

PHYLOGENY OF THE LONCHOPHYLLINI (CHIROPTERA: PHYLLOSTOMIDAE)

LILIANA M. DÁVALOS* AND SHARON A. JANSA

Division of Vertebrate Zoology–Mammalogy, American Museum of Natural History,
Central Park West at 79th Street, New York, NY 10024-5192, USA (LMD, SAJ)

Evolution, Ecology and Environmental Biology, Columbia University, New York, NY 10027-5557, USA (LMD)

Present address of SAJ: Bell Museum of Natural History, University of Minnesota,
1987 Upper Buford Circle, St. Paul, MN 55108, USA

A combination of 1,140 base pairs of the mitochondrial cytochrome *b* gene of *Platalina*, *Lionycteris*, and several species of *Lonchophylla* (Chiroptera: Phyllostomidae) with 150 morphological, sex chromosome, and restriction site characters were used in an attempt to resolve relationships among the lonchophylline taxa. In addition, the monophyly of *Lonchophylla* was tested, particularly with respect to *Platalina*. The most parsimonious hypothesis of relationships using all available characters was (*L. mordax* ((*L. chocoana* (*L. robusta*, *L. handleyi*))(*L. thomasi* (*Lionycteris*, *Platalina*))))). *Lonchophylla* appears to be paraphyletic, but this arrangement is not well supported. Our analyses suggest that *Platalina* is not simply a large *Lonchophylla*, as had been suggested by previous morphological analyses. The low support values for basal relationships found in this study are probably caused by saturation in cytochrome *b* 3rd positions. Additionally, 2 alternative explanations are viable (if improbable): unsampled lonchophyllines are necessary to confidently resolve relationships at the base of the group, or the lack of resolution at the base of the lonchophylline phylogeny might be explained by rapid speciation following the separation from other glossophagines. Future work examining the phylogenetic relationships of lonchophylline bats should focus on describing new taxa, obtaining tissue samples from unsequenced representatives, and adding nuclear loci to this mitochondrial DNA data set.

Key words: cytochrome *b*, *Lionycteris*, *Lonchophylla*, Lonchophyllinae, Lonchophyllini, mitochondrial DNA, Phyllostomidae, *Platalina*

Historically, the phyllostomid tribe Lonchophyllini Griffiths (1982) contains the genera *Platalina*, *Lionycteris*, and *Lonchophylla*. Members of the tribe are widely distributed in the Neotropics from Nicaragua south through Central America to southern Peru and southeastern Brazil (Koopman 1994). Although *Platalina* and *Lionycteris* are each monotypic, *Lonchophylla* comprises 7 recognized species (Taddei et al. 1983) and several more await description (D. Faria, pers. comm.; Dávalos, 2004). The lonchophyllines currently are classified as a monophyletic tribe in the subfamily Glossophaginae (Wetterer et al. 2000); however, their systematics has been controversial, and relationships among the 3 genera remain uncertain (Gimenez et al. 1996; Griffiths 1982, 1983).

In one of the 1st character-based phylogenetic analyses of bats to include lonchophyllines, Phillips (1971) used cranio-

dental characters to examine relationships among phyllostomid genera. Phillips (1971) found no evidence for lonchophylline monophyly and proposed instead that *Lionycteris* and *Lonchophylla* were sister taxa and closely related to *Anoura*, whereas *Platalina* was more closely related to the glossophagine genera *Hlonycteris*, *Lichonycteris*, and *Scleronycteris*. Griffiths (1982) subsequently used lingual and hyoid morphology to define a monophyletic subfamily Lonchophyllinae, within which he proposed that *Lonchophylla* and *Lionycteris* were sister taxa, with *Platalina* basal to them. Subsequent re-interpretation of Griffiths's characters supported lonchophylline monophyly, but relationships among the 3 genera could not be resolved by using these characters (Griffiths 1983; Warner 1983).

Additional attempts to resolve relationships among the 3 genera have met with limited success. Gimenez (1993) reexamined lingual morphology of phyllostomids only to discover that *Platalina* was nested among *Lonchophylla* species, with *Lionycteris* basal to this group. Parallel studies of cranial morphometrics (Solmsen 1994, 1998) concurred that *Platalina* is simply a large species of *Lonchophylla*, thereby

* Correspondent: davalos@amnh.or

TABLE 1.—Cytochrome *b* primers designed in the course of this study.

Primers	5'	3'
NEW12	GACCCRGACAACACTAMAYCCN	
BATH3	GCTAAYGGRGCCTCCATATT	
BATL5	TGCCGDGAYGTAAACTACGG	
BATH2	GCTACAGTCATYACCAACCT	
BATL4	TTCTGAGGAGCCACAGTCAT	
RU13	CAYGAAACHGGSTCHAAAYAAYCC	
BATH4	CCTACGCAATYTTACGCTC	
RNEW12	CTCCTAGGAGACCCAGACAA	
BATH5	AAGCCAATGCYTATTYTGA	
Reverse4	ACGCAATCCTACGCTCCAT	

rendering *Lonchophylla* paraphyletic. Solmsen (1994, 1998) also considered *Lionycteris* to exhibit the most primitive character states in the group. Subsequent reanalysis of lingual morphology left relationships among the genera completely unresolved (Gimenez et al. 1996). In their study of phyllostomid phylogeny, Wetterer et al. (2000) expanded the number of morphological characters to 150 and included *Lonchophylla*, *Lionycteris*, and *Platalina* among their 163 taxa. They found a monophyletic group comprising these 3 genera (tribe Lonchophyllini) with *Platalina* as the basal member of the clade. However, Wetterer et al. (2000) assumed generic monophyly for this study and therefore did not address the suggestion that *Platalina* might simply be a large *Lonchophylla*.

