ORIGINAL ARTICLE

The role of forest expansion and contraction in species diversification among galagos (Primates: Galagidae)

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ABSTRACT

Aim Here, I investigate galagid diversification and current distribution in the context of major climatic and geological events in sub-Saharan Africa. Given their widespread distribution and presence in a large range of diverse habitats, galagids represent an excellent group to investigate the role of forest contraction and expansion on biological diversification in sub-Saharan Africa.

Location Sub-Saharan Africa (Afrotropical Region).

Methods I assembled a supermatrix including 53 nuclear loci and three mitochondrial markers for 94% of the galagid species currently recognized. Bayesian and maximum likelihood methods were used to infer phylogenetic relationships and times of divergence within the family. Ancestral ranges were estimated using several methods, including 'BioGeoBEARS' and RASP.

Results Phylogenetic analyses corroborated previous results regarding the evolutionary history of this family: (1) early origin of the family soon after the Eocene-Oligocene boundary; (2) dwarf galagos (*Galagoides* spp.) represent a polyphyletic group with two well-defined clades, one in central-west Africa and one in the east; and (3) divergences within galagids are relatively old with most genera already present by the Late Miocene. The biogeographical analysis indicates central African origins and subsequent expansion to the east in the Early-Middle Miocene. An expansion to the northern and southern savannas occurred between the Late Pliocene and the Middle Pleistocene.

Main conclusions The results of this study clarify several questions related to the evolutionary history of the Galagidae in the context of sub-Saharan African biogeography. This study suggests that galagid evolution and diversification was affected by three major climatic episodes: (1) the global cooling and forest contraction in the Early Oligocene, (2) the forest expansion and the uplift of the African rifts in the Miocene and (3) the aridification and extension of open woodlands and savannas in the Late Miocene and Plio-Pleistocene.

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INTRODUCTION

Past climatic changes had a major impact on shaping biotic communities by driving extinction and speciation and affecting the distributions and abundances of species (Blois & Hadly, 2009; Figueirido *et al.*, 2012; Blois *et al.*, 2013). In tropical and subtropical regions, cyclical oscillations between warm/wet and cool/dry periods affected the distribution of different ecosystems, with alternating expansion and contraction of forested habitats (Morley, 2000; Morley & Kingdon, 2013). During dry phases, savannas and open woodlands expanded at the expense of forests, isolating populations of forest-dwelling species, whereas, during pluvial periods, forests replaced open and drier habitats, leading to the fragmentation of arid-adapted species and enabling secondary contact between forest-adapted lineages (Morley, 2000).

During the Cenozoic, palaeoenvironments in sub-Saharan Africa were influenced by multiple climatic events, including changes in global temperature due to the formation and expansion of the Antarctic ice sheet at the end of the Eocene, and of the North Hemisphere ice sheets towards the end of the Miocene and during the Plio-Pleistocene (Zachos *et al.*, 2001). Additionally, Africa was characterized by massive geological and volcanic activity that resulted in the formation of the African rift valley during the Miocene (Sepulchre *et al.*, 2006). The resulting elevated configuration of the African landmass not only disrupted connectivity between the eastern and the western part of the continent, but also provided new opportunities for colonization of new environments (e.g. high-altitude habitats). The combination of climatic and geological events over the last 60 Myr has played a key role in shaping biodiversity across Africa. Recent studies have identified very similar patterns of distribution and endemism across vascular plants and vertebrates, suggesting that, despite a complex climatic and geological history, most African taxa responded to similar environmental stimuli and their biogeographical histories were shaped by similar events (Linder *et al.*, 2012).

Members of the family Galagidae, also known as galagos or bushbabies, are an excellent group to investigate the influence of climatic change and, more specifically, the role of forest contraction and expansion on biological diversification in sub-Saharan Africa. Galagids are widely distributed across most of sub-Saharan Africa and inhabit a large range of diverse habitats including lowland rain forests, montane forests, and open woodlands and savannas (Nekaris & Bearder, 2010; Nekaris, 2013). While some genera have restricted distributions in the tropical forests of central Africa (*Euoticus* and *Sciurocheirus*), other genera are more widespread across Africa (*Galago* and *Otolemur*). Recent studies have suggested old origins for this family, dating back to roughly 30 Ma at the very beginning of the Oligocene (Pozzi *et al.*, 2014a, 2015). As the two earliest branching lineages (*Euoticus* and western *Galagoides*) are both central African lineages, Pozzi *et al.* (2014a) hypothesized a biogeographical origin in central-western Africa. Global cooling at the beginning of the Oligocene promoted major environmental change in equatorial Africa and increased aridity in the north (Zachos *et al.*, 2001; Seiffert, 2007a). The early evolution of extant galagids may have been restricted to central-western Africa where rain forests were still present at the time.

Galagids remain one of the least studied primate groups (Nekaris & Bearder, 2010; Nekaris, 2013; Pozzi *et al.*, 2015). Multiple reasons have hindered our knowledge of galagid biology, including their widespread distribution, their nocturnal habits, and their lack of obvious morphological diversity. Our understanding of galagid evolution has been historically limited to museum studies (e.g. Masters & Bragg, 2000; Masters & Brothers, 2002; Masters & Couette, 2015) or to relatively short-term field studies (e.g. Harcourt & Nash, 1986; Nash & Harcourt, 1986; Honess & Bearder, 1996; Ambrose, 2003; but see Charles-Dominique & Martin, 1977

for an exception). While much research has been conducted on the biogeography of the suborder Strepsirhini (to which the Galagidae belongs), specifically focusing on the colonization of Madagascar by lemurs (e.g. Yoder *et al.*, 1996; Stankiewicz *et al.*, 2006; Stevens & Heesy, 2006; Masters *et al.*, 2007a) and the disjunct distribution of Asian and African lorisids (e.g. Yoder, 1997; Masters *et al.*, 2005; Seiffert, 2007b), a detailed biogeographical analysis is still lacking for the galagids. Our knowledge of galagid biogeography is limited to a few studies that investigated diversification at the intrageneric level. For instance, speciation in lesser (*Galago* spp.) and greater galagos (*Otolemur* spp.) has been associated with the expansion of savanna environments following the aridification events of the Early to Middle Pleistocene (Masters, 1988, 1998).

