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Short Communication

Molecular dating of the diversification of Phyllostominae bats based on nuclear and mitochondrial DNA sequences

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ABSTRACT

Times of divergence among the three tribes included within the subfamily Phyllostominae were estimated using a Bayesian approach to infer dates of divergence based on mitochondrial and nuclear sequence data. The subfamily Phyllostominae is particularly attractive for such analysis, as it is one of the few groups of bats to have fossil specimens. Our molecular time analyses suggest that diversification among tribes and genera of phyllostominae bats occurred during the Early to Mid-Miocene, and was coincident with diversification events in two co distributed taxa: Caviomorph rodents and New World monkeys.

1. Introduction

New World leaf-nosed bats (family Phyllostomidae) constitute a large, species-rich group that is the most diverse family of mammals in terms of morphology and feeding strategy; they are a fundamental component of Neotropical communities, and represent one of the most extensively studied bat families (reviewed in Jones et al., 2002). Within the Phyllostomidae, the subfamily Phyllostominae (sensu Baker et al., 2003) includes taxa that vary considerably in feeding strategy, including carnivory (*Chrotopterus, Vampyrum*, *Trachops*), strict insectivory (*Macrophyllum*), and a combination of frugivory and insectivory (*Lophostoma*, *Mimon*, *Phylloderma*, *Phyllostomus*, *Tonatia*). Baker et al. (2003) included 9 genera with 20 species (Simmons, 2005) in the Phyllostominae, and arranged them in 3 tribes: Phyllostomini, including *Lophostoma* (7 species), *Mimon* (2), *Phylloderma* (1), *Phyllostomus* (4), and *Tonatia* (2); Vampyrini including *Vampyrum* (1) and *Chrotopterus* (1); and Macrophyllini including *Macrophyllum* (1) and *Trachops* (1). Despite advances in phyllostomid systematics provided by studies of mitochondrial and nuclear DNA sequences, relationships among the three tribes of phyllostominae bats remain unresolved (Lee et al., 2002; Porter et al., 2003; and Baker et al., 2003).

Although the fossil record of bats is notoriously poor, the subfamily Phyllostominae is among the few bat groups to include fossil representatives. Specimens assigned to phyllostomine genera are known from Mid-Miocene deposits in Colombia, northern South America (Savage, 1951; Czaplewski, 1997). The availability of fossil specimens, in combination with techniques to extract time information from multilocus sequence data, make this subfamily a natural candidate to estimate a time frame for the evolutionary history of the group based on DNA sequence data. The objective of this work is to evaluate divergence times within Neotropical bats in the subfamily Phyllostominae using combined cytochrome-*b*, 12S–16S rRNA and RAG-2 sequence data using multilocus Bayesian dating procedures.

2. Materials and methods

2.1. Specimens examined

We sequenced the complete cytochrome-*b* gene (1140 bp) for 20 specimens representing all genera in the subfamily Phyllostominae (Table 1). To facilitate comparisons with Lee et al. (2002), and Porter et al. (2003), we included specimens representing the subfamilies Desmodontinae, (*Desmodus rotundus*, *Diaemus youngi*, *Diphylla eucaudata*), Micronycterinae (*Micronycterys schmidtorum*,

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Table 1
Specimens examined and their locality information

