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Molecular phylogeny of New World *Myotis* (Chiroptera, Vespertilionidae) inferred from mitochondrial and nuclear DNA genes

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Abstract

Recent studies have shown that species in the genus *Myotis* have evolved a number of convergent morphological traits, many of which are more related to their mode of food procurement than to their phylogeny. Surprisingly, the biogeographic origins of these species are a much better predictor of phylogenetic relationships, than their morphology. In particular, a monophyletic clade that includes all New World species was apparent, but only a third of the 38 species have been analysed. In order to better understand the evolution of this clade, we present phylogenetic reconstructions of 17 Nearctic and 13 Neotropical species of *Myotis* compared to a number of Old World congeners. These reconstructions are based on mitochondrial cytochrome *b* (1140 bp), and nuclear Rag 2 genes (1148 bp). Monophyly of the New World clade is strongly supported in all analyses. Two Palaearctic sister species, one from the west (*M. brandtii*) and one from the east (*M. gracilis*), are embedded within the New World clade, suggesting that they either moved across the Bering Strait, or that they descended from the same ancestor that reached the New World. An emerging feature of these phylogenetic reconstructions is that limited faunal exchanges have occurred, including between the North and South American continents, further emphasizing the importance of biogeography in the radiation of *Myotis*. A fossil-calibrated, relaxed molecular-clock model was used to estimate the divergence time of New World lineages to 12.2 ± 2.0 MYA. Early diversification of New World *Myotis* coincides with the sharp global cooling of the Middle Miocene. Radiation of the temperate-adapted *Myotis* may have been triggered by these climatic changes. The relative paucity of species currently found in South America might result from a combination of factors including the early presence of competitors better adapted to tropical habitats.

Keywords: New World *Myotis*; Vespertilionidae; Chiroptera; Nearctic; Neotropical; Cytochrome *b*; Rag 2; Speciation; Molecular dating; Biogeography

1. Introduction

Current mammalian assemblages in the New World have been marked by successive isolation and connections of the North and the South American continents, and by global environmental changes. South American biotas evolved in isolation during most of the Cenozoic era, but a large number of its endemic taxa became extinct when the continent collided with North America in the Pliocene, and allowed faunal exchanges across the Isthmus of Panama (Cox, 2000; Flynn and Wyss, 1998). In turn, North American biotas evolved in close contact with the Eurasian fauna, as the two continents were periodically connected during low sea levels via land bridges over Greenland or the Bering Strait (Cox, 2001). Because they also share the same temperate climate, faunal similarities are greater between North America and Eurasia than with South America (Cox, 2001). North and South American continents were apparently connected three times during the past 70 million years (Marshall and Sempere, 1993), the most recent connection being accomplished by the formation of the

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Panamanian Isthmus about 3.1 million years ago (MYA). Faunal interchanges between North and South America established the background for the present mammalian assemblages in the Nearctic and the Neotropical regions. Land connections between Nearctic and Neotropics, marine transgression (Donato et al., 2003), geological events or climatic changes that could have created barriers to land colonization or promoted speciation, are described in numerous studies on mammals (e.g. Cook et al., 2004; Da Silva and Patton, 1998; Delsuc et al., 2002, 2004; Galewski et al., 2005; Smith and Patton, 1993; Steiner et al., 2005). Most of these evolutionary hypotheses were based on terrestrial mammals. Because active flight renders bats capable of long-distance dispersal, barriers that influenced the evolution of terrestrial mammals in the Nearctic and Neotropical regions might have had a less significant impact on chiropteran diversification.

