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## Molecular phylogenetic reconstructions identify East Asia as the cradle for the evolution of the cosmopolitan genus *Myotis* (Mammalia, Chiroptera)

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### ABSTRACT

Sequences of the mitochondrial cytochrome *b* (1140 bp) and nuclear Rag 2 (1148 bp) genes were used to assess the evolutionary history of the cosmopolitan bat genus *Myotis*, based on a worldwide sampling of over 88 named species plus 7 species with uncertain nomenclature. Phylogenetic reconstructions of this comprehensive taxon sampling show that most radiation of species occurred independently within each biogeographic region. Our molecular study supports an early divergence of species from the New World, where all Nearctic and Neotropical species plus a lineage from the Palaearctic constitute a monophyletic clade, sister to the remaining Old World taxa. The major Old World clade includes all remaining Eurasian taxa, most Oriental species, one Oceanian, and all Ethiopian species. Another lineage, including *M. latirostris* from Taiwan, appears at the base of these two major biogeographic clades and, because it bears nyctalodont molars, could be considered as a distinct genus. However, this molar configuration is also found in crown-group species, indicating that these dental characters are variable in the genus *Myotis* and may confound interpretation of the fossil record. Molecular datings suggest an origin of all recent *Myotis* in the early Miocene (about 21 MYA with 95% highest posterior density interval 23–20 MYA). This period was characterized by a global climatic cooling that reduced the availability of tropical habitats and favoured the development of more temperate vegetation. This sharp climatic change might have triggered the evolution of *Myotis* in the Northern continents, because *Myotis* ancestors seem to have been well adapted and successful in such temperate habitats. Ancestral area reconstructions based on the molecular phylogeny suggest that the eastern portion of the Asian continent was an important center of origin for the early diversification of all *Myotis* lineages, and involved relatively few subsequent transcontinental range expansions.

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## 1. Introduction

The evolutionary history of species is the outcome of a complex balance between processes of extinction and speciation. At any time, some groups may thus be highly diversified and successful while sister clades are depauperate (Purvis et al., 2000). The causes that may promote or inhibit these processes in a particular clade and not in others are still highly debated, but may include intrinsic key innovations; extrinsic processes linked to plate tectonics,

climatic fluctuations or vegetation changes; differential rates of gene evolution; and niche competition. One difficulty in identifying the common processes underlying the evolution of species diversification and natural history of species is the need for accurate knowledge of the phylogenetic relationships of the group considered (Paradis, 1998; Sanderson and Donoghue, 1996). In particular complete or nearly complete phylogenies including all major branches (i.e. an exhaustive phylogenetic hypothesis for the evolution of a whole group of extant species), allow for robust analysis of the factors that influenced the rates of speciation and extinction (Purvis et al., 2000). Moreover, these complete phylogenies can help to elaborate conservation plans aimed to preserve

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most of the current evolutionary history of these groups (Bininda-Emonds et al., 2000; Sechrest et al., 2002).

Bats from the order Chiroptera are part of the Laurasiatheria clade of mammals, and have diversified in all continents except Antarctica, with over 1100 currently recognized species worldwide (Simmons, 2005), a number that continues to increase with new taxonomic work. Within the superfamily Vespertilionoidea (Teeling et al., 2005), the genus *Myotis* belongs to the family Vespertilionidae, and is classified in a distinct subfamily, the Myotinae (Hoofer and Van den Bussche, 2003; Simmons, 2005). Because these bats have a rather “generalist morphology” (Horáček et al., 2000) and are very speciose, their taxonomy is difficult. In a phenetic study based on external and cranial morphology, Findley (1972) estimated that the current fauna included about 60 species of *Myotis*. This number has increased through morphological and genetic studies and recent revisions now exceed 100 species (Koopman, 1994; Simmons, 2005). Indeed, new species continue to be described regularly, even in well-known areas such as Europe (Helfersen et al., 2001; Ibáñez et al., 2006). Thus, *Myotis* is one of the most diverse genera of mammals, second only to *Crocodyrus* shrews, and offers an exceptional substrate for studying speciation and diversification at a worldwide scale. *Myotis* bats have colonized most terrestrial habitats, except polar regions, and represents the only mammal genus that is naturally distributed on every continent except Antarctica. However, the species diversity is unequal by region, with the maximum diversity found in the northern continents (see inset of Fig. 1).

To date, few molecular studies have approached the evolution of the genus *Myotis*. It has been demonstrated that the prevailing morphology-based subdivision of the genus *Myotis* into four or more subgenera (Findley, 1972; Koopman, 1993; Tate, 1941) does not reflect phylogenetic history (Hoofer and Van den Bussche, 2003; Kawai et al., 2003; Lack et al., 2010; Ruedi and Mayer, 2001). Instead, it appears that adaptive convergences have produced similar ecomorphs independently through some deterministic processes (Fenton and Bogdanowicz, 2002). By contrast, several independent biogeographic radiations emerged from phylogenetic reconstructions, including one that unites all Ethiopian taxa (Stadelmann et al., 2004b) and another that comprises all New World species (Lack et al., 2010; Stadelmann et al., 2007). However, *Myotis* from the Palaearctic and the Oriental regions have been under-represented in previous studies, impeding phylogenetic inference for the whole group (Kawai et al., 2003; Zhang et al., 2009).

By expanding the Old World sampling to include over 80% of all named *Myotis* species (Simmons, 2005) as well as several unnamed taxa, we produced a comprehensive and robust phylogeny of extant species covering all major branches. With this almost complete picture of the *Myotis* radiation, we first investigate phylogenetic relationships within Old World taxa. Second, we evaluate the timing and biogeographic evolution of *Myotis* worldwide by using a Bayesian relaxed molecular clock approach, as well as likelihood reconstructions of ancestral geographic distribution to identify the possible area of origin of this striking evolutionary radiation.

## 2. Materials and methods

### 2.1. Taxon and geographic sampling

According to the last available revision of the genus (Simmons, 2005), 103 species of *Myotis* are recognized worldwide. Unless indicated hereafter, we follow Simmons' taxonomic arrangement to refer to the nominal species analyzed in this study (see Table S1). Additional taxa recognized here include *M. aurascens* from Central and East Asia [distinct from *M. mystacinus* (Tsytsulina et al., 2012)], *M. cf. browni* from the Philippines and *M. latirostris*

