Historical Biogeography of the Antilles: Earth history and Phylogenetics of Endemic Chiropteran Taxa

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ABSTRACT

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Vicariance and dispersal are the main hypotheses used to explain the distribution and diversification of taxa on both continents and islands. Research on the origin and diversification of island biotas is particularly appealing because these have inspired classical models in biogeography. This study examined competing explanations of Caribbean historical biogeography: an Oligocene land bridge between northern South America and the West Indies, and Cenozoic dispersal from South America and/or Central America. Separate and combined phylogenetic analyses were conducted using new molecular data and published morphological characters for five monophyletic bat lineages; *Mormoops*, *Pteronotus*, a clade comprising *Brachyphylla*, *Erophylla*, and *Phyllonycteris*, the subtribe Stenodermatina, and Natalidae. The resulting phylogenies were analyzed using event-based biogeographic methods, synthesized into hypotheses of area relationships, and used to generate estimates of divergence times at critical nodes. The phylogenies of three of the five lineages are congruent with a single range expansion onto the Caribbean, whereas *Mormoops* is also consistent with this result but further studies of fossil remains are necessary, and Antillean *Pteronotus* appears to be a product of at least two separate invasions. Continental Stenodermatina and Natalidae descended from Caribbean ancestors. The ancestral areas of all the lineages are Mexico, Central America, and northern South America. The patterns of area relationships derived from phylogenies are equivocal in their support for the land bridge hypothesis. The ages of the

bat lineages appear to be too young to have used an Oligocene land bridge. Instead, lowering and rising sea levels at key transitions during the Miocene may have facilitated the spread, and subsequent isolation, of the bat lineages leading to diversification and shared biogeographic pattern. The role of the Miocene in Caribbean biogeographic history should be tested with additional phylogenies, new molecular data, as well as the fossil record. The correlation of geological history, phylogenetic patterns, and the timing of diversification in bats demonstrates congruent biogeographic patterns in the Caribbean are pervasive even among the most vagile organisms.

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Introduction

The use of phylogenies to test evolutionary hypotheses has ushered a renaissance in comparative biology over the last two decades (e.g., Avise and Wollenberg, 1997; Brooks and McLennan, 1991; Clayton and Johnson, 2003; Felsenstein, 1985; Harvey et al., 1996; Howard and Berlocher, 1998; Magallon and Sanderson, 2001; Padian, 1987; Page, 2000). Among other fields, the study of speciation and biogeography has benefited from this renewed perspective. Research on the origin and diversification of island biotas is particularly appealing because these have inspired classical models in both historical and ecological biogeography (e.g., MacArthur and Wilson, 1963; MacArthur and Wilson, 1967; Pregill, 1981; Pregill and Olson, 1981; Rosen, 1975). Phylogenies are beginning to confirm, refine, or sometimes refute these longstanding models (e.g., Baum et al., 1998; Cibois et al., 2001; Heaney, 2000; Jackman et al., 1999; Jansa et al., 1999; Klein and Brown, 1995; Losos and Schluter, 2000; Murphy and Collier, 1996; Perry et al., 1998; Raxworthy et al., 2002; Ricklefs and Bermingham, 2001; Yoder et al., 2003; Yoder et al., 1996).

This dissertation aimed to investigate the historical biogeography of the Caribbean by producing new phylogenies for five monophyletic bat taxa, and analyzing these with both single-cladogram and comparative biogeographic methods. The biogeography of Caribbean bats has been crucial in exploring links between current distributions and Earth history, and developing the equilibrium theory of island biogeography (Koopman, 1958; MacArthur and Wilson, 1967). The most recent attempt to link bat distributions to geological history is older than a decade (Griffiths and

Klingener, 1988), and species lists and hypotheses about the origin of island populations have not been updated over a longer period (Baker and Genoways, 1978; Koopman, 1989). A list of Caribbean bat species and their closest continental relatives, updated to include fossil findings (Morgan, 2001; Morgan and Woods, 1986), current taxonomy (Simmons, in press; Tejedor et al., in press; Tejedor et al., in preparation), recent phylogenies (Baker et al., 2003; Carstens et al., in press; Hoffmann and Baker, 2001; Jones et al., 2002; Villalobos and Valerio, 2002), and the findings of this dissertation is presented in Table 1.

The first chapter introduces the Cenozoic history of the Caribbean region, with particular attention to the controversial land bridge hypothesis of Iturralde-Vinent and MacPhee (1999), and presents reconciled tree analyses of published phylogenies of Antillean mammals and their continental relatives. The following four chapters are organized systematically, with new phylogenies for the mormoopids *Mormoops* and *Pteronotus*; two phyllostomid clades, the genera *Brachyphylla*, *Erophylla* and *Phyllonycteris*, and the subtribe Stenodermatina; and the family Natalidae. These phylogenies present new molecular data, and combine these with published molecular and morphological data sets to resolve relationships among species in these five clades. In some cases it became necessary to revise the species taxonomy of the chiropteran clades to reflect hierarchical relationships among basal units. Biogeographic analyses of individual phylogenies and taxonomic revisions are also presented in the systematic chapters. The sixth and final chapter revisits the geological history of the Caribbean to examine congruence with respect to the new phylogenies, and presents novel, phylogeny-based hypotheses of area relationships for the region.

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Table 1. Taxonomic list and distribution of all Caribbean bat species and their closest extant continental relatives. Taxonomy follows Simmons (in press) unless otherwise noted, chiropteran families are in **bold**. Unless otherwise noted, the phylogenetic relationships used to identify the closest extant continental relative follow Jones et al. (2002, see article for primary sources). For Greater Antilles, 'widespread' means present at least in Jamaica, Cuba, Hispaniola and Puerto Rico; for Lesser Antilles, 'widespread' means present at least in Antigua, Montserrat, Guadeloupe, and Dominica; for closest continental relative, 'widespread' means the West Indian species is distributed in one or more of the surrounding continental areas. Sources (fossil taxa): Baker and Genoways (1978), Griffiths and Klingener (1988), Koopman (1989), Morgan (2001), and Morgan and Woods (1986). CECR = closest extant continental relative, NA = North America (often refers to Mexico), CA = Central America, SA = South America, Dom = Dominica, MG = Marie Galante, Mart = Martinique, StL = St. Lucia, StV = St. Vincent. † = extinct.

Species	Greater Antilles	Lesser Antilles	Closest extant continental relative	Distribution of CECR
Noctilionidae				
Noctilio leporinus	widespread	widespread	widespread	CA SA
Mormoopidae[1]				
Mormoops megalophylla	†Cuba, †Jamaica, †Hispaniola, †Bahamas		widespread	NA CA SA
Mormoops blainvillei	widespread, †Bahamas	†Antigua	*M. megalophylla*	NA CA SA
Mormoops magna	†Cuba		*Mormoops*	NA CA SA
Pteronotus parnellii	Cuba, Jamaica, †Bahamas		*P. rubiginosus*	SA
Pteronotus pusillus	Hispaniola		*P. rubiginosus*	SA
Pteronotus portoricensis	Puerto Rico	†Antigua	*P. rubiginosus*	SA
Pteronotus pristinus	†Cuba		*P. parnellii*	NA CA SA

Table 1. Continued.

Species	Greater Antilles	Lesser Antilles	Closest extant continental relative	Distribution of CECR
Pteronotus quadridens	Cuba, Jamaica, †Bahamas		*P. personatus* or *davyi+gymnonotus +fulvus*	NA CA SA
Pteronotus fuliginosus	Hispaniola, Puerto Rico		*P. personatus* or *davyi+gymnonotus +fulvus*	CA SA
Pteronotus macleayi	Cuba, Jamaica		*P. personatus* or *davyi+gymnonotus +fulvus*	CA SA
Pteronotus davyi		MG, Dom, Mart	*P. gymnonotus*	CA SA
Phyllostomidae				
Macrotus waterhousii	widespread, †Puerto Rico	†Antigua, †Anguilla, †Barbuda	widespread	NA
Tonatia saurophila	†Jamaica		widespread	CA SA
Brachyphylla nana[2]	Grand Cayman Cuba Bahamas		*Monophyllus*+ *Glossophaga*+ *Leptonycteris*[3]	NA CA SA
Brachyphylla pumila[2]	†Jamaica, Hispaniola, Middle Caicos		*Monophyllus*+ *Glossophaga*+ *Leptonycteris*[3]	NA CA SA
Brachyphylla cavernarum[2]	Puerto Rico	widespread	*Monophyllus*+ *Glossophaga*+ *Leptonycteris*[3]	NA CA SA
Erophylla sezekorni[2]	Jamaica, Grand Cayman, Cuba, Bahamas		*Monophyllus*+ *Glossophaga*+ *Leptonycteris*[3]	NA CA SA
Erophylla bombifrons[2]	Hispaniola, Puerto Rico		*Monophyllus*+ *Glossophaga*+ *Leptonycteris*[3]	NA CA SA
Phyllonycteris poeyi[2]	Cuba, Hispaniola		*Monophyllus*+ *Glossophaga*+ *Leptonycteris*[3]	NA CA SA
Phyllonycteris major[2]	†Puerto Rico	†Antigua	*Monophyllus*+ *Glossophaga*+ *Leptonycteris*[3]	NA CA SA
Phyllonycteris aphylla[2]	Jamaica	†Antigua	*Monophyllus*+ *Glossophaga*+ *Leptonycteris*[3]	NA CA SA
Glossophaga longirostris		St. Vincent	*Glossophaga leachii*[4]	NA
Glossophaga soricina	Jamaica		widespread	NA CA western SA
Monophyllus redmani	widespread		*Glossophaga*+ *Leptonycteris*[3]	NA CA SA
Monophyllus plethodon	†Puerto Rico	widespread	*Glossophaga*+ *Leptonycteris*[3]	NA CA SA

Table 1. Continued.

Species	Greater Antilles	Lesser Antilles	Closest extant continental relative	Distribution of CECR
Sturnira lilium		Dom, Mart, StL, StV	widespread	SA
Sturnira thomasi		Guadeloupe	*S. luisi+lilium*[5]	SA
Chiroderma improvisum		Monserrat, Guadeloupe	*C. villosum*	SA
Artibeus jamaicensis	widespread	widespread	widespread[6]	NA CA SA
Artibeus anthonyi	†Cuba		Other *Artibeus*	CA SA
Artibeus lituratus		St. Vincent	widespread	SA
Phyllops falcatus[7]	Cuba Hispaniola		Stenodermatina	NA CA SA
Phyllops vetus[7]	†Cuba		Stenodermatina	NA CA SA
Phyllops silvai[7]	†Cuba		Stenodermatina	NA CA SA
Ardops nichollsi[7]		widespread	Stenodermatina	NA CA SA
Ariteus flavescens[7]	Jamaica		Stenodermatina	NA CA SA
Stenoderma rufum[7]	Puerto Rico		Stenodermatina	NA CA SA
Desmodus rotundus[7]	†Cuba		widespread	NA CA SA
Natalidae				
Natalus major[8]	Hispaniola		*N. tumidirostris*	SA
Natalus jamaicensis[8]	Jamaica		*N. tumidirostris*	SA
Natalus primus	Cuba, †Bahamas		unknown	unknown
Natalus stramineus[8]		widespread	*N. tumidirostris*	SA
Chilonatalus micropus[8]	Jamaica, Cuba, Hispaniola, Providencia		*N. tumidirostris*	SA
Chilonatalus tumidifrons[8]	Bahamas		*N. tumidirostris*	SA
Nyctiellus lepidus[8]	Cuba, Bahamas		*N. tumidirostris*	SA
Vespertilionidae				
Myotis dominicensis		Dominica	*Myotis velifer +yumanensis*	NA
Myotis martiniquensis		Martinique	Other *Myotis*	SA
Eptesicus fuscus	widespread	Dominica	widespread	NA CA SA
Eptesicus guadeloupensis		Guadeloupe	*Eptesicus fuscus*	NA CA SA

Table 1. Continued

Species	Greater Antilles	Lesser Antilles	Closest extant continental relative	Distribution of ECR
Nycticeius humeralis	Cuba		widespread	NA
Lasiurus intermedius	Cuba		widespread	NA CA
Lasiurus insularis	Cuba		*L. intermedius*	NA CA
Lasiurus pfeifferi	Cuba		*L. borealis*	NA CA SA
Lasiurus degelidus	Jamaica		*L. borealis*	NA CA SA
Lasiurus minor	Bahamas, Hispaniola, Puerto Rico		*L. borealis* or *blossevillii*	NA CA SA
Antrozous pallidus	Cuba		widespread	NA
Molossidae				
Mormopterus minutus	Cuba		*M. kalinowskii* + *M. phrudus*	SA
Tadarida brasiliensis	widespread	widespread	widespread	NA CA SA
Nyctinomops laticaudata	Cuba		widespread	NA CA
Nyctinomops macrotis	Jamaica, Cuba, Hispaniola		widespread	NA CA
Eumops auripendulus	Jamaica		*E. underwoodi*	CA
Eumops glaucinus	Jamaica, Cuba		*E. underwoodi*	CA
Eumops perotis	Cuba		*E. hansae*	CA SA
Molossus molossus	widespread	widespread	widespread	CA SA

1. See revised taxonomy and phylogeny of Mormoopidae in this dissertation.

2. See revised taxonomy in this dissertation.

3. From Baker *et al*. (2003).

4. From Hoffman and Baker (2001).

5. From Villalobos and Valerio (2002).

6. But see Carstens et al. (in press).

7. See revised phylogeny in this dissertation.

8. See revised taxonomy and phylogeny of Natalidae in this dissertation.

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Phylogeny and biogeography of Caribbean mammals

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Vicariance and dispersal hypotheses have been proposed over the last two hundred years to explain the distribution, diversity, and faunal composition of the Caribbean biota. Despite great advances in understanding the geological history of the region, recent biogeographical reviews have not used historical biogeographical methods. In this paper I review the taxonomy, distribution and phylogeny of all Cenozoic Caribbean non-volant mammals and four bat lineages, and present reconciled trees for available phylogenies. Dates available from the fossil record and hypotheses of divergence based on molecular phylogenetic studies are also included in general assessments of fit between proposed geological models and Caribbean mammal diversification. The evidence posited in mammalian phylogenies does not add to the argument of dispersal vs. vicariance. One previously unidentified temporal pattern, the colonization of the Caribbean by South American mammals between the Palaeocene and the Middle Miocene, accounts for the distribution and phylogeny of the majority of lineages studied. Choloepodine and megalocnine sloths, hystricognath rodents, and primates all arrived during this window of colonization. Of these, megalocnine sloths, hystricognath rodents, *Brachyphylla* and allied bats, Stenodermatina bats, and primates fit the pattern of divergence from the mainland implied by the Gaarlandia hypothesis. Sloths, rodents and primates also roughly fit the timing of arrival to the Caribbean implied by Gaarlandia. The remaining taxa show contradictory dates of divergence according to molecular clock estimates, and no taxa fit the predicted timing and pattern of divergence among Antillean landmasses under the Gaarlandia model. Choloepodine sloths, murid rodents, insectivorans, mormoopids, and natalids show patterns of divergence from the mainland that are inconsistent with the Gaarlandia hypothesis and seem to require taxon-specific biogeographical explanations.

ADDITIONAL KEYWORDS: Antilles – dispersal – Gaarlandia – historical biogeography – vicariance – West Indies.

INTRODUCTION

Explaining both the uniqueness and diversity of the fauna of the islands of the Caribbean Sea has been the objective of biologists for two centuries (Wallace, 1881; Woods, 2001). To this end, evidence from geology, phylogeny, and ecology has been accumulating at a particularly rapid rate over the last three decades (see papers in Woods, 1989a; Woods & Sergile, 2001). Despite these efforts the origins and patterns of diversification of the Caribbean biota are far from clear for most groups, and the debate on the historical biogeography of Caribbean vertebrates continues unabated (Williams, 1989; Woods, 2001).

The most contentious debate on the historical biogeography of the Caribbean concerns the relative roles of dispersal and vicariance in shaping its fauna. Hedges and colleagues (Hedges, Hass & Maxson, 1992, 1994; Hedges, 1996a,b, 2001) have argued that the fauna of the region shows a pattern of concordant dispersal from South America throughout the Cenozoic. Numerous others have argued that taxonomic, and more recently, phylogenetic congruence points toward a vicariant origin of the Caribbean fauna (Crother & Guyer, 1996; Rosen, 1975, 1985; Liebherr, 1988; Page & Lydeard, 1994; Guyer & Crother, 1996). While some vicariant models (e.g. Rosen, 1975) can be

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rejected given the estimated timing of emergence of Caribbean islands, the vicariant model of Iturralde-Vinent & MacPhee (1999; MacPhee & Iturralde-Vinent, 1994, 1995, 2000) represents the most serious challenge to the dispersal hypothesis of Hedges and colleagues. According to the vicariance model of Iturralde-Vinent & MacPhee (1999), derived from geological hypotheses outlined below, a land bridge between the Greater Antilles and northern South America around the Early Oligocene allowed the continental South American fauna to reach the islands. Subsequent severance of the land bridge later resulted in vicariance of different lineages from their mainland relatives and among populations in Caribbean islands. This hypothetical land bridge has been called the Gaarlandia (Greater Antilles and Aves Ridge) land span.

The geology of the Caribbean region is complex, involving the Caribbean, Pacific (including Cocos and Nazca), South American, and North American tectonic plates (Meschede & Frisch, 1998: fig. 2). Pindell (1994) and Meschede & Frisch (1998) among others have recently reviewed the early history of the region. The Antilles were formed as a result of the subduction of the North American plate beneath the Caribbean plate in the Middle to Late Cretaceous, but the precise time of their emergence as subaerial entities is not known. At the time of the Cretaceous–Tertiary boundary, about 65 Mya, an asteroid penetrated the atmosphere and the resulting dust clouds disrupted photosynthesis worldwide (Alvarez *et al*., 1980). The Chicxulub crater in the Yucatán peninsula is considered the site of impact of this asteroid (Claeys *et al*., 1998). Massive earthquakes (Claeys *et al*., 1996), continental margin failures (Norris *et al*., 2000), ejecta and tsunamis (Grajales *et al*., 2000) followed the asteroid impact in the Caribbean. This led Hedges *et al*. (1992) to propose that the asteroid impact decimated the ancient Caribbean land biota, and to explain the current faunal assemblage as a result of repeated colonization from South America thereafter.

Iturralde-Vinent & MacPhee (1999) proposed that the Aves ridge connected the already emergent Greater Antilles (Cuba, Hispaniola, Puerto Rico) to northern South America during the Late Eocene/Early Oligocene transition (35–33 Mya). This hypothesis is based on the incidence of general uplift and major sea level drops at the beginning of the Oligocene (Haq, Hardenbol & Vail, 1993; Miller *et al.*, 1996). The connection could have been an island chain (Perfit & Williams, 1989) or a dry land bridge (Iturralde-Vinent & MacPhee, 1999). By the Late Oligocene, sea level rose and land exposure declined. Emergent land areas including Cuba, Hispaniola, Puerto Rico, and the Aves ridge were further isolated as a consequence of active tectonic disruption of the northern and southern Caribbean plate boundaries (Perfit & Williams, 1989; Iturralde-Vinent & MacPhee, 1999). Jamaica had been largely emergent during the Cretaceous/Eocene transition (Portell, Donovan & Domning, 2001), but was submerged during most of the Oligocene (Robinson, 1994). From the Miocene to the Early Pliocene (between 24 and 5 Mya), the Caribbean plate continued to move eastward in relation to the American plates causing uplift and emergence of most of the islands, including Jamaica (Perfit & Williams, 1989). In the Late Miocene (9 Mya), 'stepping stone' connections between North and South America first became available.

By the Pliocene, the general configuration of the Antilles, including the Lesser Antilles and Jamaica, resembled the present, with sea level changes associated with glaciations playing a larger role in shaping subaerial margins. The Bahamas, for instance, are thought to be the product of sea level changes in the Late Quaternary (Hearty, 1998). Sea level has peaked at or above present levels at least three times over the past 0.45 Myr (Hearty & Kaufman, 2000), with a similar number of drops exposing landmasses that could have served as stepping-stones for relatively vagile land organisms. A summary of area relationships expected from geological hypotheses beginning with the Late Eocene/Early Oligocene transition is shown in Figure 1.

Both the vicariance and dispersal approaches are based on current geological hypotheses. The geological history of the Caribbean only loosely constrains the interpretation of biogeographical patterns and is the subject of much debate (Williams, 1989). In this context, the Caribbean vicariance/dispersal debate misses a fundamental question in historical biogeography: does the observed biogeographical pattern correspond to a general pattern of area interconnections, and thus reflect the history of those areas (Platnick & Nelson, 1978)? If most Caribbean lineages are monophyletic, and the arrival of their ancestors is constrained to a particular time, then the search for a common geological cause is warranted. Conversely, if most of the Caribbean fauna consists of independent lineages that reached the islands at disparate times, then the particulars of each radiation become more relevant than the pattern of interconnections among areas.

Recent reviews of the biogeography of Caribbean vertebrates (Hedges, 1996a) and mammals (MacPhee & Iturralde-Vinent, 2000) have not presented compelling phylogenetic (i.e., biological history) evidence for the preferred modes of explaining faunal assemblages. To evaluate the connection between the geological and biological history of the Caribbean, I review the taxonomy, distribution, and phylogeny of all Cenozoic non-volant West Indian mammals and four bat lineages, and evaluate the congruence between

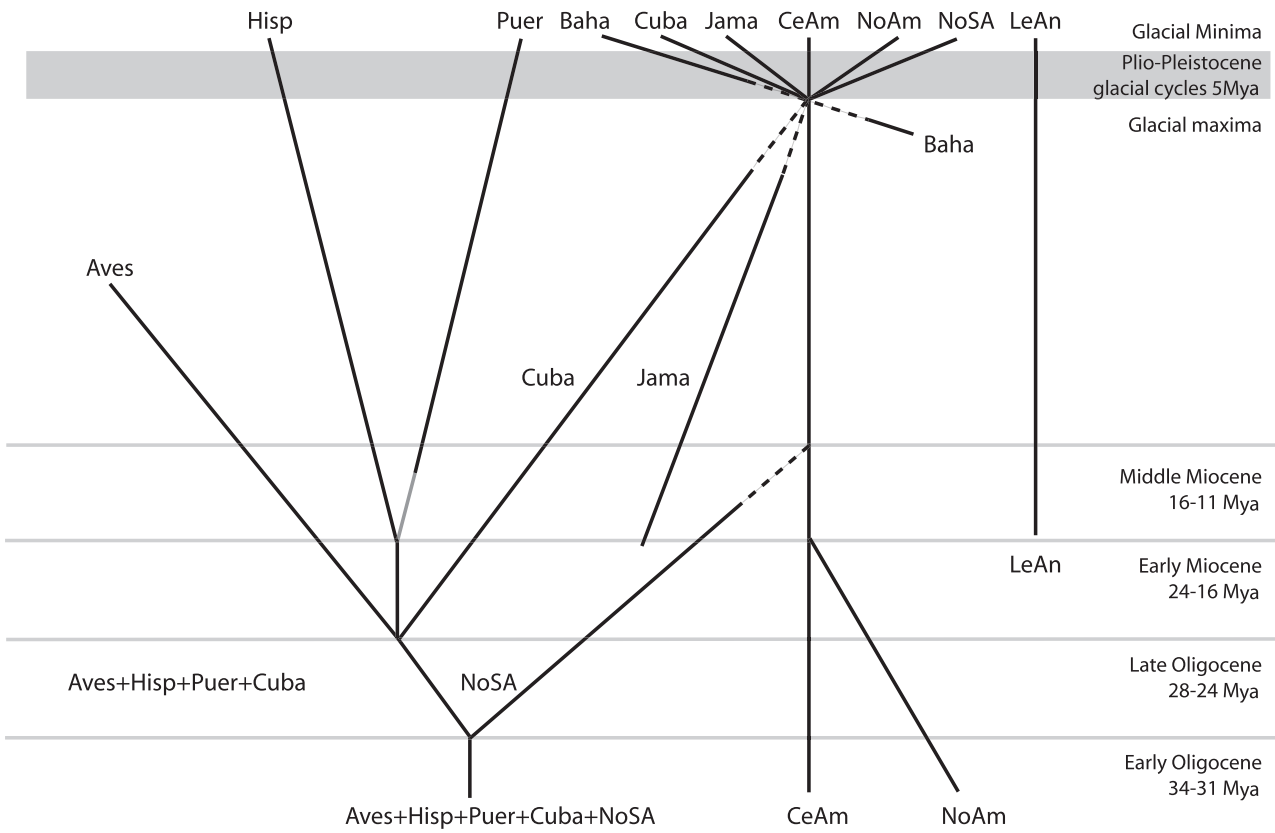

Figure 1. Expected area relationships among areas of endemism relevant to the Caribbean beginning in the Early Oligocene. Length of branches and distances among branches are not proportional to approximate timing, or distance among areas of endemism. The composite geological origin of Jamaica and Hispaniola is not depicted. Composite origins involving more than one geological unit have been proposed for western and eastern Jamaica (Robinson, 1994; Iturralde-Vinent & MacPhee, 1999), and the northern and southern part of western Hispaniola (Perfit & Williams, 1989). The hypothesized interconnection between the Aves Ridge and the Lesser Antilles via the Grenada basin is not depicted. The geological hypotheses relevant to this study correspond to the Oligocene and after because most of the Antilles was submerged or evanescent before that time (Robinson, 1994; Iturralde-Vinent & MacPhee, 1999). Aves = Aves Ridge, Baha = Bahamas, CeAm = Central America, Hisp = Hispaniola, Jama = Jamaica, LeAn = Lesser Antilles, NoAm = North America, NoSA = northern South America, Puer = Puerto Rico. Dotted line indicates connection via stepping-stone islands, grey part of branches indicates time when landmass was mostly submerged. The grey box implies cycling of isolation and near-interconnection events among islands. The pattern of area relationships expected from the Plio-Pleistocene is not resolved in strictly dichotomous form, given the short branches and repeated separations and reconnections among areas.

phylogeny and geological hypotheses by generating reconciled trees.

METHODS

Geographic scope

In this paper 'West Indies' is used in a biogeographical sense following the classification of Hershkovitz (1958), Morgan & Woods (1986), and Morgan (2001). The West Indies include the Greater Antilles (Cuba, Jamaica, Hispaniola, and Puerto Rico), Bahamas, Cayman Islands, Swan Islands, Virgin Islands and the Lesser Antilles south to St. Vincent and Barbados. Grenada and the Grenadines, Trinidad, Tobago, Margarita, and Aruba, Bonaire and Curaçao are excluded from this definition of the West Indies, as their biota is more characteristic of continental South America. The terms 'Antillean', 'Caribbean', and 'West Indian', used in a faunal sense, are intended to be synonyms, and thus encompass all the islands defined above as part of the West Indies (Morgan, 2001).

Geological area cladogram

The geological hypothesis of area relationships shown in Figure 1 was pruned to make it amenable to com-

parison with taxon-area cladograms, and modified to include two areas whose fauna is hypothesized to have arrived by dispersal given current geological evidence: Jamaica & the Lesser Antilles. Iturralde-Vinent & MacPhee (1999) proposed the Jamaican Blue Mountains were emergent and interconnected to Gaarlandia, but this hybrid geological origin is not included in the hypothesis of area relationships used hereafter (Fig. 2). In addition to the geological hypotheses presented in the Introduction, another assumption underlies the area relationships of Figure 2: that Jamaica and the Lesser Antilles were colonized by dispersal from the nearest Caribbean landmass, rather than from Central America or northern South America. All subsequent biogeographical analyses interpret these two nodes of the geological area cladogram in light of the choice described above, since there is some biological evidence for such patterns of area relationships (Griffiths & Klingener, 1988; Koopman, 1989).

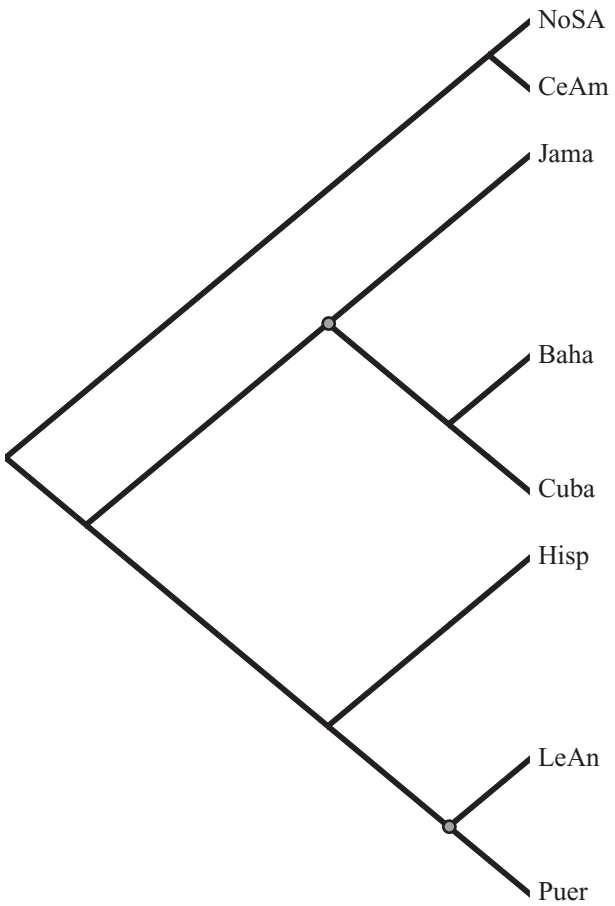

Figure 2. Simplified strictly dichotomous area relationships for Caribbean landmasses. Note the addition of Jamaica and the Lesser Antilles (nodes with grey circles) as dispersal interconnection based on distance to other Antillean areas. See 'Geological area cladogram' under Methods, for details.

TAXONOMIC SCOPE

There are *c.* 60 extant and 75 extinct species of non-introduced Caribbean land mammals from the Late Quaternary and Recent, classified in 14 families and 5 orders (Morgan & Woods, 1986). The extinction of 75 species of mammals, 67 of them non-volant, in two spasms around 10 000 and 4500 years ago (Morgan & Woods, 1986) has been attributed to climate change (Pregill & Olson, 1981), Pleistocene overkill by newly arrived humans (Martin, 1967), and/or the spread of particularly virulent diseases associated with humans and their commensals (MacPhee & Marx, 1997). The number and classification of genera and species of Cenozoic and Recent Caribbean mammals are presented in Table 1.

Taxon-area cladograms

I analysed the following taxon cladograms using a computer implementation of reconciled trees (Page, 1993): (1) the two taxon cladograms for Caribbean ground sloths resulting from a cladistic analysis of 69 skeletal characters by White & MacPhee (2001); (2) the cladogram resulting from analyses of 82 morphological characters by Woods, Borroto & Kilpatrick (2001) for Antillean hystricognaths in the families Capromyidae, Echimyidae, and Heptaxodontiidae (often referred to as West Indian Caviomorpha); (3) four different chiropteran cladograms: (a) congruent results of molecular and morphological characters analyses for the family Mormoopidae (Lewis-Oritt, Porter & Baker, 2001; Simmons & Conway, 2001; Jones *et al*., 2002; Van Den Bussche, Hoofer & Simmons, 2002), (b) the cladogram of the *Brachyphylla–Erophylla–Phyllonycteris* phyllostomid clade (Jones *et al*., 2002), (c) the cladogram of the phyllostomid tribe Stenodermatina (Jones *et al*., 2002), and (d) results of morphological character analyses for the family Natalidae (Morgan & Czaplewski, 2003); (4) the cladogram from the morphological character analysis including three extinct species of Antillean atelid primates by Horovitz & MacPhee (1999).

Finally, the taxon cladograms used in chiropteran reconciled tree analyses were updated following recent publications: extinct species *Pteronotus pristinus* and *Mormoops magna* added as sister to *P. parnellii* and *M. megalophylla* (Simmons & Conway, 2001); *Erophylla sezekorni* and *E. bombifrons* recognized as separate, sister species (Simmons, in press), and *Phyllonycteris major* added as part of a trichotomy with other *Phyllonycteris*; extinct *Phyllops vetus* added as sister to *Phyllops falcatus*; and *Natalus major* (Simmons, in press) comprising an unresolved

Table 1. Summary of genera and species of Cenozoic Caribbean mammals. Taxonomy follows McKenna & Bell (1997), unless otherwise noted. 'Endemic' means restricted to the West Indies[1]

Order	Family	Genera	Endemic	Species	Endemic
Xenarthra	Megalonychidae[2]	5	5	13	13
Rodentia	Muridae	2	1	8	8
	Echimyidae	4	4	7	7
	Capromyidae[3]	9	9	43	43
	Heptaxodontidae[3]	4	4	5	5
Lipotyphla (Insectivora)	Nesophontidae†[3]	1	1	11	11
	Solenodontidae[3]	1	1	4	4
Chiroptera	Noctilionidae	1	0	1	0
	Mormoopidae	2	0	7	3
	Phyllostomidae	15	8	26	20
	Molossidae	5	0	8	0
	Natalidae[4]	3	2	6	5
	Vespertilionidae	5	0	11	7
Primates[5]	Atelidae	3	3	4	4
Perissodactyla	Hyracodontidae†	1	0	1	0

† = extinct.

[1]Sources: Biknevicius, McFarlane & MacPhee (1993), Domning *et al*. (1997), Flemming & MacPhee (1999), Horovitz & MacPhee (1999), Koopman & Williams (1951), Koopman (1989, 1993, 1994), MacPhee & Horovitz (2002), MacPhee & Iturralde-Vinent (1994, 1995), MacPhee *et al*. (1995), MacPhee, White & Woods (2000), McKenna & Bell (1997), Morales & Bickham (1995), Morgan & Czaplewski (2003), Morgan & Woods (1986), Morgan (1993, 2001), Musser & Carleton (1993), Ramos & Borroto (2000), Silva-Taboada (1979), A. Tejedor, V. d. C. Tavares & G. Silva-Taboada (unpubl. data),White & MacPhee (2001), Williams & Koopman (1951), Woods (1989b, 1993), Woods *et al*. (2001).

[2]Includes the extant *Choloepus*; all other genera are extinct.

[3]Endemic family.

[4]Recognizing the three genera ascribed to *Natalus* by Koopman (1993) as *Natalus*, *Chilonatalus*, and *Nyctiellus* (Morgan & Czaplewski, 2003). Recognizing the three Greater Antillean subspecies of *Natalus major* (*N. stramineus* of Koopman, 1993) as the species *N. primus*, *N. major* and *N. jamaicensis* (A. Tejedor, V. d. C. Tavares & G. Silva-Taboada, unpubl. data).

[5]Ford & Morgan (1986, 1988; Ford, 1986, 1990) have described postcranial primate remains from Coco Ree Cave (St. Catherine, Jamaica) and Bahía de Samaná (Samaná, Dominican Republic) as members of the family Callitrichidae (marmosets), and a third fragment from Sheep Pen Cave (Trelawny, Jamaica) as a cebid (= atelid). A fourth set of 'unusual' postcranial remains from Long Mile Cave (the type locality for *Xenothrix mcgregori*) cannot be confidently assigned to described primate species (MacPhee & Fleagle, 1991) and although there is 'no conceivable candidate other than *Xenothrix*' for it (MacPhee & Horovitz, 2002: 191), it might not even be a primate. MacPhee & Horovitz (2002) treat the Coco Ree and Sheep Pen remains as compatible with a *Xenothrix* diagnosis, and the Bahía de Samaná specimen with *Antillothrix* (the latter originally described as *Saimiri bernensis* Rímoli, 1977). One conclusion is common to both sides of the argument: more Antillean primate species await the discovery of better-preserved material and subsequent investigation (Ford, 1990; MacPhee & Horovitz, 2002), as exemplified by the recent description of *Paralouatta marianae* from Cuba (MacPhee, Iturralde-Vinent & Gaffney, 2003).

trichotomy of the nominate *N. primus*, *N. major*, and *N. jamaicensis* (A. Tejedor, V. d. C. Tavares & G. Silva-Taboada, unpubl. data). The Caribbean distribution of many bat species (Table 1) that are widespread throughout the Americas is not analysed in this article, since reconciled tree analysis is designed to investigate association between areas and differentiated lineages.

Other sources of information

In two cases taxon cladograms for Caribbean groups were not available: (1) the relationships among the eight species of Caribbean sigmodontine murids *Oryzomys* and *Megalomys* are not resolved because they are part of the vast sigmodontine radiation (Table 1), and (2) there are no phylogenetic analyses including both lineages of Caribbean insectivores (Table 1) relevant to this biogeographical study.

Because previous hypotheses regarding these groups are relevant to this mammalian biogeographical analysis, they are discussed in a comparative context despite the lack of cladograms. The temporal context of taxon cladograms, inferred from

the age of available fossils, and/or the dating of nodes by molecular techniques, is also discussed in light of the reconciled tree analyses.

The Eocene fauna of Seven Rivers, Jamaica, comprising a ?primate petrosal (MacPhee *et al*., 1999) and the lower jaw of the perissodactyl *Hyrachyus* (Domning *et al*., 1997), is not analysed further because the island was inundated from Middle Eocene to Late Miocene (Robinson, 1994). *Hyrachyus* is found in the Eocene in Europe, Asia, and North America (McKenna & Bell, 1997), perhaps suggesting Holarctic affinities for this Jamaican fauna.

Reconciled tree analysis: component

The taxon-area cladograms listed above were mapped onto the hypothesized area cladogram obtained from geological evidence (Fig. 2) using Component ver. 2.0 for Windows (Page, 1993). The key concept in tree mapping is reconciling a tree for the taxa with a tree for the areas, under the assumption that the relationship between taxa and areas is due to 'association by descent' (Brooks & McLennan, 1991), or that areas and taxa evolve together. The analysis provides a measure of fit between taxa and area cladograms that generates hypotheses about the relative ages of divergence events for both taxa and areas, and can also be tested statistically. The taxon and area cladograms can be reconciled so that observed relationships between areas and taxa can be explained solely by 'association by descent.' To accomplish this the observed taxon cladogram is postulated as a subtree of a larger tree, a *reconciled* tree. This reconciled tree represents the complete taxon cladogram, of which the actual taxon tree is hypothesized to be a subsample or relict, given that extinction or incompleteness might have restricted taxon sampling.

Component reconciles trees by mapping the taxon cladogram to the area cladogram. The map requires that each node in the taxon cladogram is assigned a distribution in the area cladogram. The map is constructed by finding the smallest cluster in the area cladogram that contains the set representing the distribution of the taxa for each node in the taxon tree. All the nodes in the reconciled tree (a taxon tree) are therefore clusters of the area cladogram. If each node in the taxon tree maps onto a different node in the area cladogram then the associate tree is either identical with the area cladogram, or a consistent subtree of the area cladogram. If more than one taxon node maps onto the same node in the area tree then a duplication is postulated. Two kinds of duplication are recognized: those required because of incongruence between taxon and area cladograms, and those required because the descendants of a given taxon have overlapping ranges in an area (redundant nodes *sensu* Page, 1988).

Component assumes that the incongruence between area cladograms derived from different sources is due to poor taxonomic sampling and/or extinction, an idea originally proposed for area relationships by Nelson & Platnick (1981), who also counted dispersal as a source of error. Component maps all taxa onto the area cladogram, equivalent to the treatment of widespread taxa under 'Assumption 0' (Zandee & Roos, 1987), while the option of not mapping widespread taxa in the analysis implements 'Assumption 1' (Nelson & Platnick, 1981).

Component computes three measures of fit between taxon and area cladograms: the number of duplications, the number of leaves added, and the number of independent losses. Duplications are explained above, the number of leaves added is half of the difference between the number of terms and components in the reconciled tree and the original taxon cladogram (items of error *sensu* Nelson & Platnick, 1981), and losses are number of branches hypothesized to be missing in the process of reconciling the trees.

Additionally, I compared the observed number of leaves added and losses postulated in the reconciled tree with a distribution generated by mapping each taxon cladogram to 1000 random area cladograms (generated using the 'Random trees' command of the 'Generate' menu in Component). Comparisons with these distributions assessed the fit between observed taxon and area cladograms and the fit expected by chance alone.

RESULTS

Reconciled trees

Reconciled trees for the eight mammalian lineages analysed using Component are shown in Figures 3–10. All reconciled trees shown were obtained by plotting widespread distributions and assuming that absence from an area means information is missing. Because both taxon and area cladograms have to be fully resolved to use Component, the unresolved nodes present in taxon cladograms were resolved arbitrarily and this is noted here for reference in later discussion. The taxon cladograms used to generate Figures 3 and 4 (reconciled trees for megalonychids) were both fully resolved and have no known representatives in Jamaica or the Lesser Antilles (White & MacPhee, 2001). The cladogram for South American hystricognath rodents contained one Jamaican representative (*Geocapromys brownii*) and numerous unresolved nodes (Fig. 5). The cladogram of mormoopids contained five Jamaican species and two unresolved nodes (Fig. 6). The cladogram of *Brachyphylla–Phyllonycteris–Erophylla* contained

Figure 3. Reconciled tree of the distribution of choloepodine sloths (*sensu* White & MacPhee, 2001), and the extant mainland outgroup *Bradypus*. All species are extinct, except *Choloepus* and *Bradypus*. The taxon cladogram excluded *Paulocnus* from Curaçao, as did White & MacPhee (2001: fig. 2D). Nodes with black circles are duplicated to reconcile incongruence between area and taxon cladograms (*incongruent* nodes). Nodes with white circles are duplicated to explain the distribution of different species in a clade in the same area (*redundant* nodes). CH = Cuba–Hispaniola node, c = mainland–Antillean node. Black branches indicate the taxon is present in the area, grey branches mean there is no evidence to presume the clade ever ranged in the area, white branches are automatically added as part of a duplication to reconcile taxon and area cladograms.

three Jamaican species and one unresolved node (Fig. 7). The cladogram of the Stenodermatina has three unresolved nodes and a representative each in Jamaica and the Lesser Antilles (Fig. 8). The cladogram of the Natalidae has only one unresolved node, plus two species in Jamaica, and one in the Lesser Antilles (Fig. 9). Finally, the cladogram of Caribbean atelids shows one Jamaican species and no unresolved nodes (Fig. 10).

Measures of fit

Table 2 summarizes the measures of fit and statistical significance of the reconciled trees. The number of duplications caused by incongruence between taxon and area cladograms is highest in the *Brachyphylla–Phyllonycteris–Erophylla* clade, and lowest in mormoopids, sloths, caviomorphs, and primates. In nearly every case interpreting 'missing' taxa as primitively

Table 2. Mammalian lineages, biogeographical assumptions, and measures of fit for reconciled trees. Taxonomy follows McKenna & Bell (1997); taxonomy of bats follows Simmons (in press) unless otherwise noted. The interpretation of 'missing' taxa or absence of a clade from a particular area: 'missing data' = considered missing due to lack of data, 'prim. absence' = considered primitively absent. See Methods for explanation on measures of fit and obtaining significance values. I = duplication due to incongruence between taxon and area cladogram, O = duplication due to overlap among taxa. Total nodes includes all nodes seen in the figures except any CeAm-NoSA node among outgroups, codivergent nodes excludes nodes supported by taxa widespread across a node. *$P < 0.05$, **$P < 0.01$

			Duplications			Measures of fit		Measures of codivergence	
Lineage	Widespread taxa	Interpretation of missing taxa	Total	I	O	Leaves added	Losses	Total nodes	Codivergent nodes
Xenarthra									
Choloepodinae	Included	Missing data	6	1	5	14	11	9	3
	Included	Prim. absence	6	1	5	38*	20		
Megalocninae	Included	Missing data	1	1	0	0	0	4	3
	Included	Prim. absence	1	1	0	9	7		
Rodentia									
Antillean	Included	Missing data	11	1	10	19**	13**	16	4
Hystricognathi	Included	Prim. absence	11	1	10	26**	16**		
Chiroptera									
Mormoopidae	Included	Missing data	8	0	8	59*	15**	34	2
	Included	Prim. absence	8	0	8	59*	15**		
	Excluded	Missing data	8	0	8	59*	15**	34	2
	Excluded	Prim. absence	8	0	8	59*	15**		
Brachyphylla–	Included	Missing data	5	3	2	30**	10**	16	2
Phyllonycteris–	Included	Prim. absence	5	3	2	30**	10**		
Erophylla	Excluded	Missing data	3	1	2	18*	4*	15	3
	Excluded	Prim. absence	3	1	2	18*	4*		
Stenodermatina	Included	Missing data	6	2	4	28*	14*	10	2
	Included	Prim. absence	6	2	4	34	16*		
	Excluded	Missing data	6	2	4	28*	14*	9	2
	Excluded	Prim. absence	6	2	4	34	16*		
Natalidae	Included	Missing data	7	3	4	43	20*	13	1
	Included	Prim. absence	7	3	4	51	21**		
	Excluded	Missing data	6	2	4	38	16*	13	2
	Excluded	Prim. absence	6	2	4	45	17*		
Primates									
Atelidae	Included	Missing data	1	1	0	3	3	3	2
	Included	Prim. absence	1	1	0	10	6		

absent increases the number of losses and leaves added to reconcile taxon cladograms with the hypothesis of area relationships of Figure 2. For those groups including widespread taxa, removing them from analyses generally increased the fit between cladograms and area relationships. Choloepodine sloths, caviomorphs and bats showed significant measures of fit, while for other taxa the measures of fit observed were obtained by random chance between 5.3 and 35.9% of the time. The number of leaves in the reconciled area cladogram limits the measure of significance, i.e. in some cases the fit will not be significant simply because there are less than 20 permutations that reconciled the observed area with random taxon cladograms. Information on dates relevant to the reconciled cladograms (Figs 3–10) is summarized in Table 3. No molecular estimates of the divergence of relevant bats were found, and available Caribbean fossils were too recent (Pleistocene, if at all dated) to include in Table 3.

Congruence between taxon and area cladograms

Congruence varies among the taxa examined: of the non-volant mammals choloepodine sloths (Fig. 3) and

Table 3. Estimates of oldest age and latest possible date of arrival of Caribbean non-volant mammals, excluding the Eocene fauna of Jamaica (see 'Other sources of information' under Methods). Taxonomy follows McKenna & Bell (1997), unless otherwise noted

Clade	Previous dated node (Mya)	Location of node	Source	Latest possible date of arrival (Mya)	Location	Source
Choloepodinae	16–21	*Bradypus–Choloepus*	Delsuc *et al.*, 2001	29–34[2]	Puerto Rico	MacPhee & Iturralde-Vinent, 1995
	>40	*Bradypus–Choloepus*	Hoss *et al.*, 1996			
Megalocninae	51–76	Megalonychidae–Vermilingua	Delsuc *et al.*, 2001	29–34[2]	Puerto Rico	MacPhee & Iturralde-Vinent, 1995
	73–88	Cingulata–Pilosa	Hoss *et al.*, 1996	12–15	Cuba	MacPhee *et al.*, 2003
Caviomorpha	*c.* 16	*Octodon*–Other Octodontoidea	Huchon & Douzery, 2001	12–15	Cuba	MacPhee *et al.*, 2003
	31 ± 7	SA Hystricognathi radiation	Huchon, Catzeflis & Douzery, 2000			
	31	SA Hystricognathi fossil remains	Vucetich, Verzi & Hartenberger, 1999			
	33–49	SA–African Hystricognathi	Nedbal, Allard & Honeycutt, 1994			
	71–85	SA–African Hystricognathi	Mouchaty *et al.*, 2001			
Insectivora	None			17–28	Hispaniola	MacPhee & Grimaldi, 1996
Atelid primates	7	*Callicebus* radiation	Purvis, 1995	12–15[3]	Cuba	MacPhee *et al.*, 2003
	13–16	*Callicebus*–extant sister taxon	Schneider *et al.*, 1993			
	29 ± 5	Cebidae–Callitrichidae[1]	Purvis, 1995			

[1]Cebidae = *Pithecia, Cacajao, Chiropotes, Alouatta, Ateles, Lagothrix, Brachyteles*.

[2]A megalonychid fossil from Puerto Rico, unnamed genus and species B (MacPhee & Iturralde-Vinent, 1995: 12) not assigned to either subfamily would constrain the arrival of sloths to before the Early Oligocene (White & MacPhee, 2001 : 211), but it cannot simultaneously represent both groups unless a Caribbean origin for the clade containing extant sloths is postulated.

[3]An Eocene ?primate petrosal from Jamaica (MacPhee *et al.,* 1999) is not included here because this fauna has no continuity till the present. See 'Taxonomic scope' under Methods, for other sources of information.

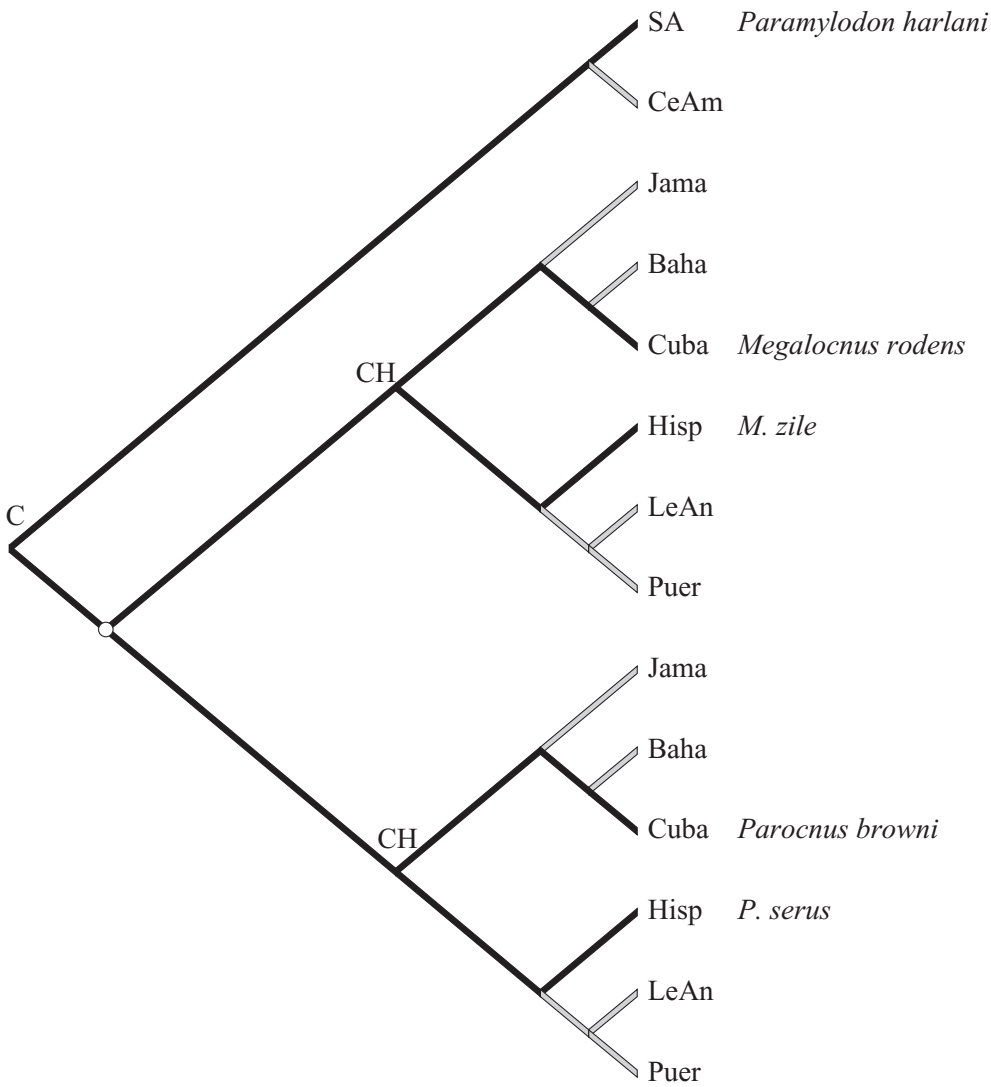

Figure 4. Reconciled tree of the distribution of megalocnine sloths (*sensu* White & MacPhee, 2001), and the extinct mainland outgroup *Paramylodon* (note that this genus is South American but not northern South American). C = mainland–Antillean node. All species are extinct.

rodents (Fig. 4) show no duplications due to incongruence among cladograms (Table 2, note that incongruence in rodents corresponds to artificially resolved node 1). Surprisingly for bats, the mormoopid phylogeny is, using the duplication criterion only, fully compatible with a geological hypothesis of (mostly) vicariant landmasses (Figs 1, 2). Megalocnine sloths (Fig. 4), the remaining bats (Figs 7–9) and primates (Fig. 10) show incongruence between phylogenies and the area cladogram, although the number of duplications is inflated by arbitrarily resolved nodes in *Brachyphylla–Phyllonycteris–Erophylla* (Fig. 7: node 1), Stenodermatina (Fig. 8: node 1), and natalids (Fig. 9: node 1).

DISCUSSION

HISTORICAL ASSOCIATION

It is clear that there is *some* historical association between taxon and area cladograms. This is a necessary but not sufficient condition to postulate cospeciation between areas and taxa because any two trees can be reconciled given an unlimited number of duplications (Page, 1994). The question would then revolve around how much of the diversification among taxa can be ascribed to the geological history. In effect, how many codivergent nodes (i.e. not redundant and not conflicting) are there in each reconciled tree? For the Caribbean, this question involves at least two steps:

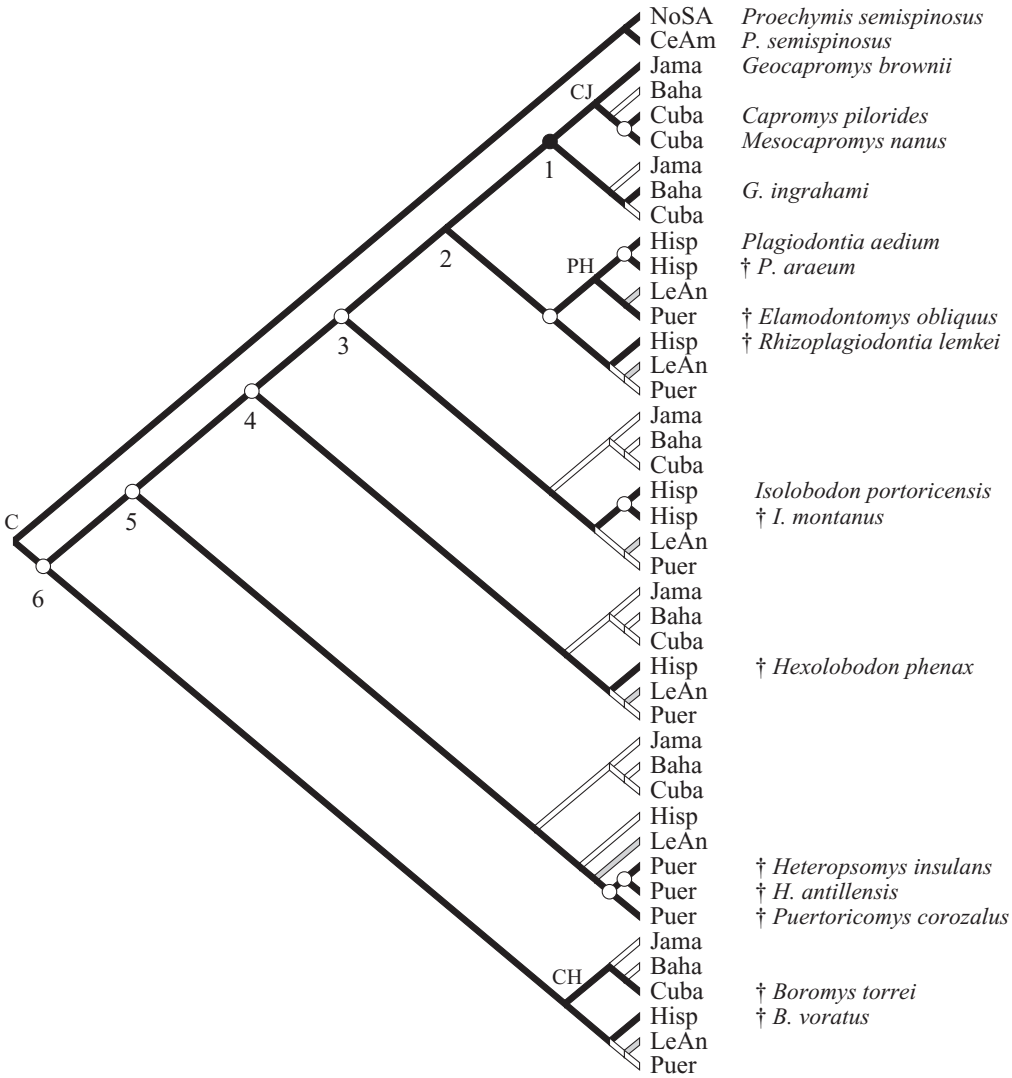

Figure 5. Reconciled tree of the distribution of Caribbean hystricognath rodents (Woods *et al*., 2001) and their mainland outgroup *Proechimys*. †Extinct species. Hereafter numbered nodes are arbitrarily resolved in the taxon cladogram to render the tree amenable to analysis with Component (Page, 1993). CJ = Cuba–Jamaica (dispersal) node, PH = Puerto Rico–Hispaniola node.

(1) nodes that connect the Antilles to the mainland, and (2) inter-island nodes.

Megalocnine sloths (Fig. 4), rodents (Fig. 5), *Brachyphylla–Phyllonycteris–Erophylla* (Fig. 7), and primates (Fig. 10) each show a unique basal nonarbitrary node connecting a monophyletic Caribbean taxon to the mainland (nodes marked C in the figures). All other groups show a mainland–Caribbean component somewhere higher in the reconciled tree (nodes marked c in the figures). There are three inter-island codivergent nodes: the first and most common is the Cuba–Hispaniola component (marked CH in the figures) seen in sloths, rodents, *Brachyphylla–Phyllonycteris–Erophylla*, and primates. The second is the Puerto Rico–Hispaniola node, and the third is the Cuba–Jamaica dispersal node, both seen in rodents (marked PH and CJ in Fig. 5). Note that arbitrarily resolved nodes (marked with numerals in the figures), and taxa widespread across the node examined were excluded from this discussion. The Cuba–Jamaica node, a hypothesized dispersal, is also supported by widespread distributions of mormoopid, *Erophylla*, *Brachyphylla*, and *Chilonatalus* bats.