Recently, DNA sequences from the nuclear genes have been applied to phyllostomid bat phylogenetics. Baker et al. (2000) analyzed DNA sequences from the recombination-activating gene 2 (*RAG2*) for almost all phyllostomid genera and recovered a sister-group relationship between *Lionycteris* and *Lonchophylla*. Unfortunately, no molecular study to date has included sequences from *Platalina*. This monotypic genus is known only from a handful of localities in dry areas of western Peru and a recent record in northern Chile (Galaz et al. 1999), and to our knowledge, no tissue samples have been collected from members of this genus.

In this study, we revisit lonchophylline systematics with 2 goals in mind: to test the monophyly of *Lonchophylla*, particularly with respect to *Platalina*, and to resolve relationships among lonchophylline taxa. To accomplish this, we combined new cytochrome *b* (*Cytb*) data for relevant taxa (including a *Platalina* sequence obtained from a museum specimen) with previously published character data from morphology, restriction sites, and sex chromosomes as described in Wetterer et al. (2000). Variation in the *Cytb* gene has been found to provide phylogenetic resolution within and among other phyllostomid genera including *Phyllostomus*, *Carollia*, *Rhinophylla*, *Artibeus*, and *Chiroderma* (Baker et al. 1994; Van Den Bussche and Baker 1993; Van Den Bussche et al. 1993; Wright et al. 1999). We believe that combining the molecular data with data from other nonmolecular sources provides the best approach for examining the phylogeny of lonchophylline bats.

MATERIALS AND METHODS

Taxon sampling.—To examine relationships among the lonchophylline bats and to test the monophyly of *Lonchophylla*, we included all 3 of the presently recognized lonchophylline genera and 4 of the 7 currently recognized species of *Lonchophylla*, as well as 1 species that is in the process of being formally described (Dávalos, 2004). Within each species, we included individuals from as many localities as possible to best capture the genetic diversity within the species. For outgroup comparison we included sequences from representatives of other phyllostomid subfamilies: Brachyphyllinae (*Brachyphylla cavernarum*), Carollinae (*Carollia perspicillata*), Glossophaginae (*Glossophaga soricina* and *Anoura caudifer*), Phyllostominae (*Phyllostomus discolor*), and Stenodermatinae (*Sturnira lilium*, *Uroderma bilobatum*, and *Artibeus obscurus*). To root the tree, we included the distantly related Mormoopidae (*Mormoops megalophylla*).

Molecular data.—For most specimens, DNA was isolated from heart, kidney, or liver tissue that had been frozen or preserved in ethanol in the field. For 2 specimens of *Lonchophylla* (AMNH 209385 and AMNH 230214), DNA was isolated from 2-mm strands of dried muscle scraped from prepared skulls that had been stored dry in a museum cabinet. For *Platalina* (AMNH 257108), DNA was extracted from a 2-mm² piece of skin and muscle taken from a formalin-fixed specimen that had been stored in ethanol. For frozen and ethanol preserved tissues, DNA was extracted by using a Qiagen DNeasy Tissue Extraction Kit (Qiagen, Inc., Valencia, California) by following the manufacturer's protocol. This protocol was modified for the dried muscle and formalin-fixed specimens as described by Iudica et al. (2001).

Extracted DNA was used as a template in polymerase chain reactions (PCRs) with primers shown in Table 1. Primer pairs MVZ05 and UMMZ04 (Jansa et al. 1999) were used to isolate the entire *Cytb* 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, 1 with MVZ05 paired with NEW12 and 1 with UMMZ13 paired with UMMZ04 (Table 1; Jansa et al. 1999). DNA extracted from the museum specimens (dried skins and formalin-fixed carcasses) was sufficiently degraded that amplifications were done as a series of overlapping 200- to 250-base pair (bp) fragments with primers listed in Table 1.

Initial amplifications with genomic DNA as a template were performed as standard 20- or 30- μ l reactions with AmpliTaq Gold polymerase (Perkin-Elmer, Inc., Wellesley, Massachusetts) and recommended concentrations of primers, unincorporated nucleotides, buffer, and MgCl₂. Reactions were performed on a Perkin-Elmer 9700 Thermal Cycler (Perkin-Elmer, Inc., Wellesley, Massachusetts) by using 37 cycles of the following conditions: denaturation at 95°C for 20 s, annealing at 50–55°C for 15 s, and extension at 72°C for 1 min. All amplifications were preceded by a 95°C soak for 10 min and followed by a 7-min extension at 72°C. For reamplifications, PCR products were purified via electrophoresis through a 2% low-melting-point agarose gel (NuSieve GTG, FMC Bioproducts, Rockland, Maine). The appropriate size band was excised from the gel by using a Pasteur pipette, and the gel plug was melted in 300 μ l of sterile water at 73°C for 20 min. The resulting gel-purified product was used as a template in 30- or 40- μ l reamplification reactions with AmpliTaq polymerase. Reactions were subjected to 30 PCR cycles by using annealing temperatures of 55–60°C. PCR products were prepared for sequencing by using the GeneClean II System (BIO 101, Inc., Carlsbad, California) and were sequenced in both directions with amplification primers and dye-terminator chemistry (dRhodamine Ready Reaction Kit, Applied Biosystems, Inc., Foster City, Califor-