The aim of this paper was to investigate the role of forest expansion/contraction and climate change in driving diversification within galagids. The main hypothesis is that major episodes of diversification within galagids coincided with cyclical oscillations between wet and dry periods in sub-Saharan Africa. To test this (1) I assembled a large molecular data set at the species level to confirm phylogenetic relationships and times of divergence within the Galagidae, and (2) I reconstructed the biogeographical history of the family, focusing on major climatic and geological events that affected the region of sub-Saharan Africa.

MATERIALS AND METHODS

Data set

I assembled a data set comprising 53 nuclear loci and three mitochondrial genes (see Appendix S1 in supporting information). Overall 17 taxa were included in the data set, representing ~94% of the galagid species described to date (Butynski *et al.*, 2013; Nekaris, 2013). Outgroups were selected to include nodes with reliable fossil calibrations within primates. They included 13 species belonging to the family Lorisidae (7), the Madagascar lemurs (1) and the catarrhine primates (5). GenBank accession numbers for all the sequences are provided in Appendix S1. Each locus was aligned independently using MUSCLE 3.8 (Edgar, 2004), and combined in a single matrix resulting in a total alignment length of 41,179 bp. PARTITIONFINDER 1.1.1 (Lanfear *et al.*, 2012) was used to identify the optimal partitioning scheme for the 56 data blocks (53 nuclear loci and three mitochondrial regions), and the best-fit nucleotide substitution model for each partition. All PARTITIONFINDER analyses used the *greedy* search algorithm, linked branch lengths in calculations of likelihood scores, and the Bayesian information criterion (BIC) for selecting among alternative partitioning strategies.

Given the different availability of sequences across taxa, the supermatrix used in this study showed a relatively high level of missing data. The impact of missing data on phylogenetic reconstructions is still debated, although recent studies suggested that even high levels of missing data do not

significantly affect the results (e.g. Wiens & Moen, 2008; Wiens & Morrill, 2011; Roure *et al.*, 2013; Zheng & Wiens, 2015). To test the influence of missing data I assembled two additional data sets: (1) one data set including only cytochrome b sequences (the only sequence available for all taxa), and (2) a subset of the original data set where only genes that were present in at least 70% of the taxa were included. This resulted in 21 genes (2 mitochondrial and 19 nuclear). Differences in topologies and branch lengths were measured based on Robinson-Foulds distances and relative scale-factor values (Soria-Carrasco *et al.*, 2007), using the full data set tree as reference.

Phylogenetic and dating analyses

Maximum likelihood (ML) analyses were run using RAxML 8.1.7 (Stamatakis, 2014). The data set was partitioned based on PARTITIONFINDER results and support for each internal node was assessed by bootstrap (BP) analyses using the bootstopping criteria autoMRE (Majority Rule Criterion). Bayesian analyses (MB) were run using MrBayes 3.2.3 (Ronquist *et al.*, 2012). Four independent runs were conducted using four MCMC (Markov chain Monte Carlo) chains for 20 million generations (25% burn-in). Convergence of MrBayes analyses was assessed using TRACER 1.6 (http://beast.bio.ed.ac.uk/Tracer) and AWTY (Nylander *et al.*, 2008).

Divergence time analyses were performed using the uncorrelated Bayesian relaxed-clock method in BEAST 1.8.0 (Drummond *et al.*, 2012). I conducted four replicate runs with four MCMC chains sampled every 5000 generations for 40 million generations each (25% burn-in). Five calibration points within primates were selected following Pozzi *et al.* (2014b) and Raaum (2014): (1) Lorisidae-Galagidae (95%: 36.9–47.0 Ma); (2) Hominoidea-Cercopithecoidea (95%: 21.0–33.9 Ma); (3) *Homo-Pongo* (95%: 12.5–18.0 Ma); (4) *Homo-Pan* (95%: 5.0–10.0 Ma); and (5) *Theropithecus-Papio* (95%: 3.5–6.5 Ma) (Appendix S2a).

Biogeographical analyses

Galagid distributions were mapped based on a recent study that used the distributions of thousands of vertebrate and plant species to identify the existence of seven statistically defined biogeographical regions in sub-Saharan Africa (Linder *et al.*, 2012). Two regions were excluded from the analyses: (1) the Ethiopian region, which was not recognized as a biogeographical area for mammals, and (2) the Saharan region, as no galagid species is found in this area. Each galagid taxon was assigned to a set of the remaining five biogeographical areas (Sudanian, Congolian, Somalian, Zambezian and Southern African) based on distribution maps and spatial data provided by the IUCN (http://www.iucnredlist.org/) (Fig. 1 and Table 1).

I employed two approaches to estimate ancestral areas. I first used the R package 'BioGeoBEARS' (Matzke, 2013) to compare alternative biogeographical models. 'BioGeoBEARS' implements the dispersal-extinction cladogenesis model (DEC), a likelihood version of the dispersal–vicariance analysis (DIVALIKE), and the Bayesian inference of historical biogeography for discrete areas (BAYAREALIKE). All the three models can also be run with the founder-event speciation parameter (+J versions). Each model allows for a different subset of biogeographical possibilities, such as dispersal, vicariance and extinction (Matzke, 2013, 2014). To take into account the role of connectivity between areas, I also ran the analyses using dispersal multipliers such that adjacent areas received a dispersal multiplier of 1 and non-adjacent areas, a value of 0.5.

The second method used for the ancestral area reconstruction was the Bayesian binary MCMC (BBM) approach implemented in RASP 3.2 (Reconstruct Ancestral States in Phylogenies; Yu *et al.*, 2015). BBM analysis was performed using an estimated model F81+G with a null root distribution. The MCMC chains were run for 10 million generations (10 MCMC chains, with sampling frequency equals to 1000 generations, a temperature of 0.1 and 25% burn-in).

In both analyses, the maximum number of ancestral areas at a given node was restricted to three, as no extant galagid occurs in more than three areas, and only one species occupies such an extensive range, the northern lesser galago, *Galago senegalensis* (Fig. 1).