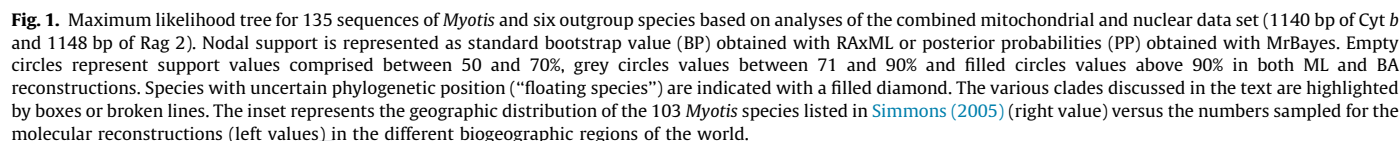
from Taiwan [both distinct from *M. muricola* (Lack et al., 2010; Stadelmann et al., 2007)], *M. escalerae* from the Iberian peninsula [distinct from *M. nattereri* (Ibáñez et al., 2006)], *M. formosus flavus* [specifically distinct from *M. formosus* (Jiang et al., 2010)], *M. gracilis* from the Far East [distinct from *M. brandtii* though *M. sibiricus* may be a senior synonym and hence the appropriate name for this species (Horáček et al., 2000; Kruskop et al., 2012)], *M. petax* from the Eastern Palaearctic [distinct from *M. daubentonii* and a senior synonym of *M. abei* (Kruskop et al., 2012; Matveev et al., 2005; Tsytsulina, 2004)], *M. phanluongi* from Vietnam [distinct from *M. sili-gorensis* (Borisenko et al., 2008)], and *M. taiwanensis* [distinct from *M. adversus* (Han et al., 2010)]. In turn the European taxon *oxygnathus* is maintained as a subspecies of *M. blythii* (Evin et al., 2008), while the Japanese *hosonoi*, *ozensis* and *yessoensis* are now all considered as synonyms of *M. ikonnikovi* (Abe et al., 2005; Kawai et al., 2003). Species for which no tissue or GenBank entries were available include *M. adversus* (Oriental), *M. aelleni* (Neotropical), *M. australis* (Oceanian), *M. bucharensis* (Eastern Palaearctic), *M. cobanensis* (Neotropical), *M. csorbai* (Oriental), *M. findleyi* (Neotropical), *M. fortidens* (Neotropical), *M. gomantongensis* (Oriental), *M. hajastanicus* (Western Palaearctic), *M. hermani* (Oriental), *M. insularum* (Oceanian), *M. melanorhinus* (Nearctic), *M. moluccarum* (Oceanian), *M. morrissi* (Ethiopian), *M. nipalensis* (Eastern Palaearctic), *M. oreias* (Oriental), *M. peninsularis* (Nearctic), *M. planiceps* (Neotropical) and *M. stalkerii* (Oriental). We also included 7 additional species of *Myotis* that diverge genetically and morphologically from other currently recognized taxa and await proper taxonomic description (see e.g. Francis et al., 2010; Larsen et al., 2012b; Salicini et al., 2011). Although not complete, we feel that this taxonomic coverage (Table S1) represents most, if not all, major lineages in the *Myotis* radiation, as most of the missing taxa appear morphologically or genetically to be part of clades that are represented in our sampling, a conclusion confirmed for New World taxa by recently published molecular surveys (Larsen et al., 2012a, 2012b).

In total, 105 taxa representing 88 nominal species, 10 distinct subspecies and 7 additional unnamed/unidentified species were analyzed (see Table S1). Because of potential concerns about species assignments and nomenclature, especially for the Asian *Myotis* (Bates et al., 1999), most sequences were derived from vouchered specimens that can be examined in public institutions. In addition to the newly sequenced specimens, some taxa were represented by published sequences (Bickham et al., 2004; Ibáñez et al., 2006; Jones et al., 2006; Kawai et al., 2003; Lack et al., 2010; Larsen et al., 2012b; Ruedi and Mayer, 2001; Stadelmann et al., 2004a, 2004b, 2007; Tsytsulina et al., 2012; Zhang et al., 2009) available in GenBank (see Table S1). We refrained from considering several other unpublished *Myotis* sequences available in public databases because they were of uncertain taxonomic origin or had experimental problems. For instance, the sequence of “*Myotis hajastanicus*” AY665138 was discarded because it proved to be a chimera between partial cytochrome *b* sequences of *M. gracilis* and of *Bos indicus*.

The Kerivoulinae and Murinae are the closest relatives of *Myotis* (e. g. Hoofer and Van den Bussche, 2003; Kawai et al., 2002). Therefore, six species representing the genera *Harpiocephalus*, *Kerivoula* and *Murina* from the Oriental region (Hoofer et al., 2003; Lack et al., 2010; Ruedi et al., 2012; Stadelmann et al., 2004b, 2007) were used as a composite outgroup (Table S1).

### 2.2. DNA amplification and sequencing

Tissue samples preserved in ethanol or DMSO were soaked for 1 h in sterile water before extraction. Total genomic DNA was then isolated following a salting out protocol (Miller et al., 1988), as detailed in Castella et al. (2001) and rediluted into 200 µl of low TE buffer. The complete mitochondrial



Phylogenetic reconstructions were conducted on the Cyt *b* (141 sequences of 1140 bp; 601 variable positions in the ingroup) and



Rag 2 datasets (122 sequences, 1148 bp, 220 variable positions) analyzed separately and in combination (141 lineages of 2288 bp) with strings of Ns replacing the missing sequences. These missing data represented 1.4% and 3.5% of the character matrix for the Cyt *b* and Rag 2 datasets, respectively. Probabilistic methods were used to reconstruct phylogenetic trees with the Maximum Likelihood (ML) approach implemented in RAXML (Stamatakis, 2006) and Bayesian inferences (BA) in MrBayes v3.2.0 (Ronquist and Huelsenbeck, 2003). All analyses were done on a fully partitioned model, where each gene and/or codon partition was allowed to have its own set of model parameters. The most appropriate model of nucleotide substitution for each partition was evaluated using MrModeltest 2.3 (Nylander, 2004) and the Akaike Information Criterion (AIC; data summarized in Table S2). The General Time Reversible (GTR) model with rate variation among sites ( $\Gamma$ ) and a proportion of invariable sites (*I*) represented the best fitting model of nucleotide substitution for the Cyt *b* and the combined Cyt *b* + Rag 2 dataset (Table S2). Topological searches were initiated from random trees.

Bayesian analyses were performed with a Markov chain Monte-Carlo technique and run for  $10 \times 10^6$  generations, with a sampling every 1000 generations. The chains were checked for convergence and appropriate effective sample size ( $ESS > 200$ ) with Tracer v.1.5 (Rambaut and Drummond, 2009). Chains converged and the log-likelihood reached an asymptote after about  $1 \times 10^6$  (Cyt *b* or Rag 2) or  $3.5 \times 10^6$  (combined) generations. These initial trees were discarded as burn-in. Posterior probabilities (PP) were subsequently computed from the consensus of the remaining sampled trees. Reliability of nodes in the ML analyses was assessed by 100 standard bootstraps (BP) with RAXML.

#### 2.4. Molecular dating analyses

We estimated the mean divergence times between taxa by a relaxed uncorrelated lognormal molecular clock as implemented in BEAST v1.7.4 (Drummond and Rambaut, 2007). Similarly to the MrBayes analyses, we used the combined dataset partitioned into 6 categories (3 codon positions each for Cyt *b* and Rag 2) with the most appropriate model of nucleotide substitution for each codon partition determined with MrModeltest (Table S2). Tree searches started from a random tree and assumed a constant lineage birth rate for each branch (Yule tree prior; Drummond et al., 2006); all other parameters were kept at default values. Chains were sampled every 1000 generations over  $10 \times 10^6$  generations, with a burn-in of 10%.

Fossil calibrations were used to place temporal constraints on two nodes, as minimum and maximum soft bounds. The choice of these calibrations was difficult as extant *Myotis* species show a combination of ancestral and derived character states and are difficult to discriminate in the fossil record (Gunnell et al., 2012; Horáček, 2001). This is especially true for the different dental remains that constitute the fossils of ancient *Myotis*-like taxa (Horáček, 2001). The first calibration constraint was the split of *M. daubentonii* and *M. bechsteini*, estimated to have diverged between 5 and 11.6 MYA (Topál, 1983). We used an exponential prior distribution (offset 5.0, mean 2.5) to encapsulate this calibration in the 95% CI. The second calibration involved the most recent common ancestor of *Myotis* estimated to have diverged in the Late Oligocene–Early Miocene (Horáček, 2001; Gunnell et al., 2012), some 20–31 MYA. If *Khonsunyeris* is also part of the early Myotinae radiation, as hypothesized by Gunnell et al. (2012), this lower boundary would be slightly older (34 MYA), but still included in the lognormal prior distribution entered in our BEAST analysis (offset 20.0, S.D. 1.6).