nia). Sequencing reactions were purified through a $MgCl_2$ -ethanol precipitation protocol and run on an ABI 377 (Applied Biosystems, Inc.) automated sequencer. Sequences were edited and compiled using Sequencher 3.1 software (GeneCodes, Corp., Ann Arbor, Michigan). Base-calling ambiguities between strands were resolved either by choosing the call on the cleanest strand or by using the appropriate International Union of Biochemistry ambiguity code if both strands showed the same ambiguity. Molecular sequences generated as part of this study have been deposited with GenBank under accession numbers AF423079–AF423101.

Morphological data.—We used the character matrix of Wetterer et al. 2000 (available electronically from <ftp://ftp.amnh.org/pub/mammalogy>, installed 1 November 2001, last accessed 31 January 2004) with the following modifications. Information from restriction sites and sex chromosomes for *Platalina* was not available and was coded as missing in the original matrix. Wetterer et al. (2000) originally coded 3 characters (characters 12, 21, and 117 in their matrix) as polymorphic within *Lonchophylla*. Wetterer et al. (2000) sampled within genera following reports of polymorphism for some characters. Because they assumed the monophyly of all phyllostomid genera except *Mimon*, *Micronycteris*, *Vampyressa*, and *Artibeus*, the polymorphism they found in *Lonchophylla* was not examined in detail in their analysis. We reexamined the states for these characters in *Lonchophylla* species and coded these polymorphic characters for the species of *Lonchophylla*; note that only characters scored differently from Wetterer et al. (2000) are discussed as follows.

Character 12: Genal vibrissae absent (0); or 1 vibrissa present in each cluster (1); or 2 genal vibrissae present in each cluster (2). Wetterer et al. (2000) coded this character as polymorphic (0 or 2) for *Lonchophylla*, noting that genal vibrissae are absent in *L. mordax* and *L. thomasi*, but that 2 genal vibrissae are present in *L. robusta*. We revised the matrix to give each of these species its unique state. We also examined this character in *L. handleyi* and the *L. choacoana* (ROM 105786), which Wetterer et al. (2000) did not include, and assigned state 2 to both.

Character 21: Central rib [of noseleaf] absent (0); or rib restricted to proximal part of spear (1); or rib extends to distal tip of spear (2). Wetterer et al. (2000) coded this character as absent in *Platalina* and described the central rib for *Lionycteris* and *Lonchophylla* as, “well defined laterally but not distally, where it loses thickness and grades into the proximal surface of the spear” (state 1; Wetterer et al. 2000:57). We reexamined the noseleaf for specimens included in our study and concluded (contra Wetterer et al. 2000) that the central rib for *L. thomasi* is best described as extending to the tip of the noseleaf spear. Therefore, we recoded this character with state 2 for *L. thomasi*, and retained state 1 for all other species of *Lonchophylla* and *Lionycteris*. We concur with Wetterer et al. (2000) that the central rib of the noseleaf is absent in *Platalina*.

Character 117: Small patch of anteriorly directed medial-posterior mechanical papillae [on tongue] always absent, all papillae oriented toward pharyngeal region (0); or medial patch present in some individuals; polymorphic within species (1); or medial patch always present (2). In some specimens of phyllostomids, a small patch of papillae in the middle of the tongue can be identified in which the papillae are oriented anteriorly rather than toward the pharyngeal region. Wetterer et al. 2000 described this patch as always present (state 2) in *Platalina* and *Lionycteris*, but noted that it was only sometimes present in *Lonchophylla* (e.g., they noted the patch in a specimen of *L. thomasi*) and coded it as polymorphic (state 1) for *Lonchophylla*. In her original description of this character, Gimenez (1993) left *Lonchophylla* unscored because the papillae were too small to score confidently, but subsequently included this character in analyses by using “some of the

best preserved tongues of *Lonchophylla* species” (Gimenez et al. 1996:49). We were unable to examine this character for all specimens included in our study; therefore, it was coded as unknown (missing) for all specimens of *Lonchophylla*.

Data analysis.—Protein-coding *Cytb* sequences were easily aligned by eye by using Sequencher 3.1 software (GeneCodes, Corp.). In order to describe the variation in *Cytb* among taxa, we calculated uncorrected pairwise (p) distances among all taxa by using PAUP* 4.0b2a software (Swofford 2002). We performed a parsimony analysis on the morphological data set and the *Cytb* data set separately and on the combined matrix by using heuristic searches as implemented in PAUP* 4.0b2a. 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 by using nonparametric bootstrap analysis (Felsenstein 1985) and the Bremer support index (Bremer 1994). All parsimony bootstrap 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 software (Eriksson 1999). Character state changes were explored with MacClade 3.01 (Maddison and Maddison 1992).