RESULTS

Phylogeny

The partition schemes identified by PARTITIONFINDER were used to reconstruct phylogenetic relationships and estimate divergence times (Appendix S2b). Phylogenetic relationships within the family Galagidae were consistent across all the analyses, with most nodes supported by BP≥70% and PP (posterior probability) ≥0.95 (Fig. 2 and Appendix S2c). The genus *Euoticus* was strongly supported as the sister taxon of all other galagids (N2; BP = 100% and PP = 1.00) and the genus *Galagoides* was not monophyletic. Two clades within 'Galagoides' were identified: one including the species *G. demidoff* and *G. thomasi* (western clade N4; BP = 100% and PP = 1.00) and another including *G. cocos*, *G. zanzibaricus*, *G. granti*, *G. orinus* and *G. rondoensis* (eastern clade N10; BP = 100% and PP = 1.00). Within this clade, the sister taxon relationship between *G. orinus* and *G. rondoensis* was the only one recovered with low support (N11; BP = 62% and PP = 0.86–0.87). All other genera were recovered as monophyletic and interrelationships among genera were highly supported. *Sciurocheirus* was inferred as the sister taxon to *Otolemur* (N14; BP = 100% and PP = 1.00), and *Galago* as the sister taxon of the eastern *Galagoides* (N6; BP = 90% and PP = 1.00).

The origin of the extant Galagidae is estimated at approximately 34 Ma (N2; 95% HPD = 30.67–38.55) (Fig. 2). Age estimates for the next divergence event within the family were much younger, dated at ~20 Ma (N3; 95%
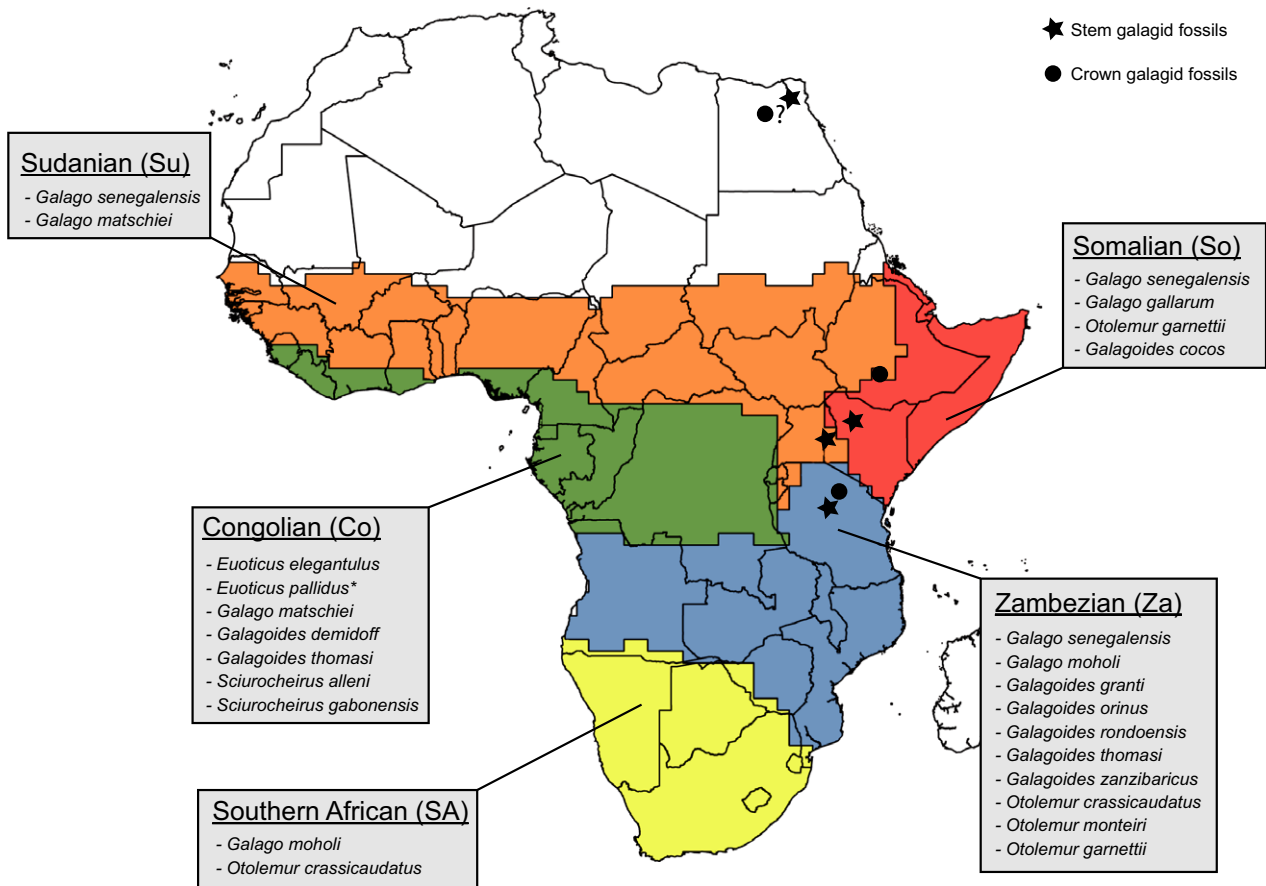
Figure 1 Map indicating the five African biogeographical regions used in this study: (a) Sudanian, (b) Congolian, (c) Somalian, (d) Zambezian and (e) Southern African, based on Linder *et al.* (2012). For each region, the galagid species present in the area are listed. *Not included in the current study. Localities for important fossil galagids are also indicated by stars (stem taxa) and filled circles (crown taxa).

Table 1 Biogeographical regions used to reconstruct the history of Galagidae, including numbers of species and genera present in each area.

Species	Code	Corresponding areas of endemism/biome	Number of species	Number of genera
Sudanian	Su	Northern savannas	2	1
Congolian	Co	Congolian and Guinean forests	6	4
Zambezian	Za	Southern savannas	10	4
Somalian	So	Somalia and the Horn of Africa dry savanna	4	3
Southern African	SA	Cape area, the Karoo–Namib and Afromontane regional centre, the Kalahari desert, the Natal region and the South-west Angola	2	2

HPD = 17.68–22.53). Overall, divergence estimates within the family were relatively old, and most genera had diverged by the Late Miocene. The only possible exception was *Sciurocheirus* that emerged between the Late Miocene and the Pliocene (N17; 3.77 Ma; 95% HPD = 1.70–6.28). Divergence estimates were especially old within the dwarf galagos: the eastern clade is dated at roughly 11 Ma (N10; 95% HPD = 8.17–13.45), while the western clade emerged in the Late Miocene, approximately 10.3 Ma (N4; 95% HPD = 7.88–12.66). Age estimates with 95% highest

probability densities (HPDs) for all nodes within the Galagidae are reported in Appendix S2d.