One node inferred from geological studies finds support only among bats: the Cuba–Bahamas relationship seems characterized by the widespread distribution of *Pteronotus*, *Mormoops*, *Phyllonycteris*, *Brachyphylla*, *Natalus*, and *Nyctiellus*. The Puerto

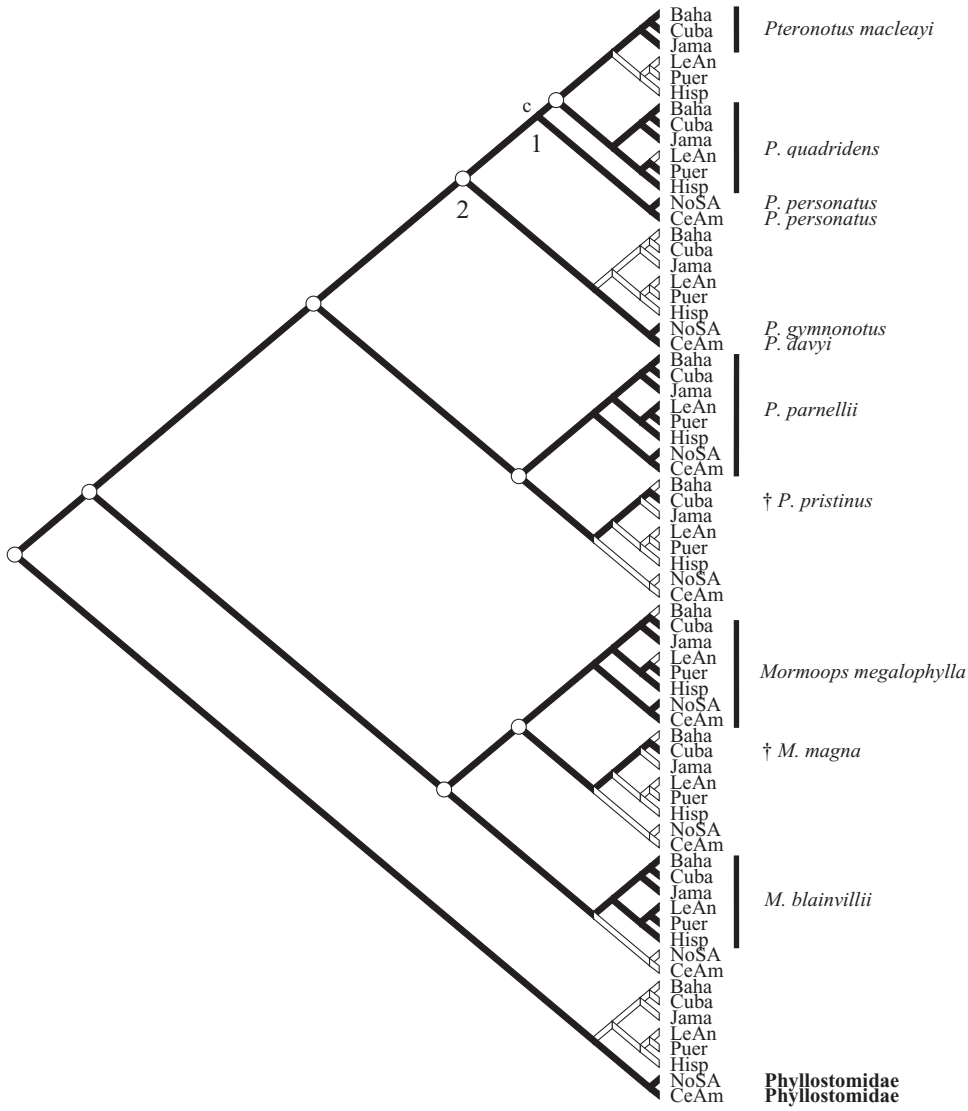

Figure 6. Reconciled tree of the distribution of mormoopids. See Methods for sources of the phylogeny.

Rico–Lesser Antilles node, another hypothesized dispersal, is only seen among bats in the widespread distribution of *Pteronotus parnellii*, *Mormoops blainvillei*, *Phyllonycteris major*, and *Brachyphylla cavernarum*. The last three species would have followed a dispersal route south from Puerto Rico, as hypothesized in the area cladogram of Figure 2, since the remainder of their populations is exclusively Antillean (Figs 6, 7). In contrast, *Natalus stramineus* shows a relationship between the continent and the Lesser Antilles pointing to dispersal from northern South America (Fig. 9), more in agreement with the biogeographical hypothesis of Hedges and colleagues.

For primates the position of *Xenothrix* as sister to other Caribbean primates contradicts the dispersal Cuba–Jamaica node proposed in Figure 2. Rather, the phylogeny (Fig. 10) implies a common ancestor present in Cuba, Hispaniola, and Jamaica, potentially compatible with the hypothesis of interconnection between the Blue Mountains (see 'Geological area cladograms' under Methods) and other Caribbean landmasses.

An overall comparison of total nodes vs. codivergent nodes shows that the highest proportion of the latter is found among non-volant mammals, between 25% and 75%. The proportion among bats only ranges from 6% to 20%, although excluding widespread taxa generally increases it (see Table 2).

For non-volant taxa, the lowest codivergence is among rodents, whose poorly resolved phylogeny may obscure greater incongruence or codivergence, particularly given the effects of outgroup choice on a clade

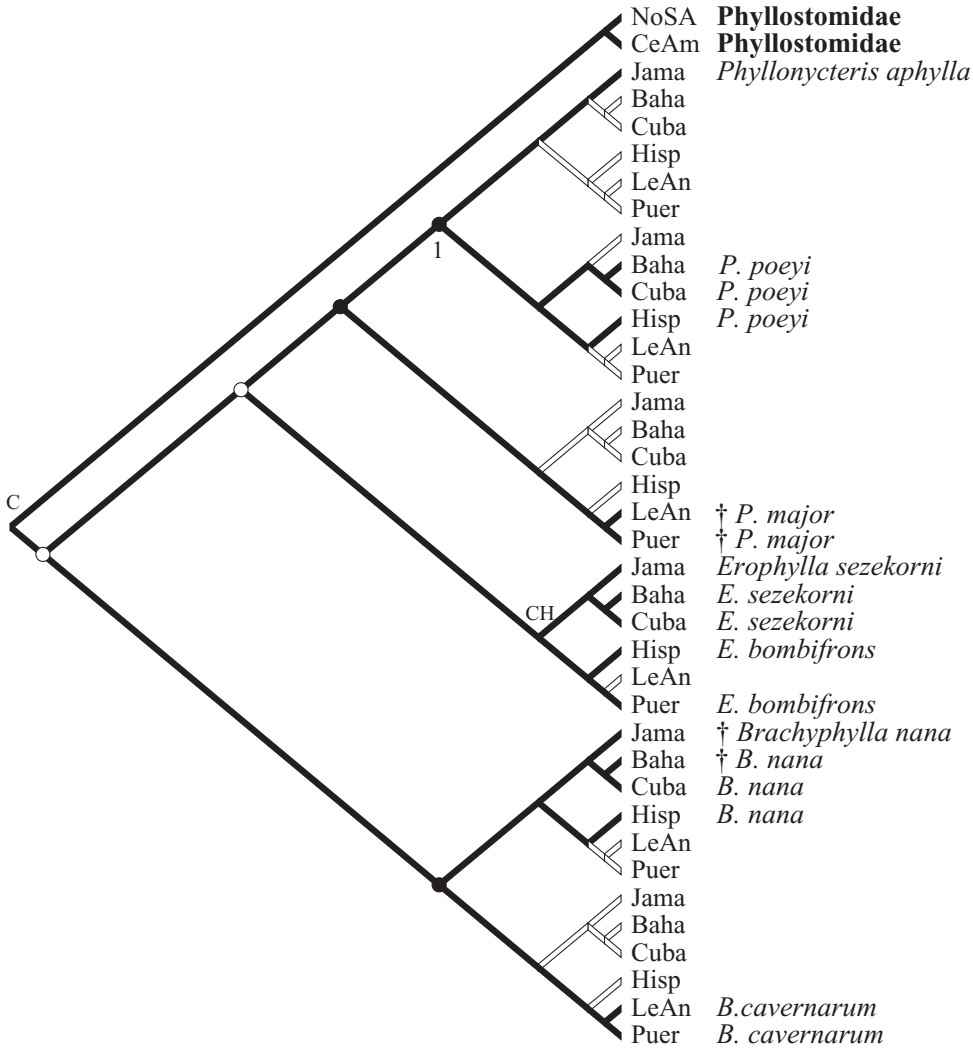

Figure 7. Reconciled tree of the distribution of *Brachyphylla*, *Phyllonycteris*, and *Erophylla*, obtained from Jones *et al*. (2002). See Discussion for alternative phylogenetic hypotheses.

with many other taxa to choose from (see Woods *et al*., 2001: 346–347). The choice of *Proechimys* is logical, but a rigorous test of the monophyly of the Caribbean group would require denser sampling from among the hystricognath rodents of South America, including the extant families Myocastoridae, Octodontidae, and Ctenomyidae, in addition to Echimyidae (Nedbal *et al.*, 1994). Only one heptaxodontiid, *Elasmodontomys obliquus*, was included so the monophyly of the giant hutias was not tested in the analyses of Woods *et al*. (2001), although Flemming & MacPhee (1996) present evidence for the monophyly of the group (but see Pascual, Vucetich & Scillato-Yané, 1990) for a dissenting view regarding *Clidomys*).

Although there is greater resolution, the phylogeny of choloepodine sloths (Fig. 3) is also unstable since there are almost 100 genera of mostly South American extinct sloths (McKenna & Bell, 1997), so taxon sampling will probably modify this phylogeny (White & MacPhee, 2001). Gaudin's (1995) cladistic analysis for 21 genera, for instance, is consistent with White & MacPhee's (2001) hypothesized sister relationship between *Choloepus* and *Acratocnus* but conflicts in every other respect with their phylogeny. Finally, the phylogeny of Antillean primates is also fluid: the description of *Paralouatta marianae* from Cuba (MacPhee, Iturralde-Vinent & Gaffney, 2003) already brings the proportion of codivergent nodes from 67% down to 50%.

For bats the proportion of codivergence is low, particularly among mormoopids, a group whose phylogeny is supported by morphological (Simmons & Conway, 2001), mitochondrial, and nuclear DNA character analyses (Lewis-Oritt *et al.*, 2001; Van Den

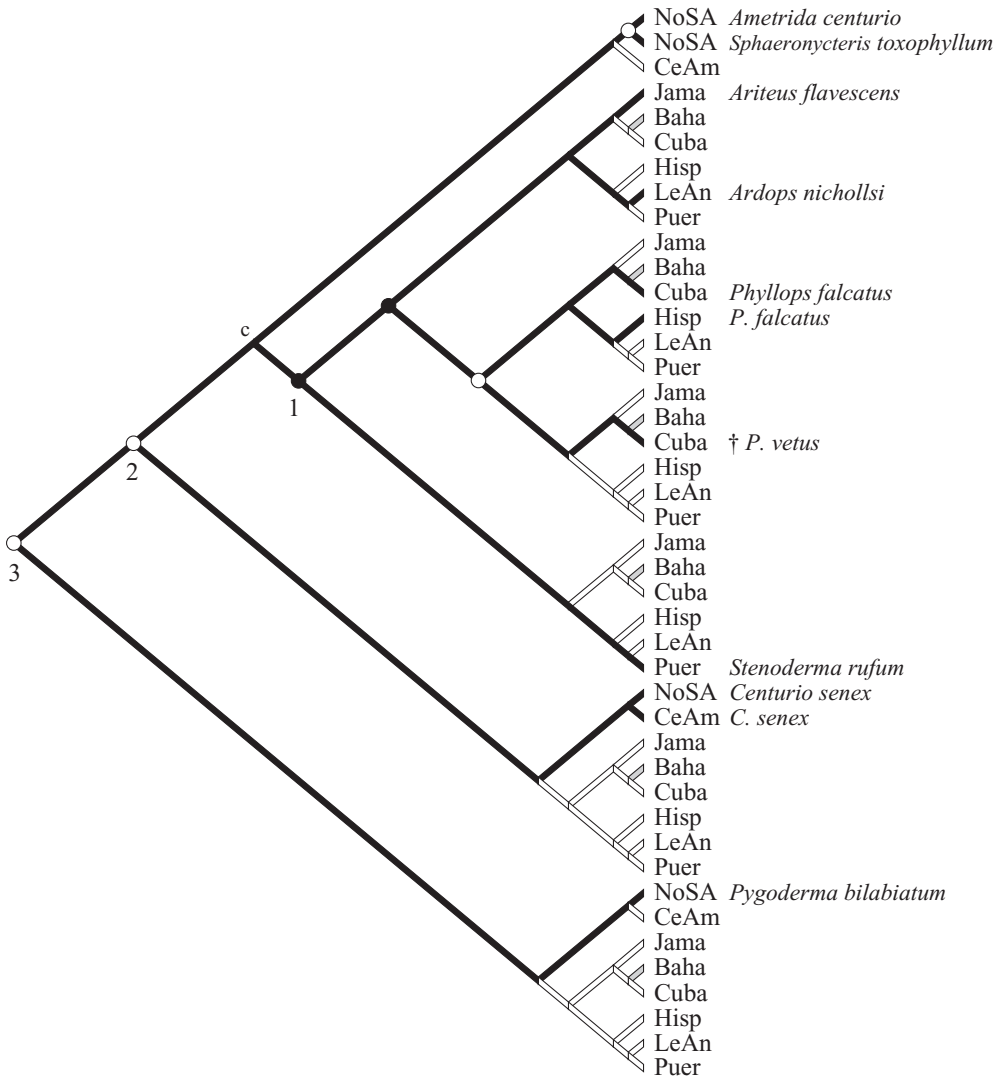

Figure 8. Reconciled tree of the distribution of the phyllostomid tribe Stenodermatina (*sensu* Wetterer, Rockman & Simmons 2000), obtained from Jones *et al*. (2002). See Discussion for alternative phylogenetic hypotheses.

Bussche, Hoofer & Simmons, 2002). Natalids show only a marginally better proportion of codivergence, despite the artefact of placing *Primonatalus prattae* in Central America rather than Florida, where it was found (Morgan & Czaplewski, 2003). This artefact is used solely to fit the cladogram to the test of the Gaarlandia geological hypothesis, because the Early Oligocene natalid remains from Florida (Morgan & Czaplewski, 2003) suggest a North American origin for the group.

The two phyllostomid clades analysed will probably be revised in the future: the taxon cladograms used to generate Figures 7 and 8 were taken from a matrix representation with parsimony supertree by Jones *et al*. (2002). The supertree analysis placed the endemic Antillean genera *Brachyphylla*, *Erophylla*, and *Phyllonycteris* within a single radiation. Recent (mostly) morphological (Wetterer, Rockman & Simmons, 2000) and molecular (Baker *et al*., 2000) phylogenetic hypotheses did not independently recover this topology. For the Stenodermatina, a monophyletic Antillean clade (Jones *et al*., 2002) was consistent with Wetterer *et al*.'s (2000) morphological hypotheses, but was not supported by Baker *et al*.'s (2000) molecular analysis (which excluded *Phyllops*).

Among all lineages the inclusion of fossils contributed to the resolution of area relationships, not only when all Antillean representatives are extinct as with sloths and primates, but also when most species are extinct, i.e. hystricognath rodents. Among bats, the inclusion of fossils alters the interpretation of missing areas substantially: mormoopids are extinct from the

Figure 9. Reconciled tree of the distribution of natalids, obtained from Morgan & Czaplewski (2003).

Bahamas and Lesser Antilles, *Phyllonycteris* from the Lesser Antilles, and *Natalus* from the Bahamas. Without these remains their absence would be interpreted as primitive (for an example of primitive absence given current knowledge see Puerto Rico in Fig. 9).

Temporal compatibility

The evaluation of congruence has hitherto focused on spatial and speciation patterns, with no regard for the temporal frame for Caribbean diversification. The geological hypotheses outlined in the Introduction, however, constrain the interconnection among landmasses as summarized in Figure 1.

The timing of divergence between *Bradypus* and *Choloepus* has been estimated (Table 3) at 16–21 Mya (Delsuc *et al*., 2001), or >40 Mya (Hoss *et al*., 1996). The former estimate would not be compatible with the Early Oligocene interconnection postulated in Figure 1, but the latter would. Delsuc *et al*. (2001) attributed the difference among age estimations to poor taxonomic sampling in previous studies, although a similar case could be made for poor taxon sampling of extinct groups altering estimations of phylogeny and divergence dates within this recent xenarthran molecular phylogeny. If megalonychid B of MacPhee & Iturralde-Vinent (1995) is a choloepodine, given the phylogeny in Figure 3, the estimate of Delsuc *et al*. (2001) must be wrong. Conversely, if megalonychid B is a megalocnine, there is no conflict between molecular divergence dates and Caribbean fossil evidence. The older limit of the divergence between Antillean megalocnines and their South American relatives, given the phylogeny of White &

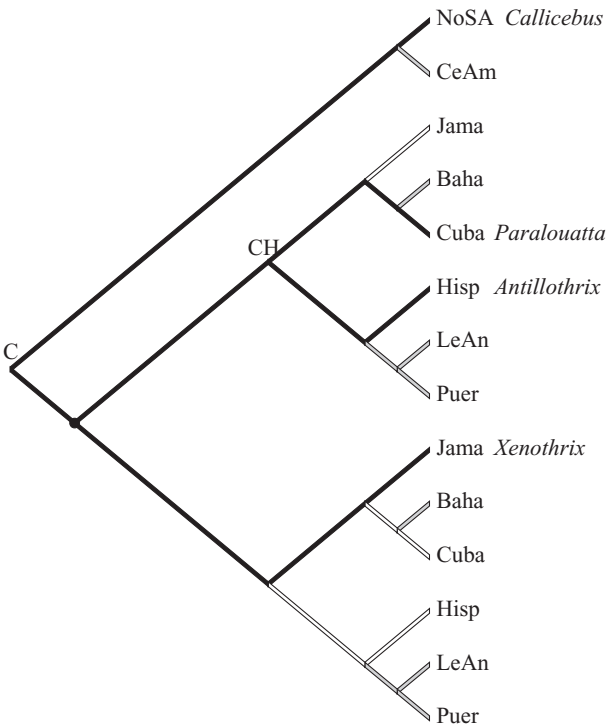

Figure 10. Reconciled tree of the distribution of Caribbean primates, obtained from Horovitz & MacPhee (1999). All Caribbean primates are extinct and *Callicebus* is extant in South America.

MacPhee (2001), would be given by the estimates of divergence among major xenarthran lineages (Table 3).

For rodents there are several, sometimes contradictory, estimates of divergence relevant to the Caribbean hystricognath clade (Table 3). Huchon, Catzeflis & Douzery (2000) dated the caviomorph radiation to the Early Oligocene (Table 3), around the same time as the hypothesized Oligocene Caribbean–South American interconnection of Figure 1. This estimate is based on a single nuclear gene and well supported by cross-calibration with higher-level mammalian divergences (Huchon *et al*., 2000), but incompatible with dating estimates from one additional nuclear and one mitochondrial gene (Honeycutt, Rowe & Gallardo, 2003). Because Honeycutt *et al*. (2003) obtain a radically different topology from that of Huchon and colleagues, estimates by the former cannot be directly compared to those of the latter and are not included in Table 3.

The Early Oligocene date for the caviomorph radiation is compatible with an Early to Middle Eocene divergence between African and South American hystricognaths (Huchon & Douzery, 2001). Other molecular estimations push the intercontinental hystricognath divergence back to the Cretaceous (Table 3, Mouchaty *et al*., 2001; but see Huchon *et al*., 2002), opening the possibility of an even earlier South American hystricognath radiation and perhaps indicating that the debate surrounding the topology and age of the diversification will continue.

The extinct Caribbean murids *Megalomys* and *Oryzomys* are thought to have reached the Lesser Antilles from South America (for a recent dispersalist argument see McFarlane & Lundberg, 2002), except for the Jamaican *Oryzomys antillarum*, a presumed Central American disperser (Woods, 1989b; McFarlane, Lundberg & Fincham, 2002). Since murids are unknown from northern South America until the Early Pliocene, their entry into the Antilles is presumed to have occurred after this time (Morgan & Woods, 1986). Until their relationships are resolved, however, the number and age of colonization events associated with these taxa remains untested.

The only recent phylogenetic analysis to include both Antillean insectivorans – *Solenodon* and *Nesophontes* – concluded that '[a]dditional data are necessary to better resolve Caribbean lipotyphlan phylogeny' (Asher, 1999: 239). This, and the ever-growing controversy over insectivoran phylogeny (Springer *et al*., 1997; Stanhope *et al*., 1998a, b; Emerson *et al*., 1999; Liu & Miyamoto, 1999; Mouchaty *et al*., 2000; Murphy *et al*., 2001; Malia, Adkins & Allard, 2002), precluded a reconciled tree analysis. There have been no attempts to date the divergence of *Solenodon* or *Nesophontes* from other insectivorans. An insectivoran fossil preserved in amber traces the occurrence of insectivores on the Antilles minimally to the Late Oligocene/Early Miocene of Hispaniola (Table 3).

Whidden & Asher (2001) reviewed the phylogenetic and biogeographical data on insectivorans, concluding that four biogeographical hypotheses are still viable: (1) overwater dispersal of an Early Tertiary North American insectivore related to *Apternodus* or the geolabidid *Centetodon*; (2) vicariance of an Early Tertiary North American insectivoran on the Western Jamaica Block with subsequent dispersal to the Greater Antilles; (3) dispersal of a Gondwanan insectivoran from Africa, and (4) dispersal of a Gondwanan insectivoran from Africa via South America.

The viability of hypothesis 2 rests exclusively on the Seven Rivers Eocene fauna of Jamaica (Domning *et al*., 1997; MacPhee *et al*., 1999). This mammal fauna has no continuity till the present (see 'Other sources of information' under Methods, above). The phylogenetic hypotheses of Liu *et al*. (2001) and Stanhope *et al*. (1998a) favour hypothesis 3 over hypothesis 4 because insectivoran remains appear in South America only after the faunal exchange linked to the closing of the Panamanian landbridge. Since the latter

phylogenies did not include extinct taxa, hypothesis 1 also remains viable.

Among bats, the dearth of Tertiary fossils and limited taxon sampling in higher-level phylogenies aimed at dating nodes have limited the dates available for analysis. For *Brachyphylla–Erophylla–Phyllonycteris* the single radiation hypothesis would imply that the ancestor of the Antillean clade and the ancestor of New World nectar-feeding bats (glossophagines and lonchophyllines) are the same age (South American glossophagines are at least 11–13 My old according to Czaplewski, 1997). Morphological (Wetterer *et al*., 2000: fig. 49) studies show the ancestor of *Phyllonycteris* and *Erophylla* to be as old as the New World nectar-feeding bats, while the unresolved position of *Brachyphylla* precludes any estimation of age for this clade. Molecular analyses (Baker *et al*., 2000: fig. 2) recover a different topology and are therefore incompatible with this date estimation. The oldest natalid is an unnamed Early Oligocene fossil from Florida, although only the Early Miocene *Primonatalus prattae* is included in phylogenetic analyses (Morgan & Czaplewski, 2003: fig. 9). The Early Oligocene natalid remains in Florida, together with the relatively novel hypothesis of phylogenetic relationships linking the Natalidae to Vespertilionidae and Molossidae (see Morgan & Czaplewski, 2003: 746 for a complete review), suggest a North American origin for this group.

Among primates, the older limit for the Caribbean radiation would be given by the Cebidae–Callitrichidae divergence (Table 3), estimated to have occurred around or after the proposed Caribbean–South America interconnection of Figure 1. Because the phylogenetic hypothesis in Figure 10 conflicts with the supertree of Purvis (1995) this date estimation might not be informative on the age of the Caribbean primate clade. In contrast, the phylogeny of Schneider *et al*. (1993) is compatible with the taxon cladogram of Figure 10, but the estimate of divergence (Table 3) is too recent to be compatible with the Gaarlandia hypothesis.

Dispersal vs. vicariance?

The evidence posited in mammalian phylogenies does not add to the argument of dispersal vs. vicariance. One pattern, the entry into the Caribbean from South America between the Palaeocene and the Middle Miocene, accounts for the distribution and phylogeny of the majority of lineages studied. Of these, few lineages fit the predictions of the Gaarlandia hypothesis in timing, and none regarding the divergence among Antillean landmasses. The remaining lineages show patterns that are inconsistent with the Gaarlandia hypothesis and require taxon-specific hypotheses.

The distribution of more than half of the groups studied – megalocnine sloths (Fig. 4), hystricognath rodents (Fig. 5), *Brachyphylla–Phyllonycteris–Erophylla* (Fig. 7), Stenodermatina (Fig. 8), and primates (Fig. 10) – can be explained by a single ancestral colonization of the Antilles from South America. In one case, megalocnine sloths, molecular divergence estimates are also consistent with the Gaarlandia hypothesis (Fig. 1). For hystricognath rodents and primates, molecular dating is not completely consistent, but could fall within the range of the Gaarlandia land bridge (Table 3). Finally, the phylogenetic instability and absence of Tertiary fossils for *Brachyphylla–Phyllonycteris–Erophylla* and Stenodermatina inhibit speculation on their time of arrival in the West Indies.

Choloepodine sloths (Fig. 3), mormoopids (Fig. 6), and natalids (Fig. 9) all show patterns that can only be partially reconciled (e.g. *Acratocnus*, Fig. 3), if at all, with the geological hypotheses underpinning Figure 1. The most recent molecular divergence estimates would make an Early Oligocene entry to the Caribbean by choloepodine sloths impossible (Table 3). The history of mormoopids (Fig. 6) points to repeated dispersal (at least twice in the *Pteronotus* clade) to the Caribbean from Central America or South America, e.g., *Pteronotus parnellii*, *Mormoops megalophylla*. Natalids invert the pattern of entry into the Caribbean: the current phylogeny points to a tropical North American ancestor whose range included Cuba and from there dispersed south to northern South America (Fig. 9).

The Cuba–Hispaniola divergence of Figure 1 dates back to the Late Oligocene, and has been suggested to be a common cause for speciation in bats (Griffiths & Klingener, 1988). This pattern of area relationships is seen in *Acratocnus* and *Neocnus* (Fig. 3), *Megalocnus* and *Parocnus* (Fig. 4), *Boromys* (Fig. 5), *Erophylla* (Fig. 7), and primates (Fig. 10), and may correspond to the resolution of Greater Antillean *Natalus* (Fig. 9). A Late Oligocene divergence, however, cannot be reconciled with the species or generic-level differentiation observed in the phylogenies and hence cannot account for this pattern. Concordant dispersal in all these taxa, or a more recent history of interconnection and separation, are more suitable explanations for this pattern.

With the exception of murid rodents, all non-volant lineages (including insectivorans: Table 3) reached the Caribbean before the Middle Miocene (MacPhee *et al*., 2003), and sloths arrived as early as the Early Oligocene (MacPhee & Iturralde-Vinent, 1995). Assuming the Chicxulub impact effectively wiped out a previous fauna, the arrival of non-volant mammals in the Antilles would be restricted to the period between the Palaeocene and the Middle Miocene. This limited window of opportunity for non-volant mammalian

lineages to colonize the Caribbean requires an explanation.

The Gaarlandia land interconnection proposed by Iturralde-Vinent & MacPhee (1999) can account for the pattern of speciation of megalocnine sloths, hystricognath rodents, two bat lineages and primates, as well as the timing of divergence from the mainland of these groups, with the exceptions of bats and perhaps hystricognath rodents and primates. The distribution of choloepodine sloths, murid rodents, insectivorans, and natalid bats has to be explained by different hypotheses: dispersal or other land interconnections, including the probable North American origin of insectivorans and natalids.

In principle, a mechanism that facilitated dispersal over the colonization period could explain the cluster of arrivals. This differs from the hypothesis of Hedges and colleagues because they have proposed numerous dispersal events into the Greater Antilles from South America throughout the Cenozoic following prevailing oceanic currents. An alternative explanation might be to accept the core of their dispersal hypothesis but find additional hypotheses to account for the lack of successful dispersal of non-volant mammals to the Caribbean between the Middle Miocene and the Present.

CONCLUSIONS

An analysis of current phylogenetic evidence finds that the geological hypotheses of Iturralde-Vinent & MacPhee (1999) can in principle account for the relationships between the mainland and the Caribbean species of megalocnine sloths, hystricognath rodents, *Brachyphylla* and allies, Stenodermatina, and primates. However, the distribution and/or proposed phylogenies of choloepodine sloths, insectivorans, mormoopids, and natalids cannot be reconciled with this model and require a different explanation for their distribution. Higher-level phylogenies for all the former groups also reveal contradictory evidence regarding the time of arrival to the Caribbean. As calibration of different molecular clocks converges, the evidence for the hypothesized Gaarlandia as an explanation of mammalian distribution will have to be reassessed.

The robustness of these biogeographical inferences is highly sensitive to the phylogenetic information, which remains incomplete for all groups examined. More taxon sampling is necessary among sloths and hystricognath rodents, and Caribbean murids and insectivorans both require species-level phylogenies. The stability of phyllostomid phylogenies seems to require further character sampling across many lineages, while the reconstruction of biogeographical history for mormoopids and natalids could benefit from population-level analyses to clarify routes of dispersal. Among primates, the examination of all available fossil remains is necessary to clarify the status of numerous lineages not included in current analyses.

The inter-island patterns of speciation, evaluated by measuring codivergent nodes, do not conform to the geological hypotheses of Iturralde-Vinent & MacPhee (1999) although improvements in phylogenetic inference (see above) will probably reveal patterns currently obscured by poor character and taxon sampling. The search for Antillean and tropical American Tertiary mammal fossils over the last decade has shown that most non-volant Caribbean mammalian lineages arrived in the West Indies before the Middle Miocene. With the interconnection hypothesis of Iturralde-Vinent & MacPhee (1999) only accounting for part of the window of colonization that seems to have existed, other hypotheses – facilitated dispersal or other means of interconnection – remain viable as explanations for this hitherto unnoticed pattern.

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Phylogeny and biogeography of the Mormoopidae

ABSTRACT

Several recent studies have attempted to resolve relationships within the neotropical family Mormoopidae. Available molecular and morphological data from these studies were used to generate a best-supported phylogeny for the group using maximum likelihood and parsimony analyses. Additionally, mitochondrial sequences were used to assess character differences among mormoopid populations currently considered conspecific. Fixed character differences in cytochrome *b* and morphological characters imply that species diversity within the mormoopids has been underestimated, with *Pteronotus parnellii* containing as many as six distinct phylogenetic units. Single-cladogram biogeographic methods were used on the resulting phylogenetic hypotheses to reconstruct ancestral distributions. These analyses suggest that the mormoopids entered South America recently, after most diversification had taken place in Mexico, Central America and the Greater Antilles. In placing the mormoopid ancestral area in the northern Neotropics, these results are congruent with recent phylogenetic hypotheses for the sister taxon to the Mormoopidae, the family Phyllostomidae.

INTRODUCTION

Mormoopidae is a neotropical family of bats characterized by flap-like outgrowths of skin below the lower lip and funnel-shaped ears (Simmons and Conway, 2001). The two extant genera, *Mormoops* and *Pteronotus*, range from Texas south through Mexico, Central America, the West Indies, northern South America to the Mato Grosso of Brazil, and west of the Andes to Peru (Koopman, 1994). The geographic ranges of all currently

recognized mormoopid species are summarized in Table 1. An undescribed fossil *Pteronotus* is known from the Pleistocene of Hispaniola (Morgan, 2001), and mormoopid remains have also been reported from the Early Oligocene of Florida (Czaplewski et al., 2003). Geographic variation within all but two of the extant species, *P. gymnonotus* and *M. blainvillei*, is recognized by partition into more than 20 subspecies (see Table 2).

Mormoopids live in habitats ranging from humid tropical forest to semiarid and arid subtropical forest and scrubland (Emmons, 1997; Handley, 1976; Smith, 1972). Mormoopids are gregarious cave dwellers, sometimes roosting in colonies of more than 40,000 individuals (Rodriguez-Durán and Lewis, 1987) and their distribution is probably limited by the availability of caves (Morgan, 2001), although *P. parnellii* also occurs in regions without them (Simmons and Voss, 1998). Mormoopid bats appear to be exclusively insectivorous (Koopman, 1994).

The long history of the taxonomy and systematics of the group has been reviewed by Simmons and Conway (2001) and Smith (1972) and will not be revisited here. Interest in the phylogeny of the Mormoopidae has recently flourished, with morphological (Simmons and Conway, 2001), molecular (Lewis Oritt et al., 2001; Van Den Bussche and Weyandt, 2003), and combined analyses (Van Den Bussche et al., 2002b) published in rapid succession. This flurry of activity follows almost two decades during which the standard reference for understanding relationships within the group was the systematic revision of Smith (1972).

The New World families Mormoopidae, Phyllostomidae and Noctilionidae, and the New Zealand Short-tailed bat *Mystacina tuberculata* (Mystacinidae), constitute the noctilionoid clade (Kennedy et al., 1999; Kirsch et al., 1998; Pierson et al., 1986; Van

Den Bussche and Hoofer, 2000). The consensus that emerged from higher-level phylogenetic studies over the last 20 years established Mormoopidae as sister to Phyllostomidae (Simmons and Conway, 2001; Teeling et al., 2003; Van Den Bussche and Hoofer, 2000). Subsequently, different sources of data have supported the monophyly of mormoopids, and of *Mormoops* and *Pteronotus* (Lewis Oritt et al., 2001; Simmons and Conway, 2001; Van Den Bussche et al., 2002b).

In this study, new mitochondrial DNA sequences were combined with published data sets to assess character differences among mormoopid populations currently considered conspecific. Morphological and molecular data sets were analyzed separately and in combination to generate a robust phylogeny for the family, and historical biogeography methods were applied to the results of those analyses. The ultimate goal was to provide a framework for subsequent comparative biogeography analyses in the Caribbean neotropical region.

MATERIALS AND METHODS

Taxon Sampling

To examine relationships among the mormoopids, both genera and 18 of the 22 currently recognized subspecies (Simmons, in press) were included in this study. A list of taxa with sequences included in this study is given in Table 2, the complete GenBank numbers and vouchers cited here are listed in Appendix 1. Most cytochrome *b* sequences, and all 12S, $tRNA^{val}$, 16S genes (the last three hereafter referred to as mitochondrial ribosomal DNA or mtrDNA) and *Rag*2 sequences were obtained from previously published studies (Lewis Oritt et al., 2001; Van Den Bussche and Hoofer, 2001; Van Den Bussche et al., 2002b). Cytochrome *b* sequences generated for this study are listed below. The first

number corresponds to the DNA sample at the Ambrose Monell Cryogenic Collection (AMCC) and the second number identifies the voucher specimen at the Mammalogy Department, both at the American Museum of Natural History (AMNH). The collecting locality follows the specimen numbers of each individual, in parentheses. *Mormoops blainvillei*— (102762, 274611): Jamaica, Westmoreland, Revival, Monarva Cave; *Pteronotus parnellii*— (110395, 269115): French Guiana, Cayenne, Paracou, near Sinnamary; (103048, 269115): Dominican Republic, María Trinidad Sánchez, La Entrada (de Cabrera); (103050, 275497): Dominican Republic, María Trinidad Sánchez, La Entrada (de Cabrera); (102714, 274627): Jamaica, St. Catherine, Polly Ground, St. Clair Cave; *Pteronotus quadridens*— (103036, 275500): Dominican Republic, María Trinidad Sánchez, La Entrada (de Cabrera); (102335, wing puncture): Puerto Rico, Arecibo, Mata de Plátano; *Pteronotus macleayii*— (102719, 274632): Jamaica, St. Catherine, Polly Ground, St. Clair Cave.

For outgroup comparison, sequences from *Mystacina tuberculata* (AF263222, AY141021, AF144068), *Noctilio leporinus* (AF263224, AF316477, AF330796), *Noctilio albiventris* (AF263223, AF330810, AF330803), and *Artibeus jamaicensis* (NC002009, AY011963) were used. To root the tree, sequences from *Saccopteryx bilineata* (AF263213, AY141015, AF044664) were included in phylogenetic analyses.

Molecular Data

For all specimens, DNA was isolated from wing clip (Rossiter et al., 2000) or liver tissue that had been frozen or preserved in ethanol or lysis buffer in the field. DNA was extracted using a Qiagen DNeasy Tissue Extraction Kit (Qiagen, Inc.) following the manufacturer's protocol. Extracted DNA was used as a template in PCR reactions with

protocols and primers for cytochrome *b* described elsewhere (Dávalos and Jansa, in press; Jansa et al., 1999). Amplification products were sequenced with the same primers used for PCR amplification and also internal primers. Sequencing reactions were purified through a $MgCl_2$-ethanol precipitation protocol and run on an ABI 3100 automated sequencer. Sequences were edited and compiled using Sequencher 4.1 software (GeneCodes, Corp.). Base-calling ambiguities between strands were resolved either by choosing the call on the cleanest strand or using the appropriate IUB ambiguity code if both strands showed the same ambiguity. Molecular sequences generated as part of this study have been deposited in GenBank under accession numbers AY604454-AY604462 (Appendix 1).

Morphological Data

The morphological character matrix of Simmons and Conway (2001) was appended to the molecular data to generate combined analyses of all characters available for the group, and investigate character conflict among different sources of data. The concatenation of molecular and morphological data is justified because Simmons and Conway (2001) examined specimens of all recognized subspecies.

Data Analysis

Protein-coding cytochrome *b* and *Rag*2 sequences were easily aligned by eye using Sequencher 4.1 (GeneCodes, Corp.). Unlike those two genes, mtrDNA does not code for protein products and sequence length varies among taxa and even individuals. Positional homology of the sequence alignment is an assumption of phylogenetic analysis (Swofford et al., 1996). CLUSTAL W (Thompson et al., 1994) was used to infer sequence homology in these sequences, with a gap/substitution penalty of 10:1. The resulting

alignment was adjusted manually based on secondary structure models that take into account the functional role of these mitochondrial regions in protein synthesis (Burk et al., 2002; Springer and Douzery, 1996). Where sequence homology could not be unambiguously established given these parameters, the characters were excluded from subsequent phylogenetic analyses.

To describe the variation in cytochrome *b* among taxa uncorrected pairwise (*p*) distances were calculated using PAUP* 4.0b10 (Swofford, 2002). Cytochrome *b* sequences were also examined for fixed character differences among putative taxonomic units, subspecies *sensu* Smith (1972). Sequences of each subspecies were compared against sequences of other subspecies in the same species, and the number of fixed character differences that distinguished them was scored.

Parsimony analyses of the morphological, cytochrome *b*, mtrDNA, and *Rag*2 datasets were performed separately and on combined matrices using branch and bound searches as implemented in PAUP* 4.0b10. For each search, phylogenetically informative characters were treated as unordered and equally weighted, and gaps were treated as missing data. Clade stability was assessed using nonparametric jackknife (Wu, 1986) and the Bremer support index (Bremer, 1994). All parsimony jackknife analyses included 1,000 replicates; searches were heuristic with 10 replicates of random taxon addition followed by TBR branch swapping. Bremer values were calculated with the aid of AutoDecay (Eriksson, 1999). Character state changes and length of alternative topologies were explored using MacClade 4.06 (Maddison and Maddison, 2003).

The Templeton (1983) test implemented in PAUP* 4.0b10 was used to assess topological incongruence among cytochrome *b*, mtrDNA, *Rag*2, and the morphological

dataset. This test assesses whether topologies differ significantly on how well they fit a data partition. Exemplars of each data set were used to generate topologies with identical terminals for each data set and compare resulting trees. Best-fit maximum likelihood models for molecular data were selected using nested likelihood ratio tests as implemented in MODELTEST (Posada and Crandall, 1998). Maximum likelihood analyses of the different molecular data sets were performed using PAUP* 4.0b10.

Finally, the constancy in rates of molecular evolution throughout the tree was evaluated. To provide the most conservative test for a clock-like model of evolution, a UPGMA tree based on Jukes-Cantor distances was calculated, and the likelihood scores of the best-fit model without enforcing the clock ($\log L_1$), and the same model forcing a clock ($\log L_2$) were compared. The significance of the difference in likelihood scores was tested by comparing $-2\log\lambda$ against a χ^2 distribution with degrees of freedom equal to the number of taxa minus 2. If the value for $-2\log\lambda$ was significant, then the molecular clock could be rejected. Subsequent to model evaluation and selection, the maximum likelihood tree was determined using a heuristic search in which the parameter values under the best-fit model were fixed and a neighbor-joining tree was used as a starting point for TBR branch swapping. Likelihood nonparametric jackknife analyses included 300 replicates, with a neighbor-joining starting tree followed by subtree pruning re-grafting (SPR) branch swapping in heuristic searches.

Biogeographic Analyses

Smith (1972) proposed that Greater Antillean mormoopids reached the Caribbean through Jamaica via Honduras, or Cuba via Yucatán, with *P. quadridens* and *P. macleayii* being the oldest clade, the Caribbean *P. parnellii* the most recent, and *M.*

blainvillei's age being somewhere in between. Subsequent authors adopted this view of continental dispersal into the Caribbean and this is the prevalent way of explaining species distribution and diversification in the region (e.g., Baker and Genoways [1978], Koopman [1989]). Smith (1972) also posited that the closest mainland relative of *P. quadridens* and *P. macleayii* was *P. personatus*. Finally, Smith (1972) restricted the ancestral area of mormoopids to southern Central America or northwestern South America.

Fossil mormoopids are known from the late Pleistocene of Florida, the Greater Antilles, Mexico and Brazil (Arroyo-Cabrales, 1992; Czaplewski and Cartelle, 1998; Morgan, 2001; Morgan, 1991), the early Pleistocene of El Salvador (Webb and Perrigo, 1984), and the late Oligocene of Florida (Czaplewski and Morgan, 2003). Czaplewski and Morgan (2003) proposed that mormoopids expanded their range to the Greater Antilles early in their history, after diversifying in Central America. Conversely, the expansion of their range into South America would have been relatively recent, after the completion of the Panama land bridge in the Pliocene (Czaplewski and Morgan, 2003). This is an alternative biogeographic scenario for mormoopid biogeography.

In this study, biogeographic hypotheses regarding mormoopid bats were compared to the results of phylogenetic analyses from different data sources by counting the number of additional steps necessary to constrain a particular result and by estimating ancestral areas for particular nodes. Two biogeographic methods were used to estimate ancestral areas: dispersal-vicariance analysis (DIVA; Ronquist [1997]), and ancestral area analysis (Bremer, 1992). Dispersal-vicariance analysis does not require an independent hypothesis of area relationships, but instead reconstructs the ancestral distribution at each

of the internal nodes of a given phylogeny. The estimation can be constrained to contain any minimum number of areas. This is accomplished by means of optimization rules and set costs for extinction (cost of 1 per area lost) and dispersal (cost of 1 per area added). Vicariant and sympatric speciation carry no cost. Species distributions are therefore explained by assigning costs for each event in a way that biogeographic explanations imply the least possible cost. Ancestral area analysis (Bremer, 1992) allows the identification of the ancestral area of a group based on its phylogeny, given geographic distribution information on the branches of the phylogeny. Each area can be considered a binary character with two states (present or absent) and optimized on the phylogeny. By comparing the numbers of gains and losses, it is possible to estimate areas most likely to have been part of the ancestral areas. Both methods were applied to the different topologies obtained from character analyses.

RESULTS

Sequence variation and saturation analysis

mtrDNA

Alignment of 12S rRNA, $tRNA^{val}$, and 16S rRNA genes resulted in 2,677 aligned positions of which 30 were excluded from phylogenetic analyses because they potentially violated hypotheses of positional homology. Within the remaining 2,647 sites, 971 (37%) of sites were variable and 677 (26%) were parsimony informative. The average base composition of sequences was skewed, with deficiency of guanine (17.8%) and overabundance of adenine (36.0%). This bias in base composition did not differ significantly across taxa (chi-square test implemented in PAUP* 4.0b10, P=0.666).

Cytochrome b

Complete cytochrome *b* sequences were obtained for all taxa, with the exceptions of *Mystacina* (AF144068) and *Saccopteryx* (AF044664), for which only 402 base pairs were available from GenBank. Since one of the objectives of this study was to assess the intraspecific variation of Caribbean species, several individuals per mormoopid species were included in the cytochrome *b* analysis (Table 2). Of these, two *Pteronotus parnellii* individuals from different localities in Jamaica (AMCC 102714 and TK27704) had identical sequences. A summary of the uncorrected pairwise divergences among individuals in different taxonomic ranks is shown in Fig. 1. Table 3 summarizes the results of sequence examination for fixed character differences among currently recognized subspecies within species *sensu* Smith (1972).

Within cytochrome *b* in the ingroup, 460 (40%) of sites were variable and 421 (37%) were parsimony informative. The distribution of the parsimony-informative sites was highly dependent on codon position: 19.2% in first, 4.8% in second, and 76.0% in third codon position. Most substitutions were synonymous, and translation of sequences to amino acids led to a matrix with only 68 informative sites (21.0% variable sites, among which 85.0% were parsimony informative). The average base composition of sequences was skewed. There is little bias at first codon position, and deficiency of adenine (20.3%) and guanine (13.7%) and overabundance of thymine (40.6%) for second position. The third position showed a strong bias: deficiency of guanine (4.0%) and thymine (14.0%), and abundance of adenine (42.1%) and cytosine (39.8%). The observed bias in base composition does not differ significantly across taxa when the whole cytochrome *b* gene was considered (chi-square test implemented in PAUP* 4.0b10,

P=0.996). Similar results were obtained for first and second positions separately, but not for third codon position, where the test was significant at the 0.1% level (*P*=0.000) among all taxa, and at the 5% level (*P*=0.048) within the ingroup.

Heterogeneity in base composition across taxa is known to affect phylogenetic reconstruction (Lockhart et al., 1994): bias in third codon position, which contains most of the sequence variation, may confound the results of sequence analyses. To examine this hypothesis, the most divergent taxon in GC content of third codon positions of cytochrome *b* was identified (GC content average for all taxa = 43.8%, SD = 4.8): *Artibeus jamaicensis* (31.6%). One can assume that the heterogeneity in base composition of this outgroup taxon is not affecting the analysis because no other taxon exhibits similar variation. No difference in base composition was found within *Mormoops* (chi-square test = 1.00) or *Pteronotus* (chi-square test = 1.00): heterogeneity in third codon base composition was confined to comparisons between the two genera. Data sources other than third codon positions in cytochrome *b* (e.g., morphology, mtrDNA, *Rag*2) all support the reciprocal monophyly of each genus. Base composition heterogeneity in third codon positions was not presumed to distort phylogenetic analyses toward recovering each of the genera. Graphs of transitions and transversions for each codon position versus uncorrected total sequence divergence were plotted to assess saturation in sequences. Those curves (not shown) indicated that first and second codon position did not experience multiple transition or transversion substitutions. Third codon positions showed saturation in transition and transversion substitutions for *Saccopteryx* and *Mystacina*, apparently because their sequences were incomplete.

Rag*2*

Of the sequences available, two *Mormoops megalophylla* individuals (CN98443 = AF338702, TK27640 = AF330818), and two *Pteronotus davyi* individuals (CN101305 = AF338691, TK25127 = AF338692) had identical sequences (Appendix 1). Of the 1,398 *Rag*2 sites, 252 (18%) were variable and 157 (11%) were parsimony informative. The distribution of the parsimony-informative sites was highly dependent on codon position: 16.6% in first, 8.9% in second, and 73.9% in third codon position. Most substitutions were synonymous, and translation of sequences to amino acids led to a matrix with only 41 informative sites (15.2% variable sites, among which 57.7% are parsimony informative). The average base composition of sequences was skewed, with deficiency of cytosine (18.1%) and thymine (20.4%) and overabundance of adenine (30.6%) in first codon position. Second positions showed deficiency of guanine (17.9%) and overabundance of adenine (35.0%), while third positions showed deficiency of guanine (16.8%) and overabundance of thymine (31.0%). The biases in base composition did not differ significantly across taxa for the whole gene, or for different codon positions (P = 1.000).

Phylogenetic analyses

Four data sets were included in phylogenetic analyses: (1) the mitochondrial cytochrome *b*, (2) mtrDNA (12S, $tRNA^{val}$, and 16S), (3) a fragment of the nuclear *Rag*2, and (4) the morphological character matrix for mormoopids published by Simmons and Conway (2001, see also Table 2 and Appendix 1). Sequences from different genes were concatenated for combined analyses, from the same individuals when possible (Appendix 1). Maximum parsimony analyses of individual data sets (Figs. 2-5) and the combined

'total evidence' data matrix (Fig. 6) were conducted with all unordered and unweighted characters. Branches that appeared in 2 of the 4 data partitions (cytochrome *b*, mtrDNA, *Rag*2, morphology) are shown in thicker lines in Fig. 6.

The Templeton (1983) tests indicated there were significant differences in: the fits of cytochrome *b* to the *Rag*2 (P=0.047) and morphology trees (P=0.020); the fits of the mtrDNA to the cytochrome *b* (P=0.019) and *Rag*2 (P=0.036) trees; the fits of *Rag*2 to the cytochrome *b* (P=0.076) and morphology trees (P=0.014); and the fits of the morphology to the cytochrome *b* (P=0.009) and *Rag*2 (P=0.023) trees. This test is not being used as a criterion for combining or excluding data, but to investigate conflict between data sets. The alternative topologies of Figs. 2-5, and the support for competing hypotheses (Table 4) further confirm these statistical results. Conflict among data sets does not appear to undermine phylogenetic resolution when combining data (Fig. 6).

Maximum-likelihood analyses were performed first using the program MODELTEST (see above). The models of molecular evolution and parameters selected for each data set are shown in Table 5. The estimates of phylogeny given the parameters of Table 5 for each molecular data partition are shown in Figs. 2-5.

Biogeographic Analyses

Table 7 presents the number of additional steps necessary to constrain: the Caribbean *P. parnellii* to be sister to the Central American *P. parnellii*, and the sister relationship between *P. quadridens* and *P. macleayii*, and of these two to *P. personatus*. The results of ancestral area estimation for particular nodes are summarized in Table 8. Optimizations using DIVA were constrained to two areas of endemism per node. This procedure is suggested in the DIVA manual (Ronquist, 1996) to optimize for a restricted distribution

rather than for all the areas occupied by descendents (the default result because vicariance carries no cost). The relative ages of Caribbean mormoopid lineages are shown in Fig. 2.

DISCUSSION

Basal units in the Mormoopidae

Phylogenetic estimation requires that the units under study be related in a hierarchical manner, i.e, that they correspond to separate species (Davis and Nixon, 1992). Fixed character combinations indicate that a given population is actually evolving independently as a species (Cracraft, 1983; Goldstein and DeSalle, 2000). The assessment of sequence variation among putative units (subspecies) within the mormoopids (Table 3) revealed multiple instances of characters that appear to be fixed in cytochrome *b*. Because sampling sizes were small, these cannot be the only data used to assess species limits. In some instances fixed character differences, high sequence divergence among presumed conspecifics (bottom two tiers of Fig. 1), distributional ranges that encompass broad areas separated by water and land barriers (Tables 1 and 2), and taxonomic limits based on morphological variation coincide (Smith, 1972) and strengthen hypotheses of evolutionary independence. These criteria (see Tables 2 and 3) apply to island populations of *P. parnellii sensu lato* or s.l. (*parnellii* Gray, *pusillus* Allen, and *portoricensis* Miller), and currently recognized subspecies of *P. davyi* (*davyi* Gray and *fulvus* Thomas) and *P. personatus* (*personatus* Wagner and *psilotis* Dobson).

Individuals of *M. megalophylla* Peters and *M. tumidiceps* Miller are distinguished by one conspicuous morphological character; a fan-shaped "cape" over the shoulders, formed by relatively long stiff hairs is present in South American specimens (Smith,

1972). In addition, 11 sites appear fixed in cytochrome *b* (Table 3). More sequences are required to test the molecular distinctiveness of these taxa because only one South American individual was included. Within *P. parnellii* s.l. and *P. personatus*, cytochrome *b* exhibits differentiation in northern South America (both taxa) and Central America and Mexico (*P. personatus*). The character differences and associated levels of divergence found among apparently continuous populations of *parnellii* and *personatus* (Fig. 1, Table 3) had not been anticipated in the morphological study of Smith (1972). Size alone distinguishes French Guianan specimens from those from the remainder of the *parnellii* s.l. range (Simmons and Voss, 1998). Additional morphological and molecular sampling (including unlinked markers) is necessary to investigate these cryptic species, or the viability of alternative hypotheses such as ancestral polymorphism of haplotypes in Central America and northern South America.

In one case, *P. quadridens*, apparently discontinuous variation in cytochrome *b* occurs along an east-west axis, rather than coinciding with the subspecies taxonomy that separates Cuban from other Greater Antillean bats (Tables 2 and 3). Finally, the Mexican and Central American samples of *P. parnellii* do not appear to be distinct, despite the size differences that inspired Smith (1972) to distinguish those populations. For the purposes of this study, each terminal that appears with a name in Fig. 2 was treated as a separate taxon in subsequent biogeographic analyses, and the results were compared to similar analyses under the current biological species taxonomy.

Phylogeny of Mormoopidae

Recent studies of mormoopid phylogeny provided most of the data analyzed here (Lewis Oritt et al., 2001; Simmons and Conway, 2001; Van Den Bussche et al., 2002b).

Although the monophyly of the family and its genera could be questioned based on some of the data partitions analyzed separately (e.g., Kennedy et al. [1999], Table 4, Fig. 2) this says more about the homoplasy of those data sets for deep divergences in the group than about the phylogeny of mormoopids. For the purposes of this study, the monophyly of mormoopids, *Mormoops* and *Pteronotus* will be assumed because this is the most parsimonious conclusion given all the evidence (Table 4, Figs. 5 and 6, but see Van Den Bussche et al. [2002b, 2003]) for two nuclear exons that do not support the monophyly of Mormoopidae).

Within *Pteronotus* there is strong support for sister taxa relationships between *P. quadridens* and *P. macleayii*, and *P. davyi* and *P. gymnonotus* (Figs. 2-6, see also Lewis Oritt et al. [2001], Van Den Bussche et al. [2002b], and Van Den Bussche and Weyandt [2003]). The position of *P. parnellii* as sister to all other *Pteronotus*, although not as well supported as previously discussed nodes (Table 4, Fig 6), is better supported than any alternative placement of *parnellii* (Table 4). Finally, relationships among *personatus* and the (*davyi*, *gymnonotus*) and (*quadridens*, *macleayii*) clades are resolved with poor support values (Fig. 6). Van Den Bussche and Weyandt's (2003) review of molecular data found high Bayesian posterior probabilities for resolving relationships among these three clades (*personatus*, ((*davyi*, *gymnonotus*), (*macleayii*, *quadridens*))), but bootstrap values were relatively low. Van Den Bussche and Weyandt (2003) pointed out that support estimated by resampling is low when few synapomorphies define a particular branch, and interpreted their results as a disadvantage of resampling methods. An alternative interpretation advocated here is that the resolution obtained is suspect precisely because few synapomorphies support it: there are only 19 unambiguous molecular changes along

the ((*davyi*, *gymnonotus*), (*macleayii*, *quadridens*)) branch (Fig. 5), less than half the number of changes in well-supported branches, e.g., number of unambiguous changes for (*macleayii*, *quadridens*) = 45, (*davyi*, *gymnonotus*) = 91.

There are three alternative hypotheses of relationships between *personatus*, (*davyi*, *gymnonotus*), and (*quadridens*, *macleayii*). One, the sister relationship between *personatus* and (*davyi*, *gymnonotus*), is not strongly supported by any individual dataset (Table 4, Figs. 2-4) and is poorly supported in parsimony analysis of the combined molecular data (Fig. 5), and can therefore be dismissed as unlikely. Of the remaining alternatives, a sister relationship between (*davyi*, *gymnonotus*) and (*quadridens*, *macleayii*) is supported by *Rag*2 and maximum likelihood analysis of mtrDNA (Table 4, Figs. 3b and 4), and was also strongly supported in Bayesian analyses published by Van Den Bussche and Weyandt (2003). A sister relationship between *personatus* and (*quadridens*, *macleayii*) is supported by morphology and this provides resolution in the total evidence tree (Fig. 6). Both topologies were examined in subsequent biogeographic analyses.

Biogeography of the Mormoopidae

Pteronotus parnellii

The species diversity of the mormoopids has been underestimated. A conservative estimate of the number of differentiated lineages using sequences from at least two individuals and morphological characters in *Pteronotus* produces at least 9 species where only 3 were counted before (Tables 2 and 3). *P. parnellii* s.l. includes *P. parnellii* (Cuba, Jamaica), *P. pusillus* (Hispaniola), *P. portoricensis* (Puerto Rico), *P. mexicanus* (Mexico

to Guyana, the status of *fuscus* and *paraguensis* was not evaluated here, see Table 2), and *P. rubiginosus* (Surinam and French Guiana). *P. personatus* includes *P. personatus* (Nicaragua to South America) and *P. psilotis* (Mexico to Honduras), and *P. davyi* includes *P. davyi* (Nicaragua to Venezuela, Trinidad, Lesser Antilles) and *P. fulvus* (Mexico to Honduras). Two hypotheses underlie competing assessments of species diversity. The first hypothesis posits that populations of *P. parnellii* (or any other polytypic species) are part of a continuum of differentiation that appears great at the extremes but is only slight between adjacent groups (Koopman, 1955). Although some populations are allopatric it is assumed that gene flow among them exists or occurred until recently. Because intergradation (used to infer gene flow) among insular and continental populations is not observed, the range of (non) resemblance permitted in a given species was widened (Smith, 1972). The molecular data support a second hypothesis: gene flow among insular and continental populations appears to have ceased even before fully recognized biological species (e.g., *P. gymnonotus* and *davyi*, Fig. 2) evolved into separate lineages. The morphological differences that were dismissed under a presumption of gene flow are in fact evidence for the isolation and independent evolution of separate lineages in widespread species such as *parnellii*, *davyi*, or *personatus*.