To assess character incongruence between the *Cytb* data set and the data sets comprising morphology, sex chromosomes, and restriction sites, we used the Mickevich–Farris character incongruence metric (Mickevich and Farris 1981). Statistical significance of incongruence was calculated by using the incongruence length difference test (Farris et al. 1995), implemented in PAUP* as the partition homogeneity test. Each test included 1,000 replicates; searches were heuristic with 20 replicates of random taxon addition and TBR branch swapping.

In addition, we performed a separate maximum likelihood analysis of the *Cytb* data. We calculated 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, and compared this value 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). We also assessed whether allowing for Γ -distributed heterogeneity of the substitution rate across sites (Yang 1994; Γ shape parameter) or allowing for a proportion of invariant sites (I) significantly improved the fit of each model to the data. Both these methods were used to determine the best-fit maximum likelihood model and were performed with Modeltest software (Posada and Crandall 1998).

Finally, we evaluated whether enforcing a molecular clock provided a better fit to the data than allowing for different rates across the tree. To provide the most conservative test for a clocklike model of evolution, we calculated an unweighted pair group method with arithmetic mean tree based on Jukes–Cantor distances and calculated the likelihood score for the best-fit model with no clock enforced ($\log L_1$) as compared to the same model with a clock enforced ($\log L_2$). 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 would be rejected. Subsequent to model evaluation and selection, the maximum likelihood tree was determined by 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 bootstrap analysis included 300 replicates and searches were heuristic, with a neighbor-joining starting tree followed by subtree-bisection reconnection branch-swapping.

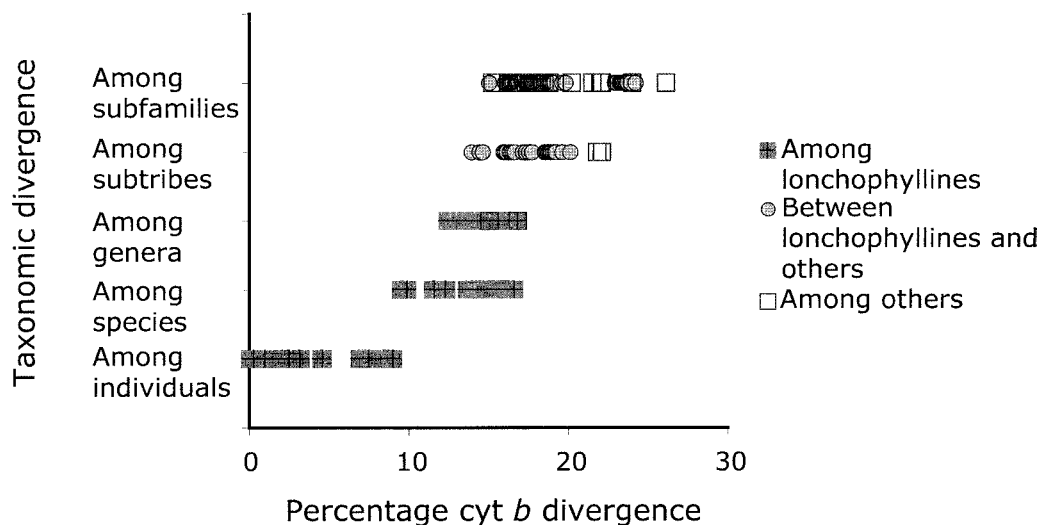


FIG. 1.—Scatter plot depicting relationship between levels of cytochrome *b* genetic divergence and levels of taxonomic divergence for taxa in this study.

RESULTS

Sequence divergence.—Sequence divergence among the studied taxa is summarized in Fig. 1. Three individuals of *L. robusta* from Colombia had identical *Cytb* sequences but differed from the 4th by 0.3% (uncorrected pairwise sequence divergence). Uncorrected sequence divergence between the Panamanian *L. robusta* and the Colombian specimens was 3.1 or 3.2%. The 2 specimens of *L. handleyi* from Peru differed by 1.0%. The 2 specimens of *L. thomasi* from Ecuador differed from each other by 2.1%, whereas sequence divergence within *L. thomasi* ranged up to 8.6% between samples from west of the Andes in Ecuador and the Guyanas. Two of the Peruvian specimens of *Lionycteris* had identical sequences, the Panamanian specimens differed from each other by 0.1%, and the Peruvian and Panamanian samples differed from each other by 4.5–4.6%. Uncorrected pairwise sequence divergence ranged from 9.5 to 16.6% among species of *Lonchophylla*, from 12.4 to 16.8% among the 3 lonchophylline genera, and from 13.9 to 24.2% between lonchophyllines and other phyllostomids (Fig. 1).

Phylogenetic analysis of the cytochrome *b* data set.—The complete *Cytb* gene (1,140 bp) was included for 29 of the 30 taxa; *Anoura* was scored only for the first 402 bp (the remaining nucleotides were scored as “missing” for this taxon). There were 517 variable characters in this matrix, of which 419 were informative for parsimony analysis. Of the parsimony informative characters, 18.4% occur at 1st codon positions, 3.6% at 2nd codon positions, and 78.0% at 3rd codon positions. Parsimony analysis of the informative *Cytb* characters resulted in 2 most-parsimonious trees with length = 1,894, consistency index (CI) = 0.42, and retention index (RI) = 0.62. A strict consensus of these 2 trees is shown in Fig. 2.