All three data sets used in this study, independent of the amount of missing data, resulted in the same topology with similar support values and age estimates for most of the nodes (Appendix S2d,e). Analyses conducted on cytochrome b showed moderately younger estimates for a few nodes (scale-factor equals to 1.0815 for cytb vs. 0.9850 for the 21-gene data set), including the origin of the extant Galagidae (~34 Ma vs. ~30 Ma). However, mean age estimates for these
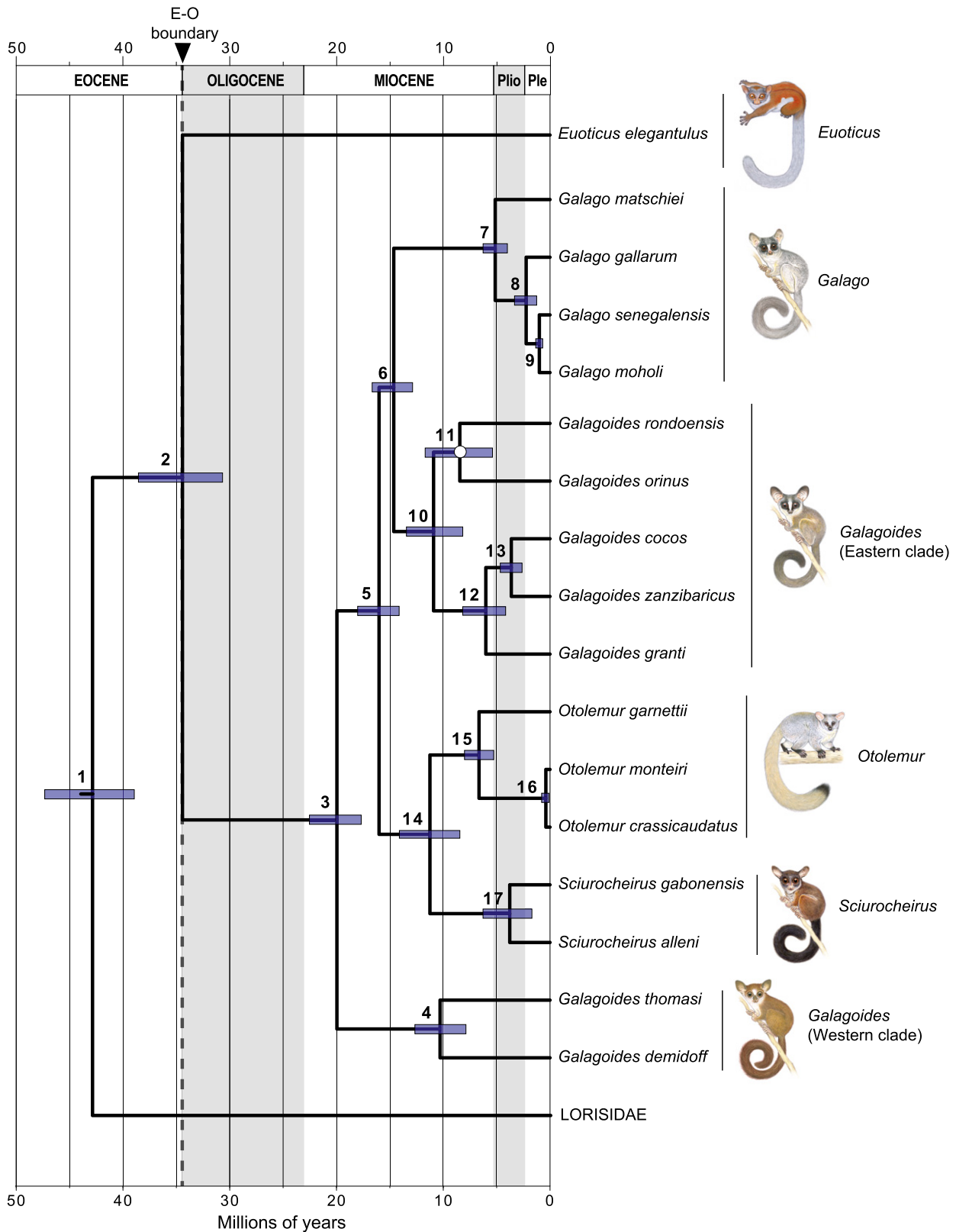
Figure 2 Estimated divergence ages with 95% highest probability densities (HPDs, blue bars). A geological time-scale is given above the tree. For detailed information on estimated divergence ages see Appendix S2d. Numbers in the tree refer to the major nodes mentioned in Appendix S2. The only node weakly supported in the analyses (N11; BP = 62% and PP = 0.86) is reported in the tree with a white circle. Plio, Pliocene; Ple, Pleistocene; E-O, Eocene-Oligocene. Images provided by Stephen Nash and used with permission from the IUCN/SSC Primate Specialist Group.

nodes fell within the 95% HPD region of analyses conducted on both the full data set and the 21-gene data set.