The closest living relatives of Antillean *P. parnellii sensu* Smith (1972) were found in northeastern South America (Surinam and French Guiana), but support for this association is poor, making alternative topologies and geographic origins viable (Table 6, Fig. 2a). The individuals in this east Guianan continental clade (Surinam and French Guiana) are highly divergent in cytochrome *b* from each other and their close relatives

(Table 3, Fig. 1) and appear as sister to all other *parnellii* when using a model of sequence evolution (Fig. 2b). Constraining Caribbean and Central American *parnellii* s.l to be sister taxa costs 11 additional steps, but constraining a sister relationship between Caribbean *parnellii* and a widespread continental clade only costs five extra steps (Table 6, this is also the topology favored in maximum likelihood analysis Fig. 2b). The hypothesis of colonization via Central America is rejected, but this result could easily be overturned with new data (Table 6).

Support for the inclusion of Jamaican exemplars in the Antillean clade is relatively low (Fig. 2). These results do not strongly reject the possibility that *parnellii sensu* Smith (1972) reached the Greater Antilles more than once with an early range expansion into the Caribbean to Jamaica and presumably Cuba (although this remains to be tested), and a later expansion reaching Hispaniola and Puerto Rico.

The relationships of the Cuban and Floridan fossil *P. pristinus* (Morgan, 2001; Silva-Taboada, 1974) need to be reexamined in light of these results. Simmons and Conway (2001) did not find consistent differences among mormoopid subspecies with the characters they studied, perhaps because these were collected with other goals in mind (p. 12). Simmons and Conway (2001) found *pristinus* to be sister to *parnellii* s.l. in their morphological analyses, leaving open the question of which *parnellii* lineage it is most closely related to. Because the support value for this relationship was low (Bremer index = 1), alternative arrangements within *Pteronotus* remain viable, including a sister relationship to *quadridens* (Silva-Taboada, 1979).

Other Antillean Pteronotus

The hypothesis of Smith (1972) whereby *P*. *macleayii* and *quadridens* are sister to *personatus* is only rejected by the cytochrome *b* data; mtrDNA, morphology, and the total evidence analysis support it (Table 6). Because there is conflict surrounding the resolution of (*quadridens*, *macleayii*) and its closest continental relative, two alternative topologies (Figs. 4 and 6) were considered in estimating ancestral areas. The use of revised mormoopid terminal units always reduced the number of areas optimized using ancestral area analysis, but not when using DIVA (Table 7). A Central American ancestral area for the most recent common ancestor of this Caribbean clade is supported by these analyses. In fact, it is the only single-area (although non-unique) resolution to this node obtained with any method. This is because using the revised taxonomy, the Central American *P*. *davyi* (= *fulvus*) is the oldest lineage in the continental sister to (*macleayii*, *quadridens*). Although this particular phylogenetic arrangement is poorly supported, the basal lineages of *personatus sensu* Smith (1972) are also in Mexico and Central America (Fig. 2), further strengthening the hypothesis of a Central American origin for this clade. The history of near-interconnections in the region during periods of low sea level (Griffiths and Klingener, 1988; Smith, 1972) is an independent source of support for the Central American ancestral area, and both biogeographic methods recover signal from the phylogenies connecting Central America or Mexico to the western Greater Antilles.

Because both *personatus* and (*davyi*, *gymnonotus*) also contain northern South American lineages and DIVA seeks to minimize the number of dispersals, northern South America is included among the potential solutions especially when using widespread biological species as terminals. Finally, the ancestral area method optimizes island areas

for the ancestor because it does not use information from deeper nodes as DIVA does (Ronquist, 1994), and because it assumes that areas repeated among terminals have a higher probability of being ancestral (Bremer, 1995). The revised taxonomy requires that Cuba and Jamaica be acquired twice in the (*macleayii*, *quadridens*) lineage, not once as when assuming *quadridens* is a single widespread species, and this leads the ancestral area analysis to optimize for these islands at the base of the lineages (Table 7).

Mormoops *and the Mormoopidae*

M. megalophylla once ranged from Florida through the Greater Antilles to Bahia in Brazil in the Late Pleistocene (Czaplewski and Cartelle, 1998; Ray et al., 1963; Silva-Taboada, 1974). The geographic variation of this widespread species has not been evaluated in detail (Morgan, 2001), and more than one basal unit may be involved. One additional species, *M. magna*, is known only from the Late Pleistocene of Cuba (Silva-Taboada, 1974). The most parsimonious interpretation of the current distribution is a Caribbean ancestral area for the *Mormoops* lineage (Table 7) because both *M. blainvillei* and *megalophylla* are known from the Greater Antilles. This result could only be overturned by the discovery of a basal *Mormoops* lineage on the continent. Since the ancestral area of *Mormoops* is not continental, the divergence from the continental group could go as far back as the split between the two mormoopid genera (Fig. 2b).

In agreement with the hypothesis of Smith (1972) the divergence of continental and Antillean *Pteronotus* apparently occurred earlier for (*quadridens*, *macleayii*) than for *parnellii* s.l. (Fig. 2b). Both DIVA and ancestral area analysis constrain the ancestral area of mormoopids to the northern part of their range when the revised taxonomy is used (Table 7). When widespread biological species are used, the hypotheses of Smith (1972)

are supported by including northwestern South America in the ancestral area (Table 7). This is because the widespread distribution of the terminal taxa is carried back into the ancestral reconstructions, adding ambiguity to the geographic optimization along the branches.

If a recently discovered mormoopid from the Oligocene of Florida (Czaplewski and Morgan, 2003) is attached to the phylogeny before the divergence of the two extant genera, then Florida becomes part of ancestral area optimizations for the family as well (results not shown). The geographic history of mormoopids, as reconstructed from the data used here, agrees with the biogeographic hypotheses of Czaplewski and Morgan (2003). The mormoopids apparently reached South America late in their history, after diversifying in Central America, Mexico, Florida, and/or the Greater Antilles (Table 7, Fig. 2b). Populations from northwestern South America were not sampled (Table 2), but even if basal lineages of particular taxa are found in this region, the deeper divergences within mormoopids (e.g., between the genera) would still optimize to the northern Neotropics.

This finding modifies the biogeographic history of noctilionoids. Both morphology (Simmons and Conway, 2001) and large concatenated molecular datasets (Teeling et al., 2003) indicate that mormoopids and phyllostomids are each other's closest relative (this topology was not always recovered in this study, perhaps because taxon sampling among bat families was poor relative to the higher-level analyses cited above). Two phylogenetic hypotheses have been proposed to explain relationships among phyllostomids. One, based on analyses of mostly morphological data (Wetterer et al., 2000) identified the vampires (*Desmodus*, *Diaemus* and *Diphylla*) as the oldest

phyllostomid lineage. A second hypothesis based on analyses of mtrDNA and *Rag*2 (Baker et al., 2003) suggests that *Macrotus* is the basal lineage of the phyllostomids.

The identity of the basal lineage of the phyllostomids would have a disproportionate effect on ancestral area reconstructions for that family. Vampires range from Mexico to Chile and Uruguay, with fossil records from Cuba (Koopman, 1994). Because of their widespread distribution, this lineage would not constrain the ancestral area of the phyllostomids. Since the greatest diversity of phyllostomids is concentrated in northern South America and the vampires include it in their range, this would be the most parsimonious ancestral area for the family. In contrast, *Macrotus* is only known from the southwestern United States south to Guatemala, through the Greater Antilles and Bahamas (Koopman, 1994). If *Macrotus* is at the base of the phyllostomid radiation, then the ancestral areas of mormoopids (Table 7) and phyllostomids would be adjacent in the northernmost Neotropics. Phyllostomid fossil are known from the middle Miocene of La Venta (Czaplewski, 1997), indicating phyllostomids reached South America early in their history (assuming this is not their ancestral area). The geographic distribution in the early history of these closely related families might help explain the remarkable differences in taxonomic and adaptive diversity between the two groups.

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Table 1. Taxonomy and geographic ranges of currently recognized species of Mormoopidae. Taxonomy follows Simmons (in press) and Simmons and Conway (2001). † = extinct population.

Taxon	Geographic range
Mormoops megalophylla	Texas through Central America to northern South America, including Aruba, Bonaire and Curaçao
Mormoops blainvillei	Greater Antilles, †Bahamas, †Antigua
†*Mormoops magna*	Cuba (Quaternary)
Pteronotus parnellii	Greater Antilles, Mexico to the Mato Grosso and east to northeastern Brazil, Trinidad
†*Pteronotus pristinus*	Cuba (Quaternary)
Pteronotus personatus	Mexico to central and northeastern Brazil, Trinidad
Pteronotus quadridens	Greater Antilles
Pteronotus macleayii	Cuba and Jamaica
Pteronotus davyi	Mexico to northwestern Peru, east to northeastern Brazil, Trinidad, Lesser Antilles to Marie Galante
Pteronotus gymnonotus	Mexico to Guyana and central Brazil

Table 2. Taxa and molecular sequences included in this study. Most molecular data were available from previous studies as follows: 12S rRNA, $tRNA^{val}$, 16S rRNA from Van Den Bussche and Hoofer (2000) and Van Den Bussche et al. (2002b), cytochrome *b* from Lewis Oritt et al. (2001), and *Rag*2 from Lewis Oritt et al. (2001). See 'Taxon sampling' for cytochrome *b* sequences generated in this study, and Appendix 1 for GenBank accession numbers. † = extinct population. When several cytochrome sequences were used, an '=n' indicates the number available.

Species	subspecies	DNA sequences available	Distribution
M. blainvillei		Rag2 12S $tRNA^{val}$ 16S cyt *b* =3	Greater Antilles
M. megalophylla	*megalophylla*	Rag2 12S $tRNA^{val}$ 16S cyt *b* =4	Texas to Nicaragua †Greater Antilles
M. megalophylla	*tumidiceps*	cyt *b* 12S $tRNA^{val}$ 16S	Colombia Venezuela Trinidad Margarita
M. megalophylla	*intermedia*		Aruba Bonaire Curacao
M. megalophylla	*carteri*		Ecuador NW Peru
P. parnellii	*parnellii*	Rag2 cyt *b* =3	Cuba Jamaica
P. parnellii	*pusillus*	cyt *b* =2	Hispaniola †Gonave
P. parnellii	*portoricensis*	cyt *b* =2	Puerto Rico
P. parnellii	*mexicanus*	cyt *b* =2	Mexico excluding Veracruz and Yucatán
P. parnellii	*mesoamericanus*	cyt *b* =2	SE Veracruz to SW Panama
P. parnellii	*rubiginosus*	12S $tRNA^{val}$ 16S cyt *b* =4	Honduras to no. South America Trinidad Tobago
P. parnellii	*fuscus*		NE Colombia NW Venezuela
P. parnellii	*paraguensis*		Paraguana
P. personatus	*personatus*	Rag2 cyt *b* =3	Nicaragua to South America
P. personatus	*psilotis*	12S $tRNA^{val}$ 16S cyt *b* =2	Mexico to Honduras
P. macleayii	*macleayi*	Rag2 12S $tRNA_{val}$ 16S cyt *b*	Cuba
P. macleayii	*griseus*	cyt *b* =2	Jamaica
P. quadridens	*torrei*	Rag2 12S $tRNA^{val}$ 16S cyt *b*	Cuba
P. quadridens	*quadridens*	cyt *b* =4	Greater Antilles excluding Cuba
P. davyi	*davyi*	Rag2 =3 12S $tRNA^{val}$ 16S cyt *b* =4	Nicaragua to Venezuela Trinidad Dominica Martinique
P. davyi	*fulvus*	cyt *b* =3	Mexico to Honduras
P. davyi	*incae*		Peru
P. gymnonotus		Rag2 12S $tRNA^{val}$ 16S cyt *b* =3	Veracruz to French Guiana and Brazil

Table 3. Fixed character differences (FCD) in base pairs of cytochrome *b* among subspecies and selected populations within each currently recognized species. Solid black lines separate each species. N = sample size. Taxonomy follows Smith (1972). See Table 2 for complete geographic distribution of subspecies. *M*. = *Mormoops*, *P*. = *Pteronotus*.

Taxon 1	N_1	Taxon 2	N_2	FCD
M. megalophylla megalophylla	4	*M. megalophylla tumidiceps*	1	11
M. blainvillei Cuba	1	*M. blainvillei* Jamaica	2	4
P. parnellii parnellii Cuba Jamaica	2	*P. parnellii* other localities	23	19
P. parnellii pusillus	2	*P. parnellii* other localities	23	8
P. parnellii portoricensis	2	*P. parnellii* other localities	23	6
(*P. parnellii parnellii*, *P.p. portoricensis*)	4	*P. parnellii* other localities	21	4
P. parnellii mexicanus	2	*P. parnellii* other localities	23	3
P. parnellii mesoamericanus	2	*P. parnellii* other localities	23	1
P. parnellii rubiginosus Guyana	1	*P. parnellii* other localities	24	4
(*P. parnellii mexicanus*, *P.p. mesoamericanus*, *P.p. rubiginosus* Guyana)	6	*P. parnellii* other localities	19	8
P. parnellii rubiginosus Surinam	1	*P. parnellii* other localities	22	15
P. parnellii rubiginosus French Guiana	1	*P. parnellii* other localities	22	21
P. parnellii rubiginosus Surinam & French Guiana	2	*P. parnellii* other localities	21	7
P. macleayii macleayii	1	*P. macleayii griseus*	2	11
P. quadridens quadridens	1	*P. quadridens fuliginosus*	4	1
P. quadridens Cuba and Jamaica	3	*P. quadridens* Puerto Rico and Hispaniola	2	8
P. personatus personatus	3	*P. personatus psilotis*	2	9
P. personatus Guyana	1	*P. personatus* other localities	4	10
P. personatus Guatemala	1	*P. personatus* other localities	4	15
P. personatus Mexico	1	*P. personatus* other localities	4	41
P. davyi davyi	4	*P. davyi fulvus*	3	55
P. davyi	7	*P. gymnonotus*	3	20

Table 4. Support for monophyly and relationships among mormoopids. Relationships are represented using the Newick format. Values are jackknife/Bremer support; when only one number is shown it corresponds to jackknife. Cytochrome *b* = cyt *b*, morphology = morph.

Node/Dataset	cyt *b*	mtrDNA	*Rag*2	morph	Total	Supported by
(*Mormoops*, *Pteronotus*)	5	83/5	59/1	98/9	98/20	All but cyt *b*
Mormoops	51/0	100/46	98/8	100/20	100/101	All
Pteronotus	96/12	100/20	97/6	99/10	100/68	All
(*davyi*, *gymnonotus*)	100/18	100/43	99/8	90/4	100/75	All
(*quadridens*, *macleayii*)	46	92/11	94/6	48/1	100/25	All but cyt *b*
parnellii sister to all other *Pteronotus*	29	55/4	91/5	33	79/9	*Rag*2 mtrDNA
(*parnellii*, *personatus*)	23	29	2	19	17	none
(*parnellii*, (*davyi*, *gymnonotus*))	4	1	0	19	0	none
(*parnellii*, (*quadridens*, *macleayii*))	7	3	1	1	5	none
((*davyi*, *gymnonotus*), (*quadridens*, *macleayii*))	0	23	65/2	20	19	*Rag*2
(*personatus*, (*davyi*, *gymnonotus*))	36/2	18	24	0	20	none
(*personatus*, (*quadridens*, *macleayii*))	1	36	3	53/1	44/3	morph

Table 5. Models of molecular evolution and parameters selected for each molecular data set, see Table 2 for sequences. GTR = general time reversible model; R-matrix = rate matrix parameter (with respect to G-T transversion); Γ = shape parameter, I = proportion of invariant sites; -2logλ = 2[logL_1 - logL_2], where L_1 = likelihood without clock and L_2 = likelihood with clock.

Data	Model	R-matrix	Γ	I	-2logλ	df	*P*
mtrDNA	GTR+I+Γ	1.0, 3.3, 1.0, 1.0, 10.7	0.4090	0.3347	21.1	16	>0.05
cyt *b*	GTR+I+Γ	0.5, 9.7, 0.5, 0.3, 10.4	0.9773	0.5040	52.4	43	>0.05
*Rag*2	GTR+Γ	1.0, 5.1, 1.0, 1.0, 7.8	0.2433	-	26.2	18	>0.05
combined	GTR+I+Γ	6.5, 11.7, 4.1, 1.0, 37.0	0.4505	0.3820	42.2	15	<0.001

Table 6. Number of extra steps necessary to constrain results to conform to the biogeographic hypotheses of Smith (1972), given different data sets studied. Relationships are represented using the Newick format. Molec. = combined molecular, 0 = no extra steps are necessary to obtain the result in parsimony analysis, – = data unavailable.

Node/Dataset	cyt *b*	mtrDNA	*Rag*2	Molec.	Total
parnellii Caribbean + Central America	11	–	–	–	–
parnellii Caribbean + Central America+Guyana	5	–	–	–	–
(*quadridens*, *macleayii*)	4	0	0	0	0
((*quadridens*, *macleayii*), *personatus*)	11	0	3	0	0

Table 7. Ancestral areas of mormoopid lineages estimated using DIVA and ancestral area analysis. Ancestral area optimizations in DIVA were constrained to a maximum of two areas. Taxonomy (T): 1 if hierarchical relationships were estimated based on the biological species of Smith (1972), 2 if the assessment of cytochrome *b* variation was used to delimit taxa (Table 2). Greater Antillean fossil *M. megalophylla* were assigned to *megalophylla* (rather than *tumidiceps*) when mapping distribution under taxonomy 2. The fossils *M. magna* and *P. pristinus* were not included in any analysis. Relationships are represented using the Newick format. The 'x' indicates the areas preceding it should be combined with each of the areas after it to generate multiple two-area regions. MRCA = most recent common ancestor.

Node/Estimated ancestral area	T	DIVA	Ancestral area analysis
MRCA of *parnellii* Caribbean and continent	2	NE South America	All areas
MRCA of ((*macleayii*, *quadridens*), *personatus*)	1	Any combination of Cuba or Jamaica x Central America or NW South America	All areas but Hispaniola and Puerto Rico
MRCA of ((*macleayii*, *quadridens*), *personatus*)	2	Any combination of Cuba or Jamaica x NW South America, NE South America, Central America, or Mexico	Cuba Jamaica
MRCA of ((*macleayii*, *quadridens*), (*davyi*, *gymnonotus*)	1	Any combination of Cuba or Jamaica x Central America or NW South America	Equal probability to Cuba, Jamaica, NW South America or Central America
MRCA of ((*macleayii*, *quadridens*), (*davyi*, *gymnonotus*)	2	Central America, Central America + Jamaica, or Central America + Cuba	Cuba Jamaica
MRCA of *Mormoops*	1, 2	Each of the Greater Antilles or each of the Greater Antilles + Central America	Greater Antilles
MRCA of all mormoopids if ((*macleayii*, *quadridens*), *personatus*)	1	Central America or NW South America or any combination of each x each of the Greater Antilles	Central America Mexico

Table 7. Continued.

Node/Estimated ancestral area	T	DIVA	Ancestral area analysis
MRCA of all mormoopids if ((*macleayii*, *quadridens*), *personatus*)	2	Central America or Central America + each of the Greater Antilles	Central America followed by Cuba Jamaica
MRCA of all mormoopids if ((*macleayii*, *quadridens*), (*davyi*, *gymnonotus*)	1	Central America or NW South America or any combination of each x each of the Greater Antilles	Central America NW South America
MRCA of all mormoopids if ((*macleayii*, *quadridens*), (*davyi*, *gymnonotus*)	2	Central America or Central America + each of the Greater Antilles	Central America followed by Cuba Jamaica

FIGURE LEGENDS

Figure 1. Scatter plot of uncorrected sequence divergence in cytochrome *b* against taxonomic rank. Taxonomy follows Smith (1972). Numerals indicate cytochrome *b* distance outliers as follows: 1: with respect to *Saccopteryx*; 2: between *Mystacina* and *Noctilio*; 3: between *Mormoops* and *Artibeus*; 4: between *Noctilio albiventris* and *N. leporinus*; 5: between *Pteronotus davyi* and *P. gymnonotus*; 6: between currently recognized subspecies of *P. quadridens*, *P. macleayii*, and *M. megalophylla*, also between *P. parnellii* from Mexico, Guatemala and Honduras classified in the subspecies *mesoamericanus* and *mexicanus*; 7: between *P. parnellii* from Puerto Rico and Hispaniola, and among samples from Guyana, Mexico and Honduras; 8: between *P. personatus* from Surinam, and individuals from Venezuela and Guyana; 9: between *P. parnellii* individuals from French Guiana and Surinam; and 10: between *P. parnellii* individuals from Guyana, and Surinam and French Guiana.

Figure 2. A. Strict consensus of 8 most parsimonious cladograms resulting from analysis of cytochrome *b* (L=1792 steps, CI=0.439, RI=0.775). Numbers above branches are percent of 1000 jackknife replicates, below branches are Bremer support values. Names of outgroups are in **bold**, see Appendix 1 for sequence data. B**.** Phylogram resulting from maximum likelihood analysis using a rate-constant GTR+I+Γ model of DNA evolution (-ln*L* = 9181.23). Numbers above branches are percent of 300 jackknife replicates.

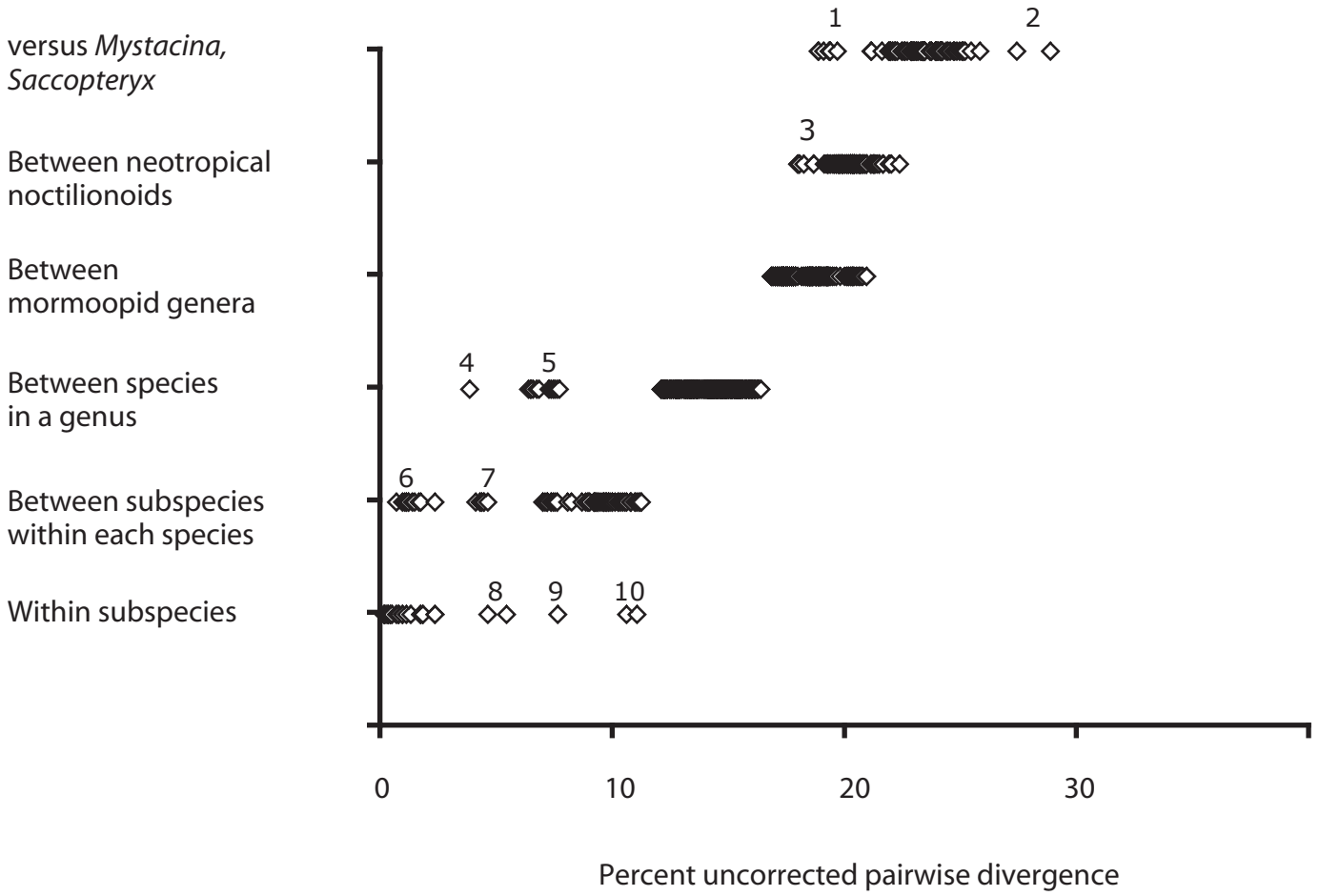
Figure 3. A. Most parsimonious cladogram resulting from analysis of mtrDNA (L=2320, CI=0.621, RI=0.666). Numbers above branches or with arrows are percent of 1000 jackknife replicates, below branches are Bremer support values. Names of outgroups are

in **bold**, see Appendix 1 for sequence data. B. Phylogram of one of two trees obtained from sequence analysis using a rate-constant GTR+ I + Γ model of DNA evolution (-ln*L* = 13600.27). Numbers above branches or with arrows are percent of 300 jackknife replicates.

Figure 4. A. Most parsimonious cladogram resulting from analysis of *Rag*2 (L=332, CI=0.840 RI=0.884). Numbers above branches are percent of 1000 jackknife replicates, below branches are Bremer support values. Names of outgroups are in **bold**, see Appendix 1 for sequence data. B. Phylogram obtained from sequence analysis using a rate-constant GTR+ Γ model of DNA evolution (-ln*L* = 3833.81). Numbers above branches are percent of 300 jackknife replicates.

Figure 5. A. Most parsimonious cladogram resulting from analysis of concatenated mtrDNA, cytochrome *b*, and *Rag*2 sequences (L=3937, CI=0.612 RI=0.640). Numbers above branches are percent of 1000 jackknife replicates, below branches are Bremer support values. Names of outgroups are in **bold**, see Appendix 1 for sequence data. B. Phylogram obtained from analysis of concatenated mtrDNA, cytochrome *b*, and *Rag*2 sequences using a GTR+I+ Γ model of DNA evolution (-ln*L* = 24736.88). Numbers above branches or with arrows are percent of 300 jackknife replicates.

Figure 6. Single most parsimonious tree obtained from analysis of concatenated sequences and morphology, 'total evidence,' (L= 4090, CI=0.647, RI=0.557). Numbers above branches are percent of 1,000 jackknife replicates, below branches are Bremer support values. Names of outgroups are in **bold**. Thicker lines show branches that appeared in at least 2 data partitions (cytochrome *b*, mtrDNA, *Rag*2, morphology).


versus *Mystacina, Saccopteryx*
Between neotropical noctilionoids
Between mormoopid genera
Between species in a genus
Between subspecies within each species
Within subspecies
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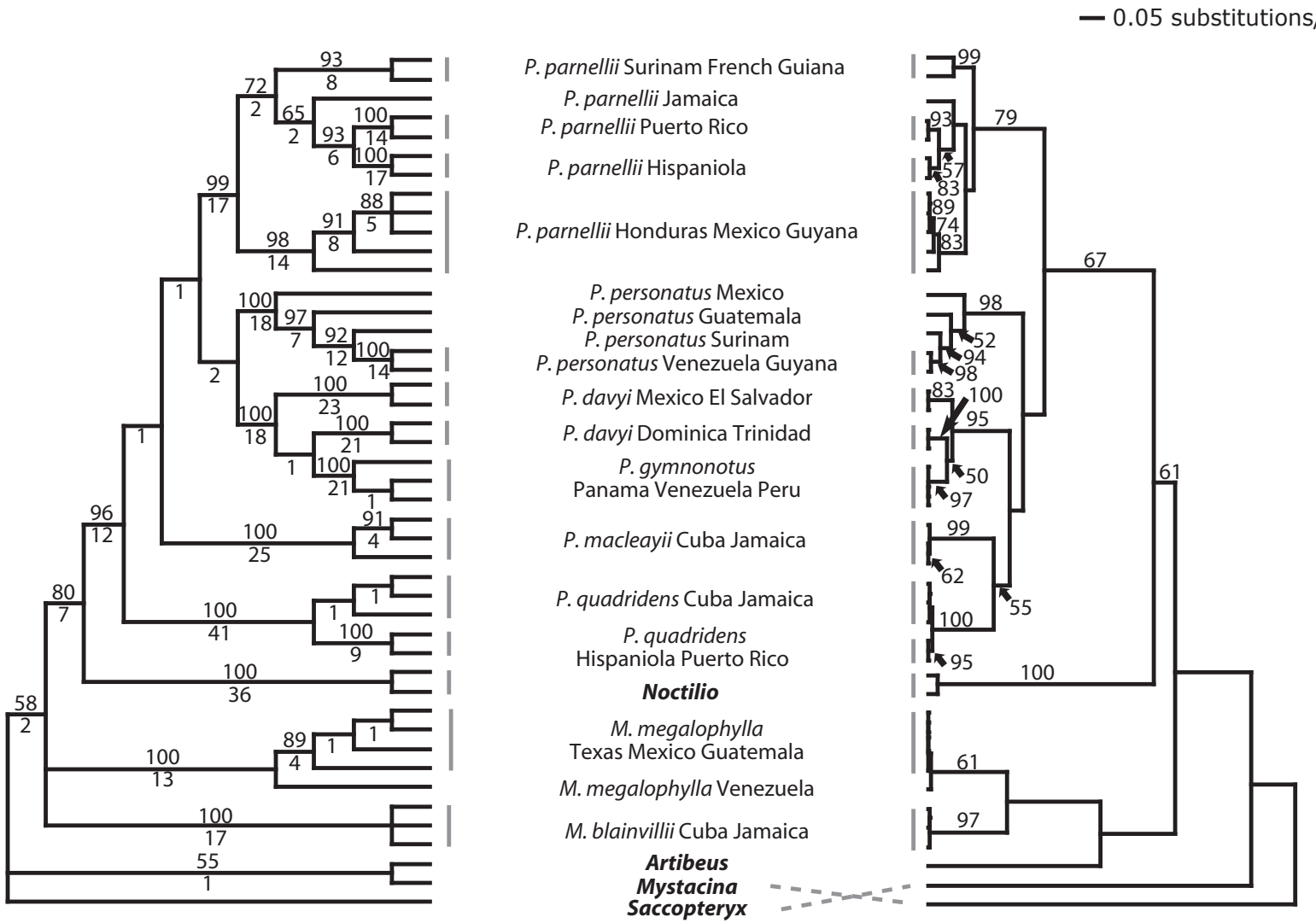
20
30
Percent uncorrected pairwise divergence

Figure 1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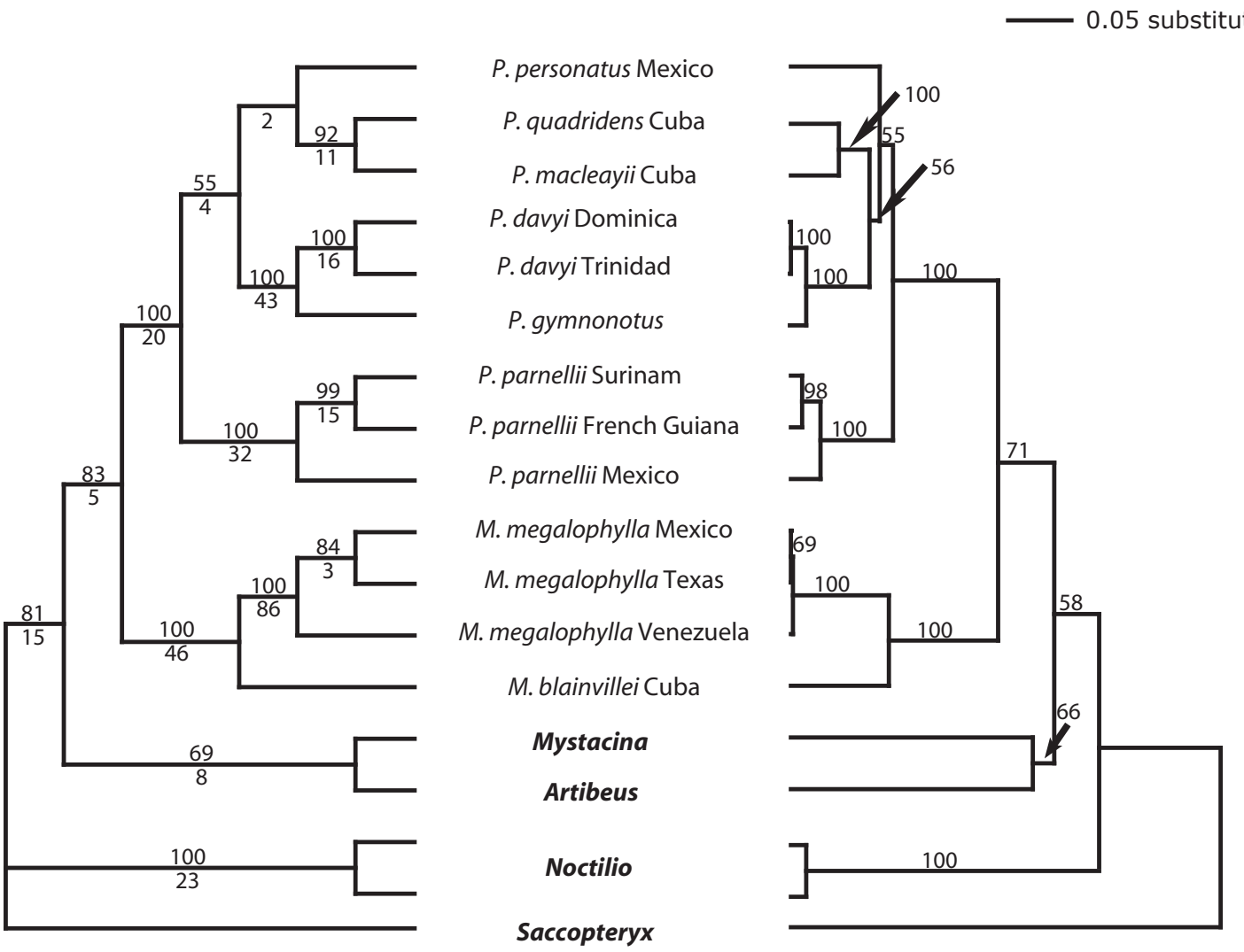
P. parnellii Hispaniola
P. parnellii Honduras Mexico Guyana
P. personatus Mexico
P. personatus Guatemala
P. personatus Surinam
P. personatus Venezuela Guyana
P. davyi Mexico El Salvador
P. davyi Dominica Trinidad
P. gymnonotus
Panama Venezuela Peru
P. macleayii Cuba Jamaica
P. quadridens Cuba Jamaica
P. quadridens
Hispaniola Puerto Rico
Noctilio
M. megalophylla
Texas Mexico Guatemala
M. megalophylla Venezuela
M. blainvillii Cuba Jamaica
Artibeus
Mystacina
Saccopteryx
A
B

Figure 2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P. gymnonotus
P. parnellii Surinam
P. parnellii French Guiana
P. parnellii Mexico
M. megalophylla Mexico
M. megalophylla Texas
M. megalophylla Venezuela
M. blainvillei Cuba
Mystacina
Artibeus
Noctilio
Saccopteryx
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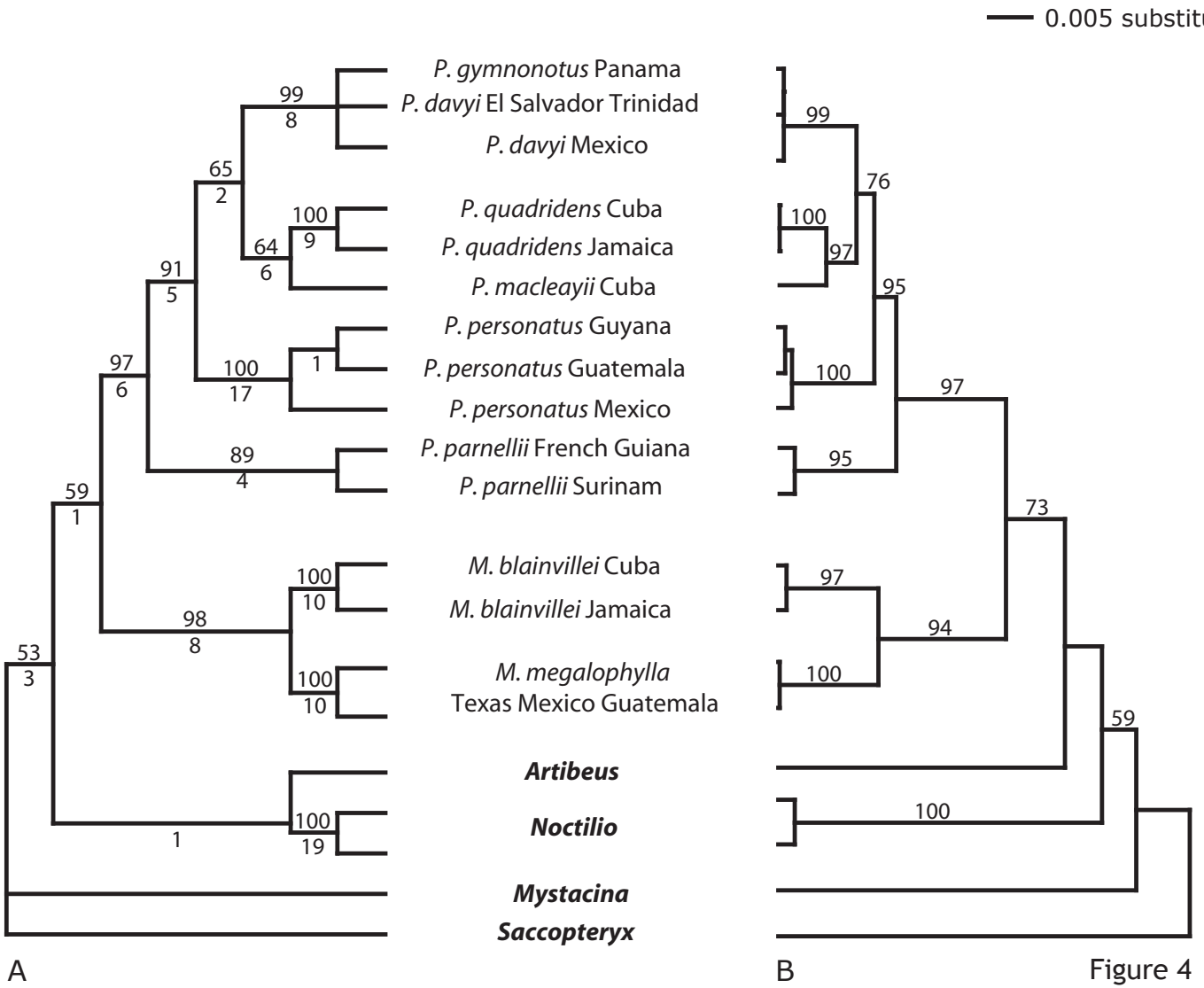
Figure 3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P. personatus Guatemala
P. personatus Mexico
P. parnellii French Guiana
P. parnellii Surinam
M. blainvillei Cuba
M. blainvillei Jamaica
M. megalophylla
Texas Mexico Guatemala
Artibeus
Noctilio
Mystacina
Saccopteryx
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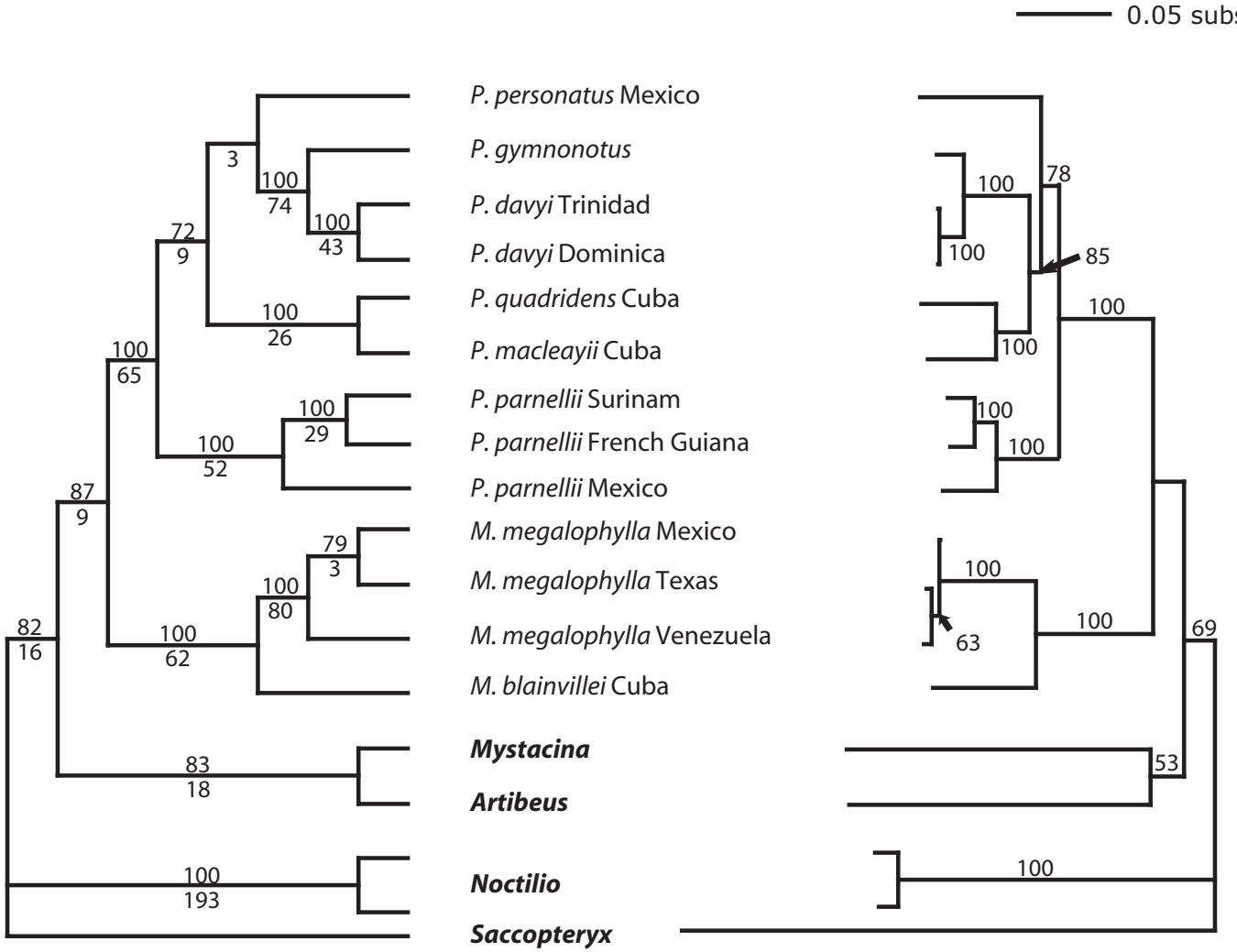
Figure 4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P. macleayii Cuba
P. parnellii Surinam
P. parnellii French Guiana
P. parnellii Mexico
M. megalophylla Mexico
M. megalophylla Texas
M. megalophylla Venezuela
M. blainvillei Cuba
Mystacina
Artibeus
Noctilio
Saccopteryx
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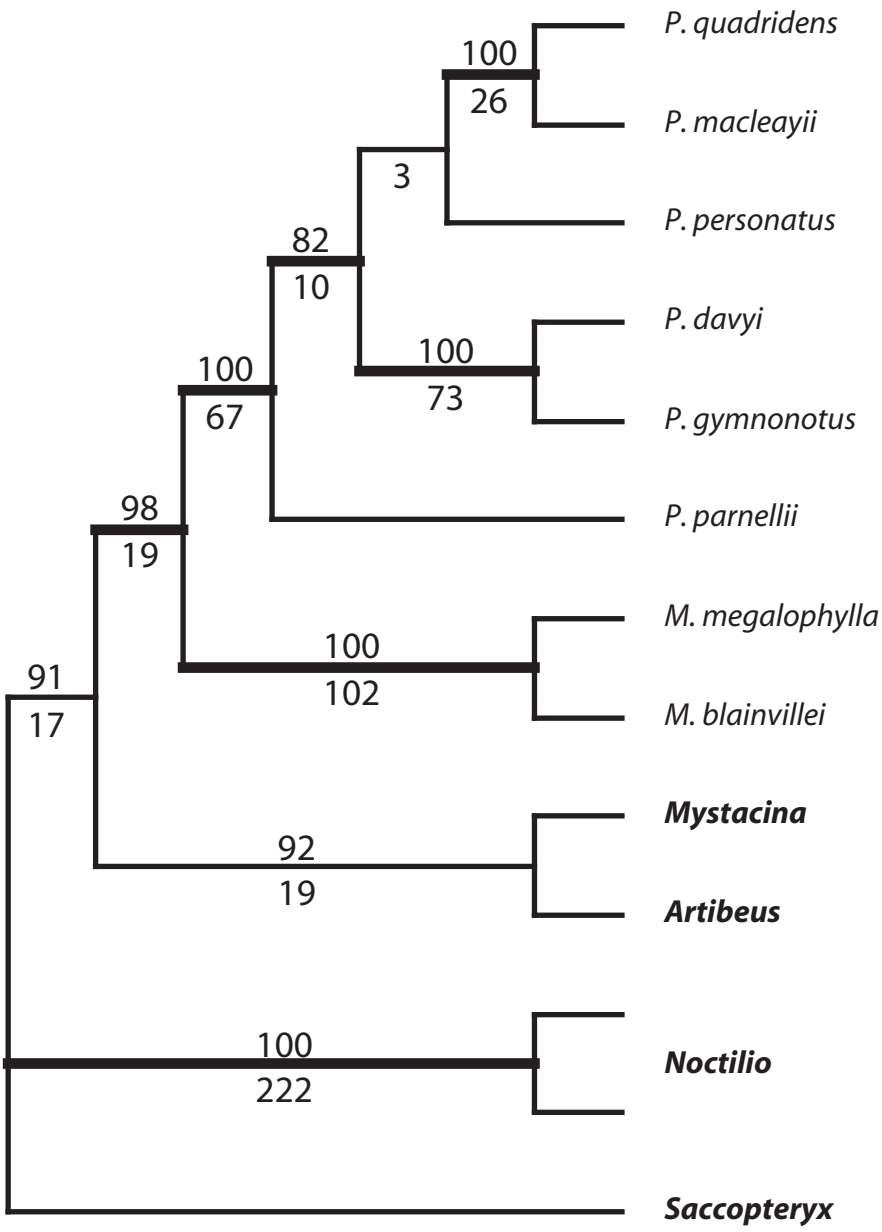
Figure 5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M. blainvillei
Mystacina
Artibeus
Noctilio
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222

Figure 6

Appendix 1. Taxa, localities, tissue vouchers and GenBank accession number for sequences used in this study. See Table 2 for subspecies classification. *S.*= *Saccopteryx*, *My.*= *Mystacina*, *N.*= *Noctilio*; *A.*= *Artibeus*; *P.*= *Pteronotus*; *M.*= *Mormoops*. AMCC = Ambrose Monell Cryogenic Collection of the American Museum of Natural History, AMNH = cadaver voucher at the Mammalogy Department of the American Museum of Natural History; CN = Royal Ontario Museum; TK = tissue collection of the Museum of Texas Tech University; UWZM = University of Wisconsin Zoological Museum. Sequences are cytochrome *b* unless otherwise noted.

Taxon	Locality	Country	Tissue voucher	Sequence(s)
S. bilineata				AF044664
S. bilineata			AMNH267842	AF263213 mtrDNA AY141015 *Rag*2
My. tuberculata	Little Barrier Island	New Zealand		AF144068
My. tuberculata		New Zealand	UWZM-M27027	AF263222 mtrDNA AY141021 *Rag*2
N. leporinus	0.5 km E Confer, Chemin, St. George	Grenada	TK18513	AF330796
N. leporinus			TK18515	AF263224 mtrDNA
N. leporinus	Craigston estate, Carriacou Island	Grenada	TK18701	AF316477 *Rag*2
N. albiventris	5840.99N, 57851.529W, 41 m elevation, Dubulay Ranch, Berbice District	Guyana	TK86633	AF330803, AF330810 *Rag*2
N. albiventris			TK46004	AF263223 mtrDNA
A. jamaicensis		Puerto Rico		AF061340 mtDNA
A. jamaicensis				AY011963 *Rag*2
P. parnellii	24 km W St. Ann's Bay, St. Ann's Parish	Jamaica	TK27704	AF338661
P. parnellii	St. Clair Cave, Polly Ground, St. Catherine	Jamaica	AMCC102714	AY604456
P. parnellii	La Entrada (de Cabrera), María Trinidad Sánchez	Dominican Republic	AMCC103048	AY604455
P. parnellii	La Entrada (de Cabrera), María Trinidad Sánchez	Dominican Republic	AMCC103050	AY604454
P. parnellii	Caribbean National Forest, Naguabo	Puerto Rico	TK21800	AF338665

Appendix 1. Continued.

Taxon	Locality	Country	Tissue voucher	Sequence(s)
P. parnellii	Caribbean National Forest, Naguabo	Puerto Rico	TK21806	AF338666
P. parnellii	19°19.19 N, 100°27.89 W Benito Juarez, Cerro Colorado 1350 m, Durango	Mexico	TK45500	AF338667, AF407181 mtrDNA
P. parnellii	25 mi. W Ciudad Valles, San Luis Potosi	Mexico	TK14517	AF338663
P. parnellii	Rio de Atoyac, Ojo de Agua, 14 km N 22 km E Cordoba, Veracruz	Mexico	TK13108	AF338664
P. parnellii	8.5 mi. SSW San Lorenzo, Valle	Honduras	TK40197	AF338662
P. parnellii	7°22.179N, 50°29.459 W, 142.0 m elev. Baramita, NW District	Guyana	TK86526	AF338668
P. parnellii	Oelemarie, Marowijn	Suriname	TK17953	AF330807, AF407180 mtrDNA, AF330817 *Rag*2
P. parnellii	near Sinnamary, Paracou, Cayenne	French Guiana	AMCC110395	AY604457
P. parnellii	near Sinnamary, Paracou, Cayenne	French Guiana	AMNH269115	AF263221 mtrDNA, AY245416 *Rag*2
P. personatus	Tehuantepec, Oaxaca	Mexico	TK12043	AF338680, AF407182 mtrDNA, AF338699 *Rag*2
P. personatus	Grutas de Lanquin, Alta Verpaz [sic]	Guatemala	CN98438	AF338677, AF338697 *Rag*2
P. personatus	0.5 km E El Manteco, Bolivar	Venezuela	TK19079	AF338678
P. personatus	Karanambo, Upper Takutu	Guyana	CN97943	AF338676, AF338698 *Rag*2
P. personatus	Grassalco, Nickerie	Suriname	TK10336	AF338679
P. davyi	1 mi. above mouth of Layou River, St. Joseph Parish	Dominica	TK15571	AF338669, AF407175 mtrDNA
P. davyi			TK155751	AF407175 *Rag*2
P. davyi	3 mi. S 3.0 mi. W Cumuto, Arena Reserve, Nariva	Trinidad	TK25127	AF338671, AF407176 mtrDNA, AF338692 *Rag*2
P. davyi	Chamela, Jalisco	Mexico	TK27642	AF338672, AF338693 *Rag*2
P. davyi	El Refugio, El Imposible, Ahuachapan	El Salvador	CN101305	AF338670, AF338691 *Rag*2
P. gymnonotus	Parque Nacional Altos de Campana, Panama	Panama	CN104265	AF338673, AF338694 *Rag*2
P. gymnonotus	35 km ESE of Caicara, Hato La Florida, Bolivar	Venezuela	CN107925	AF338675

Appendix 1. Continued.

Taxon	Locality	Country	Tissue voucher	Sequence(s)
P. gymnonotus	1 km S Tingo Maria, Leoncia Prado, Huanuco Department	Peru	TK22845	AF338674, AF407177 mtrDNA
P. macleayii	Guantanamo Bay Naval Station, Guantanamo Province	Cuba	TK32162	AF338700, AF407178 mtrDNA, AF338700 *Rag*2
P. macleayii	St.Clair Cave, St. Catherine Parish	Jamaica	TK11008	AF338684
P. macleayii	St. Clair Cave, Polly Ground, St. Catherine	Jamaica	AMCC102719	AY604461
P. quadridens	Guantanamo Bay Naval Station, Guantanamo Province	Cuba	TK32171	AF338683, AF407179 mtrDNA, AF338695 *Rag*2
P. quadridens	St. Clair Cave, St. Catherine Parish	Jamaica	TK9487	AF338682, AF338696 *Rag*2
P. quadridens	La Entrada (de Cabrera), María Trinidad Sánchez	Dominican Republic	AMCC103036	AY604459
P. quadridens	Mata de Plátano, Arecibo	Puerto Rico	AMCC102335	AY604460
M. blainvillei	Guantanamo Bay Naval Station, Guantanamo Province	Cuba	TK32166	AF338685, AF407172 mtrDNA, AY028169 *Rag*2
M. blainvillei	Monarva Cave, Revival, Westmoreland	Jamaica	102762	AY604462
M. blainvillei	St. Clair Cave, St. Catherine Parish,	Jamaica	TK9469	AF338686, AF338701 *Rag*2
M. megalophylla	Presidio Co., Texas	United States	TK48165	AF338687, AY141020 *Rag*2
M. megalophylla	Black Gap Wildlife Management Area, Brewster Co, Texas	United States	TK78661	AF263220 mtrDNA, AY141020 *Rag*2
M. megalophylla	Chamela, Jalisco	Mexico	TK27640	AF330808, AF407174 mtrDNA, AF330818 *Rag*2
M. megalophylla	8.2 mi. S Pina Blanca, Queretaro	Mexico	TK4833	AF338689
M. megalophylla	Grutas de Lanquin, Alta Verapaz	Guatemala	CN98443	AF338690, AF338702 *Rag*2
M. megalophylla	7 km NW Barinitas, Barinas	Venezuela	TK19311	AF338688, AF407173 mtrDNA

The origin of Caribbean nectar-feeding bats (Chiroptera: Phyllostomidae)

ABSTRACT

Brachyphylla, *Erophylla*, and *Phyllonycteris* are New World leaf-nosed bats confined to the West Indies. New cytochrome *b* sequences were combined with existing data to estimate phylogenetic relationships among the genera. The three genera form a clade in analyses combining molecular and morphological data, but relationships with respect to other phyllostomids are not well resolved. Mitochondrial sequences of *Brachyphylla* also revealed two non-sister lineages in *B*. *nana*, and negligible character differences between subspecies of *B*. *cavernarum* sampled. Published molecular phylogenies place the clade containing *Brachyphylla* and its allies firmly within the phyllostomid nectar-feeding radiation, whereas estimates based on morphology do not, instead placing these taxa as successive sister taxa to all other nectar-feeding phyllostomids. The conflict between these estimates involves changes in a single suite of morphological characters that might be related to diet. Regardless of the topology used, dispersal vicariance analyses suggest the most recent common ancestor of *Brachyphylla*, *Erophylla*, *Phyllonycteris* and close relatives expanded into the Caribbean from the continental Neotropics through Mexico or Central America.

INTRODUCTION

Brachyphylla, *Erophylla*, and *Phyllonycteris* are phyllostomid bats restricted to the West Indies (Koopman, 1994). The three genera range throughout the Greater Antilles and the Bahamas, Middle Caicos, Isla de Pinos, and Cayman Islands, with *Brachyphylla* reaching through the Lesser Antilles south and east to St. Vincent and Barbados (Koopman, 1989;

Morgan, 2001). Seven species are currently recognized: *Brachyphylla nana* is extant on Cuba and Hispaniola and known as a fossil from Jamaica, *B*. *cavernarum* encompasses the remainder of the range for the genus, *Erophylla sezekorni* ranges through Cuba, the Bahamas, Middle Caicos and Jamaica, while *E*. *bombifrons* is restricted to Hispaniola and Puerto Rico (Koopman, 1989; Morgan, 2001). *Phyllonycteris major* is only known from fossils on Puerto Rico and Antigua, *P*. *poeyi* ranges through Cuba and Hispaniola, and *P*. *aphylla* is restricted to Jamaica (Koopman, 1989; Morgan, 2001). Geographic variation within all but two of the species, *Brachyphylla nana* and *Phyllonycteris aphylla*, is recognized by designation of subspecies, 11 in total (Koopman, 1994).

Brachyphylla, *Erophylla*, and *Phyllonycteris* have been collected in localities ranging from forested protected areas (Jones et al., 2001) to dry thorn scrub or xeric woods (Klingener et al., 1978; Timm and Genoways, 2003), agricultural patches on forest edges (Howe, 1974), and small patches of trees near abandoned buildings (Timm and Genoways, 2003). In contrast with this apparent tolerance for different habitats, all species with the exception of *Brachyphylla cavernarum* require caves as day roosts (Silva-Taboada, 1979; Swanepoel and Genoways, 1983a). *Phyllonycteris* in particular apparently requires hot, thermally stable environments available only in long, poorly ventilated cave systems (Goodwin, 1970; Silva-Taboada, 1979). The extinction of *Phyllonycteris major* and the extirpation of *Phyllonycteris* and *Brachyphylla* from the Bahamas and Cayman Islands has been linked to environmental change leading to the loss of appropriate microenvironments in caves (Morgan, 2001). *Brachyphylla*, *Erophylla*, and *Phyllonycteris* roost in colonies of up to 27,000 individuals, although the average colony is probably in the low thousands, and may exhibit dramatic seasonal

decline probably tied to food availability (Dávalos and Eriksson, 2003; Silva-Taboada, 1979). All species have been reported to consume pollen, nectar, and small quantities of fruits and insects (Klingener et al., 1978; Silva-Taboada and Pine, 1969; Swanepoel and Genoways, 1983a; Swanepoel and Genoways, 1983b), and *Brachyphylla nana* and *Phyllonycteris poeyi* are cactophilic (Silva-Taboada, 1979; Simmons and Wetterer, 2002).