With about 100 species (Koopman, 1994; Simmons, 2005) distributed throughout the world except in the polar regions, the genus *Myotis* represents one of the most diverse and successful radiations among mammals and offers an exceptional model for investigating speciation and diversification on a worldwide scale. Several molecular studies (Hoofer and Van Den Bussche, 2003; Kawai et al., 2003; Ruedi and Mayer, 2001; Stadelmann et al., 2004a,b), including one that explored relationships between external morphology and foraging behaviour (Fenton and Bogdanowicz, 2002) have shown that the current morphology-based subdivision of the genus *Myotis* into four or more subgenera (e.g., Findley, 1972; Koopman, 1994) does not reflect phylogenetic groupings, but rather represents adaptive convergences that produced the same ecomorphs independently through deterministic processes (Losos et al., 1998). Instead, the biogeographic evolution of this genus appears to include strongly imprinted phylogenetic relationships of current species. Indeed molecular studies have demonstrated that morphologically divergent species of *Myotis*, from the same continent tend to group into well-supported clades (Ruedi and Mayer, 2001; Stadelmann et al., 2004a). Such biogeographic clades include one uniting all Ethiopian taxa (Stadelmann et al., 2004a) and another comprised of New World species (Hoofer and Van Den Bussche, 2003; Ruedi and Mayer, 2001). This New World clade thus far includes all *Myotis* from the Nearctic and Neotropical regions tested and one Palaearctic species *Myotis brandtii*, supported by several mitochondrial genes (Hoofer and Van Den Bussche, 2003; Ruedi and Mayer, 2001; Stadelmann et al., 2004b).

However, only 15 of the extant New World species were considered in previous studies, which represent less than half of the 38 species currently known from the Nearctic and Neotropical regions (Simmons, 2005). To further test the validity of the New World clade, we expanded the taxon sampling of North, Central, and South American *Myotis* species using both mitochondrial (Cyt *b*) and nuclear (Rag 2) genes.

With this expanded dataset, we tested the biogeographic hypothesis that all sampled New World species originated from a single common ancestor (i.e. are monophyletic; Stadelmann et al., 2004a). We further explore the biogeographic evolution of New World *Myotis* species in relation to current distributions in the Nearctic and Neotropical regions, using a Bayesian relaxed molecular-clock approach and likelihood reconstruction of ancestral geographic distribution. These analyses provide insight into the pattern and timing of colonization of the New World by bats of the genus *Myotis*.

2. Materials and methods

2.1. Taxon and geographic sampling

According to Simmons (2005), 38 species of *Myotis* are known to occur in the New World. Unless stated explicitly, we follow her taxonomic arrangement in referring to the species analysed. Thirty of the 38 New World species were analysed in the present study (Table 1). Taxa for which no tissue was available include very rare taxa or species with highly restricted distributions (*M. aelleni*, *M. cobanensis*, *M. findleyi*, *M. fortidens*, *M. nesopolus*, *M. peninsularis*, and *M. planiceps*), plus *M. melanorhinus* considered as a subspecies of *M. ciliolabrum* (Holloway and Barclay, 2001). When possible, several individuals from the same species, but from different locations, were analysed to validate taxonomic consistency. However, to keep datasets more tractable, only sequences differing by more that 1% were used in the final analyses. Our sampling was not designed to estimate the extent of intraspecific variability, but was intended to capture major components at the supra-specific level (Ruedi and McCracken, in press). The 30 New World species were compared to 36 other species of *Myotis* from other regions of the world (Table 1). The current distribution of the sampled species was classified based on designations of classical zoogeographical regions (Cox, 2001), as listed in Table 1. For Nearctic and Neotropical regions, we used the limits established by Ortega and Arita (1998). The 36 Old World species of *Myotis* used for comparisons include 20 species from the Palaearctic, 10 species from the Oriental, one from the Oceanian, and five from the Ethiopian regions (Table 1). One sample from Japan was originally referred by Kawai et al. (2003) as *M. mystacinus*, but it is currently recognized as a subspecies of *M. brandtii* (Benda and Tsytsulina, 2000; Simmons, 2005), or as a species of its own, *M. gracilis* (Abe et al., 2005; Horácek et al., 2000). Likewise, *M. muricola browni* from Luzon in the Philippines, mentioned by Ruedi and Mayer (2001), probably does not belong to the species *M. muricola* (L. R. Heaney, pers. comm.) and is thus provisionally referred to as *M.* cf. *browni*. Based on previous genetic evidence from multiple sources, the Kerivoulinae and Murininae are the closest relatives of *Myotis* (Hoofer and Van Den Bussche, 2003; Kawai et al., 2002; Stadelmann et al., 2004b). Thus, *Harpiocephalus mordax* and *Kerivoula* cf. *papillosa* were used to root the tree of *Myotis*.