#### 2.5. Biogeographic reconstructions

Species distributions were obtained from the geographic information available in Simmons (2005) and were coded in seven biogeographic regions (bioregions): Nearctic, Neotropical, Western and Eastern Palearctic, Oriental (= Indomalayan, Corbet and Hill, 1992), Ethiopian and Oceanian regions (see inset of Fig. 1 and Table S1 for details of geographic assignments). The dispersal–extinction–cladogenesis (DEC) likelihood model implemented in LAGRANGE (Ree and Smith, 2008) was used to investigate the biogeographic evolution of the genus *Myotis*. Following a probabilistic approach, this program traces from the tips to the root of a phylogenetic tree changes in distribution and reconstructs the ancestral state by giving probabilities of each state at each node. We used the ultrametric tree issued from the BEAST analysis as a template topology for all biogeographic reconstructions. Although *Myotis* bats can be strong flyers, areas separated by large stretches of open sea represent important, if not impassable barriers to dispersal (Castella et al., 2000; García-Mudarra et al., 2009; Larsen et al., 2012b), while adjacent land masses can be reached more easily. Hence, we performed biogeographic reconstructions assuming two dispersal models, one without any constraint on dispersal across the planet (all regions can be reached from any area;  $H_0$ ), and one where possible dispersals were restricted to adjacent land masses ( $H_1$ ). In this constrained model, an adjacency matrix of connections between regions was implemented as following: any dispersal was allowed within the Old World, or within the New World, but between those continents, only dispersals from/to the Eastern Palearctic and the Nearctic were allowed (i.e. across the Beringian Strait). Similarly, connections with the Oceanian region was only allowed to/from the Oriental region (i.e. across Wallacea), as suggested by previous genetic studies of *Myotis* species living in this region (Kitchener et al., 1995). Because of the global scale of these analyses, we assumed that ancestral areas could not cover more than two bioregions, and set the analyses accordingly. The best fitting results were chosen for interpretation by comparing their log-likelihoods (see Ree and Smith, 2008). The influence of the biogeographic origin of the outgroup species (here they were all sampled within the Oriental region, see Table S1) may introduce a bias in the reconstruction of the deepest nodes of the ingroup (personal observation). To avoid this potential problem, we artificially assigned both the Ethiopian and Oriental regions to the geographic range of *Kerivoula*, and the Oriental and Eastern Palearctic regions to the distribution of *Murina*, in order to reflect the global distribution of these outgroup genera (Simmons, 2005).

### 3. Results

#### 3.1. mtDNA and nuclear diversity

Thirty-nine sequences of the Cyt *b* gene were newly obtained, which represent 32 Oriental, 6 Palearctic and one Ethiopian lineages of *Myotis* (Table S1). These sequences were deposited in GenBank under accession numbers KF312497–KF312535. The final dataset for the Cyt *b* gene thus consisted of 141 sequences of 1140 bp (135 ingroup and 6 outgroup taxa). Whenever possible, we ensured that the same individual was sequenced for the Cyt *b* and Rag 2 genes, but this was not possible for all species. Furthermore, because tissue sample was not available for all species used in the Cyt *b* dataset, the taxon sampling of the Rag 2 dataset was reduced to 122 sequences (including 6 outgroups). The 48 new partial Rag 2 sequences obtained in this study were deposited in GenBank under accession numbers KF312536–KF312583. A complete list of all specimens and sequences is provided in Table S1.

### 3.2. Phylogenetic reconstructions

Phylogenetic trees based on ML or BA reconstructions were very similar, although nodes tended to be less well supported by bootstraps (BP) when compared to posterior probabilities (PP, Table 1), a well-known bias inherent to the different methods used to estimate nodal support (Alfaro et al., 2003; Douady et al., 2003). The overall levels of support were also higher towards the terminal clades than for deeper nodes, indicating that additional mitochondrial and nuclear markers are required to resolve the entire *Myotis* radiation with high statistic support. Deep nodes that were well supported in most analyses include the monophyly of all current *Myotis* species and of the New World clade (Table 1). As expected at low taxonomic levels, trees reconstructed with the more variable Cyt *b* gene (Fig. S1) were much more resolved when compared with those generated with the Rag 2 gene alone (Fig. S2). Phylogenetic incongruences between gene partitions include the position of *latirostris* or the clade *mystacinus-ikonnikovi*, both of which appear within the New World clade (albeit with low bootstrap) in the nuclear tree (Fig. S2), whereas it is basal in reconstructions including the Cyt *b* data set (Figs. 1 and S1). Another study including many more nuclear characters (2352 variable positions; Lack et al., 2010) also places with strong support *latirostris* as the sister-group relative to the remaining *Myotis*, not within the New World clade, suggesting that inference from the nuclear gene tree of Fig. S2 might require many more characters or a larger sampling of nuclear genes to be more reliable. The combination of both gene datasets resulted in significant improvement of most nodal supports (Table 1). We therefore report only phylogenetic reconstructions resulting from this combined Cyt *b* + Rag 2 dataset and representing 141 species or lineages (Fig. 1). Phylogenetic tree can be accessed in TreeBase (<http://purl.org/phylo/treebase/phylogenetics/study/TB2:S14471>).

Three major monophyletic groupings are statistically supported (PP and BP 72–100%) in the early radiation of *Myotis*: the *latirostris* lineage, the New World clade, and the Old World clade (Fig. 1). The *latirostris* lineage comprises *M. latirostris* from Taiwan and an unidentified taxon (*M. sp* 4) from South China. This lineage is clearly basal to the *Myotis* radiation. The monophyletic and strongly supported New World clade includes all sequenced New World species of *Myotis* plus the Palaearctic *brandtii* lineage. The latter is represented by the Western Palaearctic *M. brandtii* and the Eastern Palaearctic *M. gracilis*. The New World clade is comprised of two further, well-supported subclades, one containing exclusively Nearctic species, while the other is composed of both

Nearctic and Neotropical species, as detailed in Stadelmann et al. (2007).

The Old World clade encompasses the greatest species diversity with over 60 distinct taxa, and is structured in two major groups: the Ethiopian clade, and the Eurasian assemblage, the latter with low nodal support (Fig. 1, Table 1). Such low support can be due to a succession of short internodes or to the uncertain position of two species, *M. alcathoe* and *M. dasycneme* (called here “floating” species) that branch close to the base of this Eurasian radiation, but with little statistical support. The Ethiopian clade is highly supported with all methods of reconstruction, and has been described in Stadelmann et al. (2004b). It unites all species from sub-Saharan Africa and islands of the western Indian Ocean (Weyeneth et al., 2011), plus the circum-Mediterranean *M. emarginatus*, and two Asian species, *M. cf. formosus* and *M. formosus flavus*. Finally, the Eurasian assemblage includes all remaining species from the Palaearctic, Oriental and Oceanian regions (Fig. 1). This assemblage can be divided in several clades already described in previous studies (Clade II, III and IV, Ruedi and Mayer, 2001) and in five new ones, as indicated by continued Roman numerals (Fig. 1 and Table 1). Some of these clades are composed exclusively of lineages from the Oriental region (e.g. Clade VI or IX), while others, such as Clade II, are geographically more heterogeneous. The European *M. capaccinii* and the South-East Asian *M. annectans* are monotypic lineages with phylogenetic positions unsettled within this Eurasian assemblage.

Interestingly, species endemic to the Western Palaearctic region, such as *M. bechsteini*, *M. escalerae*, *M. capaccinii*, *M. alcathoe* or those from the Far East (e.g. *M. pruinusos*, *M. macrodactylus*, *M. yanbarensis*, *M. ikonnikovi*) are largely unrelated and dispersed across the entire phylogenetic tree (Fig. 1), while those from Africa or from the Americas are much more closely related to their respective geographic congeners. Species from the Oriental region are either closely related within an endemic clade (e.g. Clade VII or IX) or part of geographically more widespread assemblages (e.g. Clade II or V). The most dramatic endemic radiation involves species from the Nearctic and Neotropical subclades (Stadelmann et al., 2007) that largely speciated within their biogeographic regions.

