some populations may have been even more recent. For example, Bowie *et al.* (2006) used coalescent methods to estimate times of divergence between populations; these authors found that populations in the southern Eastern Arc and the Malawian mountains may have diverged as recently as 0.07 million years ago (Mya). Without external calibrations points or known rates of diversification, we cautiously apply a molecular clock to our data. Assuming a constant rate of sequence divergence similar to that of mitochondrial DNA of other vertebrates (i.e. between 0.6–2.0% per million years; Shields & Wilson 1987; Avise *et al.* 1992; Mulcahy & Mendelson 2000), then the

most recent common ancestor of *A. xenodactyloides* occurred between 2.6 and 13.2 Mya. By the same method of inference, populations spread between the southern Eastern Arc (i.e. Udzungwa) and southern Malawi (i.e. Mulanje) between 0.2 and 2.0 Mya. Our estimates of divergence, which should be viewed with the utmost caution, concur with most of those of Bowie *et al.* (2006) and Kahindo *et al.* (2007), indicating that the Pleistocene was an important period of intraspecific diversification in the Eastern Arc. During the Pleistocene, Africa's climate became more sensitive to high-latitude climatic changes, which resulted in patterns of cyclical climatic change and several aridification events (deMenocal 1995). Furthermore, these estimates of divergence times indicate that both bird and frog montane populations diverged well before the Last Glacial Maximum (e.g. Gasse 2000).

Explaining the large geographic range of A. xenodactyloides

The phylogeographic pattern observed within *A. xenodactyloides* may reflect a larger historical phenomenon in which cyclical climatic change within the Eastern Arc drives diversification and dispersal from this centre when climatic regimes favour expansion. Yet, this does not explain why *A. xenodactyloides* is so much more widespread than its congeners. The restriction of most species to the mountains of the Eastern Arc indicates that species-level adaptations are likely necessary to facilitate geographic range expansion. Two such adaptations appear to have facilitated the widespread distribution of *A. xenodactyloides*: tolerance of a wide elevational range and ability to survive outside forested environments. Physiologically, the tolerance of a wide elevational range is very important for dispersal of montane taxa, especially in the tropics in which the temperature regimes exhibit little annual variation at a given site and elevation (Janzen 1967; Ghalambor *et al.* 2006). In support of our hypothesis that adaptation facilitated this dispersal, most latitudinal change in *A. xenodactyloides* appears early in its evolutionary history rather than late. *Arthroleptis xenodactyloides* is found in forests, but, unlike many of its East African congeners, it is also known from grasslands and has one of the largest elevational ranges of any Eastern Arc frog (Poynton 2003a). In addition, contemporary gene-flow within *A. xenodactyloides* has been demonstrated across a hot and arid valley between neighbouring mountains in the Taita Hills (Measey *et al.* 2007), suggesting that this species is able to disperse across areas that may otherwise be considered barriers. We propose that physiological adaptation early in the history of *A. xenodactyloides* led to a larger ecological breadth that facilitated geographic expansion onto Pemba Island as well as to the mountains of the southern Rift of Malawi and the eastern Highlands of Zimbabwe.

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This study complements previous work by D. C. Blackburn on the evolutionary history of leaf-litter frogs of the genus *Arthroleptis*. Past and ongoing studies of diversification of amphibians across space and time are providing new insight into the history of Africa's faunas since the Pleistocene as well as at deeper temporal scales. G. J. Measey has long standing interests in dispersal and vicariance and their role in the origins of biodiversity of African amphibians. Both authors are interested in integrating phylogeographic data with historical climatic reconstructions and in utilizing phylogenetic analyses to test hypotheses about the biogeographic history of Africa's faunas.