Biogeographical history

The ancestral area reconstruction analyses performed using 'BioGeoBEARS' and RASP showed very similar results. In the 'BioGeoBEARS' analyses, the DEC+J model produced the best statistical fit to the data (Table 2, Appendix S3a). However, no significant difference with the simpler DEC model was found (ΔAIC = 0.4; P = 0.12). The other four models showed significant lower likelihoods (Table 2). Under all reconstructions, the ancestor of all extant galagids originated in the Congolian region. Subsequent dispersal events into the

Zambezian region characterized the distribution of the ancestor of all galagids, with the exclusion of *Euoticus*, restricted to Central Africa (Appendix S3a). In the DEC+J model, a major vicariant event characterized the split between the western clade of *Galagoides* (*G. thomasi* and *G. demidoff*) and the rest of the galagids, around 20 Ma (Fig. 3a). The eastern clade of *Galagoides* probably originated subsequent to this split in the Zambezian region where most of the species are found today. In the Late Miocene (~11 Ma), another important vicariant event between the Zambezian and the Congolian regions led to the split between *Otolemur* in the east and *Sciurocheirus* in the west. While *Sciurocheirus* remained within a restricted distribution in central Africa, greater galagos (*Otolemur*) underwent range expansions to occupy Southern Africa (*O. crassicaudatus*) and eastern Africa (*O. garnettii*). The ancestral area of origin for *Galago* was more difficult to reconstruct, possibly because of the current widespread distribution of two species, *Galago senegalensis* and *Galago moholi*. Both the DEC and the DEC+J models suggested a colonization event from the Zambezian region to the Sudanian region and a subsequent expansion into the Somalian and back to the Zambezian region (Fig. 3a). However, under the other models, the most likely ancestral area for the lineage leading to *Galago* was the Congolian region (Appendix S3a). All models agreed in supporting the recent diversification (~1 Ma) and range expansion into the southern savannas for *Galago moholi* and into the northern savannas for *Galago senegalensis*. The overall scenario depicted by

Table 2 Comparisons of likelihood values (LnL) and the Akaike information criterion (AIC) score from each of the analyses in 'BioGeoBEARS'.

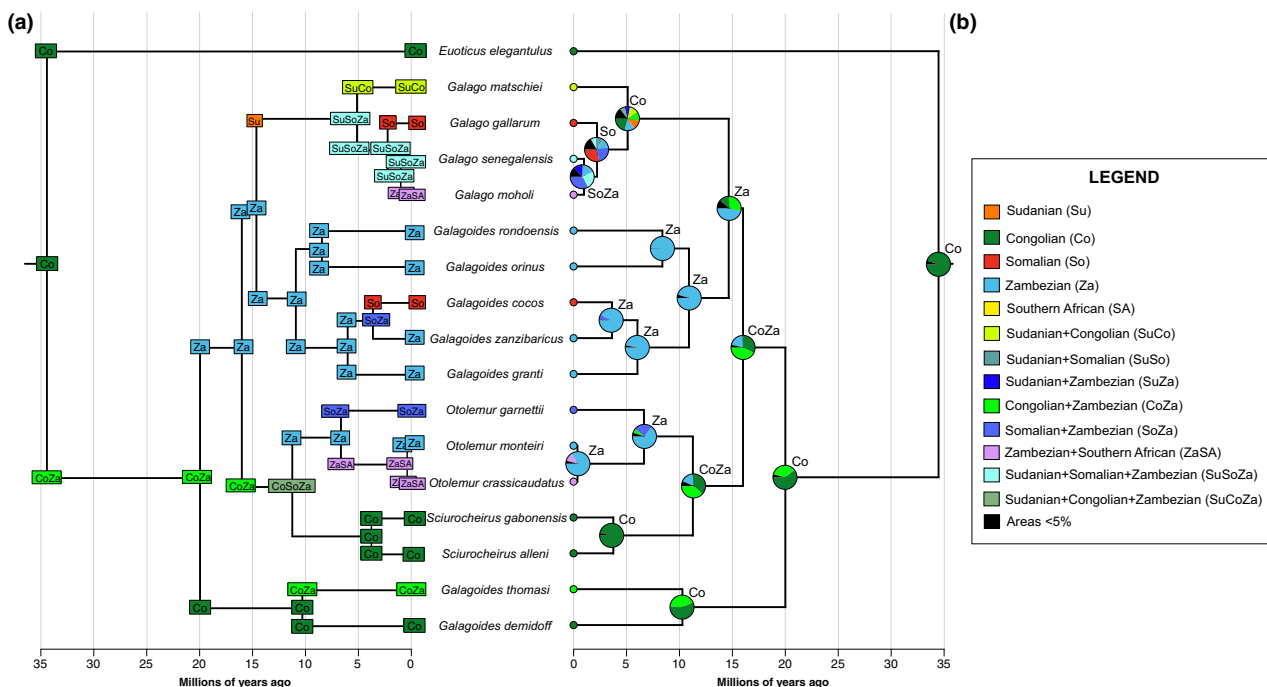| Model	LnL	AIC	ΔAIC	P-value*
DEC	−44.72	93.44	0.40	–
DEC+J	−43.52	93.04	0	0.12
DIVALIKE	−48.37	100.7	7.63	–
DIVALIKE+J	−47.5	101	7.96	0.19
BAYAREALIKE	−54.16	105.5	12.1	–
BAYAREALIKE+J	−49.74	112.3	19.26	0.003

*chi-square test between likelihood values (LnL).

Figure 3 Ancestral area reconstruction based on (a) 'BioGeoBEARS' analyses (model selected: DEC+J) on the left, and (b) RASP 3.2 (model selected: BBM) on the right. Pie charts at nodes represent 95% confidence intervals of the relative frequencies of ancestral area optimizations across the (entire) Bayesian tree.

the analyses run using dispersal multipliers was congruent with the one described above (see Appendix S3a).

Results of the BBM analysis suggest a relatively complex biogeographical history for the Galagidae including 16 dispersal and four vicariant events (Fig. 3b). BBM analysis also recovered the Congolian region as the ancestral area for the Galagidae with very high support (Co: 95.28%). Most dispersal events originated in either the Congolian (4 events) or the Zambezian region (five events). Interestingly, the Sudanian and the Southern African domains were only characterized by immigration events. BBM analysis also identified the Zambezian region as the one with the highest number of 'speciation events within areas' (7), followed by the Congolian region (4) and the Somalian region (1). No speciation events were inferred within either the Sudanian or the Southern African regions (Appendix S3b).