### 3.3. Divergence times

Divergence time estimates obtained with a relaxed molecular clock on the combined dataset are reported on Fig. 2 and suggest that all *Myotis* diverged from their closest sister group (represented

**Table 1**

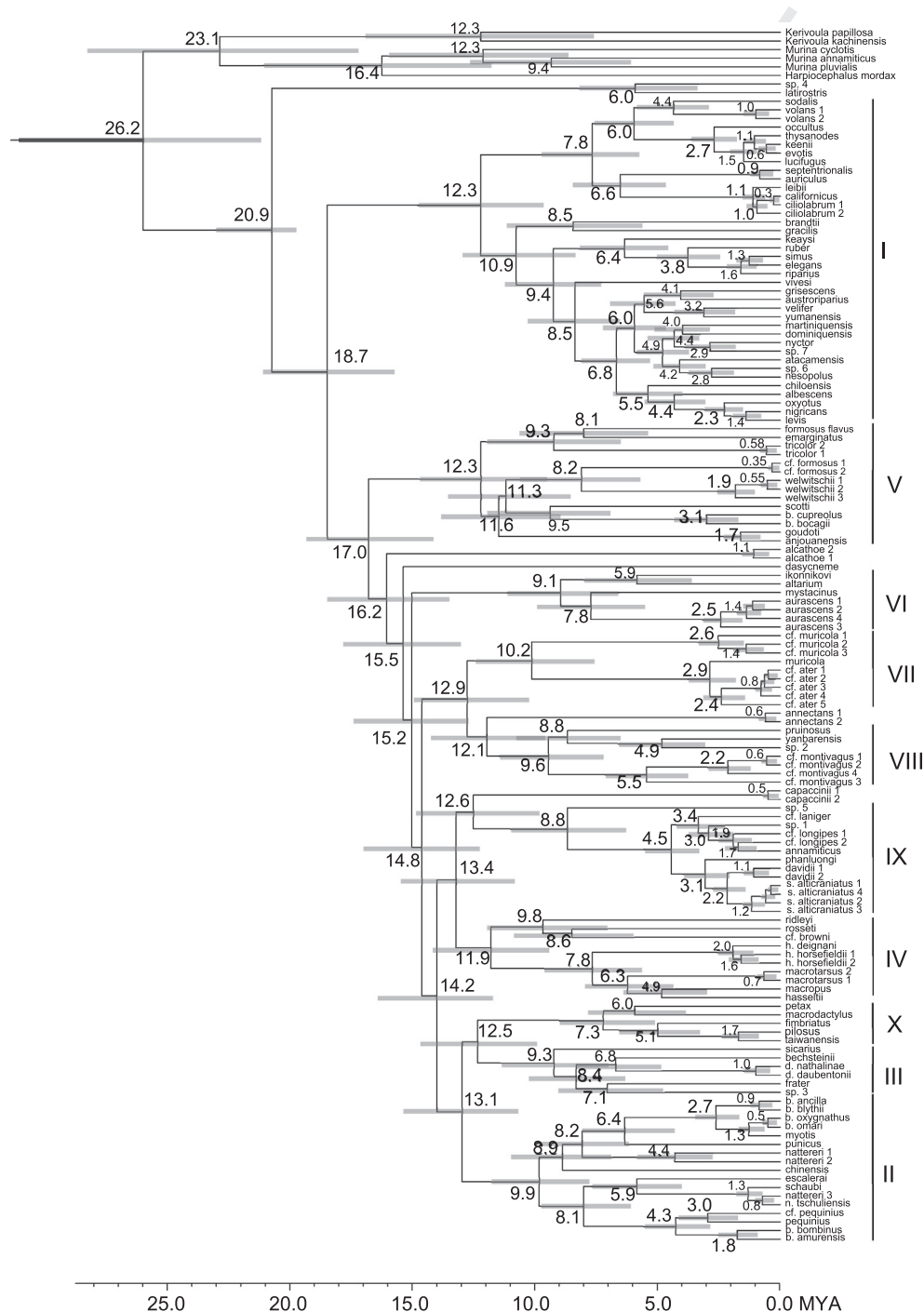
Clade name, level of support (over 50%) and inferred age estimated from the different molecular analyses. Supports were recovered from a maximum likelihood (ML) or a Bayesian approach (BA), and data sets consisted of either Cyt *b* or Rag 2 sequences analyzed separately or in combination. Values are expressed as percent bootstraps (for ML) or posterior probabilities (for BA). Nodes age are expressed in million years ago (MYA) followed by the 95% highest posterior density interval (HPD).

Clade name	Cyt <i>b</i>		Rag 2		Combined		Age (HPD) (MYA)
	ML	BA	ML	BA	ML	BA	
Myotis (incl. <i>latirostris</i> )	96	100	75	100	99	100	20.94 (23–20)
Myotis (excl. <i>latirostris</i> )	76	100	–	–	80	100	18.66 (21–16)
I New World clade	93	100	–	–	97	100	12.34 (15–10)
II–X Old World clade	62	100	–	–	72	100	16.96 (20–14)
II–III + VI–X Eurasian clade	–	51	–	–	–	65	16.22 (19–14)
II Large Myotis clade	92	100	–	–	98	100	9.93 (12–8)
III Myotis clade	100	100	–	–	100	100	9.33 (12–7)
IV Oriental Myotis clade	92	100	–	–	90	100	11.92 (14–10)
V Ethiopian clade	94	100	–	–	98	100	12.33 (15–10)
VI Whiskered Myotis clade	85	100	–	–	88	100	9.06 (11–7)
VII <i>muricola</i> clade	72	100	–	–	76	100	10.24 (13–8)
VIII Eastern Myotis clade	100	100	–	–	97	100	9.55 (12–7)
IX Asian Myotis clade	87	100	52	96	87	100	8.77 (11–6)
X Trawling Myotis clade	95	100	–	–	99	100	7.30 (9–5)

here by several Murinae and Kerivoulinae species) about 26 MYA (HPD 31–21 MYA). The *latirostris* lineage diverged about 21 MYA from the lineage leading to all other modern *Myotis*. The early divergence between the New World clade and the Old World clade represents the second major split within the *Myotis* radiation and occurred about 19 MYA. Then, the Nearctic subclade started diverging about 12 MYA, while the *brandtii* lineage split from the Neotropical subclade 11 MYA. The most recent common ancestor shared by the Ethiopian clade and the Eurasian assemblage is about 17 MYA. The lineages that have further diversified in the seven corresponding clades of the Eurasian assemblage originated

within a narrow time frame in the middle Miocene, between 16 and 11 MYA (Fig. 2).