As of 2002, one third of the literature on bat systematics had been devoted to the study of phyllostomids, often including *Brachyphylla*, *Erophylla*, and *Phyllonycteris* (Jones et al., 2002). These efforts will not be reviewed here, since Wetterer et al. (2000) have published a thorough account of the subject in the introduction to their phylogenetic study. The biogeographic history of these taxa is intriguing because they are restricted to the Caribbean and appear to have no close relatives on the continent, unlike all other Antillean bats (Baker and Genoways, 1978; Koopman, 1989). Their geographic origin is deemed uncertain, and might be as ancient as the Miocene (Koopman, 1981; Koopman, 1989). Through the 1980s, phylogenetic analyses corroborated a close relationship between *Brachyphylla*, *Erophylla*, and *Phyllonycteris*, and their exclusion from the clade of other nectar feeding phyllostomids (Baker and Bass, 1979; Griffiths, 1985; Smith, 1976). As the scope and taxonomic coverage of phylogenetic studies broadened, the relationships among these genera became less certain: the sister relationship between *Erophylla* and *Phyllonycteris* was confirmed, but *Brachyphylla* was found to be sister to all other nectar feeding bats and most internal branches of the nectar feeding clade were unresolved (Baker et al., 1989; Gimenez, 1993; Gimenez et al., 1996).

The phyllostomid phylogenies of Baker et al. (2000) and Wetterer et al. (2000) also rejected the monophyly of a *Brachyphylla*, *Erophylla*, and *Phyllonycteris* clade. Baker et al. (2000) found these genera to be part of the nectar feeding radiation based on nuclear *Rag*2 sequences, in conflict with the mostly morphological phylogeny of Wetterer et al. (2000), which placed *Brachyphylla* and (*Erophylla*, *Phyllonycteris*) as successive sister taxa to the nectar feeding subfamily Glossophaginae. The matrix representation with parsimony supertree of Jones et al. (2002) found a monophyletic *Brachyphylla*, *Erophylla* and *Phyllonycteris* clade suggesting this could be the null phylogenetic hypothesis, but found no support for the clade when analyzing studies from the 1990s only. More recently Baker et al. (2003) added mitochondrial12S, $tRNA^{val}$, and 16S sequences (hereafter mtrDNA) to their nuclear data set, but could not fully evaluate the monophyly of this Caribbean clade because *Phyllonycteris* was not included.

In this study new mitochondrial DNA sequences were combined with published data sets to test the character support for the monophyly of *Brachyphylla*, *Erophylla*, and *Phyllonycteris* with respect to selected outgroups. The results of these analyses and of published phylogenies were used to infer ancestral areas for these genera to test the hypothesis of Koopman (1989). Additionally, multiple mitochondrial cytochrome *b* sequences from most of the range of *Brachyphylla* were collected to produce a first phylogeny for this genus and resolve decades of dispute on the number of species it contains (Morgan, 2001; Silva-Taboada, 1976; Swanepoel and Genoways, 1978; Varona, 1974).

MATERIALS AND METHODS

Taxon Sampling

To examine relationships among *Brachyphylla*, *Erophylla* and *Phyllonycteris*, representatives of all three genera were included. Geographic sampling within the genus *Brachyphylla* was dense, with samples from 15 different islands (Appendix 2). Most ingroup cytochrome *b* sequences were generated for this study (Table 1), while *Rag*2 sequences were obtained from a previously published study (Baker et al., 2000). For outgroup comparison sequences from the phyllostomids *Trachops cirrhosus* (AF316490), *Carollia perspicillata* (AF187026), *Carollia brevicauda* (AF316437), *Lionycteris spurrelli* (AF423097, AF316455), *Lonchophylla thomasi* (AF187034, AF316456), *Glossophaga soricina* (AF423081, AF316452), *Anoura geoffroyi* (L19506) *Anoura caudata* (AF31643), *Artibeus concolor* (ACU66519, AF316432), *Uroderma bilobatum* (L28941, AF316491), and *Sturnira lilium* (AF187035, AF316488) were used. To root the tree, sequences from *Mormoops megalophylla* (AF338690, AF330818) were included in phylogenetic analyses.

Molecular Data

For most specimens, DNA was isolated from wing clip (Rossiter et al., 2000), or liver tissue that had been frozen or preserved in ethanol or lysis buffer in the field. For three specimens of *Brachyphylla nana* (AMNH nos. 175993, 214390, and 244916) DNA was isolated from ~5 mm ribs taken from skeletons that had been stored dry in a museum cabinet. For frozen and ethanol preserved tissues, DNA was extracted using a Qiagen DNeasy Tissue Extraction Kit (Qiagen, Inc.) following the manufacturer's protocol. This protocol was modified with methods described by Iudica et al. (2001) for the dried bone

specimens. DNA extraction and amplification of the museum specimens was conducted in the ancient DNA room of the Molecular Systematics Laboratory, a separate facility from the Cullman Laboratory for Molecular Systematics (both at the American Museum of Natural History), where all other experiments were performed. Extracted DNA was used as a template in PCR reactions with protocols and primers for cytochrome *b* described elsewhere (Dávalos and Jansa, in press; Jansa et al., 1999). Amplification products were sequenced with the same primers used for PCR amplification and also internal primers. Sequencing reactions were purified through a $MgCl_2$-ethanol precipitation protocol and run on an ABI 3100 automated sequencer. Sequences were edited and compiled using Sequencher 4.1 software (GeneCodes, Corp.). Base-calling ambiguities between strands were resolved either by choosing the call on the cleanest strand or using the appropriate IUB ambiguity code if both strands showed the same ambiguity. Molecular sequences generated as part of this study have been deposited in GenBank under accession numbers AY620438- AY620467.

Morphological Data

The morphological character matrix of Wetterer et al. (2000), as modified by Dávalos and Jansa (in press) for the outgroup *Lonchophylla*, was appended to the molecular data to generate combined analyses of all characters available for the group.

Data Analysis

Protein-coding cytochrome *b* and *Rag*2 sequences were easily aligned by eye using Sequencher 4.1 (GeneCodes, Corp.). To describe the variation in cytochrome *b* among taxa uncorrected pairwise (p) distances were calculated using PAUP* 4.0b10 (Swofford,

2002). Cytochrome *b* sequences were also examined for fixed character differences among named subspecies *sensu* Swanepoel and Genoways (1978).

Analyses were conducted on two samples of taxa: a data set consisting of morphology, cytochrome *b*, and *Rag*2 including *Brachyphylla*, *Erophylla*, *Phyllonycteris* and outgroups (hereafter the phyllonycterine data set), and a data set including cytochrome *b* of *Brachyphylla* species exemplars and outgroups (hereafter the *Brachyphylla* data set). Parsimony analyses were performed for separate data partitions and on combined matrices using branch and bound searches as implemented in PAUP* 4.0b10. For each search, phylogenetically informative characters were treated as unordered and equally weighted. Clade stability was assessed using nonparametric jackknife (Wu, 1986) and the Bremer support index (Bremer, 1994). Parsimony jackknife analyses included 1,000 replicates; searches were heuristic with 100 replicates of random taxon addition followed by TBR branch swapping. Bremer values were calculated with the aid of AutoDecay (Eriksson, 1999). Character state changes and length of alternative topologies were explored using MacClade 4.06 (Maddison and Maddison, 2003).

Best-fit maximum likelihood models for molecular data were selected using nested likelihood ratio tests as implemented in MODELTEST (Posada and Crandall, 1998). Maximum likelihood analyses of the different molecular data sets were performed using PAUP* 4.0b10. Bayesian methods were used to estimate a phylogeny using different models of molecular evolution for each partition of the molecular data. This analysis featured two partitions, mitochondrial and nuclear DNA, and the model of sequence evolution was determined using MODELTEST (see above). The values for model parameters were allowed to vary between partitions and were not specified a priori, but

treated as unknown variables to be estimated in each analysis. Bayesian analysis was conducted using MrBayes 3.0 (Huelsenbeck and Ronquist, 2001), with random starting trees without constraints, four simultaneous Markov chains were run for 2,000,000 generations, trees were sampled every 100 generations, and temperature was set to 0.20. Resulting burn in values, the point at which the model parameters and tree score reach stationarity, were determined empirically by evaluating tree likelihood scores. Analyses were repeated in 4 separate runs of MrBayes to ensure that trees converged on the same topology.

Finally, whether enforcing a molecular clock provided a better fit to the data than allowing for different rates across the tree was evaluated. To provide the most conservative test for a clock-like model of evolution, a UPGMA tree based on Jukes-Cantor distances was calculated, and the likelihood score for the best-fit model with no clock enforced ($\log L_1$) vs. the same model with a clock enforced ($\log L_2$) were compared. The significance of the difference in likelihood scores was tested by comparing $-2\log\lambda$ against a χ^2 distribution with degrees of freedom equal to the number of taxa minus 2. If the value for $-2\log\lambda$ was significant, then a molecular clock could be rejected.

Biogeographic Analyses

Koopman (1989) proposed that the ancestor of *Brachyphylla*, *Erophylla*, and *Phyllonycteris* reached the Caribbean through Central America or Mexico. This means that the ancestral area for the group and its closest continental relative should include either or both these areas. To estimate ancestral areas, two biogeographic methods were used: dispersal-vicariance analysis, DIVA (Ronquist, 1997), and ancestral area analysis (Bremer, 1992). Dispersal-vicariance analysis does not require an independent hypothesis

of area relationships, but instead reconstructs the ancestral distribution at each of the internal nodes of a given phylogeny. The estimation can be constrained to contain any minimum number of areas. This is accomplished by means of optimization rules and set costs for extinction (cost of 1 per area lost) and dispersal (cost of 1 per area added). Vicariant and sympatric speciation carry no cost. Species distributions are therefore explained by assigning costs for each event in a way that biogeographic explanations imply the least possible cost.

Ancestral area analysis (Bremer, 1992) allows the identification of the ancestral area of a group based on its phylogeny, given geographic distribution information on the branches of the phylogeny. Each area can be considered a binary character with two states (present or absent) and optimized on the phylogeny. By comparing the numbers of gains and losses, it is possible to estimate areas most likely to have been part of the ancestral areas.

RESULTS

Sequence variation and saturation analysis

Cytochrome b

Complete cytochrome *b* sequences were obtained for most taxa, with the exceptions of *Trachops cirrhosus* (Albert Ditchfield, personal communication) and *Anoura geoffroyi* (L19506), for which only 402 base pairs were available. Only 425-850 bp fragments of cytochrome *b* were sequenced from museum specimens, as these taxa were all represented by tissue exemplars. A summary of the uncorrected pairwise divergences among individuals on different islands and taxonomic ranks is shown in Fig. 1.

One of the objectives of this study was to assess the intraspecific variation in *Brachyphylla*, and cytochrome *b* from numerous individuals per species was sequenced accordingly. Of these, multiple individuals, from different localities had identical sequences and were grouped together as haplotypes (Appendix 2). A subset of these was used in the phylogenetic analyses of *Brachyphylla* (Table 1). Sequence examination for fixed characters among currently recognized subspecies *sensu* Swanepoel and Genoways (1978) uncovered a single difference between *Brachyphylla cavernarum intermedia* (Puerto Rico, n=3) and *B*. *c*. *cavernarum* (see Appendix 2 for sample size and localities included). Twenty-three fixed character differences were found between sequences of *Brachyphylla nana nana* Miller (Cuba n = 1, Grand Cayman n = 6) and *B*. *n*. *pumila* Miller (Hispaniola n = 2, Middle Caicos n = 5). A summary of the uncorrected pairwise divergences among individuals on different islands and taxonomic ranks is shown in Fig. 1.

Within cytochrome *b* of phyllonycterines, 496 (44%) of sites are variable and 350 (31%) are parsimony informative. The distribution of the parsimony-informative sites is highly dependent on codon position: 18.8% in first, 4.0% in second, and 77.1% in third codon position. Most substitutions are synonymous, and translation of sequences to amino acids leads to a matrix with only 46 informative sites (24.7% variable sites, among which 48.9% are parsimony informative). The average base composition of sequences is skewed, with little bias at first codon position, deficiency of adenine (20.3%) and guanine (14.6%) and overabundance of thymine (40.5%) for second position and a strong bias in third position: deficiency of guanine (3.2%) and thymine (20.5%), and abundance of adenine (38.9%) and cytosine (37.4%). That bias in base composition does not differ

significantly across taxa when first (chi-square test implemented in PAUP* 4.0b10, *P*=0.834) and second positions (*P*=1.000) are analyzed separately. There are significant differences in base composition among taxa in third positions (*P*=0.000), leading to significant results when the whole cytochrome *b* is analyzed (*P*=0.002).

Heterogeneity in base composition across taxa is known to affect phylogenetic reconstruction (Lockhart et al., 1994): bias in third codon position, which contains most of the sequence variation, may confound the results of sequence analyses. To examine this hypothesis, the most divergent taxa in GC content were identified for the third bases of cytochrome *b* sequences (GC content average for all taxa = 40.6%, SD = 6.7): *Lionycteris* (32.9%), *Glossophaga* (28.7%), *Phyllonycteris* (48.7%), and *Erophylla* (50.3%). Heterogeneity is presumed to mislead phylogenetic analyses because unrelated taxa with similar base composition might appear as related when using methods that do not account for this error. Of these taxa, *Lionycteris* and *Glossophaga* do not appear as sister taxa (Fig. 2), and data sources other than third codon positions in cytochrome *b*, e.g., morphology and *Rag*2, support the sister relationship between *Phyllonycteris* and *Erophylla*. This heterogeneity in third codon positions is therefore not presumed to distort phylogenetic analyses toward recovering such relationships.

Graphs of transitions and transversions for each codon position versus uncorrected total sequence divergence were plotted to assess saturation in sequences. Those curves (not shown) indicated that first and second codon positions did not experience multiple transition or transversion substitutions. Third codon positions showed saturation in transversion substitutions beyond 10% uncorrected pairwise

divergence, or for all comparisons except those within the genus *Brachyphylla*. Third codon position transitions showed saturations only with respect to partial sequences.

*Rag*2

Of the 1,363 *Rag*2 sites, 232 (17%) are variable and 71 (5.2%) are parsimony informative. The distribution of the parsimony-informative sites is highly dependent on codon position: 15.5% in first, 11.3% in second, and 73.2% in third codon position. Most substitutions are synonymous, and translation of sequences to amino acids leads to a matrix with only 19 informative sites (12.6% variable sites, among which 33.3% are parsimony informative). The average base composition of sequences is skewed, with a deficiency of cytosine (17.8%) and thymine (20.3%) and an overabundance of adenine (30.4%) in first codon position, a deficiency of guanine (17.8%) and an overabundance of adenine (35.1%) in second position, and a deficiency of guanine (18.4%) and an overabundance of thymine (30.8%) in third position. The biases in base composition do not differ significantly across taxa for the whole gene, or for different codon positions (P=1.000).

Phylogenetic analyses

Three data sets were included in phylogenetic analyses: the mitochondrial cytochrome *b*, a 1.36 kb fragment of the nuclear *Rag*2, and the mostly morphological character matrix for phyllostomids published by Wetterer et al. (2000). Molecular data from different genes were concatenated for combined analyses. Sequences for two outgroups, *Carollia* and *Anoura*, were obtained from different species (see methods). This was not considered problematic because the monophyly of these genera is uncontroversial (Wetterer et al., 2000; Wright et al., 1999). Maximum parsimony analyses of the molecular data sets

(Figs. 2a and 3a) and of the 'total evidence' data matrix (Fig. 4) were conducted with all unordered and unweighted characters.

Maximum-likelihood analyses were performed first using the program MODELTEST (see above). The models selected and parameters for each data set are summarized in Table 2. The results of maximum likelihood analysis of *Brachyphylla* cytochrome *b* are shown in Fig. 3b. Since the parameters for cytochrome *b* and *Rag*2 were considerably different, Bayesian methods were used to obtain an estimate of phylogeny that accounted for these two models while using all available molecular data. Stationarity in parameter estimation was reached after 200,000 generations (burn in = 2,000 trees). The mean parameters for the cytochrome *b* exemplar data set, followed by 95% confidence interval in parentheses, were estimated as follows: R_{matrix} = 0.783 (0.276-1.412), 6.329 (3.291-9.794), 0.573 (0.198-1.04), 0.909 (0.315-1.634), 8.343 (4.3777-13.185), 1.0, shape parameter = 0.31 (0.168-0.469). The mean parameters for the *Rag*2 data set, followed by 95% confidence values in parentheses, were estimated as follows: R_{matrix} = 3.763 (0.903-8.29), 8.212 (2.433-17.35), 1.854 (0.0189-4.132), 0.838 (0.01-2.226), 50.121 (19.331-91.44), 1.0, shape parameter = 0.208 (0.177-0.245). The resulting trees are summarized in Fig. 2b.

Biogeographic Analyses

The results of ancestral area optimization for the most recent common ancestor of *Brachyphylla*, *Erophylla*, *Phyllonycteris* and their closest continental relative are shown in Table 3. DIVA optimizations included all nectar feeding bats for the tree modified from Wetterer et al. (2000), hereafter called morphology*; *Carollia*, *Artibeus*, *Uroderma* and *Sturnira* for the tree from Fig. 4; all taxa excluding *Lonchophylla* and allies for the tree

from Fig. 5a; and all taxa for the tree from Fig. 5b. Optimizations were constrained to two areas of endemism per node following the suggestions of the author (Ronquist, 1996) for cases when an ancestral area of endemism is expected to be less than the sum of areas occupied by descendents.

DISCUSSION

How many species of *Brachyphylla*?

As many as four (Hall and Kelson, 1959) and as few as one (Buden, 1977; Varona, 1974) *Brachyphylla* species were recognized until the revision of Swanepoel and Genoways (1978). That revision encompassed more than 600 specimens from throughout the range of the genus and recognized two species, *nana* and *cavernarum*, and two additional subspecies of *cavernarum*: *intermedia* and *minor* (Swanepoel and Genoways, 1978). Most subsequent authors have adopted their taxonomic conclusions, e.g. Koopman (1994), Simmons (in press).

In this study, cytochrome *b* sequences were obtained from most of the range of *Brachyphylla* (Appendix 2). Molecular character differences support an alternative taxonomy for the genus: *Brachyphylla nana* Miller (1902) and *B. pumila* Miller (1918) are distinct, as had been suggested by Morgan (2001) based on morphological characters. The one-site difference between *intermedia* (Puerto Rico) and *cavernarum* (for included range see Appendix 2) suggests these taxa are not distinct because many other haplotypes, including some apparently restricted to particular islands, differ at single sites as well. *Brachyphylla cavernarum minor* (Barbados) is the only taxon recognized by Swanepoel and Genoways (1978) that was not studied here.

Examination of sequence variation among *Brachyphylla cavernarum* individuals (Fig. 1) shows that variation is as great within islands as it is between them, suggesting that genetic diversity is not partitioned between islands. Analyses of molecular variance for a subset of these data confirmed this result (Carstens et al., in press). This is relevant to the species taxonomy because statistical analyses of morphological characters often group individuals together for comparisons among islands, e.g., Swanepoel and Genoways (1978). It is assumed that individuals on each island are more closely related to one another than to individuals on other islands, but Carstens et al. (in press) rejected the monophyly of island populations using a parametric bootstrap. We are currently analyzing the complete data set with methods that do not generate dichotomous branching patterns among haplotypes to test these partial results.

Phylogeny of *Brachyphylla*

The relationships among species of *Brachyphylla* had not been examined with explicit phylogenetic methods until now. The study of Swanepoel and Genoways (1978) used distance phenograms to summarize comparisons of metric characters among island samples. Following that systematic revision, the phylogeny of *Brachyphylla* seemed resolved: two species can only be each other's closest relative. The phylogenetic hypothesis presented here (Fig. 3) shows that the two lineages lumped together as *Brachyphylla nana sensu* Swanepoel and Genoways (1978) are not sister taxa.

This well-supported hypothesis (Fig. 3) also helps explain the puzzling characteristics of *Brachyphylla* fossils from Andros and New Providence (Bahamas), Cayman Brac (Cayman Islands), Jamaica, and Middle Caicos (Turks and Caicos). Bahamian remains appear related to *nana*, whereas Jamaican specimens and those from

Middle Caicos are more similar to *pumila* (Koopman and Williams, 1951; Morgan, 1993; Morgan, 1989). Fossils similar to both species have been found on Cayman Brac (Morgan, 1994). Morgan (2001) pointed out that *Brachyphylla* extirpated from Jamaica, Cayman Brac, and Middle Caicos bridge the gap in size between *cavernarum* and *nana sensu* Swanepoel and Genoways (1978). *Brachyphylla pumila* and *cavernarum* diverged relatively recently (Fig. 3), and these fossils might be remains of a taxon less divergent in size from a *cavernarum*-sized ancestor.

Phylogeny of *Brachyphylla*, *Erophylla*, and *Phyllonycteris*

The question whether or not *Brachyphylla*, *Erophylla* and *Phyllonycteris* form a clade has troubled systematists for decades. Studies of allozymes, karyotypes, behavior, immunology, morphology, and parasites support a sister relationship between *Brachyphylla* and the *Erophylla-Phyllonycteris* clade (Allen, 1898; Baker and Bass, 1979; Baker and Lopez, 1970; Gardner, 1977; Griffiths, 1985; Hall, 1981; Koopman, 1984; Silva-Taboada and Pine, 1969; Smith, 1976). The clade comprising these three genera has been called Brachyphyllinae (Allen, 1898; Griffiths, 1985; Hall, 1981; Koopman, 1984) or Phyllonycterinae (Corbet and Hill, 1980; Gardner, 1977). Given the inconsistent usage of these names and the debate over the taxonomic status of *Brachyphylla*, i.e., in its own subfamily (Jones et al., 2002; Koopman, 1994; Wetterer et al., 2000) or within the subfamily glossophaginae (Baker et al., 2000; McKenna and Bell, 1997), this clade is called the *Brachyphylla*, *Erophylla*, *Phyllonycteris* clade here. Neither Wetterer et al. (2000), Baker et al. (2000), nor analyses of combined cytochrome *b* and *Rag*2 data recovered a monophyletic *Brachyphylla*, *Erophylla*, *Phyllonycteris* clade (Fig.

2). In these analyses, the sister-group relationship between *Erophylla* and *Phyllonycteris* is well supported but the position of *Brachyphylla* remmains uncertain (Fig. 2).

The total evidence analysis recovers a monophyletic *Brachyphylla*, *Erophylla*, *Phyllonycteris* clade (Fig. 4), with hidden support from the different data sources (cf. Wetterer et al. [2000]: Fig. 49; Baker et al. [2000]: Fig. 2; and Fig. 3). Five morphological characters, 1 restriction site, and 14 molecular characters change unambiguously along this branch, although it is not robust to jackknife resampling (JK <50%). Two studies including additional data partitions showed results consistent with this resolution (Baker et al., 2003; Carstens et al., 2002). Baker et al. (2003) combined mtrDNA sequences with *Rag*2 data, recovering (*Brachyphylla*, *Erophylla*) with 0.97 posterior probability and most of the support comes from the mitochondrial partition (*Phyllonycteris* was not included, see Fig. 5a). Carstens et al. (2002) added 62 skin, skull, and dentition characters to 57 soft-tissue characters from Wetterer et al.(2000) and the *Rag*2 data of Baker et al. (2000), obtaining (*Brachyphylla* (*Erophylla*, *Phyllonycteris*) with 80% bootstrap support in combined analyses (Fig. 5b). The character support and congruence in results from different combinations of independent data partitions confirm the monophyly of the *Brachyphylla*, *Erophylla*, *Phyllonycteris* clade as the null phylogenetic hypothesis given current knowledge (Jones et al., 2002).

The second question surrounding the phylogeny of the *Brachyphylla*, *Erophylla*, *Phyllonycteris* clade is which are the closest relatives of this exclusively Caribbean group. The data analyzed here support a topology where the *Brachyphylla*, *Erophylla*, *Phyllonycteris* clade is sister to all other nectar-feeding bats sampled (Fig. 4). This result is not robust: making the *Brachyphylla*+*Erophylla*+*Phyllonycteris* clade sister to

Glossophaga-Anoura (one possible resolution of Fig. 2b) costs only four extra steps. A clade consisting of *Glossophaga*, *Leptonycteris* and *Monophyllus* was sister to *Brachyphylla-Erophylla* (Fig. 5a) in analyses by Baker et al. (2003). The same clade was sister to *Brachyphylla* and allies (Fig. 5b) when characters associated with nectar feeding were excluded from the study of Carstens et al. (2002).

Two competing hypotheses are obvious: the *Brachyphylla*, *Erophylla*, *Phyllonycteris* clade is either nested within the nectar-feeding radiation (depending on the topology, with lonchophyllines Figs. 2b, 5b; or without lonchophyllines Fig. 5a), or they branch off prior to it (with lonchophyllines Fig. 4, [Carstens et al. 2002]: Fig. 1; or without lonchophyllines [Carstens et al. 2002]: Fig. 4). Estimates of phylogeny using only molecular data support the first hypothesis, and analyses of data sets including morphological characters recover the second. If morphology more closely approximates the evolutionary history of nectar-feeding phyllostomids, then the convergence in mitochondrial and nuclear sequences that leads to strong support of their inclusion in the nectar-feeding clade (Fig. 5a) needs to be explained. Future studies can test this hypothesis with additional unlinked markers.

Assuming that molecular characters provide the better estimate of phylogeny two questions arise: first, how strong is the conflicting evidence? When analyzed separately, morphological characters place *Brachyphylla* either at a polytomy at the base of the phyllostomid radiation, excluding only the vampires (Wetterer et al., 2000), or as sister to the vampire *Desmodus* (Carstens et al., 2002). This is because the morphology of *Brachyphylla* appears highly modified compared to *Erophylla* and *Phyllonycteris*: until the compelling work of Silva-Taboada and Pine (1969), this genus was placed in the

vampire subfamily Desmodontinae (e.g., Dobson, 1878) or the fruit-eating subfamily Stenodermatinae (e.g., Miller, 1907). This does not mean that the morphological evidence for the *Brachyphylla*, *Erophylla*, *Phyllonycteris* clade being basal to the nectar feeding clade is weak: nine morphological characters change unambiguously between this clade and other nectar-feeding bats in Fig. 4: characters 22, 24, 58, 84, 91, 92, 93, 96, and 97 of Wetterer et al. (2000). The quantity of characters in conflict is comparable to that supporting other uncontested phyllostomid clades *sensu* Wetterer et al. (2000), such as Desmodontinae (9), or Stenodermatinae (8).

Having shown that the conflict is strong, the second question is: can these morphological characters be explained as the result of convergence? Of the 9 characters supporting the branch that places the *Brachyphylla*, *Erophylla*, *Phyllonycteris* clade as basal to other nectar-feeding phyllostomids, three (22, 58, and 84) are also present in derived condition in unrelated phyllostomids (e.g., *Lonchorhina*, *Macrotus*, *Chiroderma*) raising the possibility that they evolved multiple times. Of the remaining six characters, five (91, 92, 93, 96, and 97) are characters of the hyoid muscles. These characters are probably associated with adaptations to feeding from flower products (Griffiths, 1982).

There is some evidence to postulate that a shift in diet has occurred in the *Brachyphylla*, *Erophylla*, *Phyllonycteris* clade in comparison to their proposed close relatives (Fig. 5). *Glossophaga* appears to be omnivorous, consuming fruit juices, insects, seeds, pollen, and nectar (Fleming et al., 1972; Goodwin and Greenhall, 1961; Nassar et al., 2003; Sosa and Soriano, 1996). Nectar, pollen, and a relatively large proportion of insects were found in samples of stomach content from *Monophyllus* (Silva-Taboada, 1979), whereas *Leptonycteris* appears to specialize in plant products, nectar, pollen and

fruit (Nassar et al., 2003; Nassar et al., 1997). The diet of the *Brachyphylla*, *Erophylla*, *Phyllonycteris* clade consists primarily of pollen, with smaller quantities of nectar, seeds, and insects (Nellis and Ehle, 1977; Silva-Taboada, 1979; Silva-Taboada and Pine, 1969). All but one of the morphological characters generating conflict with molecular estimates of phylogeny could potentially be explained as the result of change in a single suite of traits probably related to diet. These characters might have been selected for in the transition from a diverse diet to one based on pollen for the *Brachyphylla*, *Erophylla*, *Phyllonycteris* lineage. This tentative scenario remains to be tested in a rigorous comparative analysis that examines diet, morphological characters, and the interplay between the two in a phylogenetic context.

Biogeography of *Brachyphylla, Erophylla,* and *Phyllonycteris*

Ancestral area optimizations for four alternative topologies (Table 3) repeatedly recover Jamaica as part of the ancestral area. This result arises from the use of genera as terminals and the presence of *Glossophaga soricina* in Jamaica. To make phylogenies comparable, genera rather than species were used as terminals and this granted undue weight to this island as an area of endemism. Range expansion into the Caribbean appears to be recent in the history of *Glossophaga*, and Jamaican exemplars are closely related those from Mexico and Central America (Hoffmann and Baker, 2001).

Ancestral area analyses only reject strongly the Lesser Antilles as ancestral for the most recent common ancestor of the *Brachyphylla*, *Erophylla*, *Phyllonycteris* clade and its closest relatives (hereafter 'Caribbean-continent ancestor'). For this reason most of the discussion will focus on the results of dispersal vicariance analyses. DIVA provide some support for the hypothesis of Koopman (1989) by including Mexico or Central America

in ancestral area optimizations (Table 3). The optimizations for southern Central America (Panama and Costa Rica) are not equivalent to the hypothesis of dispersal from the continent via Yucatán or Honduras-Nicaragua, the "western route" of Baker and Genoways (1978). These last two areas were almost interconnected to Cuba and Jamaica during periods of low sea level (Griffiths and Klingener, 1988), whereas no similar history has been postulated for Panama and Costa Rica.

The Caribbean in the deep history of nectar-feeding Phyllostomids

The phylogenies studied challenge the conventional view of the biogeography of *Brachyphylla*, *Erophylla*, and *Phyllonycteris* by including the Greater Antilles in the range of the Caribbean-continent ancestor, rather than constraining the Caribbean expansion to the ancestor of the three genera (Table 3). Two phylogenies deserve particular attention (Fig. 5). If *Glossophaga* and *Leptonycteris* are sister taxa, a single invasion from the Caribbean is the most parsimonious interpretation of their current distribution because *Brachyphylla*, *Erophylla*, *Phyllonycteris*, and *Monophyllus* are exclusively Caribbean (Fig. 5a). If *Leptonycteris* and *Monophyllus* are sister taxa it is equally costly to place the Caribbean-continent ancestor on the continent or the Caribbean because either way two invasions are needed to explain current distributions (Fig. 5b).

Is there any additional evidence for hypothesizing that the ancestors of *Leptonycteris* and *Glossophaga* evolved in the Caribbean? The basal lineages of both genera (*G. soricina* and *L. nivalis*) overlap in Mexico, although *G. soricina* is widespread ranging south through Central America to western Peru and northern Argentina, reaching even Jamaica. These distributional ranges suggest the ancestral area of endemism for

both genera is Mexico, but this does not discriminate between a continental and a Caribbean Caribbean-continent ancestor.

Another source of evidence could be the study of species autecology: if ecological niches are conserved within lineages (Peterson et al., 1999) continental taxa descended from Caribbean ancestors should be restricted to Antillean-like environments. The 3 species of *Leptonycteris* and 3 of 5 *Glossophaga* species (*leachii*, *morenoi*, and *longirostris*) are restricted to arid mainland habitats (Arita, 1991; Handley, 1976; Koopman, 1994; Reid, 1997; Ruiz et al., 2000; Ruiz et al., 1997). The mean annual rainfall of Cuba is 1,400 mm (Silva-Taboada, 1979) and of Jamaica is 1,980 mm (Evans, 1973) whereas rainfall in Amazonian and Guianan forests is typically above 3,000 mm (Simmons and Voss, 1998; Sombroek, 2001), and some localities in the Choco record 10,000 mm (Haffer, 1970). Although this seems to support the preference for dry Antillean-like environments, two *Glossophaga* species, *comissarisi* and *soricina*, occur in rainforest habitats, with the latter reaching the Choco (Emmons, 1997; Koopman, 1981; Webster, 1993). Because *soricina* is basal in its genus, the optimization of autecology for the ancestral *Glossophaga* node is equivocal between arid habitats and rainforests. Since niches are more often conserved among sister species pairs than when comparing more distant relatives (Martínez-Meyer, 2002), autecological requirements might not provide strong tests of the West Indian Caribbean-continent ancestor hypothesis. Independent sources of biogeographic data (e.g., Earth history, congruence of area relationships with other taxa, fossils) are probably necessary to discriminate between the hypotheses for the area of endemism of the Caribbean-continent ancestor.

Here, the Caribbean origin hypothesis is bolstered mainly by a phyllostomid phylogeny that will probably inspire controversy (Fig. 5a, see the evaluation of conflict with previous phylogenies in Baker et al. [2003]). The sensitivity of the results of Baker et al. (2003) to alignment of mtrDNA, model of DNA evolution and character resampling will have to be evaluated. Subsequent studies will doubtless seek to combine the mtrDNA and *Rag*2 data with morphological and other molecular data sets, such as the ones used here. Although the hypothesis of Caribbean origin for *Glossophaga* and *Leptonycteris* is weak at present, it overturns the invasion-from-the-mainland model prevalent in neotropical biogeography. The idea that a Caribbean lineage has reinvaded the mainland and successfully diversified there had, to my knowledge, not been articulated until biogeographic analyses for the phylogeny of Short-faced phyllostomids showed as much (this dissertation). If corroborated among nectar-feeding phyllostomids, this hypothesis promises to shed new light on the evolution of roosting habits and cactophyly, among other ecological adaptations (Wetterer, 2003).

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Table 1. Species, localities and cytochrome *b* haplotypes included in phylogenetic analyses. The number of times each haplotype was observed is shown as "=n" after the locality. AMCC = Ambrose Monell Cryogenic Collection tissue voucher number, AF = GenBank number. For complete locality data and haplotype designation see Appendix 2.

Species	Locality or Island(s)	Haplotype
Brachyphylla n. nana	Grand Cayman =4	A
Brachyphylla n. nana	1 mi. E of Savannah, Grand Cayman	B
Brachyphylla n. nana	Lower Valley Forest, Grand Cayman	C
Brachyphylla n. nana	ca. 3 mi E Tapaste, Habana Province, Cuba	D
Brachyphylla n. pumila	Middle Caicos =3	A
Brachyphylla n. pumila	Conch Bar Cave, Middle Caicos	B
Brachyphylla n. pumila	Conch Bar Cave, Middle Caicos	C
Brachyphylla n. pumila	Los Patos upper cave, Barahona, Dominican Republic	D
Brachyphylla n. pumila	3 km NE Sierra de Agua, Los Haitises, San Cristóbal, Dominican Republic	E
Brachyphylla c. cavernarum	Anguilla =3 Antigua =1 Guana =1 Monserrat =4 Nevis =3 Saba =2 St. Maarten =1 St. Eustatius =1	A
Brachyphylla c. cavernarum	Nevis =2 St. Maarten =2 St. Eustatius =1	C
Brachyphylla c. cavernarum	Montserrat =2	D
Brachyphylla c. intermedia	Puerto Rico =3	U
Erophylla bombifrons	La Entrada (de Cabrera), Maria Trinidad Sanchez, Dominican Republic	AMCC 103029
Erophylla sezekorni	Windsor Great Cave, Trelawny, Jamaica	AMCC 102699
Phyllonycteris aphylla	Bluefields, Westmoreland, Jamaica	AF187033

Table 2. Models of molecular evolution and parameters selected for each molecular data set, see Methods for sequences included. GTR = general time reversible model; HKY = Hasegawa Kishino Yano model; R-matrix = rate matrix parameter (with respect to G-T transversion); ti/tv ratio = transition/transversion ratio; Γ = shape parameter, I = proportion of invariant sites; $-2\log\Lambda = 2[\log L_1 - \log L_2]$, where L_1 = likelihood without clock and L_2 = likelihood with clock.

Data	Model	R-matrix or ti/tv ratio	Γ	I	$-2\log\Lambda$	df	*P*
phyllonycterine cyt *b*	GTR+I+Γ	3.3, 8.0, 2.4, 0.7, 39.1	0.9960	0.4360	25.9	11	<0.001
Brachyphylla cyt *b*	GTR+Γ	1.0, 7.6, 1.0, 1.0, 14.7	0.2391	-	12.1	9	>0.05
phyllonycterine *Rag*2	HKY+Γ	4.4	0.2943	-	8.0	10	>0.05

Table 3. Estimation of ancestral area for the most recent common ancestor of *Brachyphylla* and allies and its continental relatives, Caribbean-continent ancestor, given alternative phylogenies proposed for the nectar-feeding phyllostomids. Morphology* is the phylogeny of Wetterer et al. (2000), modified to constrain the monophyly of *Brachyphylla* and allies (cost = 4 extra steps). Genera were used as terminals, with distribution obtained from Koopman (1994), and nodes were constrained to two areas. The 'x' indicates the area immediately preceding it should be combined with each of the areas after it to generate multiple two-area regions.

Phylogeny	Dispersal vicariance analysis	Ancestral area analysis
Morphology*	Jamaica, each of the Greater Antilles x southern Central America, northern South America, or central South America	Equal probability to Mexico, Central America, northern South America, central South America, or Jamaica
Figure 4	Each of the Greater Antilles x southern Central America, northern South America	All areas except the Lesser Antilles
Figure 5a	Each of the Greater Antilles	Jamaica followed by each of the other Greater Antilles
Figure 5b	Jamaica, or each of the Greater Antilles + Mexico	Jamaica followed by all other areas except the Lesser Antilles

FIGURE LEGENDS

Figure 1. Scatter plot of uncorrected sequence divergence in cytochrome *b* against geographic distribution or taxonomic rank. Taxonomy follows Simmons (in press), with the exception of recognizing two species in *Brachyphylla nana sensu* Swanepoel and Genoways (1978). Numerals indicate cytochrome *b* distance as follows: 1: with respect to *Erophylla*; 2: with respect to *Glossophaga*, *Sturnira*, or *Lionycteris*; 3: between partial sequences and *Sturnira*; 4: between *Phyllonycteris* and *Erophylla*; 5: between nectar-feeding genera; 6: between *Sturnira* and *Uroderma*, *Artibeus*; 7: between *Brachyphylla nana pumila* and *B*. *cavernarum*; and 8: between *B*. *n*. *nana* and *B*. *cavernarum*.

Figure 2. A**.** Strict consensus of 3 most parsimonious cladograms resulting from analysis of concatenated cytochrome *b* and *Rag*2 (L=3189 steps, CI=0.537, RI=0.187). Numbers above branches are percent of 1000 jackknife replicates, below branches are Bremer support values. Names of nectar-feeding genera are in **bold**. B. One of 18,000 phylograms (-ln*L* = 10,470; 95% confidence interval = 10,460-10,480) obtained by Bayesian analyses of concatenated cytochrome *b* and *Rag*2 sequences using separate models of evolution for each gene. Thicker branches had a posterior probability of 1.00. Values above branches are posterior probabilities.

Figure 3. A**.** Strict consensus of 18 most parsimonious cladograms resulting from analysis of cytochrome *b* sequences for selected *Brachyphylla* haplotypes (Table 1; L=537, CI=0.799, RI=0.846). Numbers above branches are percent of 1,000 jackknife replicates, below branches are Bremer support values. B**.** Phylogram of 1 of 3 trees obtained from sequence analysis using the general time reversible model of DNA evolution with

constant rates (-ln*L* = 3866.96). Numbers above branches or with arrows are percent of 300 jackknife replicates.

Figure 4. Single most parsimonious tree obtained from analysis of concatenated sequences and morphology, 'total evidence,' (L=3399, CI=0.545, RI=0.230). Numbers above branches are percent of 1,000 jackknife replicates, below branches are Bremer support values. Names of nectar-feeding genera are in **bold**.

Figure 5. A. Phylogeny based on concatenated mtrDNA and *Rag*2 sequences, redrawn from Figure 5a of Baker et al. (2003). Bayesian posterior probabilities reported in original analyses are given as follows: above branches for concatenated molecular data as in Figure 5a of Baker et al. (2003); first below branch for mtrDNA only as in Figure 4 of Baker et al. (2003); second below branch for analysis of *Rag*2 only as in Figure 3 of Baker et al. (2003). Branches are labeled not applicable (na) when absent in separate analyses. B. Phylogeny based on combined morphology and *Rag*2 sequences excluding characters thought to be associated with nectar feeding, redrawn from Figure 5 of Carstens et al. (2002). Bootstrap proportions from 1,000 pseudoreplicates reported in the original are given as follows: above branches for combined data as in Figure 5 of Carstens et al. (2002); first below branch for all morphological characters as in Figure 1 of Carstens et al. (2002); second below branch for *Rag*2 sequences as in Figure 2 of Carstens et al. (2002). Branches are labeled 'na' or not applicable when absent in separate analyses. Names of nectar-feeding genera are in **bold**.

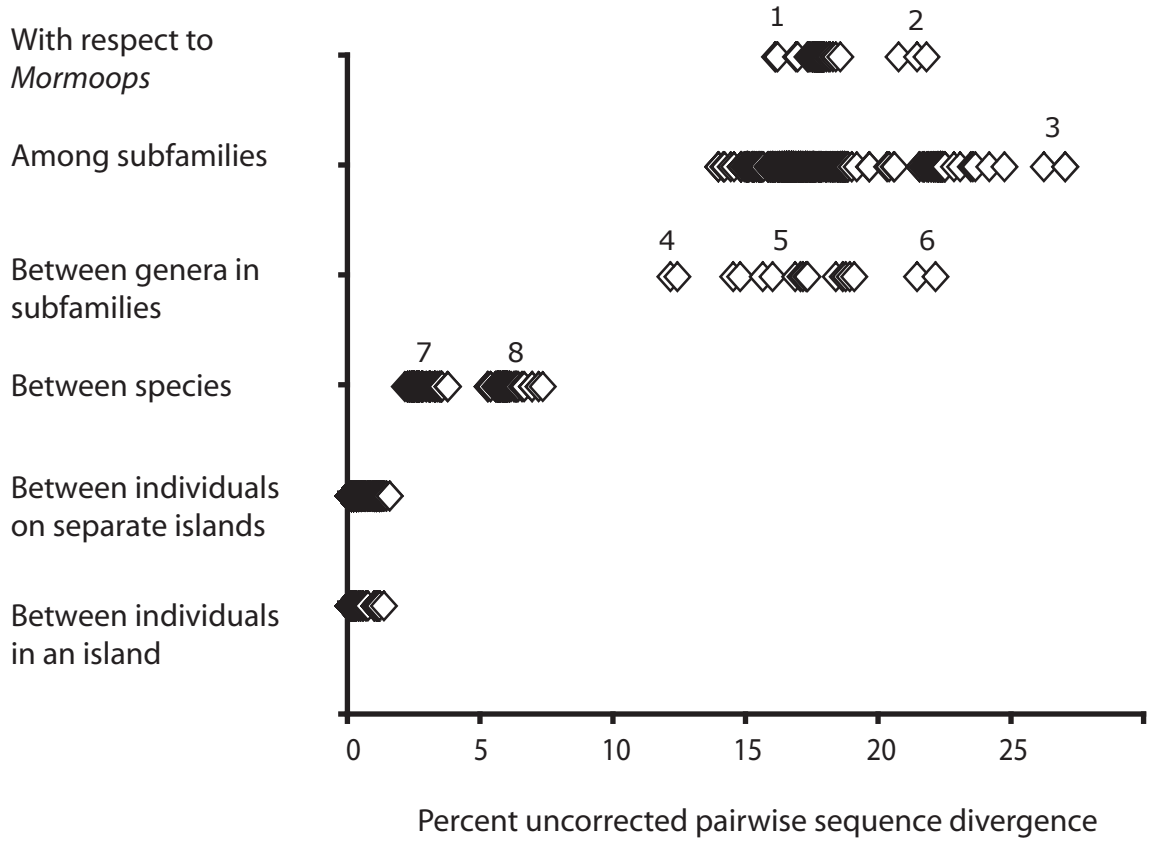

With respect to
Mormoops
Among subfamilies
Between genera in
subfamilies
Between species
Between individuals
on separate islands
Between individuals
in an island
1
2
3
4
5
6
7
8
0
5
10
15
20
25
Percent uncorrected pairwise sequence divergence

Figure 1

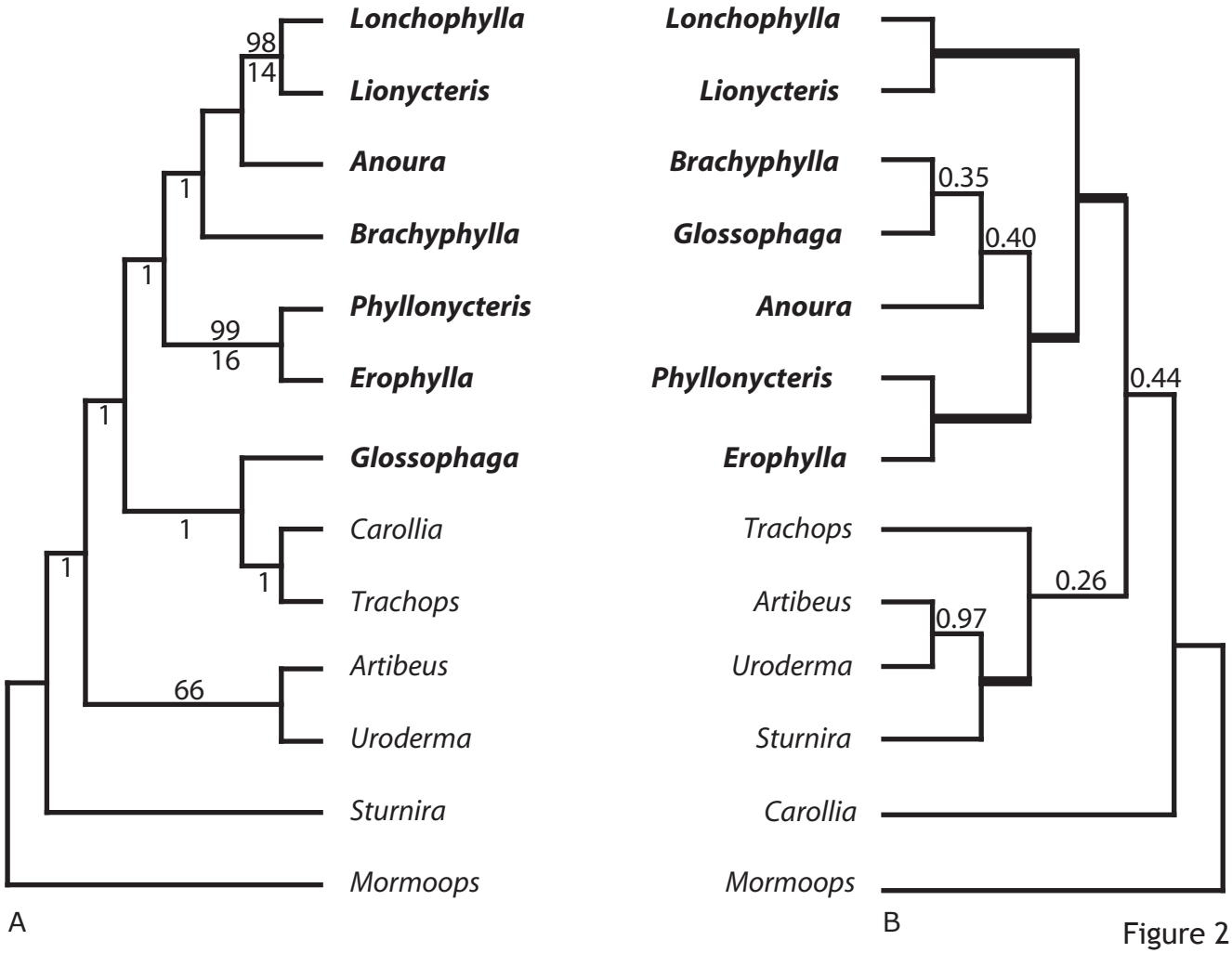
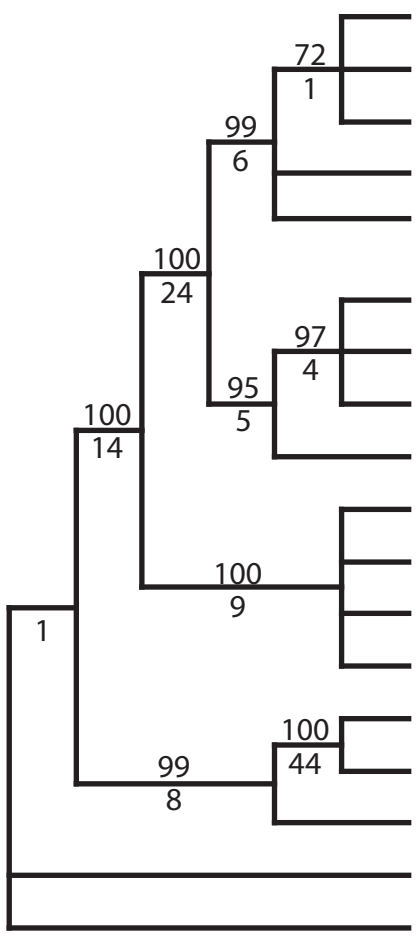

Lonchophylla
Lionycteris
Anoura
Brachyphylla
Phyllonycteris
Erophylla
Glossophaga
Carollia
Trachops
Artibeus
Uroderma
Sturnira
Mormoops
98
14
1
1
99
16
1
1
1
66
A
Lonchophylla
Lionycteris
Brachyphylla
Glossophaga
Anoura
Phyllonycteris
Erophylla
Trachops
Artibeus
Uroderma
Sturnira
Carollia
Mormoops
0.35
0.40
0.44
0.26
0.97
B

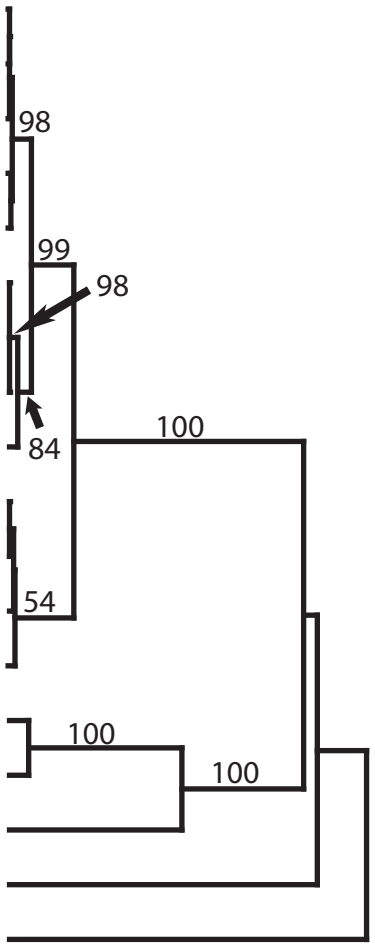
Figure 2



A

Brachyphylla pumila A
B. pumila B
B. pumila D
B. pumila C
B. pumila E

B. cavernarum A
B. cavernarum U
B. cavernarum C
B. cavernarum D
B. nana A
B. nana B
B. nana D
B. nana C
Erophylla bombifrons
E. sezekorni
Phyllonycteris aphylla
Anoura geoffroyi
Glossophaga soricina



B

Figure 3

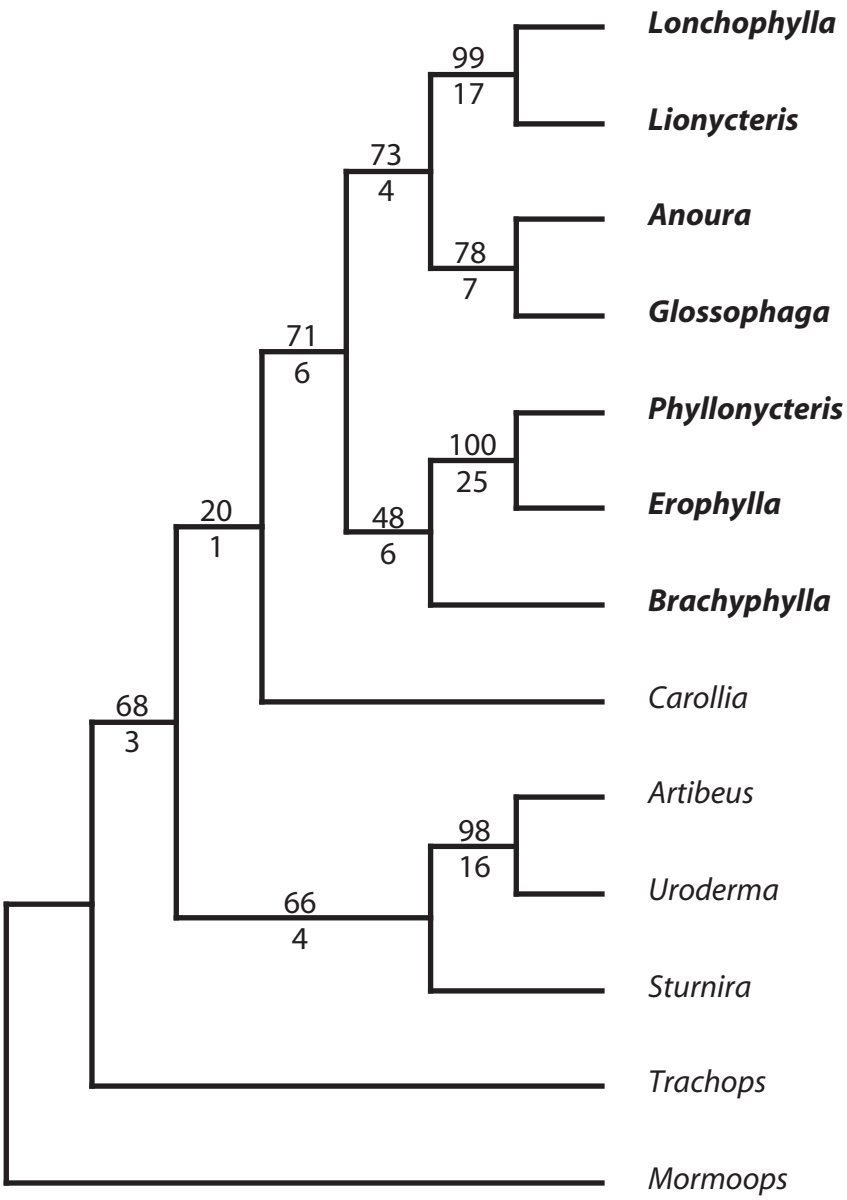

Lonchophylla
99
17
Lionycteris
73
4
Anoura
78
7
Glossophaga
71
6
Phyllonycteris
100
25
Erophylla
20
1
48
6
Brachyphylla
Carollia
68
3
Artibeus
98
16
Uroderma
66
4
Sturnira
Trachops
Mormoops

Figure 4

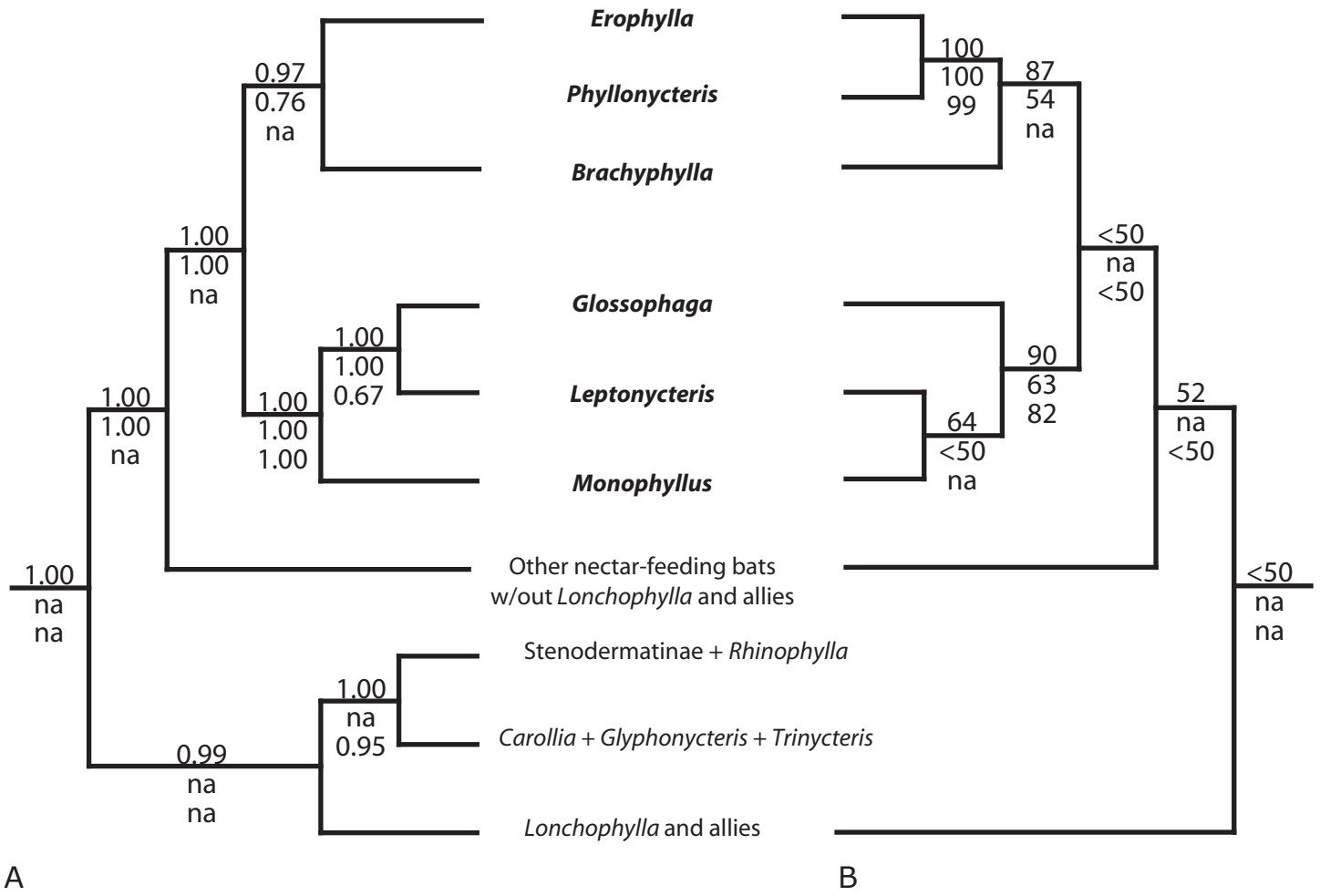

Erophylla
Phyllonycteris
Brachyphylla
Glossophaga
Leptonycteris
Monophyllus
Other nectar-feeding bats w/out Lonchophylla and allies
Stenodermatinae + Rhinophylla
Carollia + Glyphonycteris + Trinycteris
Lonchophylla and allies
0.97
0.76
na
1.00
1.00
na
1.00
1.00
0.67
1.00
1.00
1.00
1.00
1.00
na
1.00
na
na
0.99
na
na
1.00
na
0.95
A
100
100
99
87
54
na
<50
na
<50
90
63
82
64
<50
na
52
na
<50
<50
na
na
B

Figure 5

Appendix 2. Complete list of *Brachyphylla* (*B*.) individuals sequenced for this project. Identical sequences are grouped as haplotypes (Hap). When DNA was extracted from wing puncture no cadaver vouchers exist, and extracted DNA is deposited as listed. AMCC = Ambrose Monell Cryogenic Collection of the American Museum of Natural History, AMNH = Department of Mammalogy of the American Museum of Natural History, CM = Carnegie Museum of Natural History, SDSU = South Dakota State University Natural History collection, SP = tissue collection of the Carnegie Museum of Natural History, TK = TK: tissue collection of the Museum of Texas Tech University, TTU = Museum of Texas Tech University.