Major dispersal events were as follows: (1) from the Congolian region to the Zambezian region for all the lineages except for *Euoticus* and western *Galagoides*, (2) from the Zambezian region to the Southern African region and the Somalian region for *Otolemur crassicaudatus* and *O. garnettii* respectively, (3) dispersal towards the Somalian region from the Zambezian region, followed by a vicariant event for *Galagoides zanzibaricus/cocos*, and finally (4) dispersal from the Congolian/Zambezian region to the Somalian and Sudanian regions for *Galago senegalensis* and to the Southern African region for *Galago moholi*. At the genus level, the ancestral state reconstruction indicates a Congolian origin for *Euoticus*, *Sciurocheirus* (N17; Co: 97.06%), and western *Galagoides* (N4; Co: 56.62%), and a Zambezian origin for eastern *Galagoides* (N10; Za: 95.93%) and *Otolemur* (N15; Za: 64.72%). In contrast, the origin of the genus *Galago* is less certain, with a slight preference for a Congolian origin (N7; Co: 21.51%) (Appendix S3b).

DISCUSSION

Phylogenetics

Phylogenetic relationships and divergence times within galagids are consistent with recent studies using either mitochondrial (Pozzi *et al.*, 2015) or nuclear DNA (Pozzi *et al.*, 2014a). Missing data did not significantly impact the overall results, suggesting that the topology and divergence dates presented in this study are robust. The origins of the family are estimated around the time of the Eocene-Oligocene boundary (EOB; 33–34 Ma). These estimates are slightly older than those recovered by mitochondrial analyses (~30 Ma; Pozzi *et al.*, 2015; this study), but are in accordance with previous studies on nuclear DNA (Pozzi *et al.*, 2014a). As previously reported, there is a wide time gap between the emergence of *Euoticus* in the Early Oligocene and the divergence of the rest of the clade in the Early Miocene (~20 Ma). Interestingly, no galagids are found at this time in the fossil record (Seiffert, 2007a; Harrison, 2010). The genus *Galagoides* was confirmed as polyphyletic: while the western clade (*G. demidoff* and *G.*

thomasi) is the second lineage to emerge in the family, the eastern clade was consistently inferred as sister taxon of *Galago*. At the species level, the only weakly supported node was the sister taxon relationship between *Galagoides orinus* and *Galagoides rondoensis*. An earlier study using cytochrome *b* sequences reported a different topology, with *G. rondoensis* as the first lineage to emerge within the eastern *Galagoides*, and *G. orinus* as sister taxon of the Zanzibar galagos; however, in that case too, support values were relatively low (Pozzi *et al.*, 2015). To date, only limited mitochondrial data are available for these taxa and more genetic data are needed to clarify the interrelationships within eastern *Galagoides*.

Overall, divergence times within the family are relatively ancient. With the exception of *Sciurocheirus* estimated at 3.7 Ma, all the other genera emerged earlier than 5 Ma, while the two clades of *Galagoides* are older than 10 Ma. These two clades are similar in age to some of the oldest strepsirhine genera such as *Lepilemur* (7–10 Ma) or *Cheirogaleus* (7–11 Ma), but generally older than most other primate genera (Springer *et al.*, 2012). Limited research has been conducted within the two clades, and it is possible that diversity within dwarf galagos is higher than previously thought (Pozzi *et al.*, 2015). Some recent studies have suggested the presence of several undescribed species in eastern Africa, including *Galagoides nyasae* from Malawi (Grubb *et al.*, 2003). More field surveys and genetic analyses are required to clarify species diversity within eastern dwarf galagos.

Biogeography

This study represents the first comprehensive biogeographical reconstruction of African galagids. Three major phases in galagid historical biogeography can be identified (Fig. 4).

I. Early Oligocene: origins in central Africa

All the analyses performed in this study strongly indicated a biogeographical origin of extant galagids soon after the EOB in central Africa (Congolian region) (Figs 3 & 4b). The earliest fossils attributed to the extant radiation (*Otolemur howelli*, Ethiopia, ~3.0–3.2 Ma; *Galago senegalensis*, Tanzania, ~1.8 Ma; *Galagoides* cf. *zanzibaricus*, Ethiopia, ~3.0 Ma) are Middle Pliocene-Early Pleistocene in age, and are geographically restricted to eastern Africa (Harrison, 2010) (Fig. 1). One possible exception is the presence of *Galago farafraensis*, found in Egypt in 10–11 Ma sediments (Pickford *et al.*, 2006). However, the taxonomic assignment of this specimen to the genus *Galago* is debated. Given their recent age, these fossil forms are not informative regarding the biogeographical origins of the extant group. Stem galagids are known from two different regions: the youngest forms (*Komba* and *Progalago*) are found in eastern Africa in the Early-Middle Miocene (15–20 Ma) (Harrison, 2010), whereas the oldest forms derive from northern Africa, and are estimated to be 35–37 Ma in age (*Saharagalago* and *Wadilemur*) (Seiffert *et al.*, 2003, 2005) (Fig. 1).
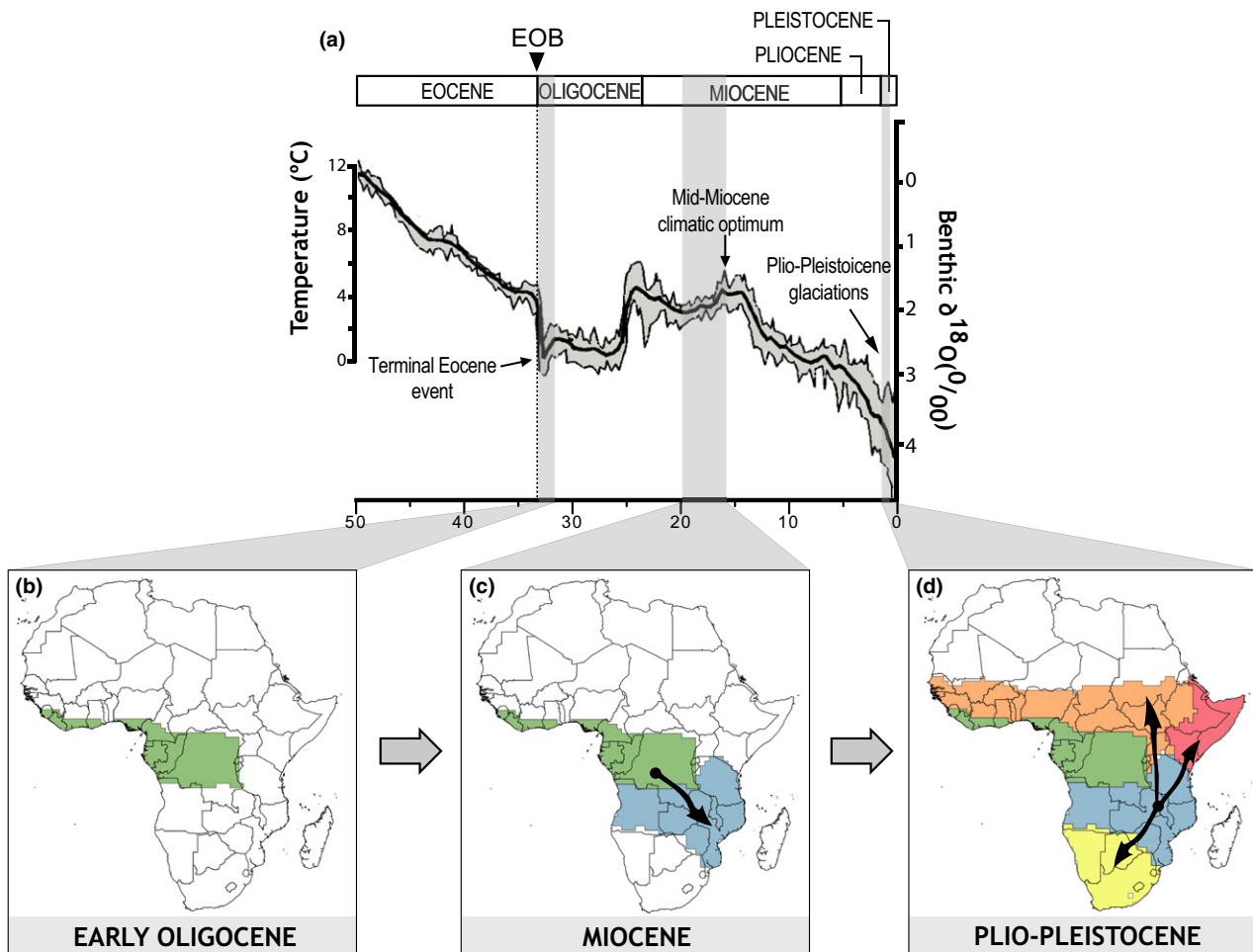
Figure 4 Proposed biogeographical scenario for African galagids. (a) A summary of important climatic events throughout the Cenozoic is presented. Benthic δ^{18}O (‰) is a proxy for global ocean temperatures, with lower values corresponding to warmer temperatures (climate figure redrawn from Morley & Kingdon, 2013). (b–d) Maps summarizing the three main events that characterized galagid biogeography: (a) origins in central Africa, (b) expansion towards eastern Africa in the Miocene and (c) expansion into northern and southern savannas in the Plio-Pleistocene.