The parsimony tree nested 1 of our outgroup taxa (*Glossophaga*) as the sister taxon to *L. mordax*. Although this grouping did not receive strong support (bootstrap = 52%;

Bremer = 6), it was novel and caused us to suspect that parsimony analysis of these data might have been misled by long-branch-attraction between these 2 taxa (Felsenstein 1978). As an initial test of this hypothesis, we reran the parsimony analysis 1st without the *Glossophaga* sequence, then without the *L. mordax* sequence. If the *Lonchophylla* sequence was spuriously attracting the *Glossophaga* sequence, then *Glossophaga* should be expected to move outside the lonchophyllines once *L. mordax* is removed. Similarly, *L. mordax* should retain its position as a basal member of a clade including all species of *Lonchophylla* except *L. thomasi* once *Glossophaga* is removed from the analysis. This is precisely what happened, and confirmed our suspicion that the sister-taxon arrangement between *Glossophaga* and *L. mordax* shown in Fig. 2 is most likely an artifact of parsimony analysis of the *Cytb* data.

Because maximum likelihood methods are purported to be less sensitive to this long-branch attraction problem (Felsenstein 1978; Huelsenbeck 1995; but see Siddall and Whiting 1999), we reanalyzed the *Cytb* data in a maximum likelihood framework. A likelihood ratio test among several competing models identified HKY + I + G as the best-fit model (estimated parameter values transition/transversion = 6.341; A = 0.334, C = 0.359, G = 0.079, T = 0.228; α = 0.910; proportion of invariant sites = 0.495). We rejected a molecular clock with $P < 0.001$. A subsequent search with optimal parameters fixed resulted in 1 tree with $-\ln L = 9,014.82$. This tree shows a monophyletic Lonchophyllini and places *Glossophaga* among the outgroup taxa (Fig. 3).

Phylogenetic analysis of the combined data set.—To ameliorate long-branch attraction between ingroup and outgroup taxa for the *Cytb* data and to provide the best estimate of phylogenetic relationships among these taxa, the *Cytb* data were added to a matrix of 150 characters from morphology, sex chromosomes, and restriction sites as previously identified by Wetterer et al. (2000). Because these characters were collected to infer relationships among phyllostomid genera, they vary