Species	Locality	Country	Tissue Voucher	Cadaver	Hap
B. nana	Lower Valley Forest	Cayman Islands	AMCC 101850		C
B. nana	Lower Valley Forest	Cayman Islands	AMCC 103023		A
B. nana	1 mi. E of Savannah, Grand Cayman	Cayman Islands	AMCC 102677		A
B. nana	1 mi. E of Savannah, Grand Cayman	Cayman Islands	AMCC 102678		A
B. nana	1 mi. E of Savannah, Grand Cayman	Cayman Islands	AMCC 102679		B
B. nana	1 mi. E of Savannah, Grand Cayman	Cayman Islands	AMCC 102680		A
B. nana	ca. 3 mi E Tapaste, Habana Province, Cuba	Cuba		AMNH 175993	D
B. pumila	Conch Bar Cave, Middle Caicos	Turks & Caicos	AMCC 102671		B
B. pumila	Conch Bar Cave, Middle Caicos	Turks & Caicos	AMCC 102672		C
B. pumila	Conch Bar Cave, Middle Caicos	Turks & Caicos	AMCC 102673		A
B. pumila	Conch Bar Cave, Middle Caicos	Turks & Caicos	AMCC 102674		A
B. pumila	Conch Bar Cave, Middle Caicos	Turks & Caicos	AMCC 102675		A
B. pumila	Los Patos upper cave, Barahona	Dominican Republic		AMNH 214390	D
B. pumila	3 km NE Sierra de Agua, Los Haitises, San Cristóbal	Dominican Republic		AMNH 244916	E
B. cavernarum		Anguilla	AMCC 121982		A
B. cavernarum		Anguilla	AMCC 121987		A
B. cavernarum		Anguilla	AMCC 121991		A
B. cavernarum		Anguilla	AMCC 121988		T
B. cavernarum		Anguilla	AMCC 121989		T

Appendix 2. Continued.

Species	Locality	Country	Tissue Voucher	Cadaver	Hap
B. cavernarum		Anguilla	AMCC 121993		T
B. cavernarum		Anguilla	AMCC 122004		T
B. cavernarum		Anguilla	AMCC 122002		V
B. cavernarum		Anguilla	AMCC 121998		Y
B. cavernarum	Bat's Cave, on the medical school campus 1.5 km east of English Harbour, St. Paul	Antigua	AMCC 122012	SDSU 1155	A
B. cavernarum	Bat's Cave, on the medical school campus 1.5 km east of English Harbour, St. Paul	Antigua	AMCC 122015	SDSU 1158	Q
B. cavernarum	Bat's Cave, on the medical school campus 1.5 km east of English Harbour, St. Paul	Antigua	AMCC 122013	SDSU 1156	X
B. cavernarum	Guana Island	British Virgin Islands		AMNH 265945	A
B. cavernarum	Pont Casse (1/4 MI NE, 1/4 MI SE), St. Paul	Dominica	TK40761		J
B. cavernarum	Stinking Hole Cave, above Cochrane, St. Paul	Dominica	TK15646	TTU 31445	L
B. cavernarum	Stinking Hole Cave, above Cochrane, St. Paul	Dominica	TK15642	TTU 31441	W
B. cavernarum	Grand-Bourg, 0.2 km WNW Auberge de Soledad, Marie Galante	Guadeloupe	SP10026	CM 112373	AB
B. cavernarum	Morne Ducos, 1.5 km NE jct rt D203 Grand-Bourg along Rt N9 50 m, Marie Galante	Guadeloupe	SP10045	CM 112374	AC
B. cavernarum	8 Km E Le Gosier (jct RT D119), Grand Terre	Guadeloupe	SP10004	CM 112371	P
B. cavernarum	Morne Ducos, 1.5 km NE jct rt D203 Grand-Bourg along Rt N9 50 m, Marie Galante	Guadeloupe	SP10046	CM 112375	V
B. cavernarum	9 Km E Le Gosier (jct RT D119), Grand Terre	Guadeloupe	SP10005	CM 112372	Z
B. cavernarum	Rendezvous Bluff Cave at Little Bay, 1 km north of the town of Cavy Hill, St. Peter	Montserrat	AMCC 102682		A
B. cavernarum	Rendezvous Bluff Cave at Little Bay, 1 km north of the town of Cavy Hill, St. Peter	Montserrat	AMCC 102683		A
B. cavernarum	Rendezvous Bluff Cave at Little Bay, 1 km north of the town of Cavy Hill, St. Peter	Montserrat	AMCC 122007	SDSU 1118	A
B. cavernarum	Rendezvous Bluff Cave at Little Bay, 1 km north of the town of Cavy Hill, St. Peter	Montserrat	AMCC 122011	SDSU 1132	A

Appendix 2. Continued.

Species	Locality	Country	Tissue Voucher	Cadaver	Hap
B. cavernarum	Rendezvous Bluff Cave at Little Bay, 1 km north of the town of Cavy Hill, St. Peter	Montserrat	AMCC 122008	SDSU 1120	AA
B. cavernarum	Lawyer's Tank	Montserrat	AMCC 102684		D
B. cavernarum	Rendezvous Bluff Cave at Little Bay, 1 km north of the town of Cavy Hill, St. Peter	Montserrat	AMCC 102685		D
B. cavernarum	Lawyer's Tank	Montserrat	AMCC 102686		E
B. cavernarum	Rendezvous Bluff Cave at Little Bay, 1 km north of the town of Cavy Hill, St. Peter	Montserrat	AMCC 102687		F
B. cavernarum	Rendezvous Bluff Cave at Little Bay, 1 km north of the town of Cavy Hill, St. Peter	Montserrat	AMCC 102688		G
B. cavernarum	Rendezvous Bluff Cave at Little Bay, 1 km north of the town of Cavy Hill, St. Peter	Montserrat	AMCC 102689		H
B. cavernarum	Rendezvous Bluff Cave at Little Bay, 1 km north of the town of Cavy Hill, St. Peter	Montserrat	AMCC 102690		I
B. cavernarum	Rendezvous Bluff Cave at Little Bay, 1 km north of the town of Cavy Hill, St. Peter	Montserrat	AMCC 102705		J
B. cavernarum	Rendezvous Bluff Cave at Little Bay, 1 km north of the town of Cavy Hill, St. Peter	Montserrat	AMCC 102709		K
B. cavernarum	Rendezvous Bluff Cave at Little Bay, 1 km north of the town of Cavy Hill, St. Peter	Montserrat	AMCC 122010	SDSU 1131	K
B. cavernarum	Rendezvous Bluff Cave at Little Bay, 1 km north of the town of Cavy Hill, St. Peter	Montserrat	AMCC 102710		L
B. cavernarum	Rendezvous Bluff Cave at Little Bay, 1 km north of the town of Cavy Hill, St. Peter	Montserrat	AMCC 102711		M
B. cavernarum	Mt. Pleasant caves	Nevis	AMCC 102712		A
B. cavernarum	New River Source	Nevis	AMCC 102722		A
B. cavernarum	Mt. Pleasant caves	Nevis	AMCC 102723		A
B. cavernarum	Mt. Pleasant caves	Nevis	AMCC 102724		C
B. cavernarum	Hamilton Estate Track	Nevis	AMCC 102725		C
B. cavernarum	Mt. Pleasant caves	Nevis	AMCC 102726		L
B. cavernarum	Mt. Pleasant caves	Nevis	AMCC 102727		N
B. cavernarum	Mt. Pleasant caves	Nevis	AMCC 102728		O

Appendix 2. Continued.

Species	Locality	Country	Tissue Voucher	Cadaver	Hap
B. cavernarum	Mt. Pleasant caves	Nevis	AMCC 102729		P
B. cavernarum	Mt. Pleasant caves	Nevis	AMCC 102730		Q
B. cavernarum	Base El Toro Trail 13.5 Km on route 192, Caribbean Natl. Forest, Naguabo	Puerto Rico	TK21798	TTU 46379	U
B. cavernarum	Base El Toro Trail 13.5 Km on route 192, Caribbean Natl. Forest, Naguabo	Puerto Rico	TK21807	TTU 46380	U
B. cavernarum	Mata de Plátano Field Station, Arecibo	Puerto Rico	AMCC 102681		U
B. cavernarum	Ravine Rainforest	Saba	AMCC 102731		A
B. cavernarum	Ravine Rainforest	Saba	AMCC 102732		A
B. cavernarum	Ravine Rainforest	Saba	AMCC 102733		B
B. cavernarum	Orangestad	St. Eustatius	AMCC 102734		A
B. cavernarum	Orangestad	St. Eustatius	AMCC 102735		C
B. cavernarum		St. Lucia	AMCC 122006		M
B. cavernarum	Loterie Farm	St. Maarten	AMCC 102736		A
B. cavernarum	Loterie Farm	St. Maarten	AMCC 102737		C
B. cavernarum	Loterie Farm	St. Maarten	AMCC 102738		C
B. cavernarum	Loterie Farm	St. Maarten	AMCC 102739		Q
B. cavernarum	Loterie Farm	St. Maarten	AMCC 102740		S

Short-faced bats (Chiroptera: Stenodermatina): a Caribbean radiation of strict frugivores

Abstract

The Stenodermatina is a small clade of New World leaf-nosed bats with four genera in the continental Neotropics and four in the West Indies. Their distribution has traditionally been explained by dispersal from the continent and subsequent diversification in the Antilles. Analyses of sequence variation at two loci, and of combined molecular and morphological data result in phylogenies well supported at most nodes. Conflict between data partitions is limited to nodes that are poorly supported in separate estimates of phylogeny. Resulting trees were analyzed using event-based biogeographic methods to test the continental origin hypothesis. While a continental origin for the clade is obtained, the West Indian representatives are not monophyletic and are successive sister taxa to a single continental lineage. These results suggest an alternative biogeographic history for the group; following diversification in the Caribbean the Stenodermatina successfully invaded and diversified in the mainland.

Introduction

The Short-faced bats, tribe Stenodermatina (Chiroptera: Phyllostomidae), comprise eight genera restricted to lowland forests and secondary habitats of the Neotropics, including the West Indies (Emmons, 1997; Howe, 1974; Silva-Taboada, 1979). *Ametrida centurio* ranges from northern South America east of the Andes to central Brazil including Trinidad, *Sphaeronycteris toxophyllum* spans from Venezuela to northern Bolivia, *Centurio senex* ranges from northern tropical Mexico to Venezuela and Trinidad, and

Pygoderma bilabiatum has two disjunct populations: one in southeastern Brazil and adjacent Paraguay, and another in southeastern Bolivia and northern Argentina (Koopman, 1994). Among the Antillean Short-faced bats *Phyllops falcatus* is endemic to Cuba, Hispaniola and Grand Cayman, *Stenoderma rufum* to Puerto Rico and the Virgin Islands, *Ariteus flavescens* to Jamaica, and *Ardops nichollsi* ranges from St. Eustatius to St. Vincent in the Lesser Antilles (Koopman, 1994). All Short-faced bats are strict frugivores and, with the exception of *Ardops* and *Ametrida* at some localities, rarely captured (Carter et al., 1981; Goodwin and Greenhall, 1961; Handley, 1976; Pedersen et al., 1996; Silva-Taboada, 1979). They have the rostrum that earns them their name, shorter than other frugivorous phyllostomids, and a diagnostic white patch on the shoulder (Wetterer et al., 2000). De la Torre (1961), Gardner (1977), and Greenbaum et al. (1975) had acknowledged the close evolutionary relationships among these 8 genera, but their monophyly was formally recognized only recently (Lim, 1993), with Wetterer et al. (2000) placing them in their own subtribe within the frugivorous subfamily Stenodermatinae.

Their biogeography is particularly interesting because they are sufficiently distinct from one another to warrant generic status and their distribution includes the West Indies. Only one other strict frugivore, *Artibeus jamaicensis*, ranges throughout the West Indies, and morphological and molecular evidence suggests it is conspecific with continental populations (Phillips et al., 1991; Phillips et al., 1989; Pumo et al., 1988; Pumo et al., 1996). The distribution of West Indian Short-faced bats has been explained as the result of dispersal (Baker and Genoways, 1978; Koopman, 1989; Morgan, 2001) or vicariance (Dávalos, 2004; Tavares and Simmons, 2000) from the continental Neotropics.

This view was based on the assumption that the Antillean Short-faced bats comprised a monophyletic group (Baker and Genoways, 1978; Koopman, 1989; Morgan, 2001), a view supported by recent analyses of many morphological characters (Wetterer et al., 2000). The monophyly of the Antillean group was not corroborated in recent molecular phylogenies (Baker et al., 2003; Baker et al., 2000), but the character support for resolving some internal nodes of the Short-faced tree was low, as it was in the earlier morphological study. In fact the monophyly of the Short-faced bats and a sister relationship between *Ariteus* and *Ardops* were the only nodes consistently well supported for these taxa in 2 recent phylogenies (Baker et al., 2000; Wetterer et al., 2000).

New mitochondrial cytochrome *b* sequences for all Stenodermtina taxa, and nuclear Recombination Activation Gene 2 (*Rag*2) sequences for *Phyllops* (complementing a previously published data set for the other taxa) were generated with two main goals: 1) to resolve the relationships among Short-faced bats with greater confidence than hitherto, and 2) to test biogeographic hypotheses, including relationships between continental and Antillean species (Dávalos, 2004). A well-supported phylogeny of the Short-faced bats can also provide a framework for future comparative studies in morphology and ecology. Cytochrome *b* was used because it is fast evolving and would provide many more characters than the published *Rag*2 data set. Cytochrome *b* also allows comparisons with many previous studies within the family Phyllostomidae (Baker et al., 1994; Dávalos and Jansa, in press; Van Den Bussche and Baker, 1993; Van Den Bussche et al., 1993; Wright et al., 1999). This mitochondrial gene is available from many more sources, such as museum specimens and poorly preserved tissue collections, than nuclear single-copy genes (Dávalos and Jansa, in press).

MATERIALS AND METHODS

Taxon Sampling

To examine relationships among the Short-faced phyllostomid bats (tribe Stenodermtina) and to test the monophyly of the biogeographic groups, 8 of the Short-faced genera (all monotypic) and 8 of the 12 currently recognized subspecies (Simmons, in press) were included. A list of taxa with sequences generated for this study is given in Table 1.

To best capture the genetic diversity within the Stenodermatina species, individuals from as many localities as possible were included. For outgroup comparison sequences from *Artibeus* (AF 316432, ACU66519) and *Dermanura* (AF316443, ACU66511), the sister taxon to the Short-faced bats according to Baker et al. (2003; 2000), were included. To root the tree, one distant relative in the subfamily Stenodermatinae (Wetterer, 2003), *Sturnira* (AF316488, AF187035), and one of the subfamily Lonchophyllinae, *Lonchophylla* (AF316456, AF187034), were included.

Molecular Data

For most specimens, DNA was isolated from muscle, wing clip (Rossiter et al., 2000), or liver tissue that had been frozen or preserved in ethanol or lysis buffer in the field (Table 1). For 4 specimens of *Sphaeronycteris*, *Centurio*, and *Pygoderma* (AMNH nos. 194213, 262637, 256330, 261759), DNA was isolated from ~5 mm ribs taken from skeletons that had been stored dry in a museum cabinet. For *Ardops nichollsi* (AMNH 212557), DNA was extracted from a 2 mm^2 piece of muscle taken from a formalin-fixed specimen that had been stored in ethanol. For frozen and ethanol preserved tissues, DNA was extracted using a Qiagen DNeasy Tissue Extraction Kit (Qiagen, Inc.) following the manufacturer's protocol. This protocol was modified using the approach of Iudica et al.

(2001) for the dried bone and formalin-fixed specimens. DNA extraction and amplification of the museum specimens was conducted in the ancient DNA room of the Molecular Systematics Laboratory, a separate facility from the Cullman Laboratory for Molecular Systematics (both at American Museum of Natural History) where all other experiments were performed. Extracted DNA was used as a template in PCR reactions with primers described elsewhere (Dávalos and Jansa, in press). Primer pairs NewM05 (5'-GACATGAAAAATCACCGTTG-3') and UMMZ04 (Jansa et al., 1999) were used to isolate the entire cytochrome *b* gene from high-molecular weight DNA extracted from frozen and ethanol-preserved specimens. To generate fragments of a suitable size for sequencing, this product was used as a template in 2 subsequent PCR reactions, reamplifications, 1 using MVZ05 paired with NEW12 and 1 using UMMZ13 paired with UMMZ04 (Dávalos and Jansa, in press; Jansa et al., 1999). One cytochrome *b* sequence was downloaded from GenBank *Centurio* (Mexico, L19509). Sequences of *Ardops* from Nevis and St. Eustatius are reported elsewhere (Carstens et al., submitted).

For 2 individuals of *Phyllops*, *Rag*2 amplification was performed following the methods described by Baker et al. (2000) using primer *RAG*2F1B. Other *Rag*2 sequences were downloaded from GenBank: *Ametrida* (AF316430), *Ardops* (AF316434), *Ariteus* (AF316435), *Centurio* (AF316438), *Pygoderma* (AF316483), *Sphaeronycteris* (AF316486), and *Stenoderma* (AF316487). DNA extracted from the museum specimens (ribs and formalin-fixed carcasses) was sufficiently degraded that amplifications were done as a series of overlapping 200-250 bp fragments using primers described by Dávalos and Jansa (in press). Initial amplifications using genomic DNA as a template were performed as standard 25 or 35 μL reactions using Hotstart Taq polymerase

(Qiagen) and recommended concentrations of primers, unincorporated nucleotides, buffer, and $MgCl_2$. Reactions were preformed on a Perkin-Elmer 9700 Thermal Cycler using 37 cycles of the following conditions: denaturation at 95º for 20 sec; annealing at 50-55º for 15 sec; extension at 72º for 1 min. All amplifications were preceded by a 95º soak for 15 min and followed by a 7-min extension at 72º. Reamplifications were only performed on DNA extracted from muscle, wing clip, or liver tissue. For reamplifications, PCR products were purified via electrophoresis through a 2% low melting-point agarose gel (NuSieve GTG, FMC). The appropriate size band was excised from the gel using a Pasteur pipette, and the gel plug was melted in 300 μL sterile water at 73º for 20 min. The resulting gel-purified product was used as a template in 30 or 40 μL reamplification reactions with Ampli-Taq polymerase (Promega). Reactions were subjected to 35 PCR cycles using annealing temperatures of 52-55º. PCR products were prepared for sequencing using a Qiagen BioRobot 3000 and were sequenced in both directions using amplification primers and dye-terminator chemistry (dRhodamine Ready Reaction Kit, Applied Biosystems, Inc.). Sequencing reactions were purified through a $MgCl_2$-ethanol precipitation protocol and run on an ABI 3100 automated sequencer. Sequences were edited and compiled using Sequencher 4.1 software (GeneCodes, Corp.). Base-calling ambiguities between strands were resolved either by choosing the call on the cleanest strand or using the appropriate IUB ambiguity code if both strands showed the same ambiguity. Molecular sequences generated as part of this study have been deposited in GenBank under accession numbers AY604431-AY604453 (Table 1).

Morphological Data

To investigate character conflict among different sources of data and character evolution, the mostly morphological (includes 12 characters from restrictions sites and Eco-R1 repeat) character matrix of Wetterer et al. (2000) was appended for relevant taxa, with the modifications for *Lonchophylla thomasi* as described by Dávalos and Jansa (in press).

Data Analysis

Protein-coding cytochrome *b* and *Rag*2 sequences were easily aligned by eye using Sequencher 4.1 (GeneCodes, Corp.). To describe the variation in cytochrome *b* among taxa of different ranks uncorrected pairwise ("p") distances among all taxa were calculated using PAUP* 4.0b10 (Swofford, 2002), excluding missing sites. Parsimony analyses of the morphological, cytochrome *b*, and *Rag*2 datasets, as well as combined analyses, were performed separately using heuristic searches as implemented in PAUP* 4.0b10. For each search, phylogenetically informative characters were treated as unordered and equally weighted. Heuristic searches consisted of 1,000 replicates of random taxon addition followed by tree-bisection-reconnection (TBR) branch swapping. Clade stability was assessed using nonparametric jackknife (Wu, 1986) and the Bremer support index (Bremer, 1994). All parsimony jackknife analyses included 1,000 replicates (or 300 for the multiple individual cytochrome *b* dataset); searches were heuristic with 100 replicates of random taxon addition followed by TBR branch swapping. Bremer values were calculated with the aid of AutoDecay (Eriksson, 1999). Character state changes were explored using MacClade 4.06 (Maddison and Maddison, 2003).

To assess topological incongruence among cytochrome *b*, *Rag*2, and the dataset comprising morphology, sex chromosomes and restriction sites, the Templeton (1983) test implemented in PAUP* 4.0b10 was used. This test assesses whether topologies differ significantly on how well they fit a data partition. Separate maximum likelihood analyses of the cytochrome *b* and *Rag*2 data sets were performed using PAUP* 4.0b10, and Bayesian analysis (Larget and Simon, 1999) of the concatenated molecular data set was performed using MrBayes 3.0 (Huelsenbeck and Ronquist, 2001). The likelihood ratio test statistic $-2\log\lambda = 2[\log L_1 - \log L_2]$, where L_1 is the likelihood under the more parameter-rich model (or one that assumes different topologies underlying the different molecular partitions) was calculated, and this value was compared to a χ^2 distribution (or a mixed distribution for invariable sites) with degrees of freedom equal to the difference in number of parameters between the 2 models (Goldman, 1993). Whether allowing for Γ-distributed heterogeneity of the substitution rate across sites, the Γ-shape parameter of Yang (1994) significantly improved the fit of each model to the data over allowing for a proportion of invariant sites (I) was also assessed. Both these methods to determine the best-fit maximum likelihood model were performed using Modeltest (Posada and Crandall, 1998). Likelihood nonparametric jackknife analyses included 300 pseudoreplicates, with a neighbor-joining starting tree followed by subtree pruning re-grafting (SPR) branch swapping in heuristic searches.

Bayesian methods were used to estimate phylogeny using a different model of molecular evolution for each partition the molecular data. This analysis featured two partitions, mitochondrial and nuclear DNA, and the model of sequence evolution was determined using Modeltest (see above). The values for model parameters were allowed

to vary between partitions and were not specified a priori, but treated as unknown variables to be estimated in each analysis. Bayesian analysis was conducted using MrBayes 3.0 (Huelsenbeck and Ronquist, 2001), with random starting trees without constraints, four simultaneous Markov chains were run for 2,000,000 generations, trees were sampled every 100 generations, and temperature was set to 0.20. Resulting burn in values, the point at which the model parameters and tree score reach stationarity, were determined empirically by evaluating tree likelihood scores. Analyses were repeated in 4 separate runs of MrBayes to ensure that trees converged on the same topology.

Finally, whether enforcing a molecular clock provided a better fit to the data than allowing for different rates across the tree was evaluated. To provide the most conservative test for a clock-like model of evolution, a UPGMA tree based on Jukes-Cantor distances was calculated, and the likelihood score for the best-fit model with no clock enforced ($\log L_1$) vs. the same model with a clock enforced ($\log L_2$) were compared. The significance of the difference in likelihood scores was tested by comparing $-2\log\lambda$ against a χ^2 distribution with degrees of freedom equal to the number of taxa minus 2. If the value for $-2\log\lambda$ was significant, then a molecular clock could be rejected. Subsequent to model evaluation and selection, the maximum likelihood tree was determined using a heuristic search in which the parameter values under the best-fit model were fixed and a neighbor-joining tree was used as a starting point for TBR branch swapping.

Biogeographic Analyses

The trees obtained by analyzing the data presented here were compared to those derived from previous biogeographic hypotheses by using a Shimodaira-Hasegawa (1999)

nonparametric test and a parametric bootstrap. The Shimodaira-Hasegawa (1999) one-tailed test resamples the data by bootstrapping to construct a distribution of log likelihoods and then compares the trees specified to this distribution. The question is how often particular trees, in this case derived from biogeographic hypotheses, fall as far below the maximum likelihood across the resampled trees as observed in reality. A significance value then allows rejecting these previous hypotheses, given statistical analyses of the data at hand.

A parametric bootstrap was used to examine the significance of differences in topology between expected and observed trees in a maximum likelihood framework (Huelsenbeck et al., 1996). First, the molecular data were used to estimate phylogenies with the maximum likelihood optimality criterion, as shown in the results. SEQ-GEN (Rambaut and Grassly, 1997) was used to simulate 1,000 data sets under the best model of sequence evolution, given the estimated topologies. Then, topological constraints equivalent to the morphology tree (Wetterer et al., 2000) were enforced on the simulated data sets. PAUP* 4.0b10 was then used to search each simulated data set for the optimal tree and the optimal tree constrained to each of the topological constraints. The null distribution was formed by the difference in maximum likelihood score ($L_{\text{constrained}}$ - $L_{\text{unconstrained}}$) for all replicates. Significance was assessed by comparing the same difference ($L_{\text{constrained}}$ - $L_{unconstrained}$) in the actual data to the null distribution.

Two other biogeographic methods were used: dispersal-vicariance analysis (Ronquist, 1997) and ancestral area analysis (Bremer, 1992). Dispersal-vicariance analysis does not require an independent hypothesis of area relationships, but instead reconstructs the ancestral distribution at each of the internal nodes of a given phylogeny

by means of optimization rules and set costs for extinction (cost of 1 per area lost) and dispersal (cost of 1 per area added). Vicariant and sympatric speciation carry no cost. Species distributions are therefore explained by assigning costs for each event in a way that biogeographic explanations imply the least possible cost. Dispersal-vicariance analysis also generates an ancestral area for each clade, which can be constrained to contain any minimum number of areas. Ancestral area analysis (Bremer, 1992) allows the identification of the ancestral area of a group based on its phylogeny, given geographic distribution information on the branches of the phylogeny. Each area can be considered a binary character with two states (present or absent) and optimized on the phylogeny. By comparing the numbers of gains and losses, it is possible to estimate areas most likely to have been part of the ancestral areas. The results of these two methods can be compared for specific nodes, such as the most recent common ancestor of Stenodermatina and its outgroup.

RESULTS

Sequence variation and saturation analysis

Complete cytochrome *b* sequences were obtained for all taxa with the exception of *Sphaeronycteris*, for which only 850 bp could be amplified. Since one of the objectives of this study was to assess the intraspecific variation of Short-faced genera (all monotypic), a few individuals per species were included in the cytochrome *b* analysis. Of these, two *Phyllops falcatus* (AMCC nos. 101762 and 101813) individuals had identical sequences. Only 650-850 bp fragments of cytochrome *b* were sequenced from museum specimens, as these species were all represented by tissue exemplars with the exception of

Sphaeronycteris. A summary of the uncorrected pairwise divergences among different taxonomic ranks, excluding missing sites, is shown in Fig. 1.

Within cytochrome *b* (all individuals) as sampled here, 438 (38%) of sites were variable and 288 (25%) are parsimony informative. The distribution of the parsimony-informative sites is highly dependent on codon position: 16.3% in first, 3.8% in second, and 78.8% in third codon position. Most substitutions were synonymous, and translation of sequences to amino acids led to a matrix with only 36 informative sites (23.4% variable sites, among which 43.8% are parsimony informative). The average base composition of sequences was skewed, with little bias at first codon position, a deficiency of adenine (20.0%) and guanine (14.5%) and an overabundance of thymine (40.6%) for second position, and a strong bias in third position: deficiency of guanine (2.3%) and thymine (18.1%), and abundance of adenine (41.8%) and cytosine (37.7%). That bias in base composition did not differ significantly across taxa, (chi-square test implemented in PAUP* 4.0b10, $P>0.05$). The same result is obtained for the complete cytochrome *b* gene, and first and second positions separately. Saturation in sequences was also assessed by plotting uncorrected total sequence divergence versus transitions and transversions for each codon position. Those curves (not shown) indicated that no codon position experienced multiple transition or transversion substitution. The sole exception is the partial *Centurio* sequence (L19509) that showed plateauing of the curve for third-position transitions and transversions.

Fragments of *Rag*2 (1.36 kb) from two *Phyllops falcatus* individuals (AMCC nos. 101769 and 103030, Table 1) were amplified to complete the *Rag*2 data set published by Baker et al. (2000). The sequences obtained were identical. As expected for protein

coding genes, cytochrome *b* and *Rag*2 sequences align without gaps. Sequences were converted to amino acids using MacClade 4.06 (Maddison and Maddison, 2003), and no stop codons were found. Within *Rag*2 120 (8.8%) of sites were variable and 48 (4%) were parsimony informative. The distribution of the parsimony-informative sites is highly dependent on codon position: 18.8% in first, 2.1% in second, and 79.2% in third codon position. The average base composition of sequences was skewed, with a deficiency of cytosine (17.6%) and thymine (20.5%) at first codon position, a deficiency of guanine (17.9%) for second position, and a deficiency of guanine (19.3%) in third position. The skewed base composition does not differ significantly across taxa (chi-squared test implemented in PAUP* 4.0b10, $P>0.05$).

Phylogenetic analyses

Four data sets were included in phylogenetic analyses: 1) the mitochondrial cytochrome *b* with multiple individuals per species, 2) cytochrome *b* for species exemplars, 3) a fragment of the nuclear *Rag*2 for exemplars (the latter two comprising the molecular data set), and 4) the mostly morphological character matrix for phyllostomids published by Wetterer et al., (2000). Because all species were shown to be monophyletic with high jackknife support (Fig. 2), molecular data were concatenated for combined analyses, although sequences were obtained from different individuals with the exceptions of some previously published outgroups. Maximum parsimony analyses for individual data sets and the combined data matrix were conducted with all unordered and unweighted characters. Branches that appeared in 2 of the 3 data partitions (cytochrome *b*, *Rag*2, morphology) are shown in thicker lines in Fig. 3.

The Templeton (1983) tests indicated there were significant differences in: 1) the fit of cytochrome *b* to the *Rag*2 (P=0.044), and the morphology (P<0.0001) trees; 2) the fit of *Rag*2 to the morphology trees (P=0.0011); and the fit of the (mostly) morphology data to the molecular (P=0.0047), *Rag*2 (P=0.0116), and cytochrome *b* (P=0.0018) trees. This test is considered too conservative for defining a threshold to combine data (Cunningham, 1997), but the conflict between the (mostly) morphological data and the molecular characters is also apparent by measuring support for particular branches (Table 2). This conflict might undermine phylogenetic resolution when combining molecular and morphological data (Fig. 3).

Maximum-likelihood analyses were performed first using the program MODELTEST under the hierarchical likelihood ratio tests option (see above). The models of sequence evolution and parameters selected for the different data sets are shown in Table 3. The cytochrome *b* data set including multiple individuals per species (Table 1) was tested for rate constancy and the molecular clock was rejected (P<0.05). A subsequent analysis without the previously published sequence from *Centurio senex* TK 13537 (GenBank accession no. = L19509) could not reject the molecular clock (P>0.05). Given this, previous results (Fig. 1), and the number of individuals available in the analysis for this taxon (Table 1), this individual was excluded and is not discussed hereafter.

Since the parameters for the two genes were considerably different, Bayesian methods were used to obtain an estimate of phylogeny that accounted for these two models while using all available molecular data. Stationarity of parameter estimation was reached after 200,000 generations (burn in = 2,000 trees). The mean parameters for the

cytochrome *b* exemplar data set, followed by 95% confidence intevals in parentheses, were estimated as follows: R_{matrix} = 4.688 (1.093-9.599), 16.098 (3.979-33.03), 4.127 (0.796-8.566), 0.751 (0.01-2.425), 48.298 (13.615-94.015), 1.0, shape parameter = 0.221 (0.186-0.262). The mean parameters for the *Rag*2 data set, followed by 95% confidence values in parentheses, were estimated as follows: R_{matrix} = 0.62 (0.133-1.222), 4.236 (1.878-7.08), 0.109 (0.01-0.307), 1.014 (0.253-1.93), 5.596 (2.534-9.48), 1.0, shape parameter = 0.00851 (0.05-0.133). The resulting trees are summarized in Fig. 5.

Biogeographic Analyses

To find the most appropriate model to calculate the likelihoods of bootstrap and comparison trees in the Shimodaira-Hasegawa test, the concatenated molecular data set was analyzed using MODELTEST, model of sequence evolution and parameters selected are shown in Table 3. The trees compared were: 1) the topology obtained by analyzing the cytochrome *b* data alone (Fig. 6a), 2) the topology obtained by analyzing the *Rag*2 data alone (Fig. 6b), 3) a topology specifying the reciprocal monophyly of Antillean and continental Short-faced bats without internal resolution (Fig. 6c), and 4) a topology specifying the monophyly of Antillean Short-faced bats with a basal polytomy of *Pygoderma* and remaining unresolved continental ingroup taxa (Fig.6d). The Shimodaira-Hasegawa (1999) test used 10,000 RELL pseudoreplicates and found no significant difference between the first two trees (P=0.2173). Trees 3 (Fig. 6c) and 4 (Fig. 6d) were rejected with P<0.001, given the molecular data.

The parametric bootstrap compared the maximum likelihood scores of trees resulting from analyses of cytochrome *b*, *Rag*2 (Fig. 4), and concatenated molecular data (Fig. 5) against the scores obtained for the same data while constraining the topologies to

that of Fig. 6d (Wetterer et al., 2000). Given the molecular data presented here, the topology of Fig. 6d (tree 4 of the Shimodaira-Hasegawa test) cannot be rejected with this parametric test, as the difference in scores was not significant in any case (cytochrome *b* P=0.098, *Rag*2 P=0.457, combined molecular P=0.052).

The dispersal-vicariance ancestral area optimizations for different topologies are summarized in Table 4. In all cases two species of *Centurio*, *senex* (Mexico and Central America) and *greenhalli* (northern South America), were included. All DIVA analyses used the phylogeny of *Artibeus* and *Dermanura* proposed by van den Bussche et al. (1998) as outgroup to the Stenodermatina, with distributional data from Simmons (in press). Estimations of ancestral areas (Bremer, 1992) for 3 topologies of Short-faced bat phylogeny are presented in Table 5. The number of gains and losses were counted only for the most recent common ancestor of Stenodermatina and *Artibeus*-*Dermanura*.

DISCUSSION

Sequence variation

Among the putative subspecies studied, cytochrome *b* divergence is greater than that among individuals in monotypic species (Fig. 1) for *Ardops nichollsi nichollsi* (Dominica) vs. *Ardops nichollsi monserratensis* (Nevis and St. Eustatius), and *Centurio senex senex* (Mexico and Central America) vs. *Centurio senex greenhalli* (Trinidad). Neither *Pygoderma bilabiatum bilabiatum* (Brazil) vs. *Pygoderma bilabiatum magna* (Bolivia), nor *Phyllops falcatus falcatus* (Grand Cayman) vs. *Phyllops falcatus hispaniolensis* (Hispaniola) exhibit divergences beyond those found within undifferentiated species (Fig. 1). Sampling within some of these populations was sparse and limited to 1 mitochondrial marker, precluding firm taxonomic conclusions. In

particular, sampling of *Centurio* populations in northern South America is necessary to establish whether or not the divergence is continuous, or if there is reciprocal monophyly between Trinidad and the mainland. *Ardops* comprises three additional subspecies in the Lesser Antilles, which remain to be sequenced, alongside more individuals of *Ardops nichollsi* from Dominica. The low divergence between samples of different named subspecies of *Pygoderma* is surprising, given both their disjunct distribution and morphological differences (Valeria Tavares, personal communication). Fresh DNA from multiple individuals of *Pygoderma bilabiatum* from Bolivia, northern Argentina and western Paraguay would be necessary to further test this result. The taxonomy of *Phyllops* has been debated, with some proposing two species, e.g., Mancina and García Rivera (2000), Silva-Taboada (1979), and others preferring to recognize subspecies only, e.g., Koopman (1994). The putative species have morphological characters that overlap broadly (Timm and Genoways, 2003). Cytochrome *b* of *Phyllops* from Grand Cayman is barely divergent from that Hispaniolan bats (Fig. 2), and the Cuban population remains to be sampled. The Virgin Islands subspecies of *Stenoderma*, and individuals from throughout the range of *Ametrida* and *Sphaeronycteris* (see below) also remain to be sampled.

Sequence divergence in cytochrome *b* is related to taxonomic rank in a linear fashion (Fig. 1) as expected from a rate-constant gene (see Table 3) and assuming taxonomic rank reflects time since divergence from the common ancestor. There are two exceptions to this linear relation: the divergence between the two *Sphaeronycteris* sampled, and the divergence between *Stenoderma* and *Phyllops*. The 2 *Sphaeronycteris* samples span the latitudinal range of the species from Venezuela to Bolivia (Table 1).

Because these are the extremes of the range, and only a fraction (~850 bp) of the gene was sequenced, additional samples are necessary to test whether northern and southern populations are distinct, or part of a continuum of genetic differentiation. Between *Stenoderma* and *Phyllops* divergence was low compared to other nominate genera, and this was also observed with *Rag*2. These two genera have been subsumed at various times (Silva-Taboada, 1979; Simpson, 1945; Varona, 1974), although they are as morphologically distinct as each of the continental Short-faced bat genera (V. Tavares, personal communication). The range of variation observed among genera is also greater than that of any other rank in Fig. 1, suggesting there is greater latitude of differentiation allowed into this taxonomic category.

Phylogeny of the Short-faced bats

There are four nodes where the different data sets, when combined, provide greater support than in separate analyses: the monophyly of the Short-faced bats, the sister relationships between *Phyllops* and *Stenoderma*, and *Ariteus* and *Ardops*, and the monophyly of continental Short-faced bats (Table 2, Fig. 3). Wetterer et al. (2000) first formally recognized the subtribe Stenodermatina, and they review the long history of hints at the monophyly of this group therein. Additional recent sources of evidence supporting both the monophyly of the Short-faced bats and the sister-group relationship between *Ardops* and *Ariteus* include more than 70 new morphological characters (V. Tavares, personal communication), and more than 2 kb of new mitochondrial sequences (Baker et al., 2003).

The sister-group relationship between *Phyllops* and *Stenoderma* and the monophyly of continental Stenodermatina are more novel propositions: neither (Wetterer

et al., 2000), nor the compilation of hypotheses summarized as a supertree by Jones et al. (2002) include it. Indeed, no morphological characters change unambiguously along the *Phyllops*-*Stenoderma* branch, despite the multiple similarities that prompted Silva-Taboada (1979) to subsume *Phyllops* under *Stenoderma*, to the exclusion of other Short-faced bats. Previous authors had synonymized these two genera (Simpson, 1945; Varona, 1974), but they also included *Ardops* and *Ariteus* in what they called "*Stenoderma*." The high support measures (Table 2) and the 28 molecular characters that unambiguously change along the *Phyllops*-*Stenoderma* branch make this a particularly robust result.

Smith (1976) first proposed the monophyly of the continental Short-faced bats, though not as part of Stenodermatina *sensu* Wetterer et al. (2000). This node is well supported (Table 2), and there are 9 morphological and 9 molecular characters that change unambiguously along this branch. Baker et al. (2003) and V. Tavares (personal communication) also recovered a well-supported continental Stenodermatina clade.

These four nodes comprise all the well-supported areas of the phylogeny. All other nodes are unstable because of character conflict, lack of data or a combination thereof. In particular: the basal branch of the Stenodermatina could either be *Ariteus*-*Ardops* (Figs. 3, 4) or *Phyllops*-*Stenoderma* (Figs. 4, 5), and there is no internal resolution among the continental Short-faced bats (Figs. 3-5). There are 10 character changes along the branch that connects *Ariteus*-*Ardops* to *Phyllops*-*Stenoderma*, but these are independent of the position of either clade. Baker et al. (2003) found high support values for the basal position of *Ariteus*-*Ardops* based on mitochondrial DNA data. These cannot be evaluated as independent sources of information, given that the mitochondrial cytochrome *b* is the single source of support for this node here (Fig. 4, Table 2).

Additional, unlinked, loci are probably necessary to resolve the basal branch of the Short-faced bats.

The main source of conflict impeding the resolution of the internal branches of continental Stenodermatina is the position of *Pygoderma*. For these nodes the topologies based on each gene (Fig. 4) are consistent with each other, with the combined molecular analysis (Fig. 5), and with the results of Baker et al. (2003; 2000) if *Pygoderma* is ignored. This does not detract from the monophyly of the continental Stenodermatina: the hypotheses of Lim (1993), where *Pygoderma* is basal to all Short-faced bats, or Wetterer et al. (2000), where *Pygoderma* is sister to a clade of Antillean stenodermatines, are unparsimonious. The optimization of the molecular data set to the Wetterer et al. (2000) hypothesis is 25 steps longer than the topology of Fig. 3b, while optimization to the Lim (1993) tree is 87 steps longer. A Shimodaira-Hasegawa (1999) test rejected Wetterer et al.'s (2000) hypothesis (Fig. 6d) in a statistical framework. Since most of the characters to resolve the internal continental Short-faced bat relationships are from cytochrome *b* (Table 2), again, additional data should be sought from unlinked markers to resolve the placement of *Pygoderma*.

Biogeography of the Short-faced bats

Previous studies of the biogeography of Short-faced bats have either sought and discussed (Dávalos, 2004) or assumed (Baker and Genoways, 1978; Koopman, 1989; Morgan, 2001) the monophyly of Antillean Stenodermatina. The underlying model was a single invasion to the Antilles, by land interconnection (or stepping-stone) vicariance or overwater dispersal of the ancestor of Caribbean Stenodermatina. Two phylogenies consistent with this biogeographic scenario, trees 3 and 4 (Fig. 6d) of the Shimodaira-

Hasegawa test, were investigated and rejected by the molecular data presented here in the nonparametric test. The lack of resolution at the base of the Short-faced bat phylogeny does not preclude a firm conclusion regarding the monophyly of Antillean taxa: it is rejected. A Caribbean Stenodermatina lineage, which one is not clear from these data, is at the base of the phylogeny (Figs. 4, 5, Table 2) and it is the continental taxa that share a relatively recent common ancestor. This well-supported node (Table 2) opens a different perspective on the biogeography of the group: the invasion of the continent by a Caribbean Short-faced bat ancestral to the 4 continental genera (Tavares and Simmons, 2000).

The dispersal-vicariance analysis of the total evidence tree postulates 3 dispersals and has only one ambiguous node, the common ancestor of the Short-faced bats (Table 4). Two of the postulated dispersals correspond to range expansions of *Centurio* into Central America and *Pygoderma* to central South America (where no other Short-faced bats have been recorded). The third dispersal is one invasion of northern South America either at the node connecting *Phyllops* and *Stenoderma* to the continent, if the ancestral Stenodermatina is restricted to the Antilles, or at the ancestor of Stenodermatina, since a hypothetical vicariant event had already separated the continental *Artibeus-Dermanura* from the ingroup (Table 4). The interpretation of the *Rag*2 and cytochrome *b* phylogenies is complicated by the assignment of ambiguous ancestors to all but one node in each tree (Table 4). Ambiguity arises from the optimization of the internal continental Stenodermatina nodes where the ranges of *Centurio* and *Pygoderma* are no longer uniquely interpreted as dispersals. Rather, Central America and central South America are postulated as part of ancestral ranges of every node beginning with the ancestors of

Centurio and *Pygoderma* (Table 4). These ambiguous optimizations highlight the importance of resolving with greater confidence the continental Short-faced radiation for biogeographic inference using this method. DIVA postulates a northern South American ancestor for the continental Stenodermatina, but does not rule out Central America or central South America at this ancestral node when confronted with the cytochrome *b*, *Rag*2, or combined phylogenies (there are multiple solutions for this node, only a few are shown in Table 4).

It may not be realistic to postulate a history of vicariance and dispersal as resulting from DIVA for the Short-faced phylogenies (Table 4). The history of land interconnections for the Caribbean would constrain the occurrence of vicariance with the northern South America to the early Oligocene (Iturralde-Vinent and MacPhee, 1999). Depending on the optimization this vicariant event would be assigned to the node that separates continental Stenodermatina from either *Stenoderma-Phyllops* (Table 4, total evidence, cytochrome *b*) or *Ardops-Ariteus* (*Rag*2), or even earlier. These scenarios seem implausible given that the Stenodermatina are hypothesized to be a relatively recent radiation within the phyllostomids in both mostly morphological (Wetterer et al., 2000) and molecular analyses (Baker et al., 2003; Baker et al., 2000). The alternative is to interpret postulated 'vicariant' events between the mainland and the Antilles as actual dispersals, range expansions of ancestral taxa across water barriers.

Koopman (1989) proposed that an *Artibeus*-like ancestor of the Short-faced bats had reached the Antilles via Central America. Unrestricted DIVA optimizations of the cytochrome *b* tree (Fig. 4a), *Rag*2 tree (Fig. 4b), and the combined molecular tree (Fig. 5), do not rule out hypothetical ancestors of the Short-faced bats in Central America

(Table 4). Thereafter the different topologies affect biogeographic interpretation: the common ancestor of Short-faced bats could reach Jamaica via Central America, or Cuba via Yucatán. The *Rag*2 (Fig. 4b) and combined molecular (Fig. 5) phylogenies fit better the latter biogeographic scenario (Table 4). The total evidence phylogeny is only consistent with an invasion of the Antilles from northern South America, followed by speciation into the deep Jamaican-Lesser Antillean lineage, the Cuban-Hispaniolan-Puerto Rican lineage, and final 'vicariant' expansion into northern South America, but not the other continental areas (Fig. 6). The most recent common ancestor of *Artibeus-Dermanura* and the Short-faced bats is hypothesized to correspond to almost the sum of the terminal ranges in all cases (Table 4).

Ancestral area reconstruction (Bremer, 1992) could provide a comparison (Table 5) for the DIVA results. Cuba, Hispaniola and Puerto Rico were interconnected to northern South America briefly in the Early Oligocene (Iturralde-Vinent and MacPhee, 1999). If ancestral area reconstructions use these island areas as a single unit, then Cuba-Hispaniola-Puerto Rico receive the second best score for ancestral distribution, after northern South America. The highest value for this composite region is found for the *Rag*2 phylogeny (similar to the combined molecular result), where the oldest lineage corresponds to that landmass. The alternative area delimitation simply uses each range category directly in the ancestral reconstruction. Northern South America is obtained as the ancestral area, with all islands being equally scored, and Central America appears as the least likely ancestral region. The reconstructions of ancestral areas, given different trees, using two methods are different but consistent. With both methods most optimizations favor a South American over a Central American ancestral area for the

most recent common ancestor of the Short-faced bats and *Artibeus-Dermanura* (Tables 4, 5).

As conclusions: 1) the most recent common ancestor of the Stenodermatina and its closest outgroup probably lived in northern South America and from there reached the Caribbean by overwater dispersal, 2) the Antillean Stenodermatina comprise two separate relatively old lineages, and 3) the continental Stenodermatina comprise a single more recent lineage that entered the continent through northern South America, or, less likely, Central America. After decades of assuming the contrary (Koopman, 1989; Morgan, 2001) the Stenodermatina are revealed to be the only Caribbean bat radiation of strict frugivores, with a single lineage on the continent.

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Table 1. List of sequences generated for this article, with number of base pairs amplified, when applicable. Tissues without museum voucher were sampled by wing puncture, identified and released in the field. When no tissue voucher is listed, DNA was extracted from parts of museum specimens. Individual AMCC 101813 was not assigned a separate GenBank number as its sequence was identical to AMCC 101762. AMNH = American Museum of Natural History, AMCC = Ambrose Monell Cryogenic Collection at the AMNH, ROM = Royal Ontario Museum, TTU = The Museum, Texas Tech University, and MVZ = Museum of Vertebrate Zoology, University of California at Berkeley.

Museum voucher	Tissue voucher	Taxon	Locality	GenBank accession number	Gene bp
AMNH 267973	AMCC 110324	*Ametrida centurio*	Near Sinnamary, Paracou, French Guiana	AY604446	cytochrome *b*
AMNH 212557		*Ardops nichollsi nichollsi*	Clarke Hall Estate, Dominica	AY604433	cytochrome *b*, 1kb
AMNH 274601	AMCC 102694	*Ariteus flavescens*	Upper entrance Windsor Great Cave, Trelawney, Jamaica	AY604434	cytochrome *b*
AMNH 274616	AMCC 102767	*Ariteus flavescens*	Portland Cave 9, Portland Cottage, Clarendon, Jamaica	AY604435	cytochrome *b*
AMNH 274610	AMCC 102761	*Ariteus flavescens*	Monarva Cave, Revival, Westmoreland, Jamaica	AY604436	cytochrome *b*
ROM 99672	F34031	*Centurio senex senex*	Rio Uyus, 5 km E San Cristóbal Acasaguastlan, Guatemala	AY604442	cytochrome *b*
ROM 99584	FN32253	*Centurio senex senex*	Biotope Cerro Cahui, El Remate, Guatemala	AY604440	cytochrome *b*
ROM 95739	FN29530	*Centurio senex senex*	44 km S Constitución, 44 km S and 70 km E Escárcega, Campeche, Mexico	AY604444	cytochrome *b*
CMNH 55731	TK13110	*Centurio senex senex*	Ojo de Agua Rio de Atoyac, Veracruz, Mexico	AY604441	cytochrome *b*
ROM 101330	F35508	*Centurio senex senex*	El Imposible, El Refugio, El Salvador	AY604443	cytochrome *b*
AMNH 256330		*Centurio senex greenhalli*	St Ann's Ward, Port of Spain, Trinidad	AY604445	cytochrome *b*, 1 kb

Table 1. Continued.

Museum voucher	Tissue voucher	Taxon	Locality	GenBank accession number	Gene bp
	AMCC 101762	*Phyllops falcatus falcatus*	Lower Valley Forest, Grand Cayman	AY604447	cytochrome *b*
	AMCC 101769	*Phyllops falcatus falcatus*	Lower Valley Forest, Grand Cayman	AY604453	*Rag*2
	AMCC 101813	*Phyllops falcatus falcatus*	Lower Valley Forest, Grand Cayman	Same as 101762	cytochrome *b*
	AMCC 101852	*Phyllops falcatus falcatus*	Lower Valley Forest, Grand Cayman	AY604450	cytochrome b
AMNH 275484	AMCC 103030	*Phyllops falcatus hispaniolensis*	La Entrada (de Cabrera), María Trinidad Sánchez, Dominican Republic	AY604449	cytochrome b
AMNH 275512	AMCC 103067	*Phyllops falcatus hispaniolensis*	Finca Don Miguel, Platanal (de Cotuí), Sánchez Ramírez, Dominican Republic	AY604448	cytochrome *b* and *Rag*2
AMNH 261760		*Pygoderma bilabiatum magna*	San Rafael de Amboró, Santa Cruz, Bolivia	AY604439	cytochrome *b*
MVZ 185903	AD 275	*Pygoderma bilabiatum bilabiatum*	Fazenda Santa Monica, Municipio Itatiaia, Rio de Janeiro, Brazil	AY604437	cytochrome *b*
MVZ 185904	AD 493	*Pygoderma bilabiatum bilabiatum*	Fazenda Guaricana, Grupo Bamerindus, Municipio Guaratuba, Parana, Brazil	AY604438	cytochrome *b*
AMNH 194213		*Sphaeronycteris toxophyllum*	Finca Aroa, 2 km W Choroní, Aragua, Venezuela	AY604452	cytochrome *b*, 850 bp
AMNH 262637		*Sphaeronycteris toxophyllum*	Independencia, Pando, Bolivia	AY604451	cytochrome *b*, 850 bp
	AMCC 102376	*Stenoderma rufum rufum*	Vieques, Puerto Rico	AY604431	cytochrome *b*
	AMCC 122019	*Stenoderma rufum rufum*	Mata de Plátano, Arecibo, Puerto Rico	AY604432	cytochrome *b*

Table 2. Summary of support values found for nodes in different analyses. Values are percent of 50% jackknife replicates for maximum parsimony (MP)/Bremer support, when only 1 number is shown it corresponds to jackknife; percent of 50% jackknife replicates using maximum likelihood (ML); or Bayesian posterior probability for the combined *Rag*- and cytochrome *b* analysis. Relationships are shown in Newick format. cyt *b* = cytochrome b, morph = morphology, mol = molecules (cytochrome *b* and *Rag*2).

Analysis	Parsimony						Maximum likelihood		Bayesian	
Node	Total evidence	cyt *b*	cyt *b* All individuals	*Rag*2	cyt *b* *Rag*2	Morph	cyt *b*	*Rag*2	cyt *b* *Rag*2	Most support
Short-faced bats	100/26	100/13	99/12	87/3	100/18	90/5	100	92	100	all
(*Ardops*, *Ariteus*) basal	55/1	58/0	71/1	12	49/0	6	68	8	18	cyt b
(*Stenoderma*, *Phyllops*) basal	40	33	24	42/1	47	<5	15	92	82	*Rag*2
Monophyly continent	95/7	79/4	86/3	26	93/8	<5	92	26	100	cyt b
Monophyly Antilles	<5	<5	<5	<5	<5	24	15	<5	<5	morph
(*Stenoderma*, *Phyllops*)	100/18	99/8	99/10	100/7	100/15	<5	100	100	100	mol
(*Ardops*, *Ariteus*)	100/13	91/4	93/5	92/4	98/9	54/1	100	99	100	all
Centurio basal (continent)	23	54/1	54/0	<5	64/2	<5	67	<5	74	cyt b
(*Centurio*, *Sphaeronycteris*)	70/3	15	<5	9	15	90/5	26	<5	<5	morph
(*Ametrida*, *Sphaeronycteris*)	<5	<5	<5	57/1	<5	7	<5	56	<5	*Rag*2
(*Ametrida*, *Pygoderma*)	88/3	97/7	100/9	<5	96/7	<5	94	6	100	cyt *b*

Table 3. Models of molecular evolution and parameters selected for each molecular data, set see Table 1 for sequences. GTR = general time reversible model; HKY = Hasegawa Kishino Yano model; R-matrix = rate matrix parameter (with respect to G-T transversion); ti/tv ratio = transition/transversion ratio; Γ = shape parameter, I = proportion of invariant sites; $-2\log\Lambda = 2[\log L_1 - \log L_2]$, where L_1 = likelihood without clock and L_2 = likelihood with clock.

Data	Model	R-matrix or ti/tv ratio	Γ	I	$-2\log\Lambda$	df	*P*
cyt *b* all individuals	GTR+I+Γ	3.6, 21.5, 3.1, 2.3, 39.4	1.2646	0.5330	53.6	33	<0.05
cyt *b* all ind. w/o L19509	same	same	same	same	43.5	32	>0.05
cyt *b* exemplars	GTR+I+Γ	7.2, 29.3, 9.1, 1.4, 104.0	1.5111	0.5488	11.7	10	>0.05
*Rag*2	HKY+Γ	3.9120	0.0153	-	9.0	10	>0.05
combined	GTR+I+Γ	2.5, 5.4, 1.4, 0.6, 23.0	0.6586	0.6160	13.0	10	>0.05

Table 4. Summary of ancestral area optimizations found by dispersal-vicariance analysis, DIVA. The first line of each topology shows the results when no limits are placed on the number of ancestral areas permitted at each node. The second line shows results when ancestral areas were restricted to only two (more dispersals are inferred). When more than one solution was found those with the least number of areas are shown. Asterisks indicate complete solution is shown. Relationships are shown in Newick format. Mex = Mexico, CeAm = Central America, noSA = northern South America, ceSA = central South America, Antilles = all island areas, Cu = Cuba, Ja = Jamaica, Hi = Hispaniola, LA = Lesser Antilles, PR = Puerto Rico.