The EOB coincided with a series of climatic changes, from wet and warm conditions in the Eocene, to cooler and drier conditions in the Oligocene. This period is associated with the development of the Antarctic ice sheet and a major fall in sea levels, that led to large-scale extinction and floral and faunal turnover in the northern continents (Zachos *et al.*, 2001). In Africa, the EOB was characterized by increased aridity in the north, and a subsequent major floristic change. In the early Oligocene, tropical forests disappeared from mid-latitudes and were probably restricted to the equatorial region (Morley, 2000). Although the African fauna was not affected by climatic changes as much as in the northern continents, the primate fossil record clearly indicates a major turnover event at the beginning of the Oligocene (Seiffert, 2007a, 2012). Most of the data available for African primates derive from northern sites, like the Fayum area in Egypt (Seiffert, 2007a). During the Eocene, warm temperatures and the presence of tropical and subtropical forests offered an ideal habitat for primates (Seiffert, 2007a). The decrease in temperature in the early

Oligocene strongly reduced primate diversity: while haplorhines persisted in the area, at least four other primate clades (adapids, djebelemurines, plesiopithecids and galagids) underwent local extinction from the Fayum sediments (Seiffert, 2007a, 2012). The persistence of lorisids and galagids across the EOB in sub-Saharan Africa is probably related to the presence of tropical refugia. It is possible that many mammalian groups, including the galagids, were restricted to rain forest habitat during the Early Oligocene (Morley & Kingdon, 2013). The absence of galagid fossils between the Late Eocene and the Early-Middle Miocene might thus be related to their restricted distribution during this period in those forested areas from which we have no fossil record.

II. Miocene: expansion to eastern Africa and splits between eastern and western clades

During the late Oligocene and the early Miocene temperatures increased globally, reaching a maximum around the

Mid-Miocene climatic optimum (Fig. 4a). At this time, rain forests expanded from coast to coast covering most of tropical and subtropical Africa (Andrews & van Couvering, 1975; Morley, 2000). The extension of tropical forests allowed clades previously confined to the equatorial forests, such as forest-dwelling galagos, to expand their distribution ranges from central Africa towards the east (Fig. 4c).

The volcanic and geological activity that affected Africa in the Neogene is likely to have played a major role in galagid evolution (Livingstone & Kingdon, 2013). The African rift valleys are the product of uplift and downthrust events that characterized the Middle to Late Miocene. It is during this time that a series of biogeographical barriers between the east and the west of the continent started to appear. The relatively small number of species in common between the Guineo-Congolian and the East African forests implies that the region of the rift valleys acted as an efficient barrier to gene flow in numerous organisms, including many species of birds, mammals and insects (Lovett & Wasser, 1993; Burgess et al., 2007; Livingstone & Kingdon, 2013). Although the timing and the separation mechanisms between the eastern and western communities are still unclear, it is likely that these events contributed to galagid diversification at three different stages: (1) the split between western Galagoides and the lineage leading to the other galagids (~20 Ma); (2) the split between the Sciurocheirus/Otolemur and Galago/eastern Galagoides lineages (16 Ma) and (3) the split between Sciurocheirus and Otolemur at around 11 Ma (Fig. 3).