Compared to our previous study based on the same markers but on a reduced taxon sampling of *Myotis* species and on a different method to calibrate nodes (see Stadelmann et al., 2007), the dates inferred here are largely concordant for the recent nodes (<8 MYA), but for the deeper ones, the dates inferred here are much older. For instance, the basal node for all modern *Myotis* is estimated here at about 20.9 MYA (with 95% highest posterior density interval, HPD = 23–20 MYA; Table 1), while it was at  $13.0 \pm 2.2$  MYA in Stadelmann et al. (2007). Another example is the origin of the New



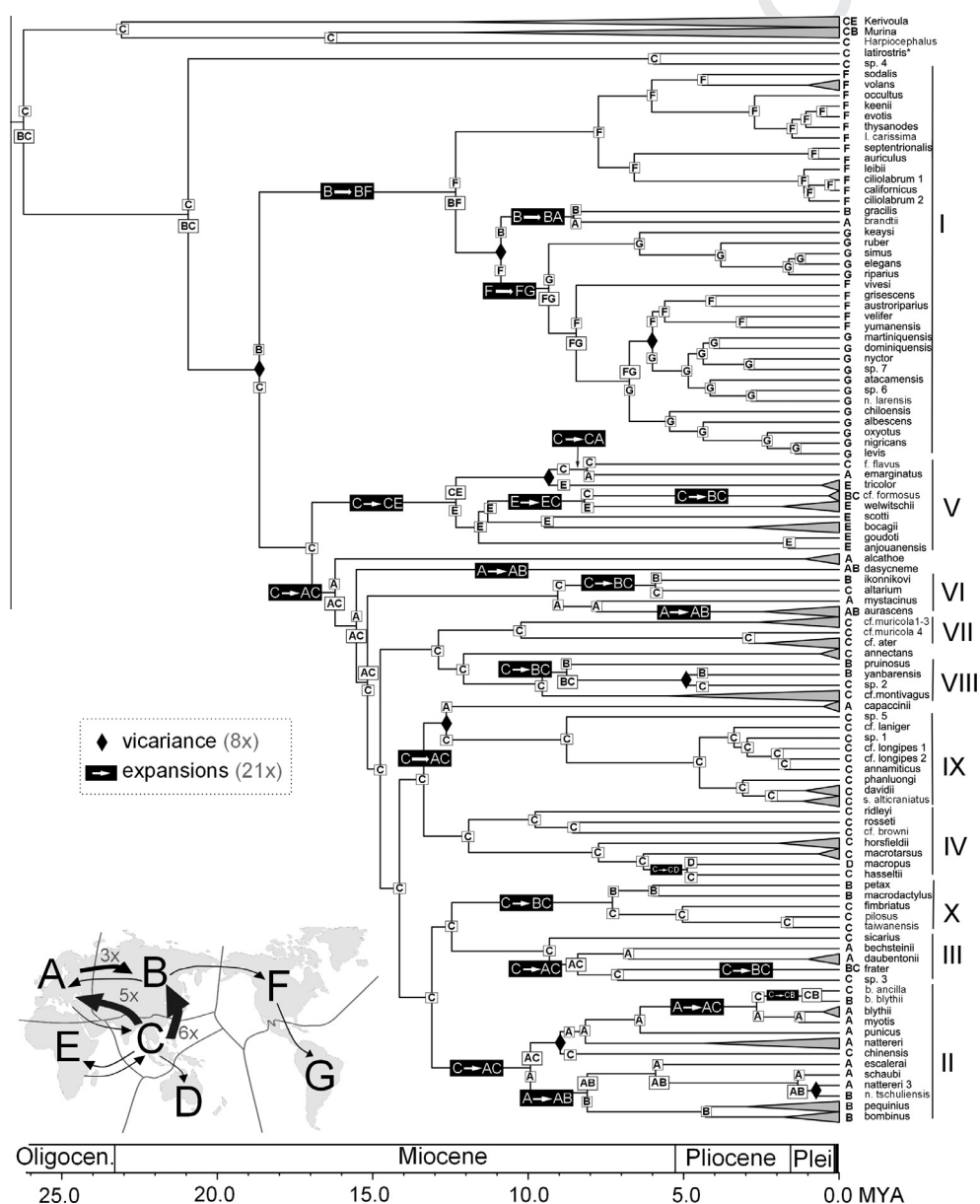
**Fig. 2.** Chronogram of *Myotis* taxa based on Bayesian dating analysis using BEAST and the combined mitochondrial and nuclear DNA gene dataset. Mean divergence values (expressed as million year ago, MYA) are given at each node and horizontal bars represent the 95% highest posterior density ranges. Clade names correspond to those given in Fig. 1.

World clade, which was estimated at  $9.9 \pm 1.7$  MYA by Stadelmann et al. (2007), while it appears about two million years older in the present estimates (12.3 MYA, HPD 15–10 MYA). Other dated molecular trees aimed to resolve the Vespertilionidae radiation, based on different genes, fossil calibration and taxon sampling (Lack et al., 2010), are also more concordant with the present estimates. We therefore feel that divergence times presented here are consistent with the best available information, even if largely imprecise due to the usual uncertainty associated with molecular tree calibrations. This calibrated phylogeny served as the basis for the following Lagrange biogeographic reconstructions.

### 3.4. Biogeographic inference

The likelihood value of biogeographic reconstructions of the unconstrained-dispersal model ( $H_0 - \ln L = 195.3$ ) was significantly worse than the constrained one ( $H_1 - \ln L = 180.3$ ; delta  $\ln L = 15$ ;

$p < 0.001$ ), and hence only this second model with dispersals limited to adjacent bioregions will be discussed further. Overall, 111 out of the 134 ingroup nodes had robust ancestral area reconstructions (AAR) with over 70% relative likelihood. For the remaining nodes some uncertainties existed due to alternative, albeit less likely, AAR. For instance, the AAR for the deepest node of all modern *Myotis* was not clearly identified, with the co-occurrence of the Oriental and Eastern Palearctic regions being more likely (35% relative probability, as shown in Fig. 3), but with either of those two bioregions alone only slightly less likely (23% and 21%, respectively). Likewise, there is considerable uncertainty about the most likely scenario for the area occupied by the ancestor leading to the Ethiopian clade. This AAR was either identified as being Oriental (46% relative probability) or Western Palearctic (45%). To ease interpretation, we recorded only the ancestral area with the highest relative likelihood on Fig. 3, but acknowledge that some of those AAR are uncertain.



**Fig. 3.** Biogeographic evolution of ancestral areas of *Myotis* species reconstructed in the chronogram of Fig. 2. The most likely ancestral areas determined with Lagrange under a model with dispersals limited to adjacent bioregions are given in boxes at each node. The inset map illustrates the seven biogeographic regions included for this study. (A) The Western Palearctic, (B) the Eastern Palearctic, (C) the Oriental, (D) the Oceanian, (E) the Ethiopian, (F) the Nearctic and (G) the Neotropical region. Inferred range expansion events are highlighted by black boxes (on the chronogram) or by arrows (on the world map), and vicariance events by filled diamonds. The bottom scale represents time (in million years before present, MYA) and the approximate subdivision of paleontological epochs.



Globally, the biogeographic scenario for the *Myotis* evolution inferred under the DEC model (Fig. 3) suggests relatively few inter-continental colonization events (noted as range expansions on the tree). These are 21 in total, 13 of which stem from the Oriental, four from the Western Palaearctic, two from the Eastern Palaearctic, and one each from the Ethiopian and Nearctic regions (see inset of Fig. 3). With only eight occurrences recorded on the tree, vicariant events leading to daughter lineages were much rarer than range expansions (Fig. 3). One such event occurred in the early ancestor of most modern *Myotis* that occupied the Eastern Palaearctic plus Nearctic regions, and which subsequently split into an American branch (leading eventually to all extant New World species (Clade I) and an Oriental branch (leading to the Eurasian assemblage, clades II to X). Another major vicariant event was identified in the Neotropical subclade (Clade I, Fig. 3) where the common ancestor of the clade comprising *M. grisescens*, *M. austroriparius*, *M. velifer* and *M. yumanensis* (all are Nearctic species) was split by vicariance from the remaining Neotropical taxa. According to the dated phylogeny of Fig. 3, these inferred colonization and vicariance events apparently occurred sporadically during the entire evolution of the *Myotis*, with no temporal aggregation.