Topology/Node	(Stenodermatina, (*Artibeus*, *Dermanura*))	Stenodermatina	(more recent Caribbean lineage, (continental Stenodermatina))	Continental Stenodermatina
Total evidence (Fig. 3)	All areas	Antilles, Antilles+noSA, Antilles+ceSA, Antilles+noSA+ceSA*	noSA+Cu+Hi+PR*	noSA*
Restricted	NoSA, ceSA, or any combination of two areas exc. PR, LA*	ceSA, or any combination of two areas of Cu, Hi, Ja x ceSA*	noSA+Cu, noSA+Hi, noSA+PR, ceSA+PR*	noSA, ceSA, or both*
cytochrome *b* (Fig. 4)	All areas	Antilles	Cu+PR+Hi+CeAm, Cu+PR+Hi+noSA	All areas or noSA
Restricted	NoSA or any combination of two areas*	noSA+LA, noSA+Ja, LA or Ja x Cu, Hi or PR*	noSA+Cu, noSA+Hi, or noSA+PR	NoSA*
*Rag*2 (Fig. 4)	All areas	Antilles	noSA+LA+Ja	noSA or Mex+CeAm+noSA
Restricted	NoSA, ceSA	noSA+Cu, noSA+Hi, noSA+PR	noSA+LA, noSA+Ja	NoSA+ceSA*
Combined molecular (Fig. 5)	All areas	Antilles or Antilles+any combination of continental areas exc. CeSA	LA+Ja or LA+Ja+any combination of continental areas exc. CeSA	NoSA or any combination of continental areas
Restricted	Any combination of two areas or Mexico	noSA+Cu noSA+Hi noSA+PR or any combination of two Antillean areas	noSA+LA, noSA+Ja*	noSA*

Table 5. Ancestral area optimization (Bremer, 1992) for three topologies of relationships among Short-faced bats. g/l= gains/losses. Above; area partition using the biogeographic hypothesis of Iturralde-Vinent and MacPhee (1999), below; area partition using taxon ranges.

Area	gains	losses	g/l	AA	gains	losses	g/l	AA	gains	losses	g/l	AA
	total evidence tree (Fig. 3)				cytochrome *b* topology (Fig. 4)				*Rag*2 topology (Fig.4)			
Northern South America	3	3	1	1	4	3	4/3	1	2	3	2/3	1
Central America	1	5	1/5	1/5	1	6	1/6	1/8	1	4	1/4	3/8
Central South America	1	5	1/5	1/5	1	4	1/4	3/16	1	5	1/5	3/10
Cuba Hispaniola Puerto Rico	1	3	1/3	1/3	1	3	1/3	1/4	1	3	1/3	1/2
Jamaica	1	4	1/4	1/4	1	4	1/4	3/16	1	4	1/4	3/8
Lesser Antilles	1	4	1/4	1/4	1	4	1/4	3/16	1	4	1/4	3/8
Northern South America	3	3	1	1	4	3	4/3	1	2	3	2/3	1
Central America	1	5	1/5	1/5	1	6	1/6	1/8	1	4	1/4	3/8
Central South America	1	5	1/5	1/5	1	4	1/4	3/16	1	5	1/5	3/10
Cuba	1	4	1/4	1/4	1	4	1/4	3/16	1	4	1/4	3/8
Hispaniola	1	4	1/4	1/4	1	4	1/4	3/16	1	4	1/4	3/8
Puerto Rico	1	4	1/4	1/4	1	4	1/4	3/16	1	4	1/4	3/8
Jamaica	1	4	1/4	1/4	1	4	1/4	3/16	1	4	1/4	3/8
Lesser Antilles	1	4	1/4	1/4	1	4	1/4	3/16	1	4	1/4	3/8

FIGURE LEGENDS

Figure 1. Scatter plot of cytochrome *b* genetic divergence and taxonomic differentiation for the taxa in this study. 1: With respect to *Centurio* (GenBank accession no. = L19509.1), between *Lonchophylla* and *Sturnira*; 2, 4, 6 and 7: with respect to *Centurio* (GenBank accession no. = L19509.1); 3: between *Phyllops* and *Stenoderma*; 5: between *Pygoderma* from Bolivia and *Pygoderma* from Brazil, and between *Phyllops* from Hispaniola and *Phyllops* from Cuba; 7: between *Sphaeronycteris* from Venezuela and Bolivia.

Figure 2. A. One of 1218 equally parsimonious cladograms (L=962, CI=0.606. RI=0781) of cytochrome *b* sequences of Short-faced bats from different localities. Values above branches are percent of 50% jackknife replicates, values below branches are Bremer support indices. B. One of 3 phylograms with equal log-likelihood value (-ln*L* = 5804.9) excluding sequence L19109 (see results for explanation). Values above branches or with arrows are percent of 50% jackknife replicates. *Sturnira* was sister to *Artibeus*-*Dermanura* in 65% of the jackknife trees.

Figure 3. A. Single most parsimonious topology (L = 1179, CI = 0.685, RI = 0.520) obtained from analyzing concatenated molecular and morphological data, 'total evidence.' Thick branches were obtained in at least 2 analyses of cytochrome *b*, *Rag*2, or morphology alone. Values above branches are percent of 50% jackknife replicates, values below branches are Bremer support indices. B. One of 2 equally parsimonious trees (L = 1004, CI = 0.675, RI = 0.510) obtained from analyzing concatenated cytochrome *b* and *Rag*2 sequences, the molecular data set. Thick branches were obtained in at least 2

analyses of cytochrome *b*, *Rag*2, or morphology alone. Values above branches are percent of 50% jackknife replicates, values below branches are Bremer support indices.

Figure 4. A. Single maximum likelihood topology (ln*L* = -5040.95322) obtained from analyzing cytochrome *b* sequences of Short-faced bat exemplars. Values above branches or with arrows are percent of 50% jackknife replicates. *Sturnira* was found to be sister to *Artibeus-Dermanura* in 79% of the jackknife replicates but not in the topology with the best score. B. Single maximum likelihood topology (ln*L* = -2832.22110) obtained by analyzing *Rag*2 sequences. Values above branches or with arrows are percent of 50% jackknife replicates.

Figure 5. Majority rule consensus of 18,000 cladograms (-ln*L* = 7931.195, 95% confidence interval = 7922.765-7940.389) obtained by analyzing concatenated cytochrome *b* and *Rag*2 sequences using separate models of evolution for each gene. Values above branches are posterior probabilities. Values below branches are percent of 300 jackknife replicates from maximum likelihood analysis using a single model for both genes (-ln*L* = 8140.596).

Figure 6. Four topologies compared in Shimodaira-Hasegawa (1999) test. A. mtDNA result, B. *Rag*2 topology, C. reciprocal monophyly of continental and Caribbean Stenodermatina, D. pruned from Wetterer et al. (2000).

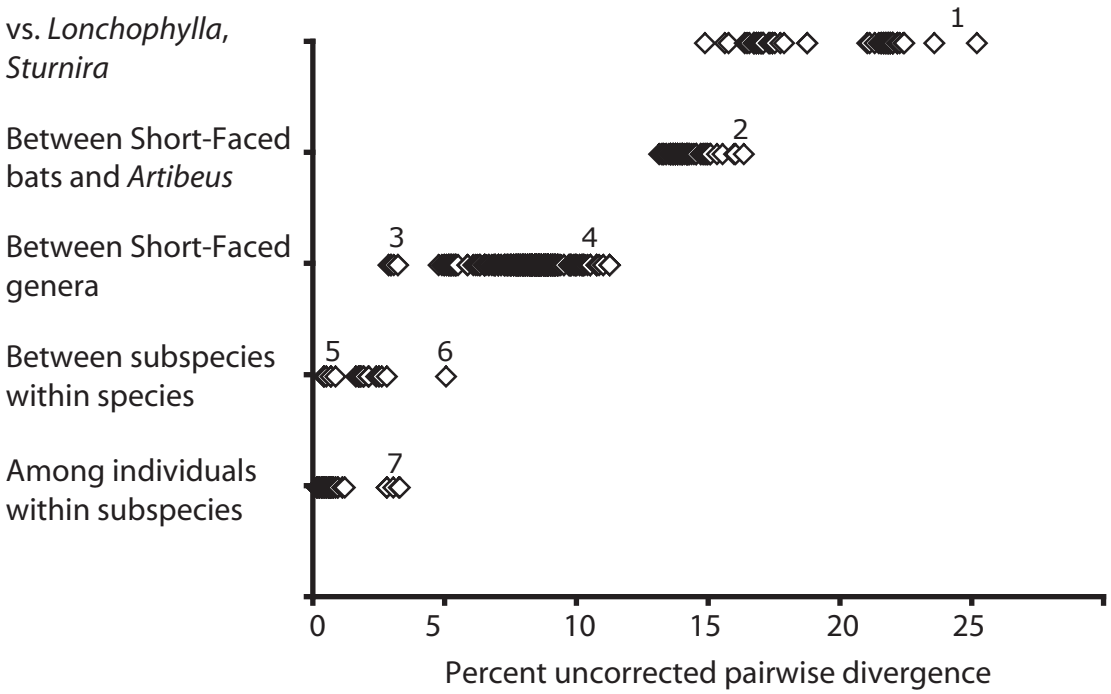
vs. *Lonchophylla*, *Sturnira*
Between Short-Faced bats and *Artibeus*
Between Short-Faced genera
Between subspecies within species
Among individuals within subspecies
1
2
3
4
5
6
7
0
5
10
15
20
25
Percent uncorrected pairwise divergence

Figure 1

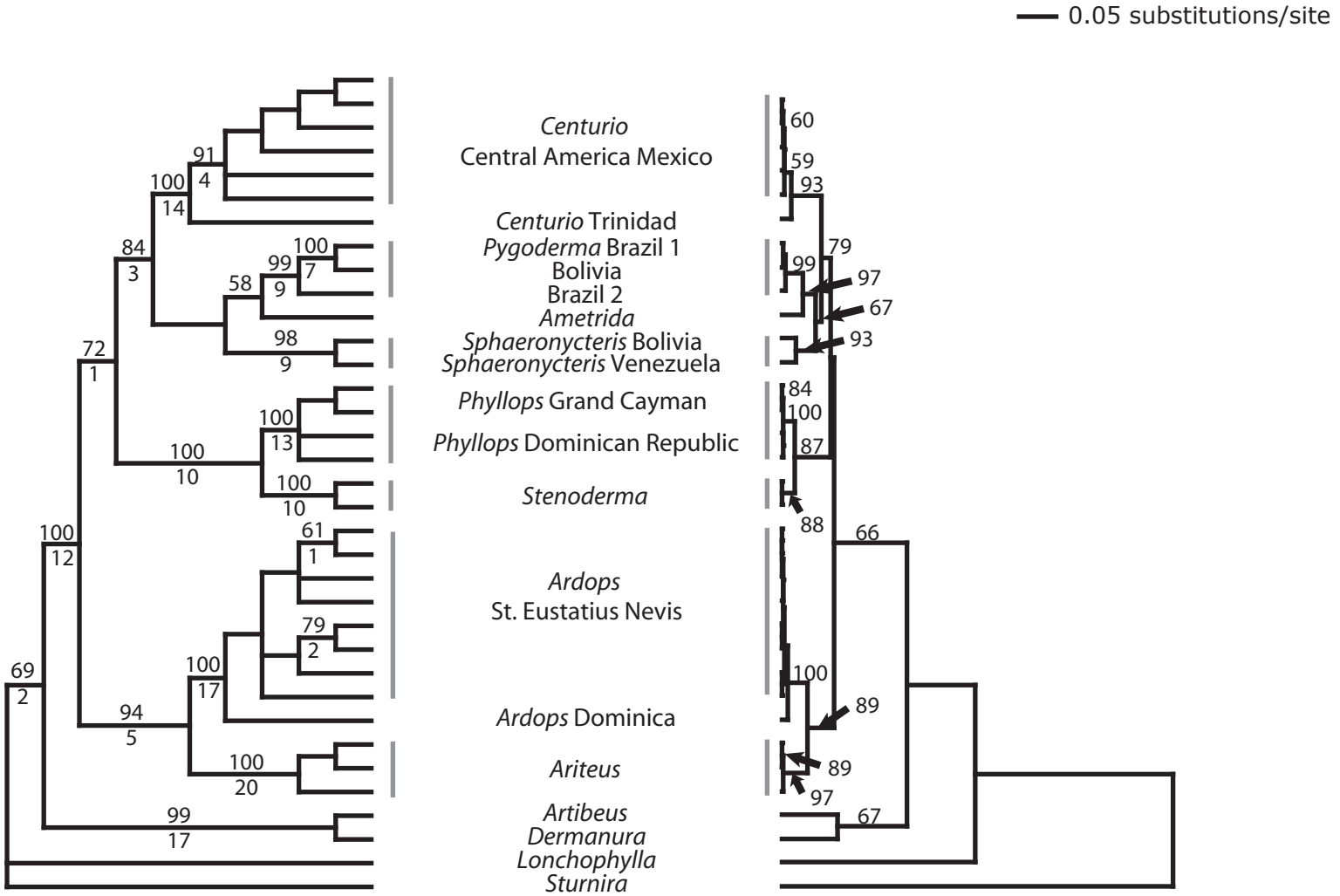
0.05 substitutions/site
Centurio
Central America Mexico
Centurio Trinidad
Pygoderma Brazil 1
Bolivia
Brazil 2
Ametrida
Sphaeronycteris Bolivia
Sphaeronycteris Venezuela
Phyllops Grand Cayman
Phyllops Dominican Republic
Stenoderma
Ardops
St. Eustatius Nevis
Ardops Dominica
Ariteus
Artibeus
Dermanura
Lonchophylla
Sturnira
A
B

Figure 2

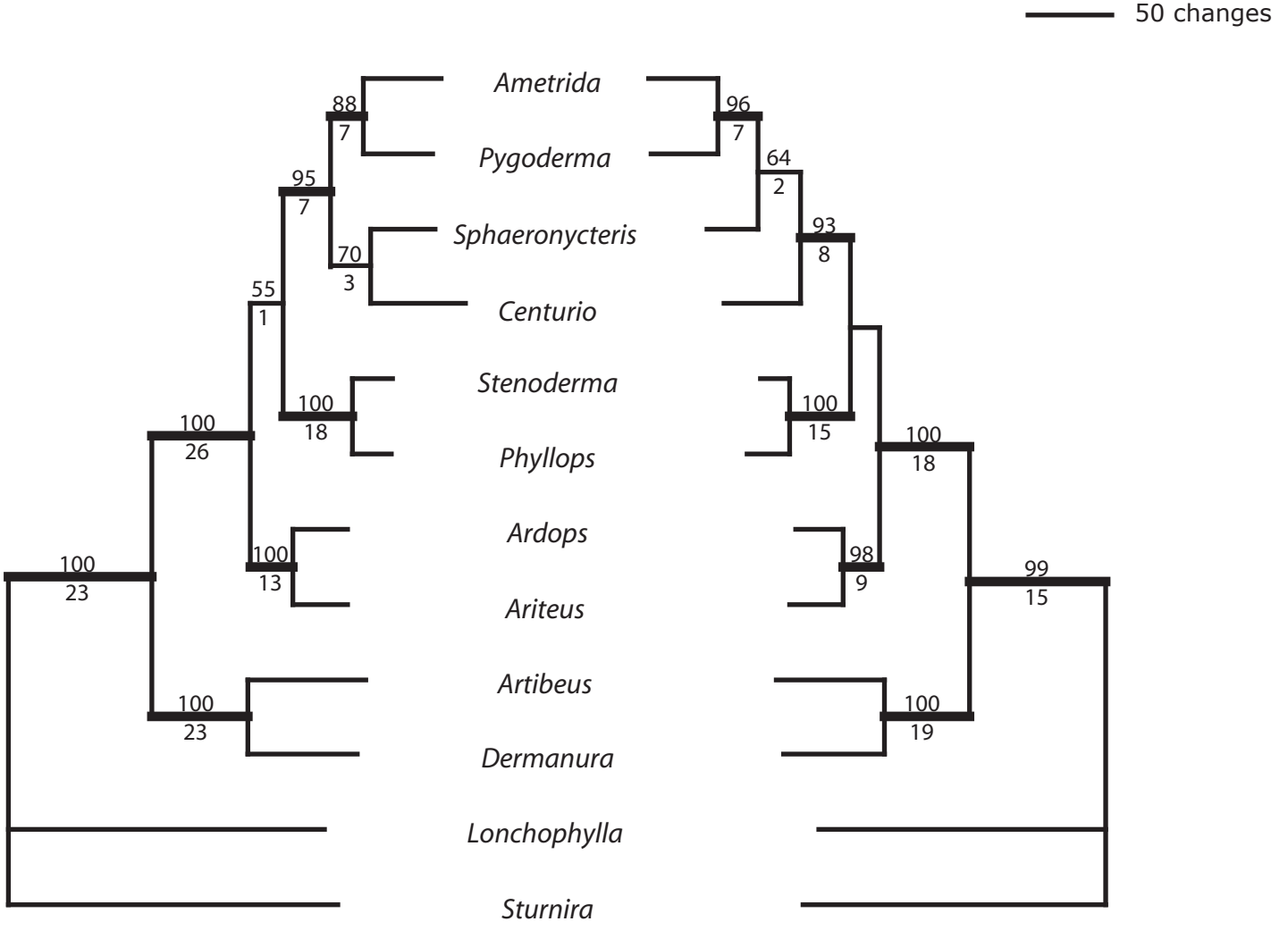
50 changes
Ametrida
Pygoderma
Sphaeronycteris
Centurio
Stenoderma
Phyllops
Ardops
Ariteus
Artibeus
Dermanura
Lonchophylla
Sturnira
88
7
95
7
70
3
55
1
100
18
100
26
100
13
100
23
100
23
A
96
7
64
2
93
8
100
15
100
18
98
9
99
15
100
19
B

Figure 3

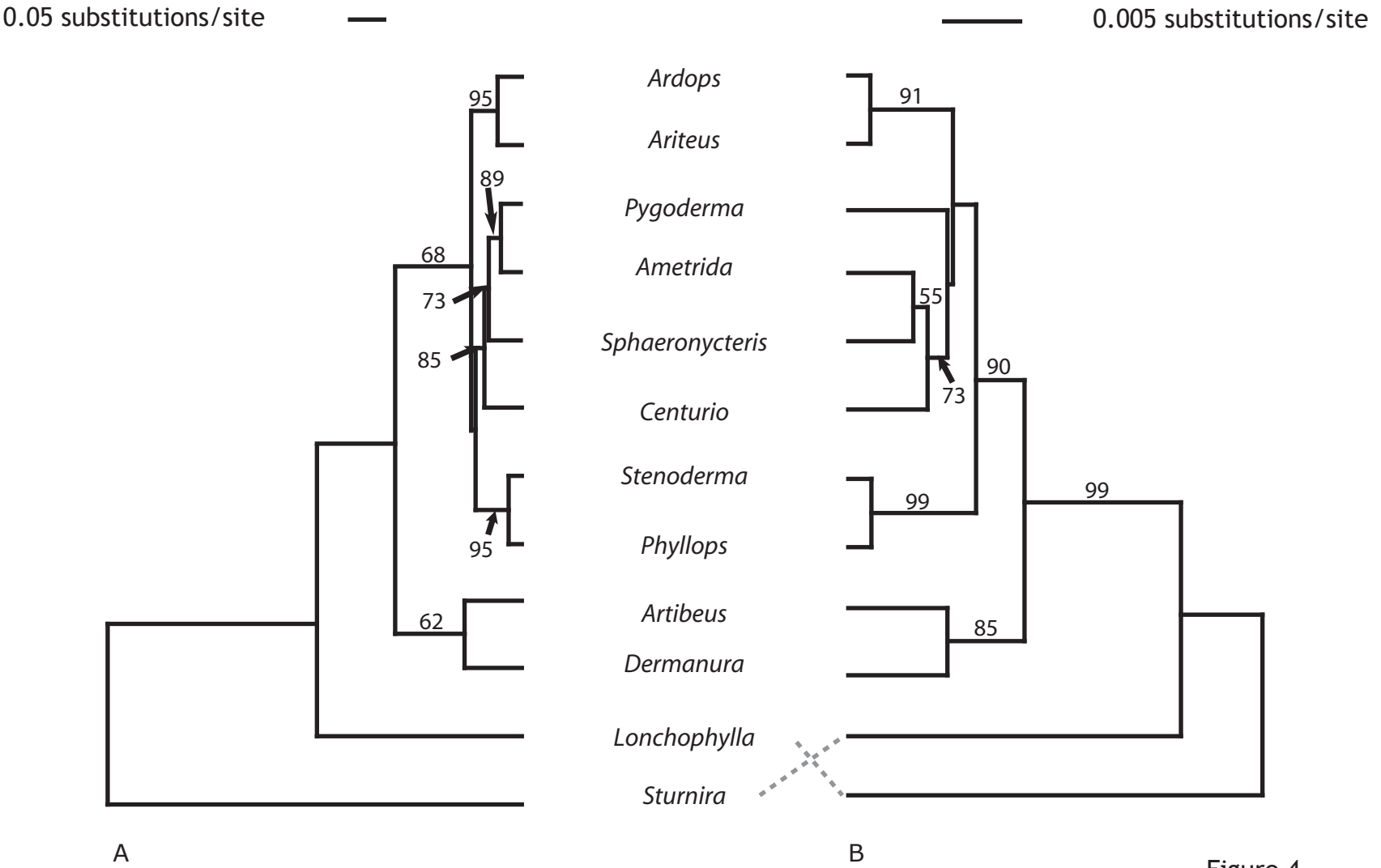
0.05 substitutions/site
0.005 substitutions/site
Ardops
Ariteus
Pygoderma
Ametrida
Sphaeronycteris
Centurio
Stenoderma
Phyllops
Artibeus
Dermanura
Lonchophylla
Sturnira
95
89
68
73
85
95
62
91
55
73
90
99
99
85
A
B

Figure 4

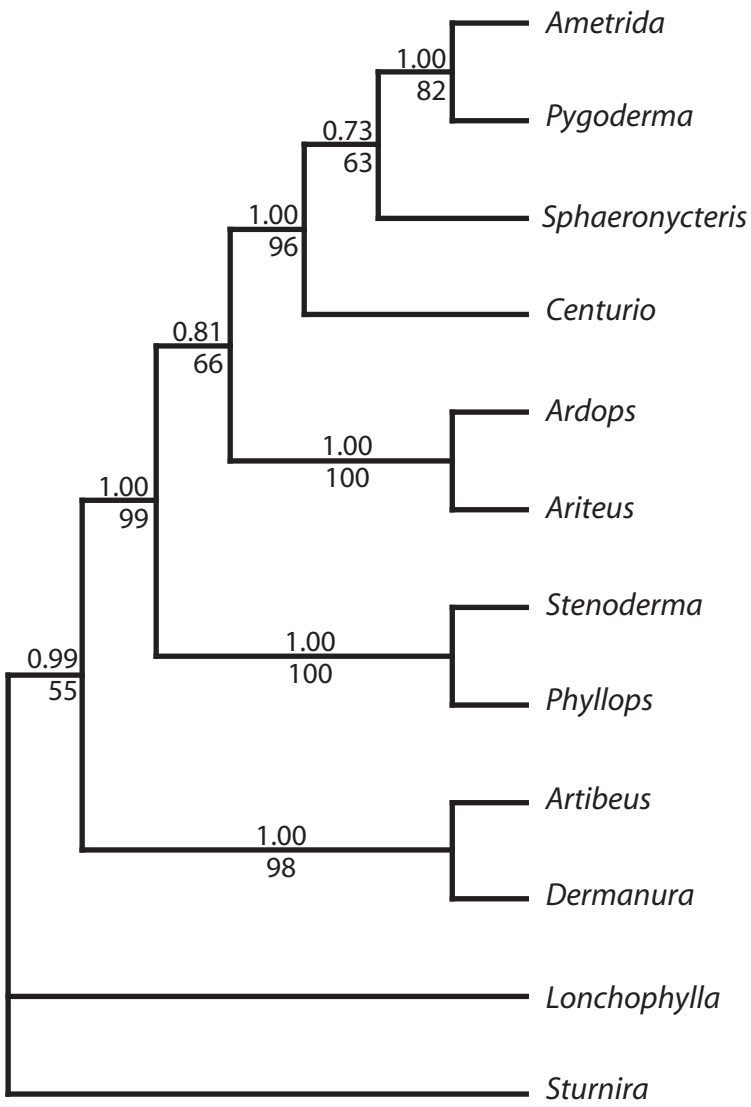
Ametrida
1.00
82
Pygoderma
0.73
63
Sphaeronycteris
1.00
96
Centurio
0.81
66
Ardops
1.00
100
Ariteus
1.00
99
Stenoderma
1.00
100
Phyllops
0.99
55
Artibeus
1.00
98
Dermanura
Lonchophylla
Sturnira

Figure 5

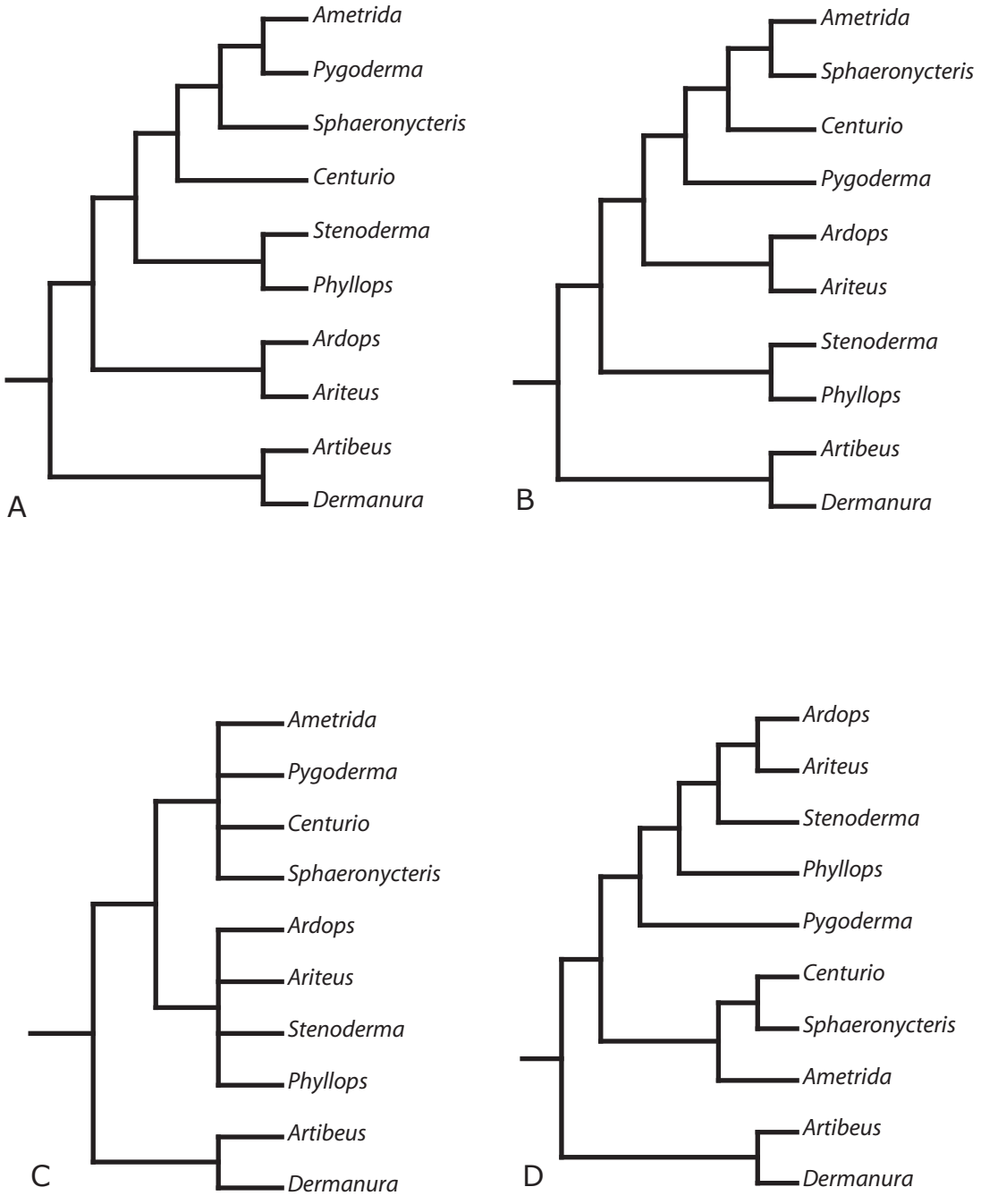
Ametrida
Pygoderma
Sphaeronycteris
Centurio
Stenoderma
Phyllops
Ardops
Ariteus
Artibeus
Dermanura
A
Ametrida
Sphaeronycteris
Centurio
Pygoderma
Ardops
Ariteus
Stenoderma
Phyllops
Artibeus
Dermanura
B
Ametrida
Pygoderma
Centurio
Sphaeronycteris
Ardops
Ariteus
Stenoderma
Phyllops
Artibeus
Dermanura
C
Ardops
Ariteus
Stenoderma
Phyllops
Pygoderma
Centurio
Sphaeronycteris
Ametrida
Artibeus
Dermanura
D

Figure 6

A first molecular phylogeny of the Funnel-eared bats (Chiroptera: Natalidae)

ABSTRACT

Nyctiellus, *Chilonatalus*, and *Natalus* form the Natalidae, a small family of neotropical bats that reaches its greatest taxonomic diversity in the West Indies. New cytochrome *b* and *Rag*2 sequences were combined with published morphological data to estimate phylogenetic relationships within this family. Monophyly of the family and the genera, and the sister relationship between *Chilonatalus* and *Natalus* are well supported. Within *Natalus*, Greater Antillean representatives form a clade, and the Lesser Antillean taxon is sister to a northern South American species. Central American *Natalus* are sister to remaining species in the genus, but this result is poorly supported. Mitochondrial sequences from throughout the range of the polytypic species *Natalus stramineus* revealed multiple instances of character fixation among allopatric populations, indicating that the species diversity within the family has been underestimated. Event-based biogeographic analyses indicate that the area of endemism of the most recent common ancestor of extant natalids is Cuba. An early Miocene fossil from Florida is sister to extant natalids, suggesting that natalids reached Cuba from North America. A North American origin for this neotropical family is consistent with the fossil record of molossids and vespertilionids, the sister group to Natalidae, but this hypothesis remains to be tested with phylogenies of these far more diverse and widespread families. Extant continental natalids originated in the Caribbean, a biogeographic hypothesis that probably explains their relative abundance, habitat preferences, and roosting ecology in the continental Neotropics.

INTRODUCTION

Natalidae is a small family of neotropical bats characterized by funnel-like ears and a tail about equal in length to the head and body (Emmons, 1997). Two genera, *Nyctiellus* and *Chilonatalus*, are restricted to the West Indies, while *Natalus* ranges from Sonora and Baja California, Mexico to Paraguay and Brazil through Central America and many Caribbean islands (Goodwin, 1959; Koopman, 1994; Taddei and Uieda, 2001). Six species are generally recognized: *Nyctiellus lepidus* is found on Cuba, Isla de Pinos, and a few Bahamian islands; *Chilonatalus tumidifrons* is confined to the Bahamas; *C. micropus* is known from Cuba, Isla de Pinos, Jamaica, Hispaniola, Providencia, and as fossil on Grand Cayman; *Natalus major* is found on Cuba, Jamaica, Hispaniola and as fossil from Middle Caicos, Grand Cayman and a handful of Bahamian islands; *N. tumidirostris* is restricted to northern South America including Trinidad, Bonaire, Curaçao, and as fossil from Tobago; and *N. stramineus* (hereafter referred to as *N. stramineus sensu lato* or s.l.) ranges from Mexico to Brazil through the Lesser Antilles (Koopman, 1994; Morgan, 2001; Ottenwalder and Genoways, 1982; Tejedor et al., in press). Geographic variation in species of *Chilonatalus* and *Natalus* is recognized in the designation of 11 (Koopman, 1994) to 13 (Dalquest, 1950; Goodwin, 1959) subspecies, most of them in the widespread species *N. stramineus* s.l.

Natalids have been collected in localities ranging from xeric woods to rainforest, gardens, and plantations (Emmons, 1997; Taddei and Uieda, 2001), but most specimens have been obtained from caves or cave-like roosts (Goodwin and Greenhall, 1961; Silva-Taboada, 1979; Timm and Genoways, 2003). Whereas *Nyctiellus* and *Chilonatalus* tolerate some variations in roost microclimate (Silva-Taboada, 1979; Timm and

Genoways, 2003; personal observation), Greater Antillean *Natalus* are restricted to hot, thermally stable environments available only in long, poorly ventilated cave systems (Goodwin, 1970; Morgan, 2001; Tejedor et al., in press). Both the extirpation of natalids from some Bahamian islands, Middle Caicos, and Grand Cayman, and the near-extirpation of *Natalus* from Cuba, have been linked to environmental change leading to the loss of appropriate microenvironments in caves (Morgan, 2001; Tejedor et al., in press). Natalids roost in colonies of up to several hundred individuals in caves often shared with other species, without forming tight clusters (Goodwin and Greenhall, 1961; Goodwin, 1970; personal observation). All species are strictly insectivorous, apparently catching prey on the wing (Goodwin and Greenhall, 1961; Silva-Taboada, 1979).

Both traditional and early cladistic classifications have placed the natalids as close allies of two other small neotropical families, the Furipteridae and the Thyropteridae (Miller, 1907; Smith, 1976). Their strictly neotropical distribution, along with morphological resemblance, seemed to support this view. An immunological study by Pierson et al. (1986) found Furipteridae as sister to Natalidae, but this clade was not closely related to Thyropteridae. Subsequent cladistic analyses of morphological and published restriction site data found support for the monophyly of a clade including the 3 neotropical families and the Old World Myzopodidae, the superfamily Nataloidea (Simmons, 1998; Simmons and Geisler, 1998). Recent molecular studies have not corroborated the results of morphology-based phylogenies. Instead, natalids appear as sister to a clade consisting of Molossidae and Vespertilionidae, and the remaining South American nataloids appear closely related to the noctilionoids; Phyllostomidae,

Mormoopidae, Noctilionidae, Mystacinidae (Hoofer et al., 2003; Teeling et al., 2003; Van Den Bussche and Hoofer, 2001; Van Den Bussche et al., 2003).

There was no formal phylogenetic hypothesis for the Natalidae until Morgan and Czaplewski (2003) analyzed 50 skeletal characters in an attempt to relate the fossil *Primonatalus prattae* to extant species. Morgan and Czaplewski (2003) also redefined the family, excluding the European and North American fossils *Ageina*, *Chadronycteris*, *Honrovits*, and *Stelihnia*, and the African fossil *Chamtwaria*, all previously classified as natalids (Beard et al., 1992; Van Valen, 1979).

In this study, new mitochondrial and nuclear DNA sequences were analyzed separately, and combined with published morphological data to define relationships among extant natalids. The results of these analyses were used to infer ancestral areas for the family and for the genus *Natalus*, and were compared to previous biogeographic hypotheses. Additionally, multiple mitochondrial cytochrome *b* sequences from the Central American and Caribbean range of *Natalus* were collected to produce a phylogeny for this genus and help resolve decades of dispute on the number of species it contains (Dalquest, 1950; Koopman, 1994; Morgan, 2001; Silva-Taboada, 1976; Varona, 1974).

MATERIALS AND METHODS

Taxon Sampling

To examine relationships among natalids, all currently recognized extant species, with the exception of the recently rediscovered *Natalus major primus*, were included (Simmons, in press; Tejedor et al., in press). At least two individuals per species from as many localities as possible were sequenced to best capture the genetic diversity of each taxon. All ingroup cytochrome *b* sequences were generated for this study (Table 1), while

two recombination activating gene 2 (*Rag*2) sequences (AY141024, AY141023) were obtained from a previously published study (Hoofer et al., 2003). For outgroup comparison and to root the tree, sequences from the vespertilionids *Myotis velifer* (AF376870, AY141033) and *Myotis riparius* (AF376866, AY141032), and the molossid *Tadarida brasiliensis* (L19734, AY141019) were included in phylogenetic analyses.

Molecular Data

For all specimens, DNA was isolated from wing clip (Rossiter et al., 2000), liver or muscle tissue that had been preserved in ethanol or lysis buffer in the field. DNA was extracted using a Qiagen DNeasy Tissue Extraction Kit (Qiagen, Inc.) following the manufacturer's protocol. Extracted DNA was used as a template in PCR reactions with protocols and primers for cytochrome *b* described elsewhere (Dávalos and Jansa, in press; Jansa et al., 1999). A 1.36 kb fragment of *Rag*2 was amplified and sequenced with primers described by Baker et al. (2000) with slight modifications as listed in Appendix 3. Amplification products were sequenced with the same primers used for PCR amplification, and also internal primers. Sequencing reactions were purified through a $MgCl_2$-ethanol precipitation protocol and run on an ABI 3100 automated sequencer. Sequences were edited and compiled using Sequencher 4.1 software (GeneCodes, Corp.). Base-calling ambiguities between strands were resolved either by choosing the call on the cleanest strand or using the appropriate IUB ambiguity code if both strands showed the same ambiguity. Molecular sequences generated as part of this study have been deposited in GenBank under accession numbers AY621006-AY6210288.

Morphological data

The morphological character matrix of Morgan and Czaplewski (2003) was appended to the molecular data to generate combined analyses of all characters available for the group. *Tadarida*, *Natalus major major, and N. stramineus stramineus* were coded as all missing, given that the original study did not include representatives of these taxa.

Data Analysis

Protein-coding cytochrome *b* and *Rag*2 sequences were easily aligned by eye using Sequencher 4.1 (GeneCodes, Corp.). To describe the variation in cytochrome *b* among taxa uncorrected pairwise (p) distances were calculated using PAUP* 4.0b10 (Swofford, 2002). Cytochrome *b* sequences were also examined for fixed character differences among named species and subspecies of *Natalus* (Table 1).

Analyses were conducted on four data sets: 1) the mitochondrial cytochrome *b* sequences; 2) the nuclear *Rag*2 sequences; 3) the concatenated cytochrome *b* and *Rag*2 data set, the molecular data set; and 4) the combined molecular and morphological data set (total evidence). Parsimony analyses were performed for separate data partitions and on combined matrices using branch and bound searches as implemented in PAUP* 4.0b10. For each search, phylogenetically informative characters were treated as unordered and equally weighted. Clade stability was assessed using nonparametric jackknife (Wu, 1986) and the Bremer support index (Bremer, 1994). Parsimony jackknife analyses included 1,000 replicates; searches were heuristic with 100 replicates of random taxon addition followed by TBR branch swapping. Bremer values were calculated with the aid of AutoDecay (Eriksson, 1999). Character state changes and length of alternative topologies were explored using MacClade 4.06 (Maddison and Maddison, 2003). The

Templeton (1983) test implemented in PAUP* 4.0b10 was used to assess the significance in length difference between topologies obtained from cytochrome *b*, combined molecular, or the morphological dataset.

Best-fit maximum likelihood models for molecular data were selected using nested likelihood ratio tests as implemented in Modeltest (Posada and Crandall, 1998). Maximum likelihood analyses of the different molecular data sets were performed using PAUP* 4.0b10. Bayesian methods were used to estimate a phylogeny using different models of molecular evolution for each partition of the molecular data. This analysis featured two partitions, mitochondrial and nuclear DNA, and the model of sequence evolution was determined using Modeltest (see above). The values for model parameters were allowed to vary between partitions and were not specified a priori, but treated as unknown variables to be estimated in each analysis. Bayesian analysis was conducted using MrBayes 3.0 (Huelsenbeck and Ronquist, 2001), with random starting trees without constraints, four simultaneous Markov chains were run for 2,000,000 generations, trees were sampled every 100 generations, and temperature was set to 0.20. Resulting burn in values, the point at which the model parameters and tree score reach stationarity, were determined empirically by evaluating tree likelihood scores. Analyses were repeated in 4 separate runs of MrBayes to ensure that trees converged on the same topology and parameters.

The molecular clock was tested by evaluating whether rate constancy provided a better fit to the data than different rates across the tree. To provide the most conservative test for a clock-like model of evolution, a UPGMA tree based on Jukes-Cantor distances was calculated, and the likelihood score for the best-fit model with no clock enforced

($\log L_1$) vs. the same model with a clock enforced ($\log L_2$) were compared. The significance of the difference in likelihood scores was tested by comparing $-2\log\lambda$ against a χ^2 distribution with degrees of freedom equal to the number of taxa minus 2. If the value for $-2\log\lambda$ was significant, then a molecular clock could be rejected.

A parametric bootstrap was used to examine the significance of differences in topology in a maximum likelihood framework (Huelsenbeck et al., 1996). First, the molecular data were used to estimate phylogenies with the maximum likelihood optimality criterion, as shown in the results. SEQ-GEN (Rambaut and Grassly, 1997) was used to simulate 1,000 data sets under the best model of sequence evolution, given the estimated topologies. Then, topological constraints equivalent to the morphology tree (Morgan and Czaplewski, 2003) and the results of parsimony analysis of cytochrome *b* (see results) were enforced on the simulated data sets. PAUP* 4.0b10 was then used to search each simulated data set for the optimal tree and the optimal tree constrained to each of the topological constraints. The null distribution was formed by the difference in maximum likelihood score ($L_{constrained} - L_{unconstrained}$) for all replicates. Significance was assessed by comparing the same difference ($L_{constrained} - L_{unconstrained}$) in the actual data to the null distribution.

Biogeographic Analyses

Koopman (1989) proposed that the ancestors of *Nyctiellus* and *Chilonatalus* reached the Caribbean through Central America or Mexico, and that the ancestor of Greater Antillean *Natalus* reached those islands from South America *via* the Lesser Antilles. In contrast, Baker and Genoways (1978) hypothesized that Greater Antillean *Natalus* descended from Mexican or Central American populations (Jones, 1989). To estimate ancestral areas, two

biogeographic methods were used: dispersal-vicariance analysis, DIVA (Ronquist, 1997), and ancestral area analysis (Bremer, 1992). Dispersal-vicariance analysis does not require an independent hypothesis of area relationships, but instead reconstructs the ancestral distribution at each of the internal nodes of a given phylogeny. The estimation can be constrained to contain any minimum number of areas. This is accomplished by means of optimization rules and set costs for extinction (cost of 1 per area lost) and dispersal (cost of 1 per area added). Vicariant and sympatric speciation carry no cost. Species distributions are therefore explained by assigning costs for each event in a way that biogeographic explanations imply the least possible cost.

Ancestral area analysis (Bremer, 1992) allows the identification of the ancestral area of a group based on its phylogeny, given geographic distribution information on the branches of the phylogeny. Each area can be considered a binary character with two states (present or absent) and optimized on the phylogeny. By comparing the numbers of gains and losses, it is possible to estimate areas most likely to have been part of the ancestral areas. This method was only used for the ancestral node of the Natalidae, since the sampled *Natalus* are allopatric and all ancestral areas are therefore equally probable.

Fossil natalids are known from the early Oligocene and early Miocene of Florida, the late Quaternary of Cuba, Isla de Pinos, Grand Cayman, Bahamas, Hispaniola, and Caicos, and the late Pleistocene of northeastern Brazil (Czaplewski and Cartelle, 1998; Morgan, 2001; Morgan and Czaplewski, 2003). This led Morgan and Czaplewski (2003) to propose that natalids originated in Florida, reached the Caribbean in the Oligocene or Miocene, and only expanded their range to South America in the Pliocene after the

formation of the Isthmus of Panama. This is an alternative biogeographic scenario for the family.

RESULTS

Sequence variation and saturation analysis

Cytochrome b

Complete cytochrome *b* sequences were obtained for most taxa, with the exception of *Tadarida brasiliensis* (L19734), for which only 402 base pairs were available. A summary of the uncorrected pairwise divergences among individuals in different taxonomic ranks is shown in Fig. 1. Sequence examination for fixed characters among currently recognized species and subspecies of *Natalus* (Table 1) uncovered fixed character differences among all named taxa (Table 2). Cytochrome *b* sequences of *Natalus major* from two distant localities were identical (Table 1).

Within cytochrome *b*, 416 (36%) of sites were variable and 349 (31%) were parsimony informative. The distribution of the parsimony-informative sites was dependent on codon position: 16.8 % in first, 3.4% in second, and 63.7 % in third codon position. Most substitutions were synonymous, and translation of sequences to amino acids led to a matrix with only 40 informative sites (15.3% variable sites, among which 69% are parsimony informative). The average base composition of sequences was skewed, with little bias at first codon position, deficiency of adenine (20.5%) and guanine (14.0%) and overabundance of thymine (40.6%) for second position, and a strong bias in third position: deficiency of guanine (2.5%) and thymine (20.2%), and abundance of adenine (45.0%) and cytosine (32.2%). That bias in base composition did not differ significantly across taxa when the whole gene (chi-square test implemented in PAUP*

4.0b10, *P* =0.252), first (*P*=1.000) and second positions (*P*=1.000) were analyzed separately. There were significant differences in base composition among taxa in third positions (*P*=0.000).

Heterogeneity in base composition across taxa is known to affect phylogenetic reconstruction (Lockhart et al., 1994): bias in third codon position, which contains most of the sequence variation, may confound the results of sequence analyses. To examine this hypothesis, the most divergent taxon in GC content for the third bases of cytochrome *b* sequences (GC content average for all taxa = 34.9%, SD = 4.4) was identified: *Nyctiellus* (22.5%). Heterogeneity is presumed to mislead phylogenetic analyses because unrelated taxa with similar base composition might appear as related when using methods that do not account for this error. This extreme bias in composition appeared only in *Nyctiellus*, and is not presumed to distort phylogenetic analyses because no other taxon exhibits similar variation. Graphs showing curves of transitions and transversions for each codon position versus uncorrected total sequence divergence were plotted to assess saturation in sequences. Those curves (not shown) indicated that first and second codon positions did not experience multiple transition or transversion substitutions. Third codon positions showed saturation in transition and transversion substitutions in comparisons with respect to and among the outgroups *Myotis* and *Tadarida*. Cytochrome *b* in all natalids examined ended with TAA, instead of AGA, the most common mammalian stop codon for this gene. This is presumed to be a synapomorphy of extant natalids.

Rag*2*

*Rag*2 fragments (~700 bp) of *C. micropus* AMCC 102718 and *N. stramineus* TK15661 were found to be identical to the published sequences available from GenBank:

AY141023 is *C. micropus* TK9454 from Jamaica, and AY141024 is *N. stramineus* TK15660 from Dominica, see Van Den Bussche et al. (2003). Of the 1,362 *Rag*2 sites, 141 (10%) were variable and 85 (6.2%) were parsimony informative. The distribution of the parsimony-informative sites was highly dependent on codon position: 10.6% in first, 12.9% in second, and 76.4% in third codon position. Most substitutions are synonymous, and translation of sequences to amino acids leads to a matrix with only 20 informative sites (8.6% variable sites, among which 51.2% are parsimony informative). The average base composition of sequences is skewed, with a deficiency of cytosine (17.7%) and thymine (20.7%) and an overabundance of adenine (29.6%) in first codon position, a deficiency of guanine (17.6%) and an overabundance of adenine (35.2%) in second position, and a deficiency of guanine (14.7%) and an overabundance of thymine (33.3%) in third position. The biases in base composition do not differ significantly across taxa for the whole gene, or for different codon positions (P=1.000).

Phylogenetic analyses

Molecular data from different genes were concatenated for combined analyses, from the same individual with the exception of the two published *Rag*2 sequences (GenBank AY141023, AY141024). Maximum parsimony analyses of the molecular data (Figs. 2-4) and the 'total evidence' data matrix (not shown, since the results were identical in topology and very similar in support values to Fig. 4a) were conducted with all unordered and unweighted characters. Maximum-likelihood analyses were performed first using the program Modeltest (see above). The models and parameters selected for the different datasets are shown in Table 3.

Since the parameters for the two genes were considerably different (Table 3), Bayesian methods were used to obtain an estimate of phylogeny that accounted for these two models while using all available molecular data. Stationarity in parameter estimation was reached after 200,000 generations (burn in = 2,000 trees). The mean parameters for the cytochrome *b* data set, followed by 95% confidence intervals in parentheses, were estimated as follows: R_{matrix} = 2.608 (0.811-5.229), 8.568 (2.911-16.361), 2.156 (0.608-4.533), 0.125 (0.01001-0.458), 34.903 (12.713-67.011), 1.0, □ = 0.203 (0.166-0.24). The mean parameters for the *Rag*2 data set, followed by 95% confidence values in parentheses, were estimated as follows: R_{matrix} = 4.987 (0.967-12.19), 14.13 (3.11-33.475), 2.272 (0.313-5.756), 3.904 (0.442-10.037), 18.493 (4.268-45.007), 1.0, □ = 10.885 (0.052264-41.951). The resulting trees are summarized in Fig. 4b.

Biogeographic Analyses

The results of dispersal vicariance optimizations for the most recent common ancestor of Natalidae and *Natalus* are shown in Table 4. Optimizations were constrained to two areas of endemism per node. *Chilonatalus micropus* was assumed to be a widespread species, and no attempt was made to place populations of South American *Natalus stramineus* s.l. or the Cuban *N. primus* in the phylogenies. Ancestral area analyses optimized the most recent common ancestor of the Natalidae to Cuba in all cases.

DISCUSSION

How many species of *Natalus*?

As many as six (Dalquest, 1950) and as few as one (Linares, 1971) species of *Natalus* have been recognized over the last few decades. In one of the most conservative

assessment of species diversity, Koopman (1994) proposed a definition of *Natalus stramineus* that made this species widespread from Baja California to Paraguay and throughout the West Indies. Although Morgan (2001; 1989) has argued for separating Greater Antillean *Natalus major* from other *N. stramineus* s.l., the conservative taxonomy is used widely (e.g., Arroyo-Cabrales et al. [1997], Timm and Genoways [2003]).

The only previous phylogenetic study of the Natalidae (Morgan and Czaplewski, 2003) recognized three species in *Natalus*: *major* encompassing Greater Antillean populations, *stramineus* for the remainder of the range, and *tumidirostris* for the northern South American (including Bonaire, Curaçao and Trinidad) populations characterized by swollen rostra and emarginated palates (Goodwin, 1959). *Natalus tumidirostris* and *stramineus* are also distinct in both cytochrome *b* and *Rag*2 sequences (Table 2), but the mitochondrial lineages are not reciprocally monophyletic (Fig. 2). Introgression, lineage sorting, and retention of ancestral polymorphism are often invoked to explain such patterns, e.g., Hoffmann et al. (2003), Patton and Smith (1994), and future studies with denser sampling of cytochrome *b* and fast-evolving nuclear and/or paternally inherited markers could distinguish among the processes involved.

Based on the study of Morgan and Czaplewski (2003) and on an unpublished study of the morphology of Greater Antillean *Natalus* (Tejedor et al., in preparation), Simmons (in press) recognized five species: *jamaicensis* (Jamaica), *major* (Hispaniola), *primus* (Cuba), *stramineus* (continent and Lesser Antilles), and *tumidirostris*. The results of DNA sequencing show that the diversity within *Natalus* is still underestimated. Fixed character differences were found among *jamaicensis*, *major*, *stramineus* (Lesser Antilles), and *saturatus* (Mexico and Central America). A previous study of allozymes

had already identified fixed differences at three nuclear loci between *jamaicensis* and *stramineus*, and at two nuclear loci between *jamaicensis-stramineus* and *saturatus* (Arroyo-Cabrales et al., 1997). Arroyo-Cabrales et al. (1997) did not reach any taxonomic conclusions partly because their assessment of character polarity was complicated by the use of *Chilonatalus micropus* (Jamaica) as the single outgroup in their analyses.

One species, *N. primus*, and four *N. stramineus* s.l. subspecies; *mexicanus* (Baja California and Sonora, Mexico), *espiritosantensis* (central Brazil to Espírito Santo), *natalensis* (NE Brazil), and *tronchonii* (northwestern Venezuela), remain to be sampled (Simmons, in press). Unassigned *N. stramineus* s.l. specimens are also known from Santa Cruz, Bolivia and Concepción, Paraguay (Taddei and Uieda, 2001). Two *Chilonatalus micropus* subspecies, *macer* (Cuba) and *brevimanus* (Providencia), and two *N. tumidirostris* subspecies, *tumidirostris* (Curaçao and Bonaire) and *continentis* (Carabobo, Venezuela and Santander, Colombia) were also omitted from this study.

Named populations of *Natalus stramineus* believed until recently to be conspecific (e.g., Arroyo-Cabrales [1997], Koopman [1994], Morgan and Czaplewski [2003]) are in fact distinct (Table 2). Given the disjunct distribution of most subspecies (Koopman, 1994), the differences in size among named taxa (Goodwin, 1959; Ottenwalder and Genoways, 1982; Taddei and Uieda, 2001), and results obtained in this sample, future studies including additional populations will likely double the number of species recognized in the family. This undertaking is urgent not only for phylogenetic estimation and biogeographic inference, but for conservation. Two natalids are included in the most recent endangered species list (IUCN, 2003): *Nyctiellus lepidus*, known in the

flesh from Cuba, Cat (formerly San Salvador), Long and Little Exuma in the Bahamas (near-threatened); and *Chilonatalus tumidifrons*, known in the flesh from San Salvador (formerly Watling Is.), Andros and Great Abaco in the Bahamas (vulnerable). In contrast, *N. jamaicensis* is not considered endangered because it has not been recognized as a distinct species. Live *N. jamaicensis* are known only from St. Clair Cave, St. Catherine Parish (Goodwin, 1959; Goodwin, 1970), and the cave is not protected in any way (Dávalos and Eriksson, 2003). A systematic revision of the family using morphological and molecular characters intends to complete taxonomic sampling for the Natalidae (A. Tejedor, pers. comm.), and this will allow for greater clarification on the phylogenetic relationships, endemism, and relative abundance of unsampled natalids.

Phylogeny of *Natalus*

There is moderate support for two pairs of sister species: *stramineus* and *tumidirostris*, and *major* and *jamaicensis* (Figs. 2-4). The relationship between these two clades, and with respect to *saturatus*, is resolved with poor support values (but see Fig. 4b). Two previous phylogenetic hypotheses for *Natalus* included three species each: *jamaicensis* (called *major* by the authors, but only Jamaican specimens were included), *saturatus* (called *stramineus*, but only specimens from Mexico and Belize were included), and *tumidirostris* (Morgan and Czaplewski, 2003) or *stramineus* (Arroyo-Cabrales et al., 1997). Morgan and Czaplewski (2003) found *jamaicensis* as sister to a clade conformed by *saturatus* and *tumidirostris*, similar to the results of maximum likelihood analysis of cytochrome *b* (Fig. 2b). This is consistent with the results of Arroyo-Cabrales et al. (1997), who found *jamaicensis* as sister to a clade conformed by *saturatus* and

stramineus. This topology is poorly supported, and is not recovered in analyses of *Rag*2 or combined molecular data (Figs. 3 and 4).

Two alternatives are viable given the molecular data: either *stramineus-tumidirostris* (Figs. 2a, 5b) or *saturatus* (Figs. 3, 4a, 5a) is sister to all other *Natalus*. The third option (Fig. 5c), *jamaicensis* sister to other *Natalus*, is well supported in analyses of morphological characters (see Morgan and Czaplewski [2003]: 93% bootstrap, Bremer decay = 10). This topology (Fig. 5c) costs five extra steps in combined molecular and cytochrome *b* analyses (Figs. 3a and 4a), two extra steps in analyses of *Rag*2 (Fig. 3a), and three extra steps in the total evidence phylogeny (not shown). These topologies are not significantly different given the molecular data presented here: $P>0.05$ among the three possible arrangements in the Templeton (1983) test, $P=0.184$ for parametric bootstrap of combined molecular tree vs. morphology topology, and $P=0.085$ for parametric bootstrap of combined molecular tree vs. cytochrome *b* parsimony topology (Fig. 5). The three topological options of relationships among clades of *Natalus* remain plausible.

Phylogeny of the Natalidae

There is strong support for the monophyly of the family Natalidae in all data partitions and types of analyses (Figs. 2-4), in agreement with phylogenies based on morphological characters, or other mitochondrial and nuclear genes (Hoofer et al., 2003; Morgan and Czaplewski, 2003; Van Den Bussche and Hoofer, 2001; Van Den Bussche et al., 2002; Van Den Bussche et al., 2003). In general, the molecular data corroborate the phylogeny of Morgan and Czaplewski (2003) for extant natalids (Fig. 4).

Chilonatalus and *Natalus* are sister taxa (Fig. 4), with hidden support from the separate data partitions (values for this branch in the total evidence topology are: 91% jackknife and Bremer = 12, compare to Figs. 2-4). The molecular data provide greater support for this clade than the morphological characters described by Morgan and Czaplewski (2003: 58% bootstrap, Bremer decay = 1). Parsimony analysis of the fast-evolving cytochrome *b* gene (Fig. 1) does not recover a sister relationship between the *Chilonatalus micropus* and *C. tumidifrons* (Fig. 2a), perhaps because these taxa diverged early in the history of the genus (see Figs. 2b and 3b). This is remarkable in light of the minimal external differentiation that prompted Hall and Kelson (1959) and Varona (1974) to suggest that *Chilonatalus* comprised a single species. The monophyly of *Chilonatalus* is moderately supported in analyses of *Rag*2 and combined molecular data (Figs. 3-4), consistent with support values obtained by Morgan and Czaplewski (2003: 75% bootstrap, Bremer decay = 5). Finally, *Nyctiellus* and *Natalus* and each of its species are monophyletic, with the exception of *N. stramineus* (Fig. 2, see above).

Biogeography of *Natalus*

DIVA optimizations constrain the ancestral area of *Natalus* to island-continent composites, depending on the resolution for the genus (Table 4). A sister relationship between Greater Antillean and Lesser Antillean populations (in this case including the northern South American *tumidirostris*, Figs. 3, 4, 5a) is better supported than Central America being sister to Greater Antillean taxa (Figs. 2a, 6b). The former would fit the biogeographic hypothesis of Koopman (1989) regarding the origin of Greater Antillean *Natalus*. The latter would fit the hypothesis of Baker and Genoways (1978). Neither of

these hypotheses can be strongly rejected with the data at hand because they do not differ significantly (see above).