Taxon	Tissue number	Museum catalog number	Country	GenBank accession number		
				12S–16S rRNA	*RAG2*	*Cyt-b*
Artibeus concolor	TK10378	CMNH63792	Suriname: Commewijne		AF316432	
Artibeus obscurus	TK17080	CMNH68951	Suriname: Nickerie	AY395805		
	TK104001	TTU84773	Ecuador: Pastaza			DQ869392
Anoura caudifer		USNM582796	Peru, Cusco	AY395835		
Anoura geoffroyi		TTU62405	El Salvador: Santa Ana		AF316431	FJ155495
Chrotopterus auritus	TK17104	CMNH68638	Suriname: Saramacca		AF316442	
	TK21039	CMNH 76767	Suriname: Para			FJ15548
	TK70457	MUSM13653	Peru: Cusco	AF411538		
Desmodus rotundusl	TK4764	TTU35582	México: Guerrero	AF263228	AF316444	
	TK40368	TTU 61104	Honduras: Atlantida			FJ155477
Diaemus youngi	TK34625	TTU62792	El Salvador: La Paz	AF411534	AF316445	FJ155475
Diphylla ecaudata	TK13514	TK13514	México: Yucatàn	AF411533	AF316447	
	TK13508	TTU 47509	Mexico: Yucatan			FJ155476
Lonchorhina aurita	TK20560	TTU36531	Mexico, Chiapas	AY395843	AF316457	FJ155494
Lampronycteris brachyotis	TK25238	TCWC55445	Trinidad: Mayaro	AF411536	AF316463	
	TK25239		Trinidad: Mayaro			AY380748
Lophostoma brasiliense	TK18834	AMNH267103	French Guyana	AF411544	AF316489	
	TK49898 (F38605)	ROM106608	Panama			FJ155486
Lophostoma evotis	TK49870	ROM95626	México: Campeche	AF411529		
	TK40341	TTU61070	Honduras: Atlantida		AF442080	
	TK49871	ROM95625	Mexico			FJ155491
Lophostoma schulzi	TK18833	AMNH267106	French Guyana: Paracou		AF442079	
	F38318					FJ155485
	TK49888	ROM101128	Locality Missing	AF411532		
Lophostoma silvicola	TK56716		Paraguay: San Pedro	AF442092	AF442081	FJ155493
Lophostoma silvicola	TK17946	CMNH77174	Suriname: Marowijne	AF263230		
	TK18832	AMNH267107	French Guyana: Paracou		AF442083	
		ROM100949	Guyana			FJ155492
Macrophyllum macrophyllum	TK19119	CMNH78289	Venezuela: Bolivar	AF411540	AF316458	FJ155484
Macrotus waterhousii	TK32030	TTU52481	Cuba: Guantanamo		AF316461	
	TK32021	TTU52478	Cuba: Guantanamo	AF263229		
	TK27889	TTU 71435	Mexico: Morelos			AY380745
Micronycteris schmidtorum	TK70447	MUSM13737	Perú: Camisea	AF411535	AF316470	AY380753
Mimon crenulatum	TK15121	TTU33287	Venezuela: Guarico		AF316472	
	TK25230	CMNH25230	Trinidad and Tobago: Trinidad	AF411534		FJ155478
Phylloderma stenops	TK10201	CMNH63614	Suriname: Saramacc	AF411542	AF316480	
	TK86685		Guyana, Berbice District			FJ155480
Phyllostomus hastatus	TK19289	CMNH19289	Venezuela: Bolivar	AF411541		
	TK19243	CMNH78333	Venezuela: Bolivar		AF316479	FJ155479
Tonatia bidens	TK56633		Paraguay: Dpto. San Pedro	AF442090	AF442087	FJ155489
Tonatia bidens	TK56519	MVZ185673	Brazil: Sao Paulo	AF442091	AF442088	FJ155490
Tonatia saurophila	TK49889	ROM103210	Guyana: Upper Takutu-Upper Essequiba		AF442084	FJ155488
	TK49892	ROM104459	Ecuador: Napo	AF411531		
Tonatia saurophila	TK46028	USNM	Perú: Quebrado		AF442085	
	TK49890	ROM103401	Guyana: Upper Demerara-Berbice	AF411530		
	TK49895	ROM104218	Panama: Canal Zone			FJ155487
Trachops cirrhosus	TK18829	AMNH267129	French Guyana: Paracou	AF411539	AF316490	
	TK19132		Venezuela: Bolivar			FJ155483
Vampyrum spectrum	TK40370	TTU61070	Honduras: Atlántida: Lancitilla	AF411537	AF316495	FJ155482

Specimen number TK49885 is also identified by University of New Mexico voucher number NK30034. Specimens with missing Museum Catalog number have yet to be cataloged.

Lampronycteris brachyotis) and Lonchorhininae (*Lonchorhina aurita*) in our phylogenies. We included samples of *Artibeus* and *Anoura* in the analyses to profit from the timescale of bat diversification estimated by Teeling et al. (2005) and added data from *Macrotus californicus* as the outgroup. We obtained 12S–16S rRNA sequences and RAG-2 sequences used by Lee et al. (2002), Baker et al. (2003), and Porter et al. (2003), as well as those from *Artibeus* and *Anoura* from GenBank. We combined sequences from different individuals within a species, as not all genes were sequenced for the same individuals, and in the cases of *Artibeus* and *Anoura* we combined sequences from closely related species (Table 1).

2.2. Molecular methods

We amplified the complete mitochondrial cytochrome-*b* gene using primers and conditions reported in Hoffmann and Baker (2001), using an additional internal sequencing primer To1L (5′- CTG CCT CTA CCT TCA TGT AGG AC-3′). We sequenced the PCR fragments using BigDye 3.0, followed by electrophoresis in an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Inc., Foster City, California). We assembled and verified fragments using Sequencer version 3.1.1 (Gene Code Corporation, Ann Arbor, Michigan) and VectorNTI (Informax Inc., Bethesda, Maryland), and performed sequence alignments in CLUSTAL X (Thompson et al., 1997). For rRNA data we followed Hoofer and Van Den Bussche (2003) to delimit ambiguously aligned sites.

2.3. Phylogenetic analyses

Wiens (1998) proposed that when analyses of individual loci produce compatible topologies, data from multiple loci may be concatenated for phylogenetic reconstruction. Trees are considered as compatible if there are no strongly supported conflicting nodes. In this study, we considered nodes to be strongly supported if they