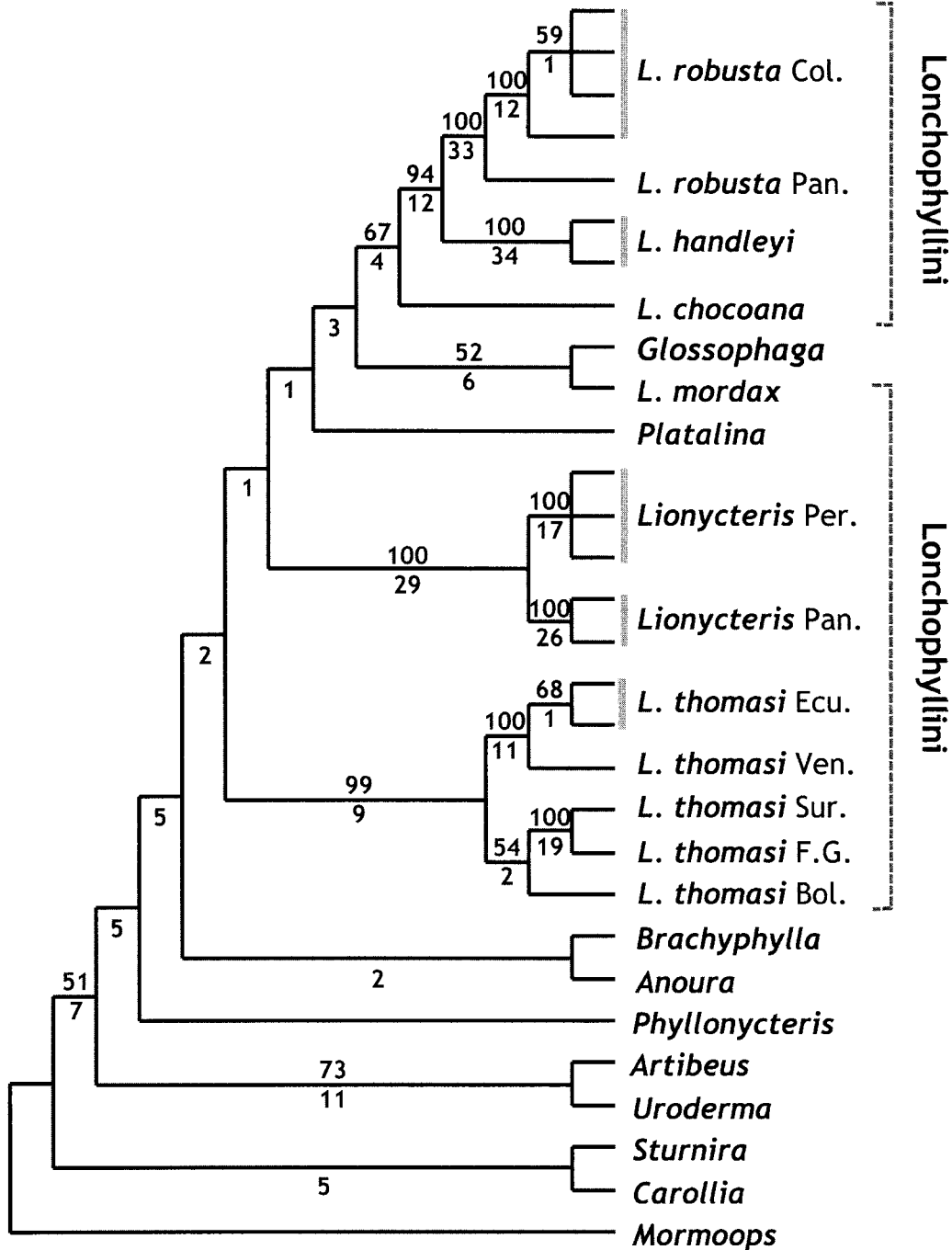


FIG. 2.—Strict consensus of 4 most-parsimonious trees (length = 1,893, consistency index = 0.42, and retention index = 0.62) based exclusively on cytochrome *b*. Numbers below branches are Bremer support values, numbers above are bootstrap values > 50% of 1,000 pseudoreplicates. *L. robusta*, *L. handleyi*, *L. chocoana*, *L. mordax*, and *L. thomasi* are species of *Lonchophylla*. Locations of samples are indicated: Col., Colombia; Pan., Panama; Ecu., Ecuador; Ven., Venezuela; Sur., Surinam; F.G., French Guiana; Bol., Bolivia; Per., Peru. Vertical gray bars indicate the label applies to the terminals behind them.

principally between the ingroup and outgroup taxa in our study. Of the 150 characters scored, 110 are variable across all taxa included in our study and 81 are phylogenetically informative. Ten characters are variable and 5 are phylogenetically informative within lonchophyllines, and 1 is variable and phylogenetically informative within *Lonchophylla*. Parsimony analysis of this data set yielded 3 minimum length trees of

length = 187, CI = 0.72, and RI = 0.79. Although these characters provide some resolution among the lonchophylline taxa (Wetterer et al. 2000:134, figure 49), we include them primarily to securely root the tree between the ingroup and outgroup taxa.

A partition homogeneity test between *Cytb* and morphological data indicated that significant incongruence occurred