In any case, as with the phyllostomid Short-faced bats, all continental *Natalus* must have descended from Caribbean ancestors because Antillean natalids are basal in the phylogeny (Table 4, Figs. 2-4). *Natalus* has expanded its range into the mainland at least twice (Fig. 2-4), and probably diversified there, although when and how remains to be tested with samples of South American *Natalus stramineus* s.l.

Biogeography of Natalidae

A Central American origin for natalids (Baker and Genoways, 1978; Koopman, 1989) is rejected in these phylogenetic analyes (Table 4). Both DIVA and ancestral area analyses optimize the ancestral node of natalids to Cuba and the Bahamas. This is because Cuba and the Bahamas are the only areas of endemism to contain 2 of 3 extant lineages in the phylogenies studied here, including the sister to all other natalids, *Nyctiellus* (Fig. 4). As noted before, fossil natalids are known from the early Oligocene and early Miocene of northern Florida (Morgan and Czaplewski, 2003). Of these, only the early Miocene *Primonatalus* was complete enough to include in phylogenetic analysis, and Morgan and Czaplewski (2003) found it was sister to extant natalids. DIVA optimizations for phylogenies modified to include *Primonatalus* as sister to extant taxa assign the ancestral area of natalids to Florida and Cuba-Bahamas (not shown). The ancestor of *Primonatalus* and the extant natalids would have been widespread across these areas of endemism prior to the early Miocene.

A North American origin is implied by higher-level bat relationships, even if fossil natalids are ignored. Most molecular studies of higher-level chiropteran

relationships find natalids as sister to a clade composed of molossids and vespertilionids (Hoofer et al., 2003; Teeling et al., 2003; Teeling et al., 2002; Van Den Bussche et al., 2002; Van Den Bussche et al., 2003). The earliest molossid fossil is *Wallia* from the middle Eocene of Saskatchewan (Legendre, 1985), and the earliest vespertilionid fossil is *Stehlinia* from the middle Eocene of Europe (McKenna and Bell, 1997).

These extinct taxa constrain the last common ancestor of natalids, molossids, and vespertilionids to the northern supercontinent of North America-Europe, and date the divergence among the three lineages prior to the middle Eocene (> 50MYA). North America and Europe were interconnected prior to the middle Eocene (Savage and Russell, 1983). The early Eocene Earth was a greenhouse, with tropical forests extending to Montana and London, and broadleaf forests within the Arctic circle (Prothero, 1994). The tropical climate prevalent at the time in North America still limits the distribution of natalids and most molossids, but not vespertilionids. By the Oligocene, when natalids first appear in the fossil record, Florida was tropical: emballonurids, mormoopids, molossids, and perhaps even phyllostomids lived in the region (Czaplewski et al., 2003; Morgan and Czaplewski, 2003). The late Oligocene-early Miocene period was one of relative global warmth followed by a transition to the middle Miocene "ice-house" world (Mutti, 2000; Pearson and Palmer, 2002). Relatives of these neotropical Florida bats survive today in the remaining Neotropics: the West Indies, Mexico, Central America, and South America.

Is this congruent with the subsequent history of molossids and vespertilionids? Because taxonomic sampling in the study of Hoofer and Van Den Bussche (2003) was

incomplete and several internal nodes were poorly supported, the authors abstained from making any biogeographic inferences. Hoofer and Van Den Bussche (2003), however, concluded that geographic distribution was a more precise predictor of phylogenetic relationships than ecology and related morphology, a conclusion echoing that of an earlier study of the speciose vespertilionid genus *Myotis* (Ruedi and Mayer, 2001). Phylogenetic hypotheses for Molossidae are incomplete, and basal relationships were not resolved in a recent supertree analysis (Jones et al., 2002). Future phylogenetic studies of molossids and vespertilionids could test the broader biogeographic implications of a North American origin for natalids and their close relatives.

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Table 1. Taxa and locality data of individuals included in this study. Taxonomy follows Koopman (1994). When only tissue vouchers are listed, DNA was extracted from wing punctures. *N.* = *Natalus*. AMCC: Ambrose Monell Cryogenic Collection of the American Museum of Natural History, AMNH: cadaver voucher at the Mammalogy Department of the American Museum of Natural History; CM: Carnegie Museum of Natural History; NHM: Natural History Museum (London); SP: tissue collection of the Carnegie Museum of Natural History; T: tissue collection of the Natural History Museum (London); TK: tissue collection of the Museum of Texas Tech University; UAMI: Universidad Autónoma Metropolitana Iztapalapa.

Taxon	Locality	Tissue voucher	Cadaver voucher
Nyctiellus lepidus	Crawling Cave, Industrious Hill, Cat Island, Bahamas	AMCC 119283	AMNH 275537
Nyctiellus lepidus	Crown Cave, Dumfries, Cat Island, Bahamas	AMCC 119271	AMNH 275535
Chilonatalus micropus	St. Clair Cave, Polly Ground, St. Catherine, Jamaica	AMCC 102717	AMNH 274630
Chilonatalus micropus	St. Clair Cave, Polly Ground, St. Catherine, Jamaica	AMCC 102718	AMNH 274631
Chilonatalus micropus	St. Clair Cave, Polly Ground, St. Catherine, Jamaica	TK9456	CM 44580
Chilonatalus tumidifrons	Reckly Maze Cave, San Salvador (Watling Island), Bahamas	AMCC 121973	
Chilonatalus tumidifrons	Reckly Maze Cave, San Salvador (Watling Island), Bahamas	AMCC 121978	
N. stramineus stramineus	0.5 mi N Toucari, St. John Parish, Dominica	TK15661	TTU 31458
N. stramineus stramineus	Morne Ducos, 1.5 km NE jct rt D203 Grand-Bourg along Rt N9 50 m, Marie Galante, Guadeloupe	SP10036	CM 112377
N. stramineus stramineus	1.1 km S Calihaut (by road) small shallow cave in slope below HWY, St. Peter Parish, Dominica	SP9393	CM 112376
N. stramineus jamaicensis	St. Clair Cave, Polly Ground, St. Catherine, Jamaica	TK9421	TTU 29110
N. stramineus jamaicensis	St. Clair Cave, Polly Ground, St. Catherine, Jamaica	TK9424	TTU 29113
N. stramineus major	La Entrada (de Cabrera), María Trinidad Sánchez, Dominican Republic	AMCC 103028	AMNH 275480

Table 1. Continued.

Taxon	Locality	Tissue voucher	Cadaver voucher
N. stramineus major	Don Miguel (Platanal de Cotui), Sánchez Ramírez, Dominican Republic	AMCC 103056	AMNH 275513
N. stramineus saturatus	Cave Las Cuevas Research Station, Belmopan, Belize	T48	NHM 2003.201
N. stramineus saturatus	Rio Uyus, 5 km E San Cristóbal Acasaguastlán, El Progreso, Guatemala	F34011	ROM 99652
N. stramineus saturatus	Laguna Noh-Bec, 2 km W of Noh-Bec, Quintana Roo, Mexico	FN30994	ROM 97519
N. stramineus saturatus	Colola (5 km N) Municipio Aquila, Michoacán, Mexico	TK43151	UAMI
N. stramineus saturatus	Rivas, Nicaragua	F48058	ROM 112172
N. tumidirostris haymani	Tamana Cave, St. Andrew, Trinidad	AMCC 119246	AMNH 275517
N. tumidirostris haymani	Tamana Cave, St. Andrew, Trinidad	AMCC 119247	AMNH 275518

Table 2. Character differences in sites of cytochrome *b* among included taxa within the genus *Natalus*. Taxonomy follows Simmons (in press). See Table 1 for localities, and text for geographic distribution. *N.* = *Natalus*.

Named taxa or samples	Compared to	Fixed character differences
N. stramineus	Other *Natalus*	15
N. jamaicensis	*Natalus* Greater Antilles	17
N. major	*Natalus* Greater Antilles	15
N. saturatus	Other *Natalus*	4
N. saturatus	*Natalus* Greater Antilles	7
N. saturatus	*N. stramineus*	21
N. tumidirostris haymani	*N. stramineus*	4

Table 3. Models of molecular evolution and parameters selected for each molecular data set, see Table 1 for sequences. GTR = general time reversible model; HKY = Hasegawa Kishino Yano model; R-matrix = rate matrix parameter (with respect to G-T transversion); ti/tv ratio = transition/transversion ratio; Γ = shape parameter, I = proportion of invariant sites; -2logΛ = 2[logL_1 - logL_2], where L_1 = likelihood without clock and L_2 = likelihood with clock.

Data	Model	R-matrix or ti/tv ratio	Γ	I	-2logΛ	df	*P*
cyt *b*	GTR+I+Γ	1.5, 6.2, 1.6, 0.0, 19.4	2.5640	0.5531	24.2	21	>0.05
*Rag*2	HKY+Γ	2.6594	0.4389	-	9.7	9	>0.05
combined	GTR+I+Γ	1.0, 3.5, 1.0, 1.0, 15.7	0.6258	0.5840	13.7	9	>0.05

Table 4. Estimation of ancestral area for the most recent common ancestor of *Natalus* and extant Natalidae, given alternative phylogenies discussed in the text. Ancestral area optimizations in DIVA were constrained to a maximum of two areas. The 'x' indicates the areas preceding it should be combined with each of the areas after it to generate multiple two-area regions. The results shown for ancestor of Natalidae are those with the least number of areas.

Topology	ancestor *Natalus*	ancestor Natalidae
saturatus basal (Fig. 6a)	Central America, Mexico x Lesser Antilles, northern South America, Hispaniola, Jamaica	Cuba or Bahamas
stramineus-tumidirostris basal (Fig. 6b)	northern South America, Lesser Antilles, Mexico, Central America x Hispaniola, Jamaica	Cuba or Bahamas
major-jamaicensis basal (Fig. 6c)	northern South America, Lesser Antilles x Mexico, Hispaniola, Jamaica	Cuba or Bahamas

FIGURE LEGENDS

Figure 1. Scatter plot of uncorrected sequence divergence in cytochrome *b* against taxonomic rank. Taxonomy follows Simmons (in press). Numerals indicate cytochrome *b* distance as follows: 1: between *Nyctiellus* and *Myotis riparius*; 2: *Natalus* vs. *Chilonatalus*; 3: *Natalus* and *Chilonatalus* vs. *Nyctiellus*; 4: *Natalus tumidirostris* vs. *N. stramineus*; 5: between two species of *Chilonatalus*; 6: between two species of *Myotis*; 7: among *Natalus saturatus*.

Figure 2. A. One of two equally parsimonious trees resulting from analysis of cytochrome *b* (L=845 steps, CI=0.634, RI=0.775). Numbers above branches are percent of 1,000 50% jackknife replicates, below branches are Bremer support values. B. Phylogram resulting from maximum likelihood analysis using a rate-constant GTR+I+G model of DNA evolution (-ln*L* = 5080.34). Numbers above branches or with arrows are percent of 300 50% jackknife replicates.

Figure 3. A. One of nine equally parsimonious trees resulting form analysis of *Rag*2 (L=154 steps, CI=0.948, RI=0.939). Numbers above branches are percent of 1,000 jackknife replicates, below branches are Bremer support values. B. Phylogram resulting from maximum likelihood analysis using a rate-constant model of DNA evolution (-ln*L* = 2815.62). Numbers above branches or with arrows are percent of 300 jackknife replicates.

Figure 4. A. Most parsimonious cladogram resulting form analysis of concatenated cytochrome *b* and *Rag*2 sequences (L=958, CI=0.699, RI=0.602). Numbers above branches are percent of 1,000 50% jackknife replicates, below branches are Bremer

support values. This topology is identical to that obtained from parsimony analysis of concatenated sequences and morphological characters, total evidence (not shown, L=930, CI=0.739, RI=0.534). B. Majority-rule consensus of 18,000 phylograms (-ln*L* = 7,572; 95% confidence interval = 7,564.106-7,582.164) obtained by Bayesian analyses of concatenated cytochrome *b* and *Rag*2 sequences using separate models of evolution for each gene. Values above branches are posterior probabilities, below branches are percent of 300 50% jackknife replicates from maximum likelihood analysis using a single model for both genes (see Results).

Figure 5. Three hypotheses of relationships in the genus *Natalus* (*N.*). A. Result of combined molecular and total evidence analyses (Figs. 3, 4). B. Result of parsimony analysis of cytochrome *b* (Fig. 2a). C. Result of phylogenetic analyses of morphology (Morgan and Czaplewski, 2003), and maximum likelihood analysis of cytochrome *b* (Fig. 2b). *Natalus major*, in gray, was not sampled in the morphological study.

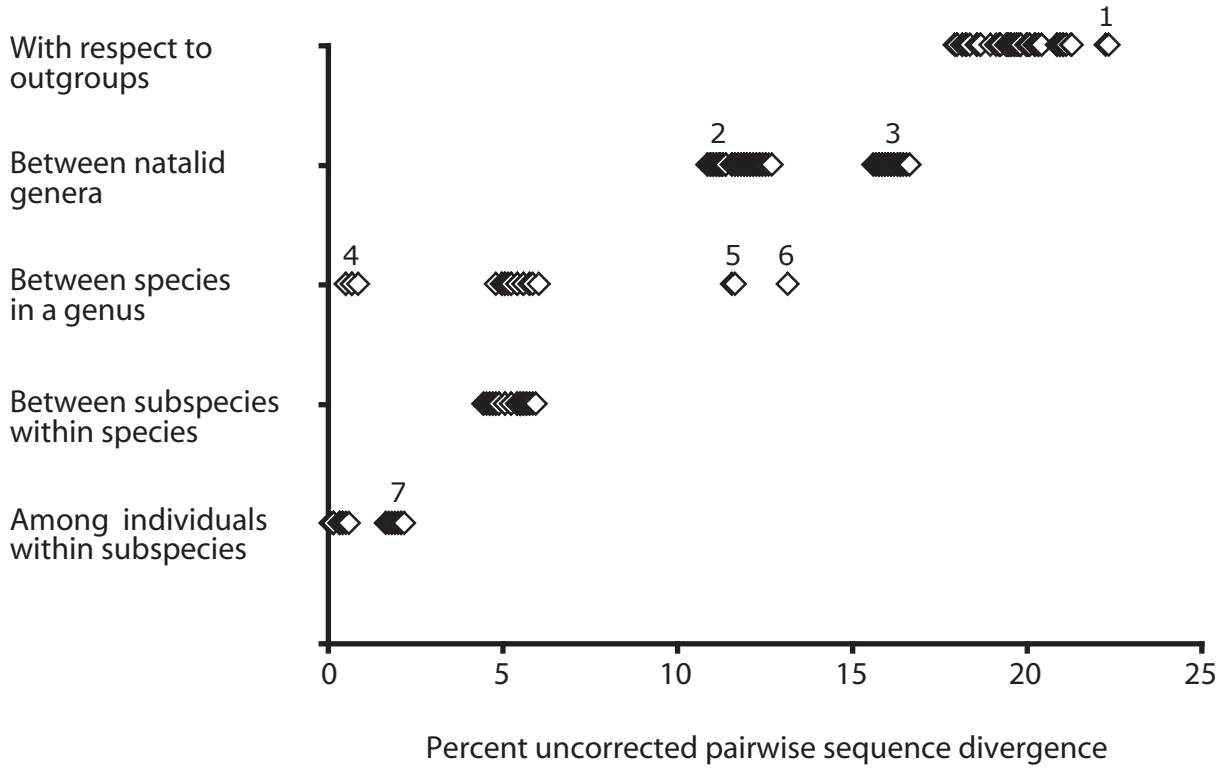
With respect to outgroups
Between natalid genera
Between species in a genus
Between subspecies within species
Among individuals within subspecies
1
2
3
4
5
6
7
0
5
10
15
20
25
Percent uncorrected pairwise sequence divergence

Figure 1

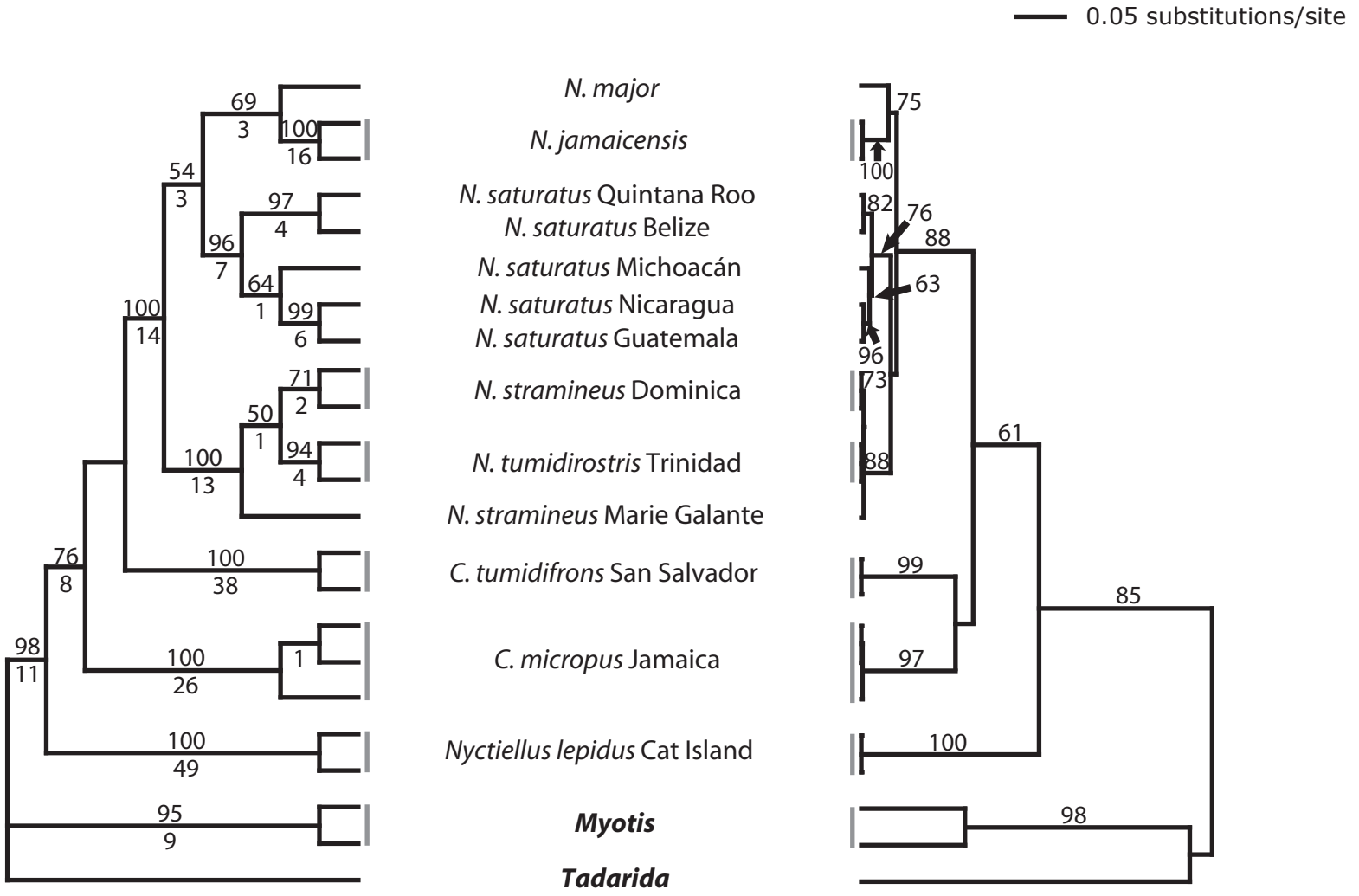
0.05 substitutions/site
N. major
N. jamaicensis
N. saturatus Quintana Roo
N. saturatus Belize
N. saturatus Michoacán
N. saturatus Nicaragua
N. saturatus Guatemala
N. stramineus Dominica
N. tumidirostris Trinidad
N. stramineus Marie Galante
C. tumidifrons San Salvador
C. micropus Jamaica
Nyctiellus lepidus Cat Island
Myotis
Tadarida
A
B

Figure 2

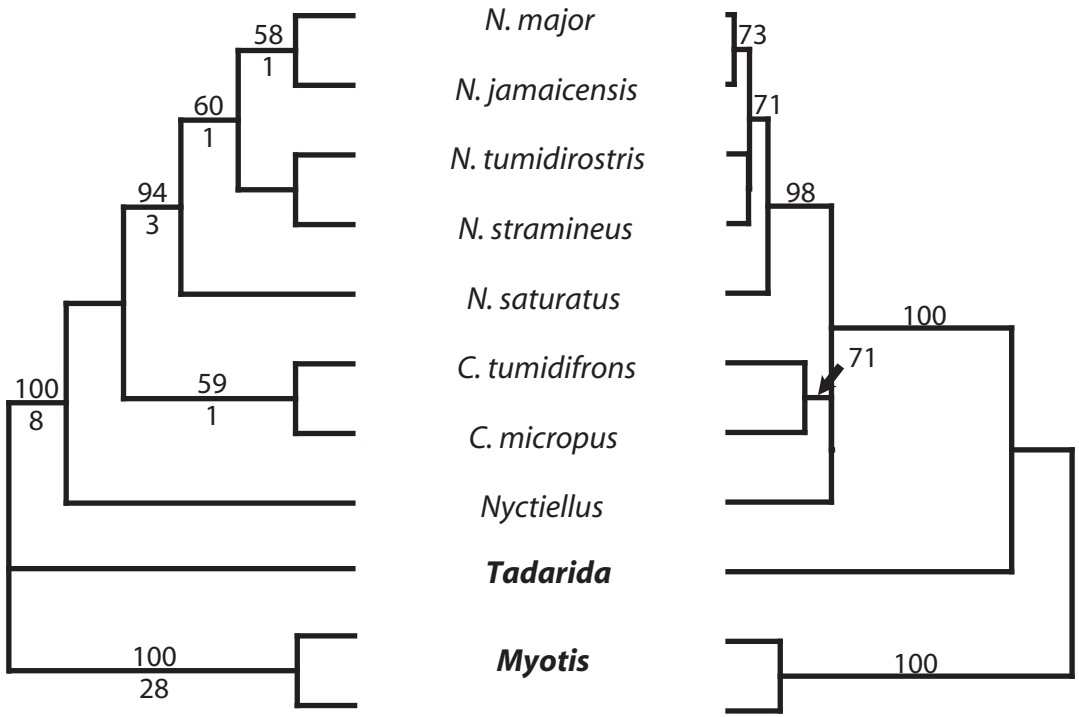
0.005 substitutions/site
N. major
N. jamaicensis
N. tumidirostris
N. stramineus
N. saturatus
C. tumidifrons
C. micropus
Nyctiellus
Tadarida
Myotis
58
1
60
1
94
3
59
1
100
8
100
28
73
71
98
100
71
100
A
B

Figure 3

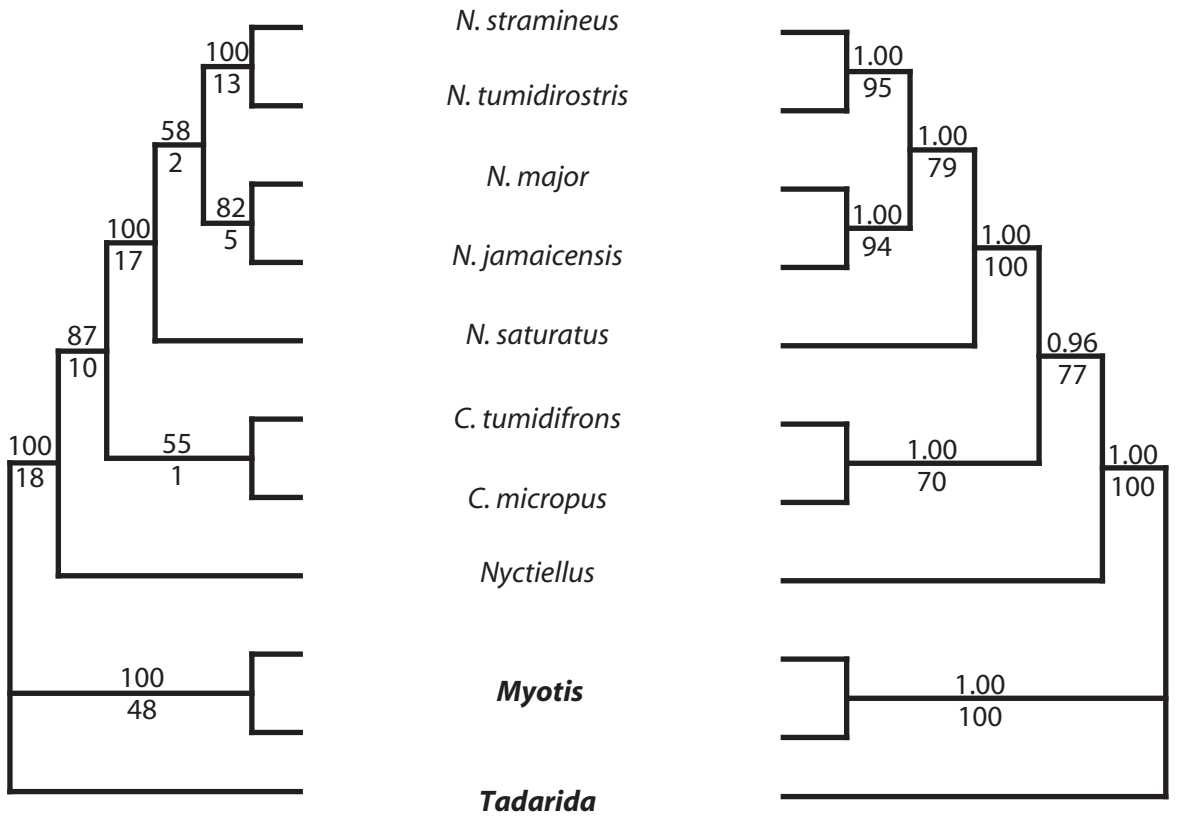
N. stramineus
N. tumidirostris
N. major
N. jamaicensis
N. saturatus
C. tumidifrons
C. micropus
Nyctiellus
Myotis
Tadarida
100
13
58
2
82
5
100
17
87
10
100
18
55
1
100
48
1.00
95
1.00
79
1.00
94
1.00
100
0.96
77
1.00
70
1.00
100
1.00
100
A
B

Figure 4

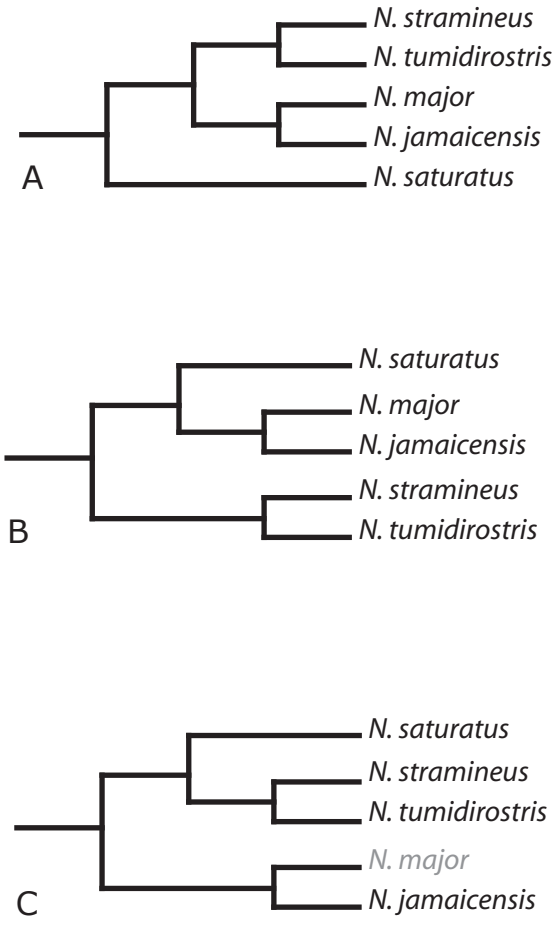
N. stramineus
N. tumidirostris
N. major
N. jamaicensis
N. saturatus
A
N. saturatus
N. major
N. jamaicensis
N. stramineus
N. tumidirostris
B
N. saturatus
N. stramineus
N. tumidirostris
N. major
N. jamaicensis
C

Figure 5

Appendix 3. Primers used in amplification and sequencing of natalid *Rag*2.

Name	5'-primer sequence-3'
NAT-RAG2F2	5'-TTTGTTATTGTGGGTGGCTATCAG-3'
NAT-RAG2F2-I	5'-GGATTCCACTCCSTTTGAAGA-3'
NAT-RAG2F1-I	5'-ATACAGTCGAGGAAAGAGCATGG-3'
NAT-RAG2R1	5'-AGCCTGTTTATTGTCTCCTGGTATGC-3'
NAT-RAG2R1-I	5'-GMGGCAGGCAGTCAGCTAC-3'
NAT-RAG2R2	5'-GGAAGGATTTCTTGGCAGGAGT-3'
NAT-RAG2R2-I	5'-ACAGCATGTAATCCAGTAGC-3'

Miocene area relationships in the Caribbean inferred from five neotropical bat phylogenies

ABSTRACT

How did the West Indies acquire their biota? Two competing hypotheses have been proposed to explain Caribbean biogeography: an Oligocene land bridge between northern South America and the West Indies (Gaarlandia), and post-Cretaceous dispersal from South America and, to a lesser extent, Central America. A previous biogeographic analysis of mammalian phylogenies found some congruence between bat phylogenies and the land bridge hypothesis, but the lack of resolution in species-level cladograms and estimates for divergence times, coupled with conservative taxonomy contributed to inconclusive results. This paper summarizes the results of biogeographic analyses and estimates of divergence times at crucial nodes in new molecular phylogenies of 3 chiropteran lineages. These phylogenies were both compared to the land bridge hypothesis and to phylogeny-based patterns of area relationships. Reconciled tree analyses for the five bat lineages show measures of codivergence supporting a close link between taxon cladograms and area relationships. The phylogenies of 3 of the 5 groups studied, *Brachyphylla* and allies, Stenodermatina, and natalids, are congruent with a single range expansion onto the Caribbean. Relationships in *Mormoops* are consistent with this result but further studies of fossil exemplars are necessary, and Antillean *Pteronotus* are the product of at least two separate invasions. Continental Short-faced bats (Phyllostomidae: Stenodermatina) and Funnel-eared bats (Chiroptera: Natalidae) descended from Caribbean ancestors. The transitions from the middle to late Miocene and from the late Miocene to early Pliocene, both characterized by low sea levels and

shoaling of the Central American seaway, correspond roughly to diversification events in Caribbean bats. Divergences in *Mormoops*, *Pteronotus*, and natalids, and divergence from the outgroup in Short-faced bats and *Brachyphylla* and allies appear to coincide with the first episode. The Caribbean range expansion of the *Pteronotus rubiginosus* and *Natalus* lineages, divergences between *Erophylla* and *Phyllonycteris*, and between *Pteronotus quadridens* and *macleayii*, and the secondary invasion of the mainland by Short-faced bats roughly coincide with the second episode. The role of the Miocene in Caribbean biogeographic history remains to be tested with additional phylogenies, new molecular data, analyses of divergence time that do not assume rate constancy, and the fossil record. The correspondence of geological history, phylogenetic patterns, and the timing of diversification in bats demonstrates congruent biogeographic patterns in the Caribbean are pervasive even among the most vagile organisms.

INTRODUCTION

Bats are the most speciose and abundant of Caribbean mammals, the survivors of a fauna that included native sloths, monkeys, rodents, and insectivorans, all now extinct or nearly so (Morgan and Woods, 1986). There are 63 Recent and late Quaternary species in 32 genera of six families (Koopman, 1989; Morgan, 2001; Tejedor et al., in press; Tejedor et al., in preparation). The bat fauna of the Antilles is unique: about 50% of the species are endemic to the region, and the proportion of endemics rises if only the Greater Antilles are considered (Baker and Genoways, 1978; Koopman, 1989). How can the diversity and distribution of this fauna be explained?

Until recently the answer to this question has been over water dispersal from mainland America, followed by speciation in the West Indies (Baker and Genoways,

1978; Koopman, 1989). As a result of this view, bats were excluded or downplayed in studies of Caribbean biogeographic patterns although their richness and endemism make them ideal for undertaking analyses of historical biogeography (Page and Lydeard, 1994). Further, the dispersal approach has little predictive power and cannot fully account for obvious examples of congruent geographic differentiation and distribution in Caribbean bats (Griffiths and Klingener, 1988; Page and Lydeard, 1994).

An initial analysis of biogeographic congruence between an area cladogram compatible with the Gaarlandia geological hypothesis (Iturralde-Vinent and MacPhee, 1999) and four bat phylogenies showed a few nodes of bat phylogenies might be explained by geological events as ancient as the Oligocene (Dávalos, 2004). The conclusions of that analysis were limited by the lack of resolution of some of the phylogenies (e.g., in the phyllostomid subtribe Stenodermatina), the absence of estimates for divergence times, the use of a conservative taxonomy that 'lumped' together populations ranging from Mexico to Brazil, including the West Indies (Koopman, 1994), and the comparison to a single geological hypothesis: Gaarlandia (Iturralde-Vinent and MacPhee, 1999).

The geological and biogeographic model of Iturralde-Vinent and MacPhee (1999) mainly concerns the area interconnections from the Eocene/Oligocene transition and the subsequent isolation of landmasses until the end of the Oligocene (for a summary see Dávalos [2004]). The history of proposed interconnections or near-interconnections between the West Indies and the continental American landmasses did not end with the Oligocene (Perfit and Williams, 1989). From the Miocene to the Early Pliocene (25-5 MYA), the Caribbean plate continued to move eastward in relation to the American

plates, causing uplift and emergence of most of the islands, including Jamaica, which had been submerged during most of the Oligocene (Perfit and Williams, 1989; Portell et al., 2001). 'Stepping stone' connections between North America and South America first became available around the middle Miocene (Duque-Caro, 1990; Pindell, 1994).

Near interconnections among areas after the Oligocene involved sea level changes exposing wider subaerial margins or submerged banks (Perfit and Williams, 1989). Exceptionally low sea levels marked the middle/late Miocene transition (c. 10 MYA), and the Miocene/Pliocene (ca. 5 MYA) (Haq et al., 1993; Miller et al., 1996; Roth et al., 2000). This might have exposed some of the shallow banks of the map in Fig. 1, and expanded the margins of the islands and continents. Sea level peaks followed the episodes of interconnection, isolating islands and even regions within islands (e.g., Cuba was trisected, Hispaniola was bisected). Earth history models such as Gaarlandia, or predictions arising from interconnections later in the history of the region, are appropriate null hypotheses in testing diversification models because it is parsimonious to assume that areas and taxa evolve together (Cracraft, 1975).

This paper summarizes the results of biogeographic analyses using the revised phylogenies of five chiropteran lineages (completely overlapping with the analyses of Dávalos [2004], but counting the family Mormoopidae as two lineages). In addition to comparing these phylogenies to the Gaarlandia hypothesis, the phylogenies themselves were used to produce patterns of area relationships. I present new estimates of the substitution rates for mitochondrial and nuclear loci, and applied these and fossil calibrations to estimate the timing of divergence in critical nodes.

MATERIALS AND METHODS

Areas of endemism

The areas of endemism included in this study are defined by the presence of at least two endemic taxa (Platnick, 1991). The continental areas of endemism are: Mexico, Central America (from Guatemala to Panama), northwest South America (Colombia, Venezuela and Guyana, including the islands of Trinidad, Tobago, Margarita, and Aruba, Bonaire and Curaçao, south to northern Peru and northwest Brazil), northeast South America (Surinam and French Guiana south to northeast Brazil), and central South America (from Peru and Bolivia west to central Brazil, south to Paraguay). Northwest South America and northeast South America only appear distinct in for *Pteronotus*, so the two areas are treated as a single unit hereafter. The distinction between the two areas is noted to highlight the possibility of separate histories, and to inspire future taxonomic revisions of taxa hitherto considered widespread across the region. Note also that the Chocó, from eastern Panama south to northwest Ecuador, is not characterized by endemic taxa in this study and not defined as a separate biogeographic unit.

The insular areas of endemism are: the Bahamas (Bahamas and Turks and Caicos), Cuba, Jamaica, Hispaniola (Haiti and the Dominican Republic), Puerto Rico, and the Lesser Antilles (from Anguilla south to St. Vincent and Barbados). The West Indies include the Greater Antilles (Cuba, Jamaica, Hispaniola, and Puerto Rico), Bahamas, Turks and Caicos, Cayman Islands, and the Lesser Antilles south to St. Vincent and Barbados (Morgan, 2001). Grenada and the Grenadines, Trinidad, Tobago, Margarita, and Aruba, Bonaire and Curaçao are excluded from this definition of the West Indies, and considered part of the continental South America area of endemism. The

terms *Antillean*, *Caribbean*, and *West Indian*, are synonyms and thus encompass all the islands defined above as part of the West Indies (Morgan, 2001).

Taxon cladograms

The following taxon cladograms were included in subsequent biogeographic analyses:

1. The two taxon cladograms for the mormoopid genera *Mormoops* and *Pteronotus*, resulting from maximum likelihood analysis of cytochrome *b*;

2. The cladogram resulting from maximum likelihood analysis of cytochrome *b* of the phyllostomids *Brachyphylla*, *Erophylla* and *Phyllonycteris* (hereafter *Brachyphylla* and allies);

3. The cladogram resulting from maximum likelihood analysis of concatenated cytochrome *b* and *Rag*2 for the phyllostomid subtribe Stenodermatina (Short-faced bats); and

4. The cladogram resulting from maximum likelihood analysis of concatenated cytochrome *b* and *Rag*2 for the family Natalidae.

These taxon cladograms were modified to reduce distortion from extinct populations and missing taxa as follows: inclusion of Cuba, Hispaniola, Jamaica and the Bahamas in the range of *Mormoops megalophylla*; inclusion of Jamaica in the range of *Brachyphylla pumila* (Morgan, 2001); addition of *Phyllonycteris major* (extinct) and *P. poeyi* (extant, except in Bahamas) as part of an unresolved trichotomy with *P. aphylla*; and addition of *Phyllops vetus* (extinct) and *P. silvai* (extinct) as part of an unresolved trichotomy with *P. falcatus* (Silva-Taboada, 1979; Suárez and Díaz-Franco, 2003). Finally, two species of *Centurio*, *senex* (Mexico and Central America) and *greenhalli* (northern South America), were mapped on the combined molecular phylogeny to include maximally endemic taxa.

This is consistent with results of phylogenetic analysis of cytochrome *b* for this genus (*Rag*2 data were available only for *senex*). The taxon cladograms analyzed are shown in Fig. 2.

Pteronotus pristinus (Cuba), *Pteronotus* sp. (Hispaniola), and *Mormoops magna* (Cuba) were not included, as their relationships to extant species are uncertain, particularly given the revised taxonomy used here (Morgan, 2001; Silva-Taboada, 1974; Simmons and Conway, 2001). The extinct Antillean populations of *Mormoops megalophylla* were referred to *megalophylla* rather than *tumidiceps*, although a study of variation among these supposed conspecifics is necessary to test this diagnosis (Morgan, 2001). Unsampled populations of *Natalus stramineus sensu* Koopman (1994) were likewise omitted, as their relationships to the five species currently recognized within *Natalus* are uncertain. *Natalus primus*, another species of the *N. stramineus* s.l. complex, was also excluded since ongoing morphological studies (Adrian Tejedor, personal communication) suggest that it is not closely related to *N. major* as previously believed (Morgan, 2001; Morgan and Czaplewski, 2003).

Geological area cladogram

A previous reconciled tree analysis (Dávalos, 2004) used a version of the Gaarlandia hypothesis of Iturralde-Vinent and MacPhee (1999) that attempted to include in the area cladogram the history of landmasses after the Oligocene. Central America was added as sister to northern South America, a pattern expected from biotic range expansion following the closing of the Isthmus of Panama (Duque-Caro, 1990) and the Bahamas were added as sister to Cuba, expected from the near-interconnections between the banks that hold those islands (Hearty and Kaufman, 2000; Hearty and Kindler, 1997).

Additionally, Jamaica was placed as sister to Cuba-Bahamas, and the Lesser Antilles were placed as sister to Puerto Rico, both based on distributional patterns for Caribbean bats (Baker and Genoways, 1978; Griffiths and Klingener, 1988).

The inclusion of all relevant areas in a geological area cladogram for this study would require the addition of two more areas, Mexico and central South America. Rather than add these areas to the geological pattern and further distort the Gaarlandia hypothesis (Iturralde-Vinent and MacPhee, 1999), this study compared the taxon cladograms to the area relationships without modifications (see Fig. 3). For comparisons with this pattern, the taxon cladograms were pruned of all but four areas; northern South America, Cuba, Hispaniola, and Puerto Rico. This assumes that all other areas where these taxa are present were colonized by post-Oligocene dispersal.

General area cladograms

Two methods were used to generate hypotheses of area relationships based on taxon cladograms: BPA, Brooks parsimony analysis (Wiley, 1988) and Component (Page, 1993), a computer implementation of component analysis (Nelson and Platnick, 1981). BPA finds the most parsimonious general area cladogram by coding all of the terminal and internal nodes of the phylogenies as binary characters with state present ("1") and absent ("0"). For the BPA widespread taxa were included and coded without modifications, although how to code redundant, missing, and widespread taxa is still the subject of debate (Brooks, 1990; Enghoff, 2000). Areas that were missing from a clade (e.g., Puerto Rico for Natalidae) were coded as missing data ("?"). These can be interpreted *a posteriori* as extinctions, lack of sampling, or primitive absence (van Veller

and Brooks, 2001). A hypothetical ancestor absent from all areas (coded by all zeros) was included to root the tree.

Component finds a general area cladogram by postulating that the observed taxon cladograms are subtrees of a larger, reconciled tree. The reconciled tree is a larger cladogram, of which the actual taxon trees are hypothesized to be subsamples or relicts, given that extinction or incompleteness might have restricted taxon sampling. Heuristic searches in Component (Page, 1993) were conducted by sub-tree pruning and re-grafting (SPR) branch swapping, with all phylogenies as the input, under three different optimality criteria: minimizing the addition of terminal branches ("leaves"), minimizing hypothetical extinctions ("losses"), and minimizing duplications. The biogeographic parameters for these searches were: 'absence is treated as missing taxa', and 'widespread associates [taxa] not mapped.'

Comparisons between taxon and area cladograms: reconciled tree analyses

Tree mapping reconciles the tree for the taxa with a tree for the areas, under the assumption that areas and taxa evolve together (Brooks and McLennan, 1991). As in the search for the general area cladogram, the taxon tree is hypothesized to be a subsample of the reconciled tree. The map between taxon and area cladogram is constructed by finding the smallest cluster in the area tree that contains the distribution of the taxa for each node in the taxon tree. Component (Page, 1993) assumes that the incongruence between area cladograms derived from different phylogenies is due to poor taxonomic sampling and/or extinction. If removing a taxon greatly improves the fit between the taxon and area trees, this can be considered evidence for dispersal (Page, 1993). The 'prune leaves' command can be used to delete each taxon and identify species that might have dispersed.

Finally, the observed number of leaves added and losses postulated in the reconciled trees were compared to a distribution generated by mapping each taxon cladogram to 1000 random area cladograms (generated using the 'Random trees' command of the 'Generate' menu in Component). These comparisons assessed the fit between observed taxon and area cladograms and the fit expected by chance alone. The pruned chiropteran taxon cladograms were mapped (reconciled) onto the Gaarlandia area cladogram (Fig. 3). Additionally, taxon cladograms were mapped onto two general area cladograms: the single tree obtained by using BPA (Fig. 3a), and the tree resulting from component analysis optimizing for a minimal number of losses (Fig. 3b).

Estimation of divergence time

Substitution rates

All the phylogenies were tested for rate constancy by calculating a UPGMA tree based on Jukes-Cantor distances, and the likelihood score for the best-fit model with no clock enforced (log$L1$) vs. the same model with a clock enforced (log$L2$) were compared by calculating $-2\log\lambda = 2[\log L_1 - \log L_2]$. The significance of the difference in likelihood scores was tested by comparing $-2\log\lambda$ against a χ^2 distribution with degrees of freedom equal to the number of taxa minus 2. If the value for $-2\log\lambda$ was significant, then a molecular clock could be rejected. If the molecular clock could not be rejected, constant substitution rates could be used to estimate divergence time among clades of interest (e.g., Caribbean versus continental lineages).

Divergences in the phylogenies of mormoopids, *Brachyphylla* and allies (*Rag*2 only), and natalids were calibrated by fixing the age of nodes with one of these two fossils:

1. Mormoopid and ?phyllostomid fossils from the late Whitneyan (32–30 MYA) and early Arikareean (28–25 MYA) of Florida constrain the mormoopid-phyllostomid divergence (Fig. 4a) to before the late Whitneyan (Czaplewski et al., 2003b; Morgan and Czaplewski, 2003).

2. The molossid fossil *Wallia* (Legendre, 1985) constrains the divergence between Molossidae and Vespertilionidae (Fig. 4c), the sister to Natalidae (Hoofer et al., 2003; Teeling et al., 2003; Teeling et al., 2002; Van Den Bussche et al., 2002; Van Den Bussche et al., 2003), to before the late Uintan (43–40.5 MYA).

Maximum likelihood pairwise distances among mormoopids and phyllostomids were used to obtain rates of molecular evolution for external calibration of the phyllostomid phylogenies, with the exception of the *Rag*2 phylogeny *Brachyphylla* and allies (Fig. 3b). The maximum likelihood parameters for cytochrome *b* and *Rag*2 were estimated using the hierarchical ratio test option of Modeltest (Posada and Crandall, 1998). The pairwise distances between mormoopids and phyllostomids were then translated into estimates of substitutions per lineage per million year as follows:

substitution rate = pairwise distance/oldest possible fossil age x 2

The average of the pairwise distances was used to convert branch-lengths into age estimates.

Confidence intervals on dates of divergence

The error associated with the divergence dates was estimated by using a parametric bootstrap based on the rate-constant phylogenies. The parametric bootstrap approach generates independent stochastic replicates of the observed data, given a tree with branch lengths, and a model of DNA substitution (Huelsenbeck et al., 1996). This allows for

calculating confidence intervals that take into account all the errors involved in estimating an age of divergence: errors arising from the substitution rate and its associated calibration point (van Tuinen and Hedges, 2001), and from estimating branch-lengths (Britton et al., 2002).

First, the optimal model of sequence evolution for each data set was selected using the hierarchical likelihood ratio test option of Modeltest (Posada and Crandall, 1998). The model parameters were then used to simulate 100 datasets using Seq-Gen (Rambaut and Grassly, 1997). The simulated datasets were based on the optimal topology (with branch lengths) estimated from observed data. Paup* (Swofford, 2002) was then used to optimize topologies and rate-constant branch lengths for each of the 100 simulated datasets under optimal maximum likelihood parameters. The resulting branch lengths were then tabulated and used to calculate the 95% confidence interval around the nodes of interest as given by:

$$\text{confidence interval} = t_{0.05(n-1)} \times s/\sqrt{n}$$

where $n = 100$; $t_{0.05(99)} = 1.987$; $s =$ standard deviation = standard error (Sokal and Rohlf, 1995).

RESULTS

General area cladograms

Analysis of the BPA data matrix (Appendix 1) resulted in a single fully resolved area cladogram shown in Fig. 3a. Heuristic searches for general area cladograms using Component optimizing for different criteria resulted in multiple trees. Table 1 shows the number of items of error observed when the phylogenies (Fig. 2) were mapped onto the general area cladograms generated using Component under different optimization

criteria. The general area cladogram obtained by minimizing the number of losses, hereafter the Component tree, was selected for subsequent reconciled tree analyses (Fig. 3b).

Tree comparisons

There were two unresolved nodes in taxon cladograms, one among species of *Phyllonycteris* (Fig. 2b) and the other among species of *Phyllops* (Fig. 2c). These two nodes were resolved arbitrarily and this produces a small increase in incongruence between the taxon cladograms and the area cladograms, although not for the reconciled trees between *Brachyphylla* and allies and Gaarlandia because the Jamaican *Phyllonycteris aphylla* was pruned (Fig. 2b).

The bat phylogenies pruned to include only taxa ranging in northern South America, Cuba, Hispaniola, and Puerto Rico were reconciled to the Gaarlandia tree, and the complete bat phylogenies (Fig. 2) were reconciled to both the BPA tree (Fig. 3a) and the Component trees (Fig. 3b). The measures of fit between taxon and area cladograms under different biogeographic parameters are summarized in Table 2. In general, an interpretation of missing taxa as primitively absent increases the number of reconstructed losses and leaves added to reconcile taxon cladograms with hypotheses of area relationships. Not mapping widespread taxa often increased the fit between cladograms and area relationships, although it also reduced information as all groups included widespread species.

There are no significant measures of fit between taxa and the Gaarlandia area cladogram, as random trees of four areas result in this topology with $P > 0.05$. Among significant values, the BPA tree shows less items of error than the Component tree for

Pteronotus and natalids, while *Mormoops* shows a better fit with the Component tree (Table 2). The measures of fit are similar for reconciled trees of *Brachyphylla* and allies and each of the complete area cladograms (Table 2). Each taxon was pruned and the resulting items of error were noted to identify the greatest proportion of incongruence. For example, when *P. falcatus* was pruned from the Stenodermatina phylogeny (Fig. 2c) and reconciled with the Component tree, the number of duplications went from six to four, the number of leaves added went from 28 to 13, and the number of losses went from 15 to seven. These differences were noted across all taxa, under all biogeographic treatments, and the results are summarized in Table 3.

Congruence and codivergence of taxa and landmasses

The Gaarlandia hypothesis is a subtree of the BPA tree (Fig. 3a), and incompatible with the Component cladogram (Fig. 3b). *Mormoops* and *Brachyphylla* and allies show no duplications due to incongruence when widespread taxa are not mapped (Table 2), although one unresolved node in *Phyllonycteris* (Fig. 2b) could result in incongruence when resolved. The arbitrarily resolved node of *Phyllops* (Fig. 2c) has not effect on the number of duplications arising from incongruence, as the three species have been found on Cuba.

The Component tree explains a greater proportion of nodes than the BPA tree in *Pteronotus*, *Brachyphylla* and allies, and Stenodermatina, while Natalidae shows greater codivergence with the BPA tree (Table 2). The measures of codivergence are equally poor for reconciled trees of *Mormoops* and each of the complete area cladograms (Table 2). Three nodes are common to both phylogeny-based area cladograms: the northern

South America-central South America node, the Mexico-Central America node, and the Cuba-Bahamas node (Fig. 3).

Substitution rates and divergence times

The results of tests for rate constancy in each phylogeny are shown in Table 4. The parameters of estimation and resulting substitution rates for different data sets used to calibrate phyllostomid phylogenies are shown in Table 5. The substitution rate for cytochrome *b* in phyllostomids, as estimated from the mormoopid-phyllostomid divergence (Fig. 4a), was equivalent to a pairwise divergence of ~3.8% per million years. The rate obtained is higher than the traditional ~1.0 x 10^{-2} rate hypothesized for mammal mitochondrial evolution (Brown et al., 1979), but lower than a recent estimate for cytochrome *b* of ~2.6 x 10^{-2} substitutions per lineage per million years (Arbogast et al., 2002). This rate was applied to the phylogenies of phyllostomids.

The substitution rate for *Rag*2 in phyllostomids, as estimated from the mormoopid-phyllostomid divergence (Fig. 4a), was equivalent to a pairwise divergence of ~0.2% per million years. This is about 16 times slower than the rate found for cytochrome *b*, or eight times slower than the rate for 12S, $tRNA^{val}$, and 16S, and consistent with the initial assessment of mitochondrial evolving 10 times faster than nuclear mammal DNA (Brown et al., 1979). More recent analyses of molecular evolution of mammalian nuclear genes used methods that do not assume a molecular clock and have not attempted to calibrate a uniform rate of substitution (Springer et al., 2003; Teeling et al., 2003). The rate obtained here was applied to the phylogeny of Stenodermatina.

Table 6 summarizes the estimates of branch lengths converted to million years using either a calibration point (Fig. 4) or an average rate (Table 5) for critical nodes in the taxon phylogenies.

DISCUSSION

Do bats and areas evolve together?

Because any two trees can be reconciled given an unlimited number of duplications, historical association should be evaluated with measures of codivergence, rather than significance in measures of fit alone (Dávalos, 2004; Page, 1994). A previous reconciled tree analysis found only 6-20% codivergent nodes for then available phylogenies of the taxa studied here (Dávalos, 2004). The systematic revisions and phylogenies analyzed have resulted in higher proportions of codivergent nodes for all groups with the exception of *Mormoops*: 14-50% for the BPA tree (a tree compatible with Gaarlandia), and 14-40% for the Component tree. The values obtained are within the range obtained for nonvolant mammals (25–75%), suggesting congruent patterns of area relationships among volant species may be more pervasive than previously thought.

At the same time, the measures of fit for the BPA and Component biogeographic hypotheses are as high as they were in a previous analysis, and generally higher than those obtained for nonvolant mammals (Dávalos, 2004). This, coupled with the consistency index of 0.71 for the BPA analysis (Fig. 3a), can be interpreted as evidence for incongruence in area relationships across the different phylogenies. This is unsurprising: there is no geological evidence of subaerial interconnections between Jamaica (probably connected to Central America in the Cretaceous/Eocene, but submerged in the Oligocene), the Bahamas, or the Lesser Antilles and other islands or

continents (Perfit and Williams, 1989; Portell et al., 1997). A strict vicariant model might not fully account for the distribution and phylogeny of some taxa because some species might have dispersed to areas without land interconnections, resulting in incongruent patterns of area relationships.

The pruning analyses in Component identified several dispersal candidates (Table 3). Not counting Gaarlandia since those areas were pruned from analyses, more than half of the candidates for dispersal range into the disconnected areas (e.g., *Mormoops megalophylla*, *Brachyphylla nana*, *Nyctiellus lepidus*). The remaining species on the list show patterns that appear to be taxon-specific. For example, all area cladograms show the Greater Antilles as a monophyletic clade (Fig. 3), suggesting an ancient separation between the continents and the larger Caribbean islands. *Natalus saturatus* (Fig. 2e) does not fit these patterns because it is continental species deep in a Greater Antillean clade, perhaps the result of dispersal from the islands to the continent. In the case of *Phyllops falcatus* (Fig. 2c, Table 3), dispersal from Hispaniola to Cuba has been suggested based on the Cuban fossil record (Suárez and Díaz-Franco, 2003).

Only five bat lineages, out of approximately 25 present in the Caribbean, are analyzed here. Future studies of other groups that include endemic Antillean taxa, e.g., *Lasiurus* and *Monophyllus*, could test these results. Analyses of this sample of the Caribbean biota suggest that the vagility of bats does not completely erase patterns of area relationships from their phylogenies.

Area relationships

The single-origin hypothesis

As mentioned before all hypotheses of area relationships show the Greater Antilles as a monophyletic group, predicting a single range expansion into these islands from the continent before diversification occurs on America or the islands (Fig. 3). Previous biogeographic studies have assumed that *Mormoops* reached the Caribbean several times, once for *blainvillei*, a second time for the Greater Antillean fossils assigned to *megalophylla*, and perhaps a third time for *magna* (Baker and Genoways, 1978; Griffiths and Klingener, 1988; Koopman, 1989). These biogeographic hypotheses assume that while a continental lineage may expand its range onto islands, the reverse is implausible.

There are two *Mormoops* lineages: the Greater Antillean endemic *blainvillei* and the widespread assemblage including the species *megalophylla*, *tumidiceps* and *magna*. This last lineage includes extinct populations from the Pleistocene of Florida, the West Indies, and northeastern Brazil (Czaplewski and Cartelle, 1998; Morgan, 2001; Ray et al., 1963; Silva-Taboada, 1974). Species limits and relationships among these populations are unresolved because no detailed analyses of these fossils have been conducted (Morgan, 2001). The Antillean fossils were placed here with *megalophylla* on the basis of their resemblance to extant populations (Silva-Taboada, 1974), without the benefit of character analysis. When such morphological studies become available, single-origin hypotheses for the Antillean members of this genus could be tested. If the continental species form a monophyletic assemblage, it is equally plausible that *Mormoops* is an ancient Caribbean lineage that invaded the mainland later in its evolutionary history (Fig. 3a).

The phylogeny of *Pteronotus* does not support the single-origin hypothesis for its Greater Antillean representatives (Fig. 2d). At least two invasions are apparent: one for *parnellii-pusillus-portoricensis*, and another for *macleayii-quadridens-fuliginosus*. The Hispaniolan *Pteronotus* sp., and the Cuban *P. pristinus* remain to be placed in a detailed phylogeny: the first is larger than any of the extant Caribbean species (and therefore similar to the continental *rubiginosus* or *mexicanus*), and the second was found to be sister to *parnellii sensu* Smith (1972) before the multiple species in this lineage were recognized as such (Simmons and Conway, 2001). Because the monophyly of the *parnellii-pusillus-portoricensis* clade is poorly supported with available data, analyses of other molecular markers in combination with morphological studies of extinct populations might reveal at least one other *Pteronotus* invasion within the lineage of *parnellii-pusillus-portoricensis*.

There is uncertainty about the identity of the sister taxon of *Brachyphylla* and allies (Baker et al., 2003; Baker et al., 2000; Carstens et al., 2002; Wetterer, 2003; Wetterer et al., 2000), and species of *Artibeus* (Fig. 4b) are more informative about area relationships on the continental Neotropics than about the Caribbean (Van Den Bussche et al., 1998). For these reasons the sister taxa of the monophyletic phyllostomid clades were not included in analyses of area relationships for these groups. The monophyly of *Brachyphylla* and allies, and of the Short-faced bats, supports the single-origin model.

The phylogeny of the natalids also support the single-origin hypothesis, and representatives of their sister group (molossids and vespertilionids) are shown in Fig. 4c. Molossids and vespertilionids have a worldwide distribution, while the sister taxa of all other groups examined are neotropical. The phylogenies of three out of the five groups

studied are consistent with a single entry into the Greater Antilles. This hypothesis cannot be tested with available data for *Mormoops*, and can be rejected for *Pteronotus*, whose history appears to include several independent colonization events.