## 4. Discussion

### 4.1. Morphological convergences and taxonomy

The first major dichotomy in molecular reconstructions is the basal split of the *latirostris* lineage that represent the sister group of all other modern *Myotis* species (Fig. 1). This phylogenetic position of the *latirostris* lineage also appeared in analyses involving a much larger character sampling (over 6000 aligned nucleotide positions) and wider taxonomic coverage (Lack et al., 2010), confirming that this outlier position is not an artefact of our sampling. Species representing this lineage (*M. latirostris* from Taiwan and an unnamed species from China, *M. sp4*; Table S1) have a lower molar configuration (nyctalodony, Fig. S3) that would exclude them from members of the Myotinae, as this subfamily is supposed to contain species with exclusively myotodont molars (Gunnell et al., 2012; Menu and Sigé, 1971). Based on those genetic and morphologic lines of evidences, the *latirostris* lineage could be treated as a distinct genus (Lack et al., 2010; Stadelmann et al., 2007). However the presence of other fully nyctalodont species among crown-group *Myotis* (e.g. *M. siligorensis alticranatus*; Fig. S3) suggest that this character state was acquired several times within the group and that other truly synapomorphic characters are needed to diagnose the unique *latirostris* lineage. Remarkably, the overall conserved morphology and very close resemblance to other small-sized, Asian *Myotis* led most authors to include *latirostris* in the synonymy of *M. muricola* (Corbet and Hill, 1992; Simmons, 2005), which is clearly inappropriate owing to their paraphyletic position (Fig. 1).

The second dichotomy within the *Myotis* radiation separates all New World (Clade I) from most Old World species (clades II to X), the only exceptions being two Palaearctic taxa embedded within Nearctic species (Fig. 1). These two boreal species, *M. brandtii* and *M. gracilis* [for which *M. sibiricus* may be the appropriate name according to Kruskop et al. (2012)] are morphologically almost indistinguishable from each other and were long considered as synonyms of *M. mystacinus*. They differ from *mystacinus* by subtle penial and dental characteristics, yet appear as distantly related on the *Myotis* tree (Fig. 1). This further illustrates that close morphological resemblance in this difficult genus does not necessarily imply close phylogenetic relationships (Ruedi and Mayer, 2001). This observation also holds for other chiropteran taxa, for example the Phyllostomidae genera (Davalos et al., 2012).

The evolution of the New World clade was already described in a previous paper (Stadelmann et al., 2007). Since the current sampling only adds a few more species (*M. nesopolus* and *M. nyctor*) which do not alter the main phylogenetic relationships within this clade, we will not discuss it in more detail here. We do note that the multiple new lineages or cryptic species recently evidenced in South America (Larsen et al., 2012) are all nested within the Nearctic or the Neotropical subclades, as predicted by their geographic origins. This supports our supposition that our sampling of *Myotis* species, although not complete, is representative of the major lineages existing in this continent.

The Old World clade is fairly well supported in our reconstructions (Table 1) and includes all species from the remaining continents in the Western Hemisphere (clades II to X, Fig. 1). This large assemblage unites morphologically disparate species, including the smallest (*M. siligorensis*, less than 4 grams) and the largest species (*M. chinensis*, over 30 g), orange (*M. anjouanensis*) and solid black species (*M. macropus*), short-eared (*M. daubentonii*) and long-eared species (*M. bechsteini*) or short-footed (*M. frater*) and long-footed species (*M. pilosus*). Conversely, examples of morphologically very similar species that appear distantly related within this large clade are also numerous. For instance the Eastern Palaearctic *M. petax* was long considered a single species with the Western Palaearctic *M. daubentonii*, but multiple lines of evidence (Datzmann et al., 2012; Kruskop et al., 2012; Matveev et al., 2005) clearly show that they are not even sister-species (they appear in clades X and III, respectively, on Fig. 1). Even species sharing a remarkable black-and-orange pigmentation (e.g. *M. welwitschii*, *M. cf. formosus* and *M. formosus flavus*) are not each other's closest relatives, although they do all belong to the Ethiopian clade (Clade V, Fig. 1). Phylogenetic relationships rather indicate that the striking black-and-orange patterning of these *Myotis* is either a plesiomorphic feature among members of the Ethiopian clade, or is a convergent character. These examples confirm that convergence in several morphological characters can mislead the systematics of *Myotis* bats.

Strong, misleading resemblances of unrelated taxa may also explain the difficulties in classifying properly many species. In some cases, species that clearly fit within the phylogeny of *Myotis* were originally classified in different genera [e.g. *M. annectans* and *M. ridleyi* were originally classified as *Pipistrellus* and *M. roseti* as *Glischropus* because they often lack the middle upper and lower premolars, although other morphological and genetic characters clearly indicate they belong in *Myotis* (Hill and Topál, 1973; Topál, 1970)]. In other cases, multiple unrelated lineages with similar morphology have been classified as the same species (e.g. *M. nigricans* Larsen et al., 2012a). Hence several of the specimens included in our molecular sampling, although keying out to known species, bear “cf.” in their name to warn that they are not assigned confidently to a particular taxonomic name. This is particularly evident in the *M. horsfieldii*, *M. montivagus* and *M. muricola* species groups that are certainly composed of several cryptic taxa (Francis et al., 2010; Görföl et al., 2013; Volleth and Heller, 2012). Other unnamed taxa are more divergent, both morphologically and genetically, and await formal description (e.g. unknown species 1–7). Such descriptions will require integrated approaches combining molecular and morphological characters to ensure that they are accurately described in relation to currently recognized taxa.

The next major phylogenetic subdivision of the *Myotis* tree separates the Ethiopian Clade V from the Eurasian clade (clades II to IV and VI to X, Fig. 1). The former is well supported in all analyses and was described by Stadelmann et al. (2004b). We add to this clade *M. anjouanensis* from the Comoros and both Oriental species of the *formosus* species complex (Jiang et al., 2010). Interestingly, the latter are not each other closest relatives as *M. formosus flavus* is sister to the circum-Mediterranean *M. emarginatus*, while *M. cf.*



*formosus* is more closely related to the African *M. welwitschii*. The Ethiopian clade is thus composed of all sampled sub-Saharan species (*M. morrissi* remains unsampled) plus at least three Eurasian and Oriental species.

The Eurasian assemblage is the most speciose and includes at least 57 recognized species and a number of undescribed taxa (Fig. 1). The monophyly of this Eurasian assemblage is not supported statistically (Table 1) probably due to the four “floating” species that are variably placed near the base or in a more internal position within this assemblage. These “floating” species include *M. alcathoe*, *M. dasycneme*, and *M. capaccinii*, all distributed mainly in Europe, and the Asian species *M. annectans* (Fig. 1). It is unclear if the phylogenetic uncertainty reflects hard polytomies (i.e. episodes of rapid radiation), or merely soft polytomies that could be better resolved with increased character sampling (Lack and Van Den Bussche, 2010). Published DNA barcode sequences for *M. goman-tongensis* suggest that it may be allied to *M. annectans* (Francis et al., 2010) and hence to this Eurasian assemblage, but we were not able to include it in our analysis as we lacked the matching genes.

Our molecular results further demonstrate the existence of at least eight strongly supported clades within this Eurasian assemblage. Clade IX is composed exclusively of species endemic to the Oriental region. Clade II is geographically more heterogeneous as it includes species endemic to Europe, to Central or to Eastern Asia, all within Eurasia. Clade III is a composite of geographically and ecomorphologically very distinct *Myotis*. It includes the surface gleaner *M. bechsteini*, the aerial feeder *M. frater*, the trawling bat *M. daubentonii*, as well as species living in mountain forests of the Himalayas (*M. sicarius*) or found in lowland bamboo forests in Taiwan (*M. sp3*). Similarly, the Oriental Clade IV includes several trawling species with large feet (e.g., *macrotarsus*, *horsfieldii*, and *hasseltii*) as well as some unusual small species with small feet and only two premolars (*M. ridleyi* and *M. rossetti*). This diversity illustrates the rapid morpho-anatomical and/or ecological changes that can occur within these clades.