The formation of the African rift and the concomitant global cooling were the main drivers of the expansion of open woodland and savanna at the expense of rain forests in the Late Miocene (Morley, 2000; Morley & Kingdon, 2013). Between 8 and 6 Ma there is clear evidence of a transition from woodlands to grasslands (Sepulchre et al., 2006) with bovids becoming increasingly predominant (Vrba, 1985). The consequent fragmentation of forests led to the speciation of many equatorial mammals (Morley & Kingdon, 2013). For instance, important speciation events within guenons roughly coincide with these aridification events in the Late Miocene and Pliocene (Kamilar et al., 2009; Guschanski et al., 2013). Diversification within galagids in the Late Miocene included the genus Otolemur and the eastern Galagoides. Based on the biogeographical analyses, both clades have unambiguous eastern African origins (Fig. 3) and it is likely that climatic events in this area strongly affected their diversification. Speciation within greater galagos (Otolemur) is likely the consequence of the expansion of open woodland and savanna areas at the end of the Miocene (~6 Ma) that led to the split between O. crassicaudatus, found in well-developed woodlands and savannas, and O. garnettii, restricted to lowland and coastal forests and thickets. For dwarf galagos (Galagoides), the relatively high species diversity in the east is probably to be related to climatic and ecological changes during the Late Miocene and Early Pliocene in the Eastern Arc Mountains and coastal forests. This region was influenced by major geotectonic events, including the

uplift of the central African plateau that formed a barrier between the eastern and the Guineo-Congolian forests, reducing the faunal interchange between the two areas (Lovett & Wasser, 1993; Burgess et al., 2007). During the Pleistocene, this area was subjected to climatic shifts resulting from the alternation of glacial periods, characterized by cooler temperatures and an expansion of grasslands, and pluvial periods, more humid and warmer, with consequent forest expansion. Palynological, palaeoenvironmental and population genetic studies support the presence of a mosaic of Pleistocene refugia in eastern Africa, probably due to the repeated fragmentation of forested habitat during the glacial periods (deMenocal, 1995, 2004; Lorenzen et al., 2010). Important biogeographical barriers, such as the Rufiji River, might also have played a role as faunal barriers for many lowland taxa that occupy this region, including the dwarf galagos (e.g. Galagoides granti) (Kingdon, 1974; Butynski et al., 2006).

III. Late Pliocene-Pleistocene: expansion to northern and southern savannas

The radiation of lesser galagos (Galago) took place in the Pliocene (~5 Ma). Ancestral range reconstructions are, however, ambiguous. Interestingly, the first lineage to emerge, Galago matschiei, is the only one confined to wet forested habitat. The early Pliocene was characterized by moist climate with the consequent expansion of rain forests and the contraction of savannas. Wooded habitats still existed in northern and southern Kenya and in Ethiopia until 5–3.4 Ma (Sepulchre et al., 2006). The current distribution of Galago matschiei might then represent a relict of the original distribution of the Galago common ancestor.

The colonization of drier environments by lesser galagos (Galago) only took place during the Late Pliocene and Pleistocene (Masters, 1998; Masters et al., 2007b; Pozzi et al., 2014b). The drying phase started at approximately 3–3.2 Ma, when the onset of glaciation in the Northern Hemisphere coincided with the expansion of dry areas in the Afrotropics (Morley & Kingdon, 2013). Galago gallarum colonized the African horn at around 2.2–2.3 Ma (Figs 3 & 4d). The presence of numerous species of mammals adapted to open grasslands in Eastern Africa, including australopiths, baboons, modern giraffes and white rhinos, clearly indicates that aridity increased in eastern Africa at ~2.5 Ma (Sepulchre et al., 2006). The divergence and range expansion of Galago senegalensis and Galago moholi are more recent (~1–1.5 Ma) and probably associated with increased aridity during the Pleistocene. Northern (Galago senegalensis) and southern (Galago moholi) lesser galagos inhabit dry woodlands and savannas, and occupy different areas of floral endemism. Masters (1998) suggested that their speciation took place alongside the radiation of their plant hosts in the Middle Pleistocene. Two migration routes were inferred for these species: (1) a northward expansion for Galago senegalensis, colonizing the northern savannas, woodlands and thorn

shrubs, and (2) a southward expansion for *Galago moholi*, distributed across the semi-arid habitats and woodlands of Southern Africa. These colonization events were probably facilitated by the repeated opening up of the north–south corridors in east Africa during the glacial periods (Morley & Kingdon, 2013).

CONCLUSIONS

I investigated the historical biogeography of one of the least studied groups of primates, the African galagids. My results strongly indicate ancient origins in central Africa for the family, likely to be a consequence of the climatic events that affected forest distribution at the EOB. The biogeographical analyses suggest the importance of forest expansion/contraction and the influence of the African uplift on the diversification and distribution of galagids. Ancestral galagids were probably forest-adapted primates; adaptations to arid environments are of recent origin, as a consequence of the expansion of open woodlands and savannas that took place in the Plio-Pleistocene. These results further indicate the critical role of climatic changes as drivers of mammal diversity in sub-Saharan Africa during the Cenozoic.

Although this study only provides a large-scale reconstruction of galagid biogeography, it represents a critical starting point for future research that aims to understand diversification within this family. Species diversity with the galagids is probably underestimated and the discovery of cryptic lineages could affect the proposed biogeographical scenario. However, current evidence indicates that cryptic diversity is likely to be restricted to the western and the eastern clades of *Galagoides*, biogeographically confined to the Congolian and the Zambezian regions respectively. New cryptic species within these two groups would not affect the overall biogeographical scenario, but only increase the number of speciation events within each area. Future studies should include more geographically dense sampling of different species, to address the factors that characterized diversification at the generic and specific levels. For instance, phylogeographical studies that include all species within the eastern *Galagoides* would be critical to an understanding of how climatic change, forest transgression-regression, local topography and rivers affected the high level of species diversity in the area.

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SUPPORTING INFORMATION

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

Appendix S1 GenBank accession numbers.
Appendix S2 Phylogenetic and molecular dating results.
Appendix S3 Results of biogeographical analyses.

BIOSKETCH

Luca Pozzi is an evolutionary biologist and molecular anthropologist interested in primate evolution, biodiversity and conservation. His current work mainly focuses on Strepsirhine primates (galagos, lorises and lemurs) and aims at documenting primate biodiversity and at exploring the ecological and evolutionary factors that drive diversification in time and space.

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