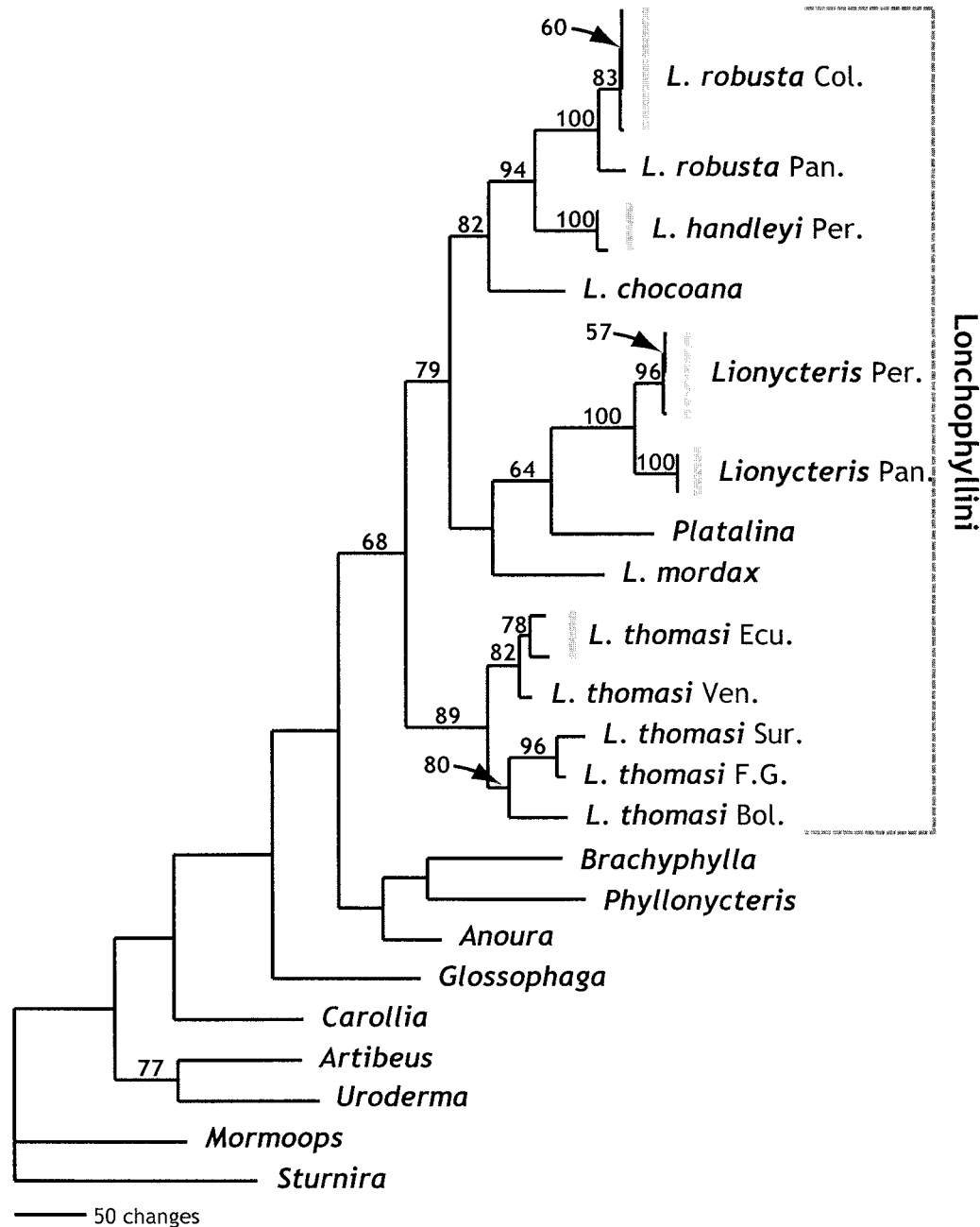


FIG. 3.—Topology of a maximum likelihood analysis ($-\ln L = 9,014.82$) of cytochrome *b* obtained using HKY + I + G as the best-fit model (estimated parameter values transition/transversion = 6.341; A = 0.334, C = 0.359, G = 0.079, T = 0.228; $\alpha = 0.910$; proportion of invariant sites = 0.495). Numbers above branches are bootstrap values > 50% of 300 replicates.

between these 2 data sets ($P = 0.01$), which might suggest that they should not be combined in a single analysis (but see Barker and Lutzoni 2002). However, most of the character incongruence appears to occur among the distantly related outgroup taxa. When we confine the test to include only the outgroup taxa, the test returns a result of significant incongruence ($P = 0.051$); however, when the test is performed on only the lonchophylline taxa, we find no significant incongruence between the *Cytb* and morphological data sets ($P = 0.97$). Our study was not designed to test relationships among phyllostomid bats but to address relationships within

the lonchophyllines, and this should be kept in mind when interpreting all our results. Given that no incongruence occurs between the morphological and *Cytb* data for the lonchophyllines, we maintain that the combined data tree is the best estimate of phylogeny for these taxa but caution that it should not in any case be used to interpret relationships among the outgroup taxa.

Parsimony analysis of the combined *Cytb* and morphological data sets resulted in 4 trees of length = 2,110, CI = 0.44, and RI = 0.62. The strict consensus of these 4 trees (Fig. 4) shows a monophyletic lonchophyllini that is recovered in 62% of