#### 4.2. Biogeographic evolution of the genus *Myotis*

Among placental mammals, molecular characters have shown that there is a widespread pattern of phylogenetic relationships matching with the geographical origin of the members of the corresponding major clades (Murphy et al., 2001). At lower taxonomic scales within laurasiatherians, we here recover a similar pattern for *Myotis* bats as continental assemblages of species, e.g. those found in Africa, in North and South America, or in Eurasia tend to appear in monophyletic groups (Fig. 1). This suggests that most speciation events and diversification occurred within each continent independently. The strong influence of the biogeographic origin of the *Myotis* species in shaping global phylogenetic relationships corroborate one earlier study based on less complete taxonomic sampling (Stadelmann et al., 2007). Indeed, the vast majority of species segregate in clades with a strong geographic component, especially if we consider that the Eurasian continent is a huge landmass dissected by rather loose biogeographic boundaries. For instance, the northeastern portion of the Oriental region has no major topological discontinuity that would separate its faunal elements from those in the Eastern Palaearctic region. This is reflected by a broad transition zone comprising faunal elements typical of both regions (Corbet and Hill, 1992), and in the phylogenetic relationships of *Myotis* by a mixture of endemics from both regions in the same clades (Fig. 1). The same is true between the Western and the Eastern Palaearctic regions where the limits are fuzzy and not bounded to major geographic discontinuities. The evolution of ancestral areas reconstructed onto the phylogenetic relationships of *Myotis* (Fig. 3) reflect these fuzzy biogeographic boundaries within the

Eurasian continent, as the vast majority (16 out of 21) of ancestral range expansions recorded worldwide occurred within this huge land mass (see inset of Fig. 3).

Barriers between the other biogeographic regions, such as mountain ranges, large deserts or sea channels, appear to be much more effective at reducing dispersal of *Myotis* species (Castella et al., 2000; García-Mudarra et al., 2009). For instance, none of the species found on either side of the Sahara seem to have a common ancestor that lived across this desert. Likewise no current *Myotis* species have a Holarctic distribution, and AAR suggest that a single expansion across the Beringian Strait in the Middle Miocene could explain the occurrence of *Myotis* species in the New World (Fig. 3). This scenario is strongly supported by the Lagrange analysis (relative probability >98%) and contradicts the alternative scenario of a back colonization of a putative common ancestor of *brandtii/gracilis* lineage coming from the Nearctic region.

If we assume that all current species living on the Australian continent form a monophyletic assemblage (Cooper et al., 2001), including *M. macropus* represented here in Clade IV, the colonization of this southern landmass can also be traced back to a single event that occurred at the end of the Miocene epoch (Fig. 3). Interestingly, *Myotis* species from the Japanese Archipelago (Kawai et al., 2003) appear in various independent clades (e.g. *M. macrodactylus*, *M. yanbarensis* or *M. ikonnikovi*), suggesting that these islands were colonized independently by several lineages before evolving eventually into endemic taxa.

The high proportion of expansion/colonization events originating from the Oriental region clearly highlights the pivotal importance of this region in the diversification of the *Myotis* species worldwide. AAR (Fig. 3) also suggests that only eight ancestral ranges were split by vicariance into descendent lineages, whereas most other diversification events took place within the bioregions. However, as the geographic scale of each bioregion is very large (i.e. continent-level) and probably many more vicariant events may have occurred within each of these large and heterogeneous regions, our data are not sufficient to infer more precisely which mode of speciation (sympatric versus allopatric) prevailed during the evolution of *Myotis*.

The global number of species contained in each biogeographic region is very unequal, with the northern continents much more diversified than the southern ones. For instance, the number of species currently known to inhabit the Ethiopian region (up to 6 species, excluding Madagascar and the Comoros; Happold and Happold, 2013) is in sharp contrast with the species diversity found on other continents (except Australia; see inset of Fig. 1). This suggests that *Myotis* species from the African continent did not radiate into many endemic species despite the fact that genuine *Myotis* apparently entered into this region relatively early (Middle Miocene; Fig. 3) during the evolution of modern species. Given the poor record of Tertiary fossils of vespertilionids in Africa (Gunnell et al., 2012), it is not possible to know if this paucity of species is due to an increased rate of extinction or a decreased rate of diversification. By contrast, the Oceanian region was likely the last part of the world that was reached by *Myotis* species, about 5.0 MYA (Fig. 3), suggesting that a late arrival might have contributed to low current diversity of *Myotis* species found in Australia.

#### 4.3. Fossils and molecules in the *Myotinae*

The *Myotis* species are rather abundant and already diverse in the European fossil record of the Miocene through the Pleistocene, and recent studies (Gunnell et al., 2012; Horáček, 2001) suggest that their ancestry might date back to the Late Oligocene. The difficulty in assigning the most ancient fossils, often represented by isolated tooth (Menu et al., 2002) or dentary fragments, is that the genus *Myotis* itself is defined by a

combination of plesiomorphic characters (e.g. three upper and lower premolars), as well as more derived ones shared with other genera (e.g. single-rooted third premolars). The most prominent feature believed to be characteristic of the genus *Myotis* is the myotodont configuration of lower molars (Menu and Sigé, 1971). According to these dental characters, several of the more ancient fossils initially attributed to *Myotis* (e.g. the Early Oligocene “*Myotis*” *misonnei*) are now assigned to other genera (e.g. *Quietia* or *Stehlinia*) (Horáček, 2001) that are not related to the Myotinae. By relying mainly on these dental characters, Gunnell et al. (2012) recently revised the fossil origins of the Myotinae and stressed that there is a large gap between the divergence times estimated from the molecules and the first appearance of fossil *Myotis* on the different continents. The dated molecular phylogeny of Fig. 2 and divergence dates independently obtained by Lack et al. (2010) now suggest that the most recent common ancestor of all modern *Myotis* (including *latirostris*) is older than previously estimated, with a divergence of 21 MYA (95% HPD 23–20 MYA, Table 1) or 18 MYA (HPD 23–13 MYA, Lack et al., 2010). As a lower bound these molecular reconstructions also suggest that the closer outgroups to the Myotinae (the Kerivoulinae–Murininae subfamilies) diverged in the Late Oligocene (about 26 MYA, HPD 31–21 MYA; Fig. 3 and Lack et al., 2010). These molecular estimates are still over 10 million years younger than the presumed oldest Myotinae fossil (*Khonsunysteris*, 34 MYA, Gunnell et al. (2012)). A plausible explanation for these large temporal discrepancies between molecular and fossil interpretation is that the dental characteristics used to assign fossil fragments to the Myotinae are convergent characters that do not identify unambiguously members of this subfamily. For instance, it is now clear that species in the *siligorensis* group are nyctalodont or semi-nyctalodont (Borisenko et al., 2008; Tjornov et al., 2011), including all specimens of *M. s. alticraniatus* analyzed here (Fig. S3 and Table S1). These specimens are nested within the Asian clade IX (Fig. 1), but on a paleontological point of view, their cranial characteristics would have excluded them from the Myotinae. *Myotis latirostris* and the unknown species 4 both have entirely nyctalodont molars (Fig. S3) and are basal to, but clearly part of the early Myotinae radiation (Fig. 1), yet have diverged only about 21 MYA from other recent species of *Myotis* (Table 1). These modern taxa share striking dental and cranial similarities with a fossil genus, *Submyotodon* that was described recently from the Upper part of the Middle Miocene deposits in Europe, but classified away from *Myotis* lineages owing to its dental features (Ziegler, 2003). Finally, members of the genus *Cistugo* have all the dental characteristics of Myotinae, including myotodont molars (Fig. S3), yet they differ by the presence of unique wing glands and a distinctive karyotype (Rautenbach et al., 1993), and are also genetically so divergent from any other Vespertilionidae that they are now classified in their own family (Lack et al., 2010). Again, paleontologically, *Cistugo* species would have been classified within the genus *Myotis* as all those distinguishing features would have been invisible on fossils. Given these morphological ambiguities, we therefore postulate that the most ancient, Oligocene fossils of *Myotis*-like bats (including *Khonsunysteris* or *Leuconoe* = *Myotis*) are stem taxa not directly related to extant *Myotis*, nor even part of the subfamily Myotinae. Their exact phylogenetic affinities should therefore be reevaluated. This problematic paleontological assignation of dental remains might also explain the great apparent gap between the Early Miocene (about 20 MYA) presence of *Myotis*-like bats in Australia, as suggested by Menu et al. (2002), and our molecular reconstructions that date their earliest arrival on this continent in the Pliocene (Fig. 3), about 5 MYA. Such a late, Pliocene, arrival of Myotinae in Australia is also compatible with a

scenario based on chromosomal evolution as shown for Australian Vespertilionini (Volleth and Tidemann, 1991).