Relationships among islands

As noted before, the BPA and Component trees share three nodes, and only the Cuba-Bahamas node (Fig. 3) is relevant to relationships among islands. The widespread distribution of three *Pteronotus* species (Fig. 2d), *Phyllonycteris*, *Erophylla* (Fig. 2b), and *Nyctiellus* (Fig. 2e) support the Cuba-Bahamas node. In this analysis no pair of sister taxa exhibit a Cuba-Bahamas relationship, perhaps because the two banks are separated by shallow water, and sea level drops have exposed enough land to virtually connect the areas (Hearty, 1998; Hearty and Kaufman, 2000), see Figure 1.

The BPA and Component trees differ in inferring the relationships of the Lesser Antilles: linked to the continent in BPA (Fig. 3a), and linked to Puerto Rico in Component (Fig. 3b). The phylogeny of *Pteronotus* shows examples of both patterns; links to the continent for *davyi*, and links to Puerto Rico for *portoricensis* (Fig. 2d). The only pair of sister taxa (rather than widespread distribution) supporting relationships for the Lesser Antilles are *Natalus stramineus* and *N. tumidirostris* (Fig. 2e), a pattern compatible with the BPA tree. In all other instances (i.e., *Mormoops blainvillei*, *Phyllonycteris major*, *Ardops*), widespread distributions support the Component pattern (Fig. 2a and 2b), or reject both patterns (Fig. 2c). Detailed analyses of *Brachyphylla* genetics found no differentiation between populations from Puerto Rico and the northern Lesser Antilles. Population studies of fossil *Pteronotus* and *Phyllonycteris*, as well as

molecular analyses of other co-distributed taxa (e.g., *Monophyllus*) could test the Puerto Rico-Lesser Antilles relationship further.

The size of the Lesser Antilles and the distance between the islands have varied from the Miocene to the early Pliocene (25-5 MYA) as the eastward movement of the Caribbean plate generated tectonism along the eastern margins, and with changes in sea level from the Miocene until the present (Haq et al., 1993; Miller et al., 1996; Perfit and Williams, 1989). The conflict in hypothesized area relationships for the Lesser Antilles is consistent with studies that find these islands to be a faunal hybrid of Caribbean and northern South American taxa (Baker and Genoways, 1978; Carstens et al., in press; Lovette et al., 1999; Phillips et al., 1989; Pregill et al., 1994; Ricklefs and Bermingham, 2001), and with the geological history of the region that rejects a historical interconnection with surrounding landmasses (Perfit and Williams, 1989).

The remaining differences between the two phylogeny-based area cladograms concern the relative position of Hispaniola and Jamaica in the Greater Antilles (Fig. 3). BPA places Jamaica with Cuba-Bahamas, a hypothesis that had been proposed before based on bat distributions (Griffiths and Klingener, 1988). The widespread distribution of *Mormoops*, *Pteronotus quadridens*, *P. macleayii*, *P. parnellii*, *Erophylla sezekorni*, and *Chilonatalus micropus* (Fig. 2) supports the BPA pattern. No pair of sister taxa recover a Cuba-Jamaica relationship.

Component recovers a Jamaica-Hispaniola sister relationship on the basis of a single species pair, *Natalus major* and *jamaicensis* (Fig. 2e). The Cuban *Natalus primus* was not included in phylogenetic analyses, and it is unlikely to be sister to either of the species defining the Jamaica-Hispaniola node because its morphology is primitive within

Natalus (A. Tejedor, personal communication). The widespread distribution of *Brachyphylla pumila* (Fig. 2b) and *Chilonatalus micropus* (Fig. 2e) is consistent with the Jamaica-Hispaniola pattern. Jamaican fossil *Brachyphylla* have been studied in detail by an early proponent of species distinction for *nana* and *pumila* (Morgan, 1993; Morgan, 2001), suggesting this widespread distribution is correct. In contrast, a systematic revision of *Chilonatalus micropus* using molecular data might clarify relationships among Caribbean populations currently considered conspecific based on morphometry (Ottenwalder and Genoways, 1982).

BPA recovers a sister relationship between Hispaniola and Puerto Rico (Fig. 3a), a node common to the Gaarlandia hypothesis, while Component recovers a Hispaniola-Jamaica node from the natalid phylogeny (Fig. 3b). One pair of sister species, *Pteronotus portoricensis* and *pusillus*, and the widespread distribution of *P. fuliginosus* (Fig. 2d), support the BPA pattern. Support for the Hispaniola-Jamaica pattern found in Component was discussed before.

As with the conflict regarding the area relationships of the Lesser Antilles using BPA and Component, the competing hypotheses might be explained in part by the history of the landmasses. Both Jamaica and Hispaniola appear to be geological composites (Iturralde-Vinent and MacPhee, 1999; Perfit and Williams, 1989; Robinson, 1994). The Eocene Jamaican fauna, when the island was apparently interconnected to an insular Central America, has no continuity through the present because this island only became emergent at the beginning of the Miocene (Portell et al., 2001; Robinson, 1994). Southwestern Hispaniola accreted onto the rest of the island well into the Tertiary (Mann et al., 1995; Perfit and Williams, 1989). Additionally, sea level drops have exposed lands

around Honduras, Yucatán, Cuba, and Hispaniola (Fig. 1). Jamaica and Puerto Rico were relatively unchanged in size, but the distance between the former and Central America and Cuba, and the latter and Hispaniola and Lesser Antilles decreased. Rising sea levels have at times further isolated the Greater Antilles from the continent, trisected Cuba, and bisected Hispaniola (Fig. 1). The patterns of area relationships recovered by different methods result not only from the relative role of widespread distributions (included in BPA, excluded in Component) and other methodological issues, but also from the historical reality of hybrid faunas arising from a reticulate history of the islands.

The hypotheses of area relationships presented here can serve as starting points for subsequent systematic studies of taxa that were not sampled exhaustively within their range, or extinct populations (e.g., within *Mormoops*, *Phyllonycteris*, *Chilonatalus*). The relatively well-sampled *Pteronotus* shows two nodes that support the BPA relationships: *quadridens-fuliginosus* and *parnellii-pusillus-portoricensis* (Fig. 2d). In these two clades, phylogenetic relationships suggest speciation has occurred between the western Caribbean (Cuba, Jamaica, and satellites), and the eastern Antilles (Hispaniola, Puerto Rico, and the Lesser Antilles). This fits better the predictions of the Gaarlandia hypothesis (Fig. 3), than the north-to-south splits of the Component tree (Fig. 3b).

Caribbean to continent: a novel pattern of area relationships

The continental *Mormoops* and the nectar-feeding genera *Glossophaga* and *Leptonycteris* might be descendents of Caribbean lineages (see above for *Mormoops*). A recent analysis of ~4.0 kb of nucleotide data suggests the Antillean genus *Monophyllus* is sister to *Glossophaga* and *Leptonycteris*, and this clade is sister to *Brachyphylla* and allies (Baker et al., 2003), see Fig. 5. The most parsimonious interpretation of the current distribution

of these taxa would be a single invasion of the Caribbean by the most recent common ancestor of *Brachyphylla* and *Leptonycteris*, followed by the return to the continent of the ancestor of *Glossophaga* and *Leptonycteris*. Because the robustness of that particular topology has not been investigated, this interpretation remains tentative.

The phylogenies of Short-faced bats and natalids show Caribbean lineages deep in the phylogeny, with one (Fig. 2c), or at least two (Fig. 2e) expansions into the mainland. Although both phylogenies have a few short internal nodes, this does not affect the biogeographic results (see previous chapters). For Stenodermatina, a sister relationship between the Caribbean lineages was statistically rejected with a Shimodaira-Hasegawa test (1999), and the short internodes in natalids occur only within the well-supported *Natalus* clade.

There is no fundamental principle or process that precludes island species from colonizing the mainland, although the equilibrium theory of island biogeography (MacArthur and Wilson, 1963; MacArthur and Wilson, 1967) has been incorrectly cited in this regard (Lazell and Koopman, 1985). Terborgh and Faaborg (1980) proposed that an upper limit of tolerance to interspecific competition restricted endemic Caribbean birds to the islands. The role of competition in structuring West Indian bat communities is open to debate (McFarlane, 1991; Rodríguez Durán and Kunz, 2001), and even if a limit applied to some bats, it certainly does not apply to Short-faced bats and natalids. *Centurio*, *Ametrida*, *Sphaeronycteris*, and *Natalus* are all known from lowland Central American and Amazonian forests whose richness is >50 species (Simmons and Voss, 1998), and *Pygoderma* is known from the Atlantic forest and Cerrado of Paraguay and

Brazil, again in sympatry with >50 species (Marinho-Filho, 1996a; Marinho-Filho, 1996b; Willig et al., 2000).

The Caribbean origin of Short-faced bats and *Natalus* might help explain some of their ecological characteristics. Unfortunately, little is known about the habits of either group: the best reference on the ecology of the Short-faced species *Phyllops falcatus* and all natalid lineages is still Silva-Taboada's (1979) monograph. The one known ecological synapomorphy of Stenodermatina, their short face, sets them apart from their continental sister group (*Artibeus*), but its relationship to Caribbean environments or to the function of these bats is unknown. The continental *Natalus* appear restricted by the availability of caves for roosting, where they have been found alongside mormoopids, phyllostomids, and emballonurids (Goodwin and Greenhall, 1961; Linares and Lobig, 1973; Taddei and Uieda, 2001). This preference can probably be traced to the Caribbean, where cave roosting seems advantageous in the face of hurricanes (Jones et al., 2001).

The avian passerine family Parulidae is another clade with basal Caribbean branches whose resolution might support a Caribbean-to-mainland pattern (Lovette and Bermingham, 2002). The Greater Antillean *Spindalis* appears as sister to the Parulidae (the typical wood warblers) in recent analyses of mtDNA, albeit with low support values. Two other Antillean lineages are part of an unresolved polytomy basal to the Parulidae+*Spindalis* node: a clade of the Hispaniolan endemics *Microligea*, *Xenoligea* and perhaps *Phaenicophilus*, and a clade of continental genera plus the Cuban endemic *Teretistris*. The lack of resolution at the base of this phylogeny and the need for further taxonomic sampling preclude any conclusion at this time (Lovette and Bermingham,

2002), but suggest an early role of the Caribbean in the expansion of the range and diversification of this New World radiation.

The timing of diversification

Estimates of divergence times are remarkably congruent across different data partitions (Table 6), with few exceptions. For Short-faced bats the difference in estimates between data partitions appears confined to the two basal ingroup branches (Fig. 2c). Topological incongruence between data partitions can explain part of the discrepancy in branch lengths: *Ariteus-Ardops* is basal in cytochrome *b* estimates, and *Rag*2 optimizes for *Stenoderma-Phyllops* as the basal branch. The Stenodermatina is one of two cases in which the *Rag2* estimates are higher than the cytochrome *b* results. The second instance of *Rag*2 estimates slightly older than mtDNA is the divergence of *Natalus saturatus* (Fig. 2e).

Divergence times resulting from analysis of *Rag*2 exhibit broad confidence intervals (Table 6). In general, branch lengths obtained from maximum likelihood analysis of *Rag*2 were much shorter than those obtained in studies of mtDNA, i.e., there are fewer synapomorphies to define branches with the nuclear gene. This results in great variability in simulated branch lengths, and broad confidence intervals reflect this variation. Future studies should test these results by integrating the different genes into a multiple-loci estimate of divergence time, such as is possible through Bayesian analyses (Kishino et al., 2001). Bayesian methods offer the additional advantage of separating the estimation of divergence time from the rate of molecular evolution, i.e., relaxing the clock (Thorne and Kishino, 2002; Yang and Yoder, 2003), and allowing for more realistic models of evolution than those used here.

An ideal study would also include several fossil calibrations, perhaps by incorporating mammalian outgroups with a better fossil record than bats (e.g., Teeling et al. [2003]). The estimates presented here must be interpreted as minimum age of divergence between groups because the calibration points correspond to the minimum date of divergence, without a close upper limit. The divergence between mormoopids and phyllostomids, for example, has been placed around 40 MY in multiple-loci analyses (Teeling et al., 2003). This is 20% older than the age of calibration used here, but still within the confidence interval of most mtDNA divergence estimates (Table 6).

The Gaarlandia hypothesis

The remainder of the discussion will focus on mtDNA estimates of divergence times. The island-continent divergences for the bat lineages cannot be traced back to the late Eocene/early Oligocene, as predicted by the Gaarlandia hypothesis, even allowing for a 20% increase in the age of every divergence in Table 6. The divergence between natalids and their relatives appears to be older than the Oligocene, although multiple loci analyses place it ~35 MYA (Teeling et al., 2003). That result is close to the first appearance of natalids in the fossil record (Morgan and Czaplewski, 2003) and almost 10 MY *after* the first appearance of fossil vespertilionids (Figure 4c), implying conflict between phylogeny, the time estimation, and/or the fossil record. Even if the basal divergence of natalids is placed 30–38 MYA (Teeling et al., 2003), the pattern of area relationships for the group does not correspond to the Gaarlandia hypothesis (Fig. 2e). Neither the distribution and phylogeny of the extant sister taxa, or the distribution of the fossils in natalids and their sister taxa suggest a South American origin for the Natalidae (Hoofer and Van Den Bussche, 2003; McKenna and Bell, 1997; Morgan and Czaplewski, 2003;

Teeling et al., 2003). In contrast other groups whose phylogenies are consistent with Gaarlandia are all incontrovertibly South American, e.g., Antillean sloths, primates, and hystricognath rodents (Horovitz and MacPhee, 1999; White and MacPhee, 2001; Woods et al., 2001).

The Miocene and the history of Caribbean bats

The earliest mormoopid, phyllostomid, and natalid fossils are from the Oligocene of Florida (Czaplewski et al., 2003b; Morgan and Czaplewski, 2003), and there are several phyllostomid remains (including a relative of nectar-feeding bats) in the middle Miocene of Colombia (Czaplewski, 1997; Czaplewski et al., 2003a). Both phylogenies and the fossil record suggest mormoopids were endemic to the northern Neotropics (Fig. 2a and 2d), natalids were endemic to North America (Morgan and Czaplewski, 2003), and phyllostomids were widespread in the Americas (Baker et al., 2003; Wetterer et al., 2000). The divergences between the two main lineages of *Mormoops*, two lineages of *Pteronotus* (*macleayii* and *davyi*), *Brachyphylla* and allies with respect to their outgroup, Short-faced bats with respect to their outgroup, and species of *Chilonatalus* are roughly contemporary to the middle/late Miocene transition (Figs. 2 and 4, Table 6).

The middle/late Miocene transition in the Caribbean was characterized by the seaway opening along the northern Nicaraguan rise, the gradual closure of the Central American seaway (Roth et al., 2000), and a major sea level drop ~10.0–9.5 MYA (Haq et al., 1993; Miller et al., 1996). Abrupt changes in the benthic fauna signal uplift along the Isthmus of Panama, and perhaps a temporary closure of the isthmus 10.71–9.36 MYA (Duque-Caro, 1990; Roth et al., 2000). Decreases in sea level probably exposed stepping-stone islands in the Caribbean banks, although this does not automatically mean these

islands harbored terrestrial environments (Iturralde-Vinent and MacPhee). Around 9 MYA, sea levels returned to average, isolating the islands and submerging the banks (Fig. 1). The near-interconnections among landmasses allowed bats in some lineages to expand their range onto the Caribbean, or onto the islands that were Central America perhaps for *Mormoops*. The relatively narrow window of opportunity and subsequent isolation not only explains the current distribution and phylogenetic patterns, but also the short internodes among major lineages of *Pteronotus* and at the base of the *Brachyphylla* and allies clade. It is possible that another lineage of Short-faced bats evolved at this time; a new undescribed genus from Cuba apparently shows primitive *Artibeus* characters alongside derived Stenodermatina features (A. Tejedor, pers. commun.; Carlos Mancina in Suárez and Díaz Franco [2003]). If extant, this lineage would shorten the basal Stenodermatina internode (Fig. 4b) and make this phylogeny more similar to those of *Pteronotus* and *Brachyphylla* and allies than it now seems (see phylograms in previous chapters).

The divergences between *Pteronotus mexicanus* and *portoricensis*, *Phyllonycteris* and *Erophylla*, the Caribbean and continental Short-faced bats (i.e., *Ardops* and *Centurio*), and *Natalus saturatus* and *jamaicensis* are roughly contemporary to the late Miocene/early Pliocene transition (Fig. 2, Table 6). The biogeochemical record indicates the Central American seaway was shallow enough to cause the reorganization of circulation in the Caribbean Sea at the end of the Miocene around 4.6 MYA (Haug and Tiedemann, 1998). The shoaling of the seaway was preceded by a sea level drop ~5.5 MYA, and followed by a rise >100 m beyond current levels c. 4.5 MYA (Haq et al., 1993). The exposure of landmasses would have allowed populations of the *Pteronotus*

mexicanus lineage to expand onto the Caribbean, the ancestor of *Phyllonycteris* and *Erophylla* onto the eastern Caribbean islands, the Short-faced bats onto the mainland, and *Natalus* onto Central America (Fig. 1). Unlike the mid-Miocene range expansion, many islands already harbored close relatives of these taxa. Extirpation or extinction probably culled some of the lineages (e.g., *Pteronotus pristinus* in Cuba, *Pteronotus* sp. on Hispaniola), allowing for relatively long internal branches.

As noted before, the timing of divergence based on molecular evolution is subject to debate, and the biogeographic scenario formulated here is not robust to changes in rates of molecular evolution. A 20% increase in the estimated ages of mormoopids and phyllostomids would shift older divergences to the early/middle Miocene transition and more recent divergences to the middle/late Miocene period. Although lowered sea levels have also been recorded for the early/middle Miocene transition, these are not as dramatic as those recorded later in the Miocene (Haq et al., 1993; Miller et al., 1996). Given the fossil constraints used here, the timing of divergence between most Caribbean lineages and their continental relatives lies firmly in the Miocene. This result is consistent with the observation that most Caribbean mammals reached the region before the middle Miocene (Dávalos, 2004).

CONCLUSION

Although previous studies have linked speciation in New World bats to geological processes (Hoffmann and Baker, 2003; Hoffmann et al., 2003), this is the first analysis to explore the deep history of neotropical bats. As such, direct tests with other chiropteran lineages are currently unavailable. The phylogenies of other Caribbean groups, additional molecular data for all lineages, the inclusion of fossil populations in future systematic

studies, and the use of methods that do not assume a molecular clock can further test the patterns and timing of the Miocene biogeographic scenario developed here. For example, the exposure of landmasses during the Miocene could account for the distribution of hystricognath rodents and primates: their fossil record in the Caribbean only dates these taxa to as recently as 12 MYA (MacPhee et al., 2003). The correlation of geological history, phylogenetic patterns, and the timing of diversification in bats demonstrates congruent biogeographic patterns in the Caribbean are pervasive even among the most vagile organisms. Dispersal is neither random, nor capable of completely obscuring such historical patterns.

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Table 1. Items of error observed when reconciling the phylogenies (Fig. 2) with area cladograms generated by Component (Page, 1993) under different optimization criteria. All area cladograms were obtained by heuristic searches with absence treated as missing data, and without mapping widespread taxa, followed by SPR branch swapping. Items of error were estimated for all phylogenies with absence treated as missing data, and with (bottom) and without (top) mapping widespread taxa.

Optimizing criterion	Number of trees obtained	Observed duplications	Observed leaves added	Observed losses
Duplications (A)	>1,000	21	142-163	57-100
Leaves added (B)	147	21-22	142	55-62
Losses (C)	1	23	154	52
A	-	28-32	201-242	89-143
B	-	30-32	191-218	85-108
C	-	28	183	69

Table 2. Chiropteran lineages, biogeographic assumptions, and measures of fit for reconciled trees. Measures of fit and codivergence are not equivalent for Gaarlandia area cladogram because trees were pruned of all areas except northern South America, Cuba, Hipaniola and Puerto Rico. Dup. = duplications, I = duplication due to incongruence between taxon and area cladogram, O = duplication due to overlap among taxa, cod. = codivergent nodes. Interpretation of 'missing' taxa (absence of a clade from a particular area): 'missing data' = lack of data, 'prim. absence' = primitively absent. * = $P < 0.05$, ** = $P < 0.01$.

Lineage	Area cladogram	Widespread taxa	Missing taxa	Measures of fit				Codivergence	
				dup.		leaves added	losses	cod.	total
				I	O				
Mormoops	Gaarlandia	mapped	missing data	0	1	5	1	1	2
	Gaarlandia	mapped	prim. absence	0	1	5	1	1	2
	Gaarlandia	not	prim. absence	0	1	5	1	1	2
	Gaarlandia	not	missing data	0	1	5	1	1	2
	BPA	mapped	missing data	1	1	24	7*	0	2
	BPA	mapped	prim. absence	1	1	27	8*	0	2
	BPA	not	prim. absence	0	1	17	5	0	1
	BPA	not	missing data	0	1	15	4	0	1
	Component	mapped	missing data	1	1	24	5	0	2
	Component	mapped	prim. absence	1	1	27	6	0	2
	Component	not	prim. absence	0	1	17	3**	0	1
	Component	not	missing data	0	1	15	2**	0	1
Pteronotus	Gaarlandia	mapped	missing data	0	6	9	3	5	11
	Gaarlandia	mapped	prim. absence	0	6	9	3	5	11
	Gaarlandia	not	prim. absence	0	6	9	3	5	11
	Gaarlandia	not	missing data	0	6	9	3	5	11

Table 2. Continued.

Lineage	Area cladogram	Widespread taxa	Missing taxa	Measures of fit				Codivergence	
				dup.		leaves added	losses	cod.	total
				I	O				
Pteronotus	BPA	mapped	missing data	4	7	76**	27**	2	14
	BPA	mapped	prim. absence	4	7	76**	27**	2	14
	BPA	not	prim. absence	3	7	66**	23**	2	14
	BPA	not	missing data	3	7	66**	23**	2	14
	Component	mapped	missing data	4	7	78**	28**	3	14
	Component	mapped	prim. absence	4	7	78**	28**	3	14
	Component	not	prim. absence	3	7	72**	24**	3	14
	Component	not	missing data	3	7	72**	24**	3	14
Brachyphylla and allies	Gaarlandia	mapped	missing data	1	2	5	3	3	6
	Gaarlandia	mapped	prim. absence	1	2	6	3	3	6
	Gaarlandia	not	prim. absence	0	2	3	0	3	5
	Gaarlandia	not	missing data	0	2	2	0	3	5
	BPA	mapped	missing data	3	3	33*	16*	1	7
	BPA	mapped	prim. absence	3	3	57**	20*	1	7
	BPA	not	prim. absence	0	3	32	9**	1	5
	BPA	not	missing data	0	3	16	5	1	5
	Component	mapped	missing data	3	3	34	13*	1	7
	Component	mapped	prim. absence	3	3	38**	13**	1	7
	Component	not	prim. absence	1	3	26*	7**	2	6
	Component	not	missing data	1	3	22**	7*	2	6
Stenodermatina	Gaarlandia	mapped	missing data	1	4	8	5	1	6
	Gaarlandia	mapped	prim. absence	1	4	8	5	1	6
	Gaarlandia	not	prim. absence	1	4	8	5	1	6
	Gaarlandia	not	missing data	1	4	8	5	1	6
	BPA	mapped	missing data	3	7	35**	18**	3	10
	BPA	mapped	prim. absence	3	7	41**	21*	3	10
	BPA	not	prim. absence	3	4	41*	21*	3	10
	BPA	not	missing data	3	4	35*	18*	3	10

Table 2. Continued.

Lineage	Area cladogram	Widespread taxa	Missing taxa	Measures of fit				Codivergence	
				dup.		leaves added	losses	cod.	total
				I	O				
Stenodermatina	Component	mapped	missing data	2	4	28**	15**	4	10
	Component	mapped	prim. absence	2	4	33**	18**	4	10
	Component	not	prim. absence	2	4	33**	18**	3	10
	Component	not	missing data	2	4	28**	15**	3	10
Natalidae	Gaarlandia	mapped	missing data	0	2	5	4	1	3
	Gaarlandia	mapped	prim. absence	0	2	8	6	1	3
	Gaarlandia	not	prim. absence	0	2	8	6	1	3
	Gaarlandia	not	missing data	0	2	5	4	1	3
	BPA	mapped	missing data	2	2	28*	12**	3	7
	BPA	mapped	prim. absence	2	2	37**	15**	3	7
	BPA	not	prim. absence	1	2	32	11**	3	6
	BPA	not	missing data	1	2	24	8**	3	6
	Component	mapped	missing data	3	2	48*	19	2	7
	Component	mapped	prim. absence	3	2	48	19	2	7
	Component	not	prim. absence	2	2	42	15*	2	6
	Component	not	missing data	2	2	32	13*	2	6

Table 3. Taxa that maximally increase the incongruence between taxon and area cladograms, as identified by pruning the reconciled trees using Component (Page, 1993). See Fig. 2 for phylogenies and geographic distribution.

Area cladogram	*Mormoops*	*Pteronotus*	*Brachyphylla* and allies	Stenodermatina	Natalidae
Gaarlandia	*megalophylla blainvillei*	*rubiginosus mexicanus*	-	*P. falcatus*	-
BPA	*megalophylla blainvillei*	*portoricensis gymnonotus*	*P. major* *Brachyphylla* spp.	*P. falcatus*	*N. saturatus* *Ny. lepidus*
Component	*megalophylla blainvillei*	*davyi gymnonotus*	*E. bombifrons* *B. nana B. pumila*	*P. falcatus*	*N. tumidirostris*

Table 4. Model of DNA evolution, parameters, and results of test for rate constancy used to generate rate-constant phylogenies.

Mormoopidae includes *Mormoops*, *Pteronotus*, and outgroups.

Clade	Data	Model	ti/tv or R-matrix	Γ	I	-2log	df	*P*
Mormoopidae	mtrDNA	GTR+I+Γ	1.0, 3.3, 1.0, 1.0, 10.7	0.4090	0.3347	21.1	16	>0.05
	cyt. *b*	GTR+I+Γ	0.6, 11.5, 0.8, 0.4, 17.6	1.0592	0.5361	14.3	21	>0.05
	*Rag*2	GTR+Γ	1.0, 5.1, 1.0, 1.0, 7.8	0.2433	-	26.2	18	>0.05
Brachyphylla and allies	cyt. *b*	GTR+Γ	1.0, 7.6, 1.0, 1.0, 14.7	0.2391	-	12.1	9	>0.05
	*Rag*2	HKY85+Γ	4.4	0.2943	-	8.0	10	>0.05
Stenodermatina	cyt. *b*	GTR++I+Γ	7.2, 29.3, 9.1, 1.4, 104.0	1.5111	0.5488	11.7	10	>0.05
	*Rag*2	HKY85+Γ	3.9	0.0153	-	9.0	10	>0.05
Natalidae	cyt. *b*	GTR+I+Γ	1.0, 4.2, 1.0, 1.0, 17.4	0.8229	0.4833	3.0	8	>0.05
	*Rag*2	HKY85+Γ	2.6	0.4389	-	8.2	9	>0.05

Table 5. Molecular data used to estimate substitution rates for phyllostomids, with results of rate constancy tests within each data set.

See Methods.

Taxa	Data	Model	R-matrix	Γ	I	-2log	df	*P*	Substitution rate
Mormoopidae-Phyllostomidae[1]	cyt. *b*	GTR+I+Γ	0.38, 9.10, 0.59, 0.79, 12.00	0.6007	0.4662	36.1	25	>0.05	$1.91 \pm 0.08 \times 10^{-2}$
Mormoopidae-Phyllostomidae[2]	*Rag*2	GTR+Γ	1.00, 3.31, 0.34, 0.34, 5.36	0.2246	-	24.5	25	>0.05	$1.2 \pm 0.03 \times 10^{-3}$

[1]Sequences used to calibrate rate: *Mormoops blainvillei* AF338685, *M. blainvillei* AF338686, *M. megalophylla* AF330808, *M. megalophylla* AF338690, *Pteronotus rubiginosus* Surinam AF330807, *P. psilotis* Mexico AF338680, *P. psilotis* Central America AF338677, *P. personatus* Guyana AF338676, *P. gymnonotus* AF338673, *P. fulvus* AF338672, *P. davyi* AF338671, *P. davyi* AF338670, *P. quadridens* AF338681, *P. quadridens* AF338682, *P. macleayii* AF338683, *Lonchophylla thomasi* AF187034, *Lionycteris spurrelli* AF423097, *Anoura caudifer* L19506, *Glossophaga soricina* AF423081, *Phyllonycteris aphylla* AF187033, *Erophylla bombifrons* AY620438, *Brachyphylla cavernarum* AY572365, *Artibeus concolor* ACU66519, *Uroderma bilobatum* L28941, *Sturnira lilium* AF187035, *Carollia perspicillata* AF187026.
[2]Sequences used to calibrate rate: *Mormoops blainvillei* AY028169, *M. blainvillei* AF338701, *M. megalophylla* AF330818, *M. megalophylla* AF338702, *M. megalophylla* AY141020, *Pteronotus psilotis* Mexico AF338699, *P. psilotis* Mexico AF338698, *P. psilotis* Central America AF338697, *P. rubiginosus* AF330817, *P. gymnonotus* AF338694, *P. fulvus* AF338693, *P. davyi* AF338692, *P. fulvus* AF338691, *P. quadridens* AF338695, *P. quadridens* AF338696, *P. macleayii* AF338700, *Lonchophylla thomasi* AF316456, *Lionycteris spurrelli* AF316455, *Anoura geoffroy* AF316431, *Glossophaga soricina* AF316452, *Phyllonycteris aphylla* AF316478, *Erophylla sezekorni* AF316450, *Brachyphylla cavernarum* AF316436, *Artibeus concolor* AF316432, *Uroderma bilobatum* AF316491, *Sturnira lilium* AF316488, *Carollia brevicauda* AF316437.

Table 6. Estimated divergence time in million years with 95% confidence interval, and data sources for nodes critical to Caribbean biogeography. Horizontal lines separate the different clades. See Figs. 2 and 4 (marked by *) for position of relevant nodes.

Divergence between:		Data used to estimate topology and branch lengths		
Taxon 1	Taxon 2	mtrDNA	cytochrome *b*	*Rag*2
Mormoops megalophylla	*M. blainvillei*	14 (11-17)	11 (9-13)	11 (2-20)
Pteronotus personatus	*P. davyi*	16 (13-19)	15 (14-17)	13 (4-21)
Pteronotus macleayii	*P. davyi*	12 (9-14)	12 (10-14)	9 (1-16)
Pteronotus macleayii	*P. quadridens*	8 (5-10)	9 (8-10)	6 (0-12)
Pteronotus mexicanus	*P. portoricensis*	-	7 (5-8)	-
Brachyphylla	*Glossophaga**	-	12 (10-14)	11 (3-20)
Brachyphylla	*Anoura**	-	10 (9-12)	14 (6-22)
Brachyphylla	*Erophylla*	-	10 (9-11)	12 (4-20)
Phyllonycteris	*Erophylla*	-	6 (5-7)	5 (0-12)
Stenoderma	*Artibeus**	-	9 (8-11)	9 (0-16)
Stenoderma	*Ardops*	-	3 (3-4)	6 (0-13)
Ardops	*Centurio*	-	4 (3-4)	5 (0-10)
*Myotis**	*Nyctiellus*	-	50 (45-55)	43 (26-60)
Chilonatalus micropus	*C. tumidifrons*	-	14 (11-16)	10 (0-20)
Natalus saturatus	*N. jamaicensis*	-	3 (2-5)	5 (0-13)

FIGURE LEGENDS

Figure 1. Relief map of Central America, northwest South America, and the Caribbean. Deeper waters are indicated by darker hues, while shallow banks are lighter color. The shallower areas of the banks of Yucatán, the Nicaraguan plateau, Cuba, the Bahamas, Jamaica, and Hispaniola would have been exposed when sea levels dropped >100 m below current levels beginning in the Miocene. Increases in sea level >100 m would have submerged most of the Bahamas, and low-lying areas of Cuba, Hispaniola, and the Lesser Antilles. During these periods Cuba was trisected and Hispaniola was bisected, while Jamaica and Puerto Rico only became slightly smaller.

Figure 2. Chiropteran taxon cladograms and distributions analyzed in this study. Mex = Mexico, CeAm = Central America, noSA = northern South America, ceSA = central South America, Baha = Bahamas, Jama = Jamaica, Hisp = Hispaniola, Puer = Puerto Rico, and LeAn = Lesser Antilles. A. *Mormoops* (*M*.). B. *Brachyphylla* (*B*.), *Erophylla* (*E*.), and *Phyllonycteris* (*P*.). C. Short-faced bats (Stenodermatina), *C*. = *Centurio*, *P*. = *Phyllops*. D. *Pteronotus* (*P*.). E. Natalid bats, *N*. = *Natalus*, *C*. = *Chilonatalus*, *Ny*. = *Nyctiellus*.

Figure 3. Hypotheses of area relationships. Unmarked trees on the right are area cladograms expected from the Gaarlandia hypothesis (Iturralde-Vinent and MacPhee, 1999). A. Single general area cladogram (L = 116, CI = 0.716, RI = 0.641) resulting from branch and bound parsimony analysis of the BPA matrix (BPA, see Appendix 4). A. Single general area cladogram resulting from reconciled tree analysis of five chiropteran taxon cladograms using Component (Page 1993). Searches were heuristic, optimality

criterion minimizing losses, followed by SPR branch swapping. Widespread taxa were not mapped and areas without taxa were interpreted as missing data.

Figure 4. Schematic cladograms depicting relationships among study taxa and outgroups, black dots indicate calibration point (in million years) for estimates of divergence times. A. Mormoopids and phyllostomids; B. Stenodermatina and its continental sister taxon; C. Natalids and their closest living relatives.

Figure 5. Summary cladogram of a subset phyllostomid nectar-feeding bats (from Baker et al. [2003]). Continental taxa are shown in gray (one species, *Glossophaga soricina*, appears to have recently dispersed to Jamaica), *Phyllonycteris* was not included in the original study but there is high support for its sister relationship to *Erophylla* with cytochrome *b* and *Rag*2 (see previous chapter).


Gulf of Mexico
North Atlantic Ocean
Caribbean Sea
North Pacific Ocean
UNITED STATES
Tampa
St. Petersburg
Miami
Key West
Grand Bahama
Freeport
Great Abaco
THE BAHAMAS
Nassau
Eleuthera
Cat Island
San Salvador
Rum Cay
Long Island
Samana Cay
Crooked Island
Mayaguana
Acklins Island
Great Inagua
Turks and Caicos Islands (U.K.)
Grand Turk
Tropic of Cancer
Havana
Matanzas
Santa Clara
Pinar del Rio
Cienfuegos
CUBA
Nuevitas
Camagüey
Holguín
Manzanillo
Santiago de Cuba
Guantánamo
Isla de la Juventud
Campeche Bank
Yucatan Channel
Mérida
Cancún
Isla Cozumel
Bahía de Campeche
Campeche
Ciudad del Carmen
MEXICO
Chetumal
Villahermosa
Belize City
Belmopan
BELIZE
Flores
ISLAS DE LA BAHIA
GUATEMALA
Guatemala
Tapachula
Quetzaltenango
Puerto Barrios
San Pedro Sula
La Ceiba
HONDURAS
Tegucigalpa
Puerto Lempira
Choluteca
San Salvador
EL SALVADOR
Santa Ana
San Miguel
NICARAGUA
León
Managua
Granada
Matagalpa
Bluefields
Puerto Cabezas
Cayos Miskitos
ISLAS DEL MAIZ
COSTA RICA
San José
Puntarenas
Puerto Limón
Bocas del Toro
Colón
Panama
PANAMA
Balboa
David
Santiago
Middle America Trench
George Town
Cayman Islands (U.K.)
Cayman Trench
SWAN ISLANDS (HONDURAS)
Montego Bay
Kingston
JAMAICA
Navassa Island (U.S.)
Cap-Haïtien
Port-au-Prince
HAITI
DOMINICAN REPUBLIC
Santiago
Santo Domingo
Isla Mona
San Juan
Puerto Rico (U.S.)
Puerto Rico Trench
St. Croix
British Virgin Is. (U.K.)
Virgin Is. (U.S.)
Basseterre
ST. KITTS AND NEVIS
ANTIGUA AND BARBUDA
St. John's
Plymouth
Montserrat (U.K.)
Guadeloupe (FRANCE)
Basse-Terre
Marie-Galante
DOMINICA
Roseau
Martinique (FRANCE)
Fort-de-France
Castries
ST. LUCIA
BARBADOS
Bridgetown
Kingstown
ST. VINCENT AND THE GRENADINES
GRENADA
St. George's
Isla Aves (VENEZUELA)
Tobago
Port-of-Spain
TRINIDAD AND TOBAGO
Trinidad
Aruba (NETH.)
Oranjestad
Netherlands Antilles (NETH.)
Curaçao
Bonaire
Willemstad
Isla de Margarita
Isla la Tortuga
Santa Marta
Barranquilla
Cartagena
Maracaibo
Lago de Maracaibo
Golfo de Venezuela
Barquisimeto
Valencia
Maracay
Puerto Cabello
Caracas
Barcelona
Cumaná
Maturín
Ciudad Bolívar
Ciudad Guayana
San Fernando
Mérida
San Cristóbal
Cúcuta
Bucaramanga
Arauca
VENEZUELA
Georgetown
GUYANA
Puerto Ayacucho
GUIANA HIGHLANDS
BRAZIL
Boa Vista
Montería
Medellín
Pereira
Ibagué
Bogotá
Cali
Buenaventura
COLOMBIA
ANDES
Isla de Providencia (COLOMBIA)
Isla de San Andrés (COLOMBIA)
Isla del Coco (COSTA RICA)
Scale 1:12,500,000
Lambert Conformal Conic Projection, standard parallels 9°N and 17°N
0 100 200 300 Kilometers
0 100 200 300 Miles
Boundary representation is not necessarily authoritative.

Figure 1

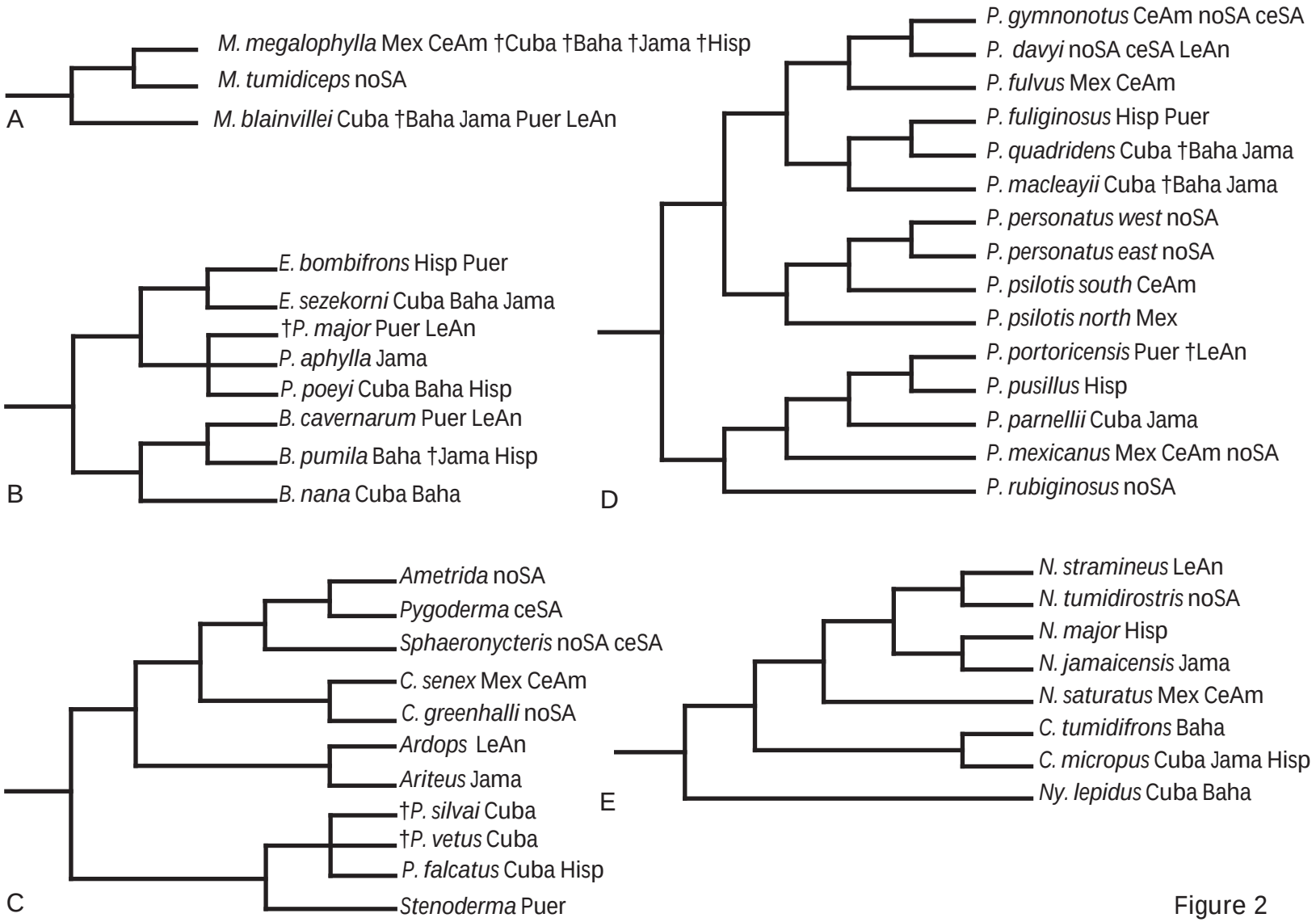
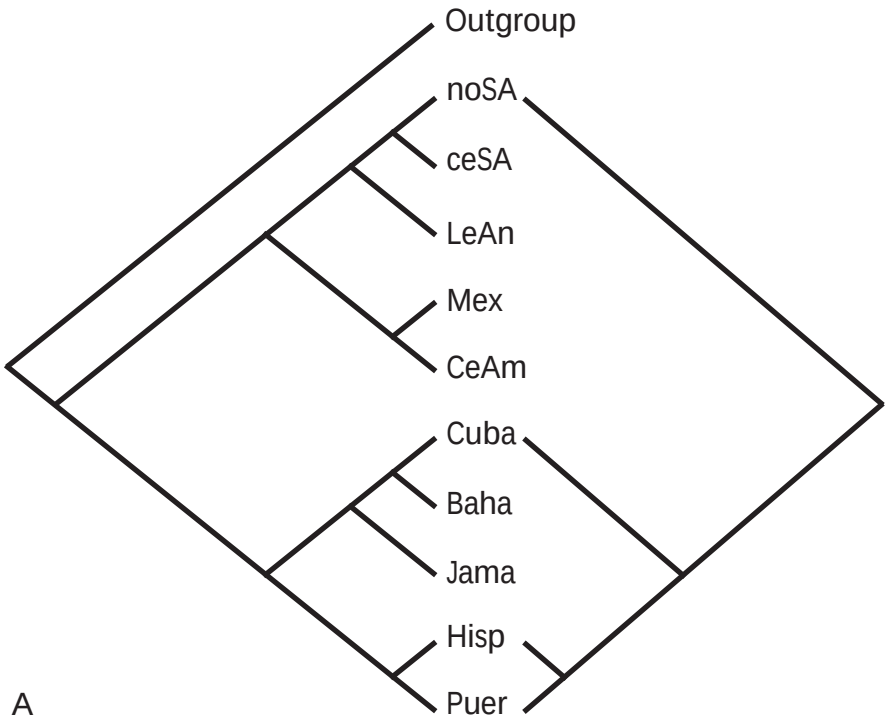

A
M. megalophylla Mex CeAm †Cuba †Baha †Jama †Hisp
M. tumidiceps noSA
M. blainvillei Cuba †Baha Jama Puer LeAn
B
E. bombifrons Hisp Puer
E. sezekorni Cuba Baha Jama
†P. major Puer LeAn
P. aphylla Jama
P. poeyi Cuba Baha Hisp
B. cavernarum Puer LeAn
B. pumila Baha †Jama Hisp
B. nana Cuba Baha
C
Ametrida noSA
Pygoderma ceSA
Sphaeronycteris noSA ceSA
C. senex Mex CeAm
C. greenhalli noSA
Ardops LeAn
Ariteus Jama
†P. silvai Cuba
†P. vetus Cuba
P. falcatus Cuba Hisp
Stenoderma Puer
D
P. gymnonotus CeAm noSA ceSA
P. davyi noSA ceSA LeAn
P. fulvus Mex CeAm
P. fuliginosus Hisp Puer
P. quadridens Cuba †Baha Jama
P. macleayii Cuba †Baha Jama
P. personatus west noSA
P. personatus east noSA
P. psilotis south CeAm
P. psilotis north Mex
P. portoricensis Puer †LeAn
P. pusillus Hisp
P. parnellii Cuba Jama
P. mexicanus Mex CeAm noSA
P. rubiginosus noSA
E
N. stramineus LeAn
N. tumidirostris noSA
N. major Hisp
N. jamaicensis Jama
N. saturatus Mex CeAm
C. tumidifrons Baha
C. micropus Cuba Jama Hisp
Ny. lepidus Cuba Baha

Figure 2


Outgroup
noSA
ceSA
LeAn
Mex
CeAm
Cuba
Baha
Jama
Hisp
Puer
A

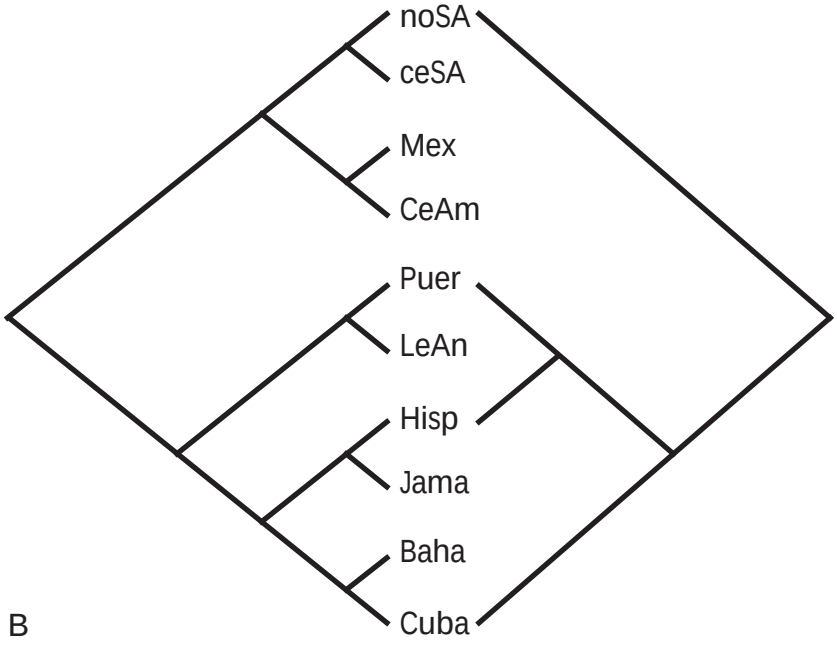

noSA
ceSA
Mex
CeAm
Puer
LeAn
Hisp
Jama
Baha
Cuba
B

Figure 3

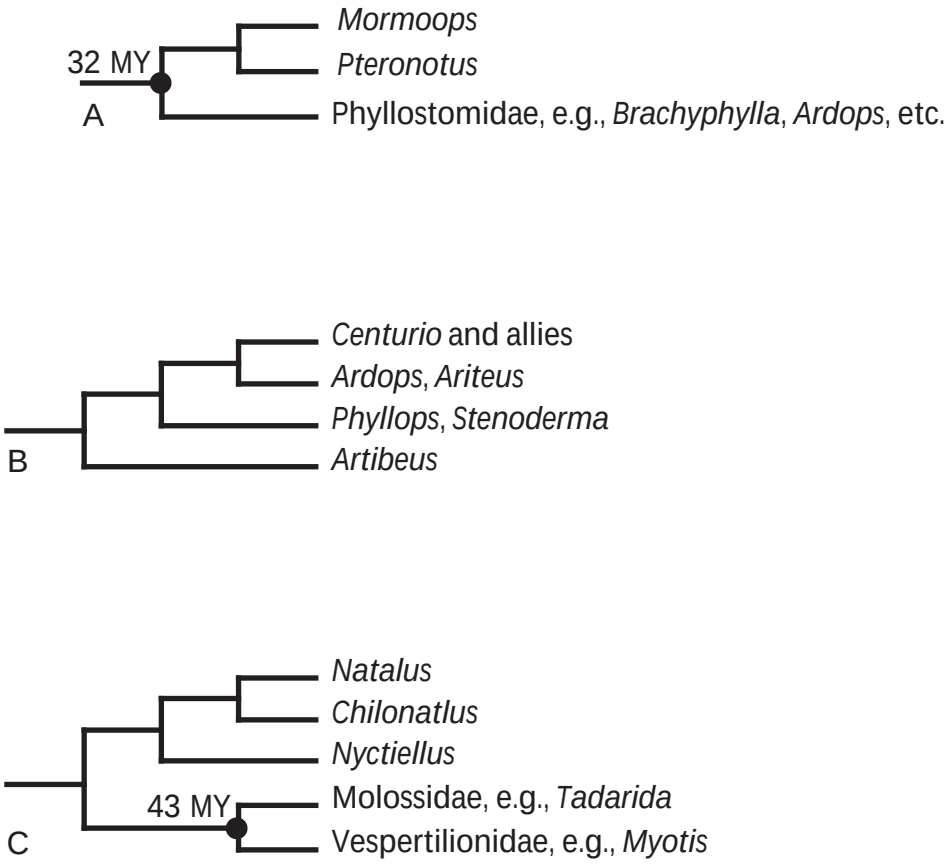

Mormoops
32 MY
Pteronotus
A
Phyllostomidae, e.g., Brachyphylla, Ardops, etc.
Centurio and allies
Ardops, Ariteus
Phyllops, Stenoderma
B
Artibeus
Natalus
Chilonatlus
Nyctiellus
43 MY
Molossidae, e.g., Tadarida
C
Vespertilionidae, e.g., Myotis

Figure 4

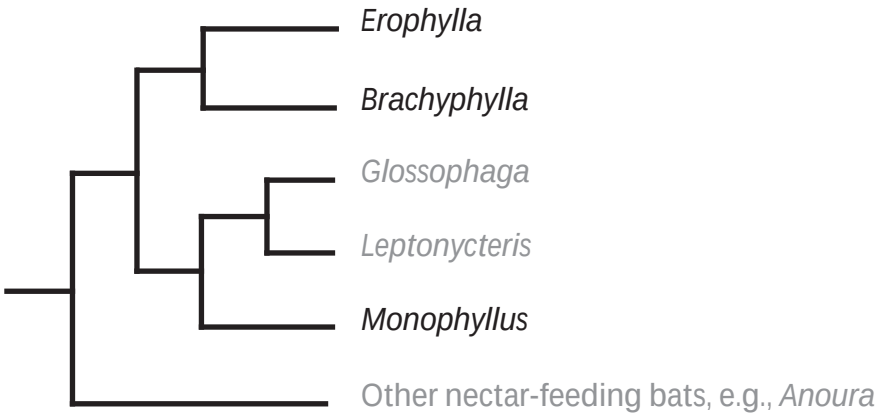

Erophylla
Brachyphylla
Glossophaga
Leptonycteris
Monophyllus
Other nectar-feeding bats, e.g., *Anoura*

Figure 5

Appendix 4. Brooks parsimony analysis (BPA) matrix. Node numbers correspond to those marked on the figure.

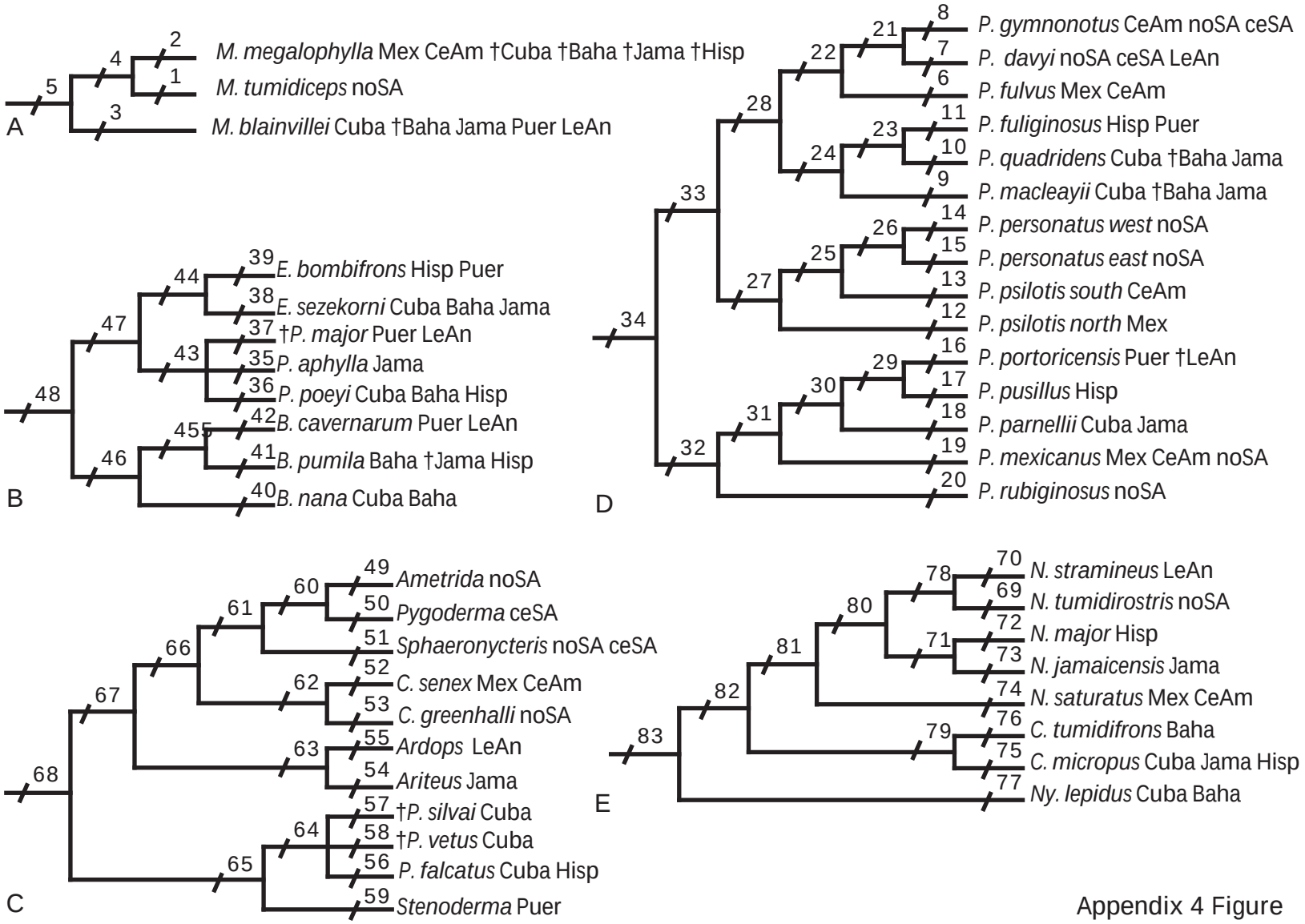
Area/Node	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Ancestor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mexico	0	1	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	1	0	0	1	1	1	1
Central America	0	1	0	1	1	1	0	1	0	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0	1	0	1	1	0	0	1	1	1	1
n. South America	1	0	0	1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	1	1	1	1	0	0	1	1	1	1	0	0	1	1	1	1
c. South America	?	?	?	?	?	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	1
Cuba	0	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	1	0	1	1	1	1	1
Bahamas	0	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	1	0	1	1	1	1	1
Jamaica	0	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	1	0	1	1	1	1	1
Hispaniola	0	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	1	1	1	1	1	1	1
Puerto Rico	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	1	1	1	1	1	1
Lesser Antilles	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	1	1	1	1	1	1	1

Area/Node	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68
Ancestor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mexico	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1
Central America	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1
n. South America	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	0	1	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	1
c. South America	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	1
Cuba	0	1	0	1	0	1	0	0	1	1	0	1	1	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	0	0	1
Bahamas	0	1	0	1	0	1	1	0	1	1	1	1	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Jamaica	1	0	0	1	0	0	1	0	1	1	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	1
Hispaniola	0	1	0	0	1	0	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1
Puerto Rico	0	0	1	0	1	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1
Lesser Antilles	0	0	1	0	0	0	0	1	1	0	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	1

Appendix 4. Continued

Area/Node	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83
Ancestor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mexico	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1
Central America	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1
n. South America	1	0	0	0	0	0	0	0	0	1	0	1	1	1	1
c. South America	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Cuba	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1
Bahamas	0	0	0	0	0	0	0	1	1	0	1	0	0	1	1
Jamaica	0	0	1	0	1	0	1	0	0	0	1	1	1	1	1
Hispaniola	0	0	1	1	0	0	1	0	0	0	1	1	1	1	1
Puerto Rico	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Lesser Antilles	0	1	0	0	0	0	0	0	0	1	0	1	1	1	1

Appendix Figure. Chiropteran taxon cladograms and distributions analyzed in this study with terminals and nodes marked as coded in the matrix above. Mex = Mexico, CeAm = Central America, noSA = northern South America, ceSA = central South America, Baha = Bahamas, Jama = Jamaica, Hisp = Hispaniola, Puer = Puerto Rico, and LeAn = Lesser Antilles. A. *Mormoops* (*M.*). B. *Brachyphylla* (*B.*), *Erophylla* (*E.*), and *Phyllonycteris* (*P.*). C. Short-faced bats (Stenodermatina), *C.* = *Centurio*, *P.* = *Phyllops*. D. *Pteronotus* (*P.*). E. Natalid bats, *N.* = *Natalus*, *C.* = *Chilonatalus*, *Ny.* = *Nyctiellus*.



Appendix 4 Figure