Molecular divergence times of younger nodes are more concordant with paleontological data. Indeed, fossil deposits show a burst of diversification during the Middle Miocene, in particular in Europe (Ziegler, 2003), which corroborates the emergence of most basal lineages within the Eurasian assemblage (Fig. 2).

#### 4.4. Paleoenvironmental changes and *Myotis* evolution

The Middle Miocene is characterized by a major climatic transition where global cooling provoked a drop in sea level, and the development of more extensive temperate habitats. These major environmental changes correspond to faunal turnovers (Janis, 1993) that coincide with the basal radiation of crown-group *Myotis*, including the divergence of the Old World versus New World clades (Fig. 2) that presumably occurred after a range expansion of an ancestral Eastern Palaearctic taxon into North America, some 12.3 MYA (HPD 15–10 MYA, Table 1).

The northward drift of the African–Arabian plate resulted about 18–19 MYA in the gradual closure of the Tethyan Seaway which had previously separated Africa from Eurasia. Some mammals experienced faunal interchanges at that early time, but *Myotis* (and other groups such as horses) apparently dispersed much later between these two continents, as indicated by the colonization date of ancestors of the Ethiopian clade (12.3 MYA, HPD 15–10 MYA, Table 1, which corresponds to the Astracian age). It seems that besides the creation of the land bridge, a change in habitat, probably driven by climatic factors, was necessary to the establishment of dispersal routes suitable for *Myotis*. Our biogeographic reconstructions suggest that the Ethiopian clade is most probably linked to an Oriental stock (Fig. 3). This supposed Oriental origin together with the fact that the Sahara and its adjacent regions have gradually shifted from a tropical to an arid environment since the Neogene warmth climax, suggest that *Myotis* took advantage of these paleoenvironmental changes to reach Africa from Asia (Fig. 3). The Asian *M. cf. formosus* represents the sister lineage of the African *M. welwitschii* and colonized secondarily the Oriental region out of Africa (Fig. 3). Likewise the biogeographic interpretation of the AAR of Fig. 3 suggests that *M. emarginatus*, now confined to the Mediterranean region, descended from widespread ancestors that ranged across Asia and Africa, then became isolated in the Oriental region, and finally reached Europe through a range expansion (Fig. 3).

The Late Miocene epoch coincides with further cooling of the climate and aridification, resulting in a continuing reduction of both tropical forests, and expansion of more open and temperate habitats. This might have triggered the diversification of the Old World *Myotis* lineages, especially the species-rich assemblage of Eurasian taxa. Indeed, temperate forests, whether present in high latitude regions or in mountain ranges of intertropical regions, characterize the habitats occupied today by most species of *Myotis* (La Val, 1973).

#### 4.5. Geographic origins of the Myotinae

The fossil record of Tertiary vespertilionids in tropical Asia (Mein and Ginsburg, 1997) or Africa (Butler, 1984) is poorly documented compared to that found in Europe (e.g. Horáček, 2001; Ziegler, 2003) or North America (Gunnell and Simmons, 2005). Hence any firm biogeographic inference based solely on the relative abundance or on the first occurrence of *Myotis* fossils in sediments is difficult. The presumed African origin for the subfamily Myotinae proposed by Gunnell and colleagues (Gunnell et al., 2012) strongly relies on the dentary fragments of *Khonsunysteris* (a Late Eocene fossil found in Egypt) being part of that subfamily. We however

showed that this interpretation is questionable, as taxa unrelated to the *Myotinae*, such as the endemic African *Cistugo*, can share all dental characteristics believed to be unique to the genus *Myotis*. Contrary to the current interpretation of the fossil record, our molecular phylogeny (Fig. 1) and likelihood biogeographic reconstructions (Fig. 3) identify eastern Asia as the cradle of *Myotis* evolution. There is some uncertainty whether the Oriental region alone or in combination with the Eastern Palearctic (as shown on Fig. 3) is the origin of early *Myotinae*, but alternative bioregions are clearly less likely. In addition, 13 of the 21 intercontinental range expansions originated from the Oriental region (inset of Fig. 3) suggesting that this area is a center of origin for the radiation of *Myotis* species. The deepest lineage identified in molecular reconstructions, the *latirostris* lineage, that is sister to all remaining modern *Myotis* (Fig. 1 and Lack et al., 2010), is also endemic to this region, further supporting an ancient, East Asian origin for this radiation. Finally, the *Myotis* species diversity in the Oriental region (inset of Fig. 1) is already the highest in the world based on currently recognized taxa, and is likely strongly underestimated (Francis et al., 2010), again pointing to this area as a cradle of evolution for the genus.

## 5. Conclusions

Reconstruction of the biogeographic origin of *Myotis* (Fig. 3) suggests that the East Asian region is the center of origin for the radiation of modern *Myotis* and is thus probably also the cradle for the entire *Myotinae*. The Indo-Malayan, Indo-Chinese, Sino-Himalayan, and East Asiatic floras and faunas (Corbet and Hill, 1992) meet in this region and engendered the highest biodiversity hotspots on earth (Myers et al., 2000). This geographical area, which has been subjected to gradual uplifting of the Tibetan plateau (the trans-Himalayan range) during the last several million years, includes several high mountain ranges exhibiting sharp topographical complexity, and shows climates ranging from tropical to arctic. Such high environmental and topographic complexity could have promoted speciation.

Considering the potential vagility of bats, and their current worldwide distribution, the overall number of transcontinental migrations in the *Myotis* radiation is relatively low (Fig. 3). This illustrates the relative inability of *Myotis* species to cross some physical barriers. In turn, *Myotis* species can occupy a variety of ecological niches, and it is not exceptional to find up to 12 species coexisting in sympatry. This and the high ecological and morphological diversity found within each *Myotis* assemblage at a worldwide scale support the central impact of biogeography on the *Myotis* evolutionary history. Climatic and vegetation changes since the Middle Miocene might also have favored speciation during the evolution of *Myotis*.

Recent molecular surveys of *Myotis* taxa from different parts of the world identified that several species currently diagnosed by morphological characters certainly contain species complexes: *M. muricola*, *M. siligorensis*, *M. horsfieldii* (Francis et al., 2010), *M. montivagus* (Görföl et al., 2013; Volleth and Heller, 2012), *M. nigricans* (Larsen et al., 2012a), and even European species such as *M. nattereri* (Salicini et al., 2011). Many more cryptic species are present in the Oriental region, as suggested by the presence of numerous unnamed taxa in our reconstructions (Table S1). Here, phylogenetic tools based on the comparison of polymorphic mitochondrial and nuclear molecular markers will be of central interest to identify cryptic species, refine the *Myotis* systematics, and help choosing among priorities for biological conservation purposes. Unfortunately, this region of high biodiversity is also facing high rates of habitat destruction, and is in urgent need of protection to maintain this evolutionary diversity (Sodhi et al., 2004).

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2013.08.011>.

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