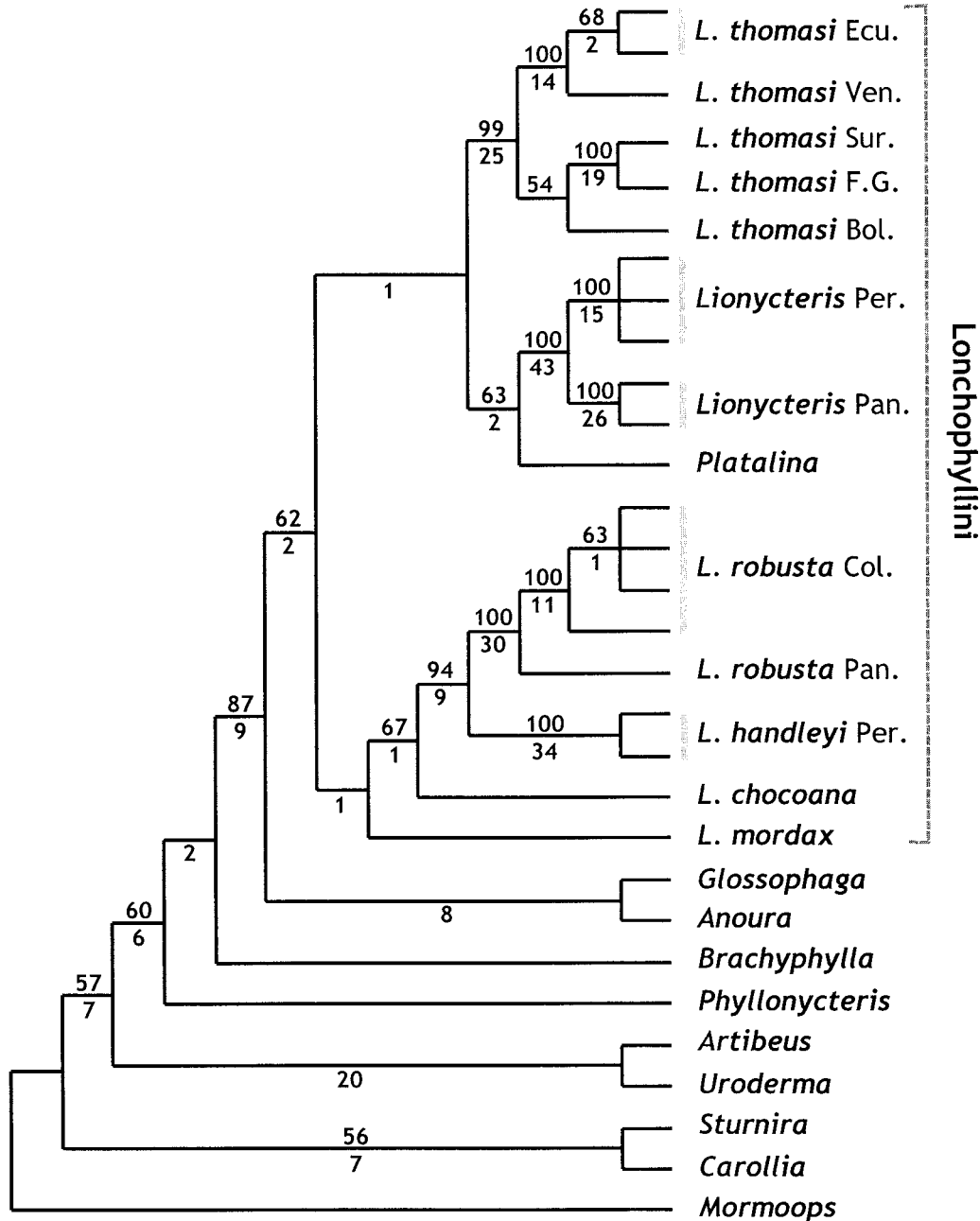


FIG. 4.—Strict consensus of 2 most-parsimonious trees (length = 2,110, consistency index = 0.44, and retention index = 0.62) based on cytochrome *b* and morphological data. Numbers below branches are Bremer support values, numbers above are bootstrap values > 50% of 1,000 pseudoreplicates when using random addition sequence of 100 replicates each.

bootstrap replicates with a Bremer support value of 2. Moreover, this topology is substantially more parsimonious, 20 steps shorter, than the length obtained by optimizing molecular and morphological characters on the most parsimonious *Cytb* consensus topology (Fig. 2).

Within lonchophyllines, a monophyletic *Lonchophylla* was not recovered. Rather, *L. thomasi* is sister to a clade containing *Lionycteris*, and *Platalina* with low bootstrap (<50) and Bremer (1 step) support, and this group is sister to the remaining species of *Lonchophylla* with low bootstrap (<50) and Bremer (2 steps) support (Fig. 4). Determining the position of *Platalina* relative to other lonchophyllines was one of the

primary goals of our study. The combined data tree shows *Platalina* as the sister taxon to *Lionycteris* to the exclusion of *Lonchophylla*, but only with moderate bootstrap (63%) and Bremer (2 steps) support.

DISCUSSION

Phylogeny of the Lonchophyllini.—Most of the support for lonchophylline monophyly (Figs. 2 and 4) comes from the morphological data set. This is not surprising: of the 8 character state changes in the morphological data set that can be mapped to this node, 6 are uniquely derived and unreversed (character numbers 13, 112, 114, 127, 146, and 148 as described in

Wetterer et al. [2000]). In addition, 14 state changes in the *Cytb* data set can be mapped to this node, but only 1, a silent A → C transversion at position 717, is uniquely derived and unreversed. This lack of molecular character support might be explained by the saturation of 3rd positions in *Cytb* at deeper phylogenetic levels.

Within Lonchophyllini, we found weak support for parphyly of *Lonchophylla* (Fig. 4). Analyses recovered *L. thomasi* as the sister taxon to *Lionycteris* and *Platalina*, but support for this relationship is negligible (bootstrap < 50%; Bremer = 1), and support for the sister-taxon relationship between *Platalina* and *Lionycteris* is similarly low (bootstrap = 61%; Bremer = 2). Although this particular arrangement of lonchophylline genera has not been suggested previously, parphyly of *Lonchophylla* is not an unprecedented result. Studies of tongue morphology (Gimenez 1993) and cranial morphometrics (Solmsen 1994, 1998) suggested that *Platalina* is a large species of *Lonchophylla*, rendering the latter genus paraphyletic.

Constraining the monophyly of *Lonchophylla* requires 6 additional steps, and given the low support values at the *L. thomasi*–*Lionycteris*–*Platalina* node, additional data might overturn this result. Whether *Platalina* is a large *Lonchophylla* is better resolved: forcing *Platalina* to be part of a clade with species of *Lonchophylla*, with *Lionycteris* sister to this (Gimenez 1993; Solmsen 1994, 1998) requires an additional 15 steps. However, the hypothesis that *Platalina* is the most basal member of the Lonchophyllini (Wetterer et al. 2000) is only slightly less parsimonious than our tree; forcing *Platalina* to be the basal member of the Lonchophyllini requires only 3 additional steps. Therefore, although it seems unlikely that *Platalina* is simply a large *Lonchophylla* (contra Gimenez 1993; Solmsen 1994, 1998), where this genus fits relative to *Lionycteris* and species of *Lonchophylla* remains uncertain.

The position of *L. mordax* is more contentious. Parsimony analysis suggested long-branch attraction between *Glossophaga* and *L. mordax* (Fig. 2). In the combined data parsimony analysis we found a monophyletic clade comprising *L. mordax*, *L. chocoana*, *L. robusta*, and *L. handleyi* but with low support values (<50% bootstrap, Bremer = 1; Fig. 4). This result also is consistent with support values for the maximum likelihood analysis of the *Cytb* data alone (Fig. 3). Fourteen character-state changes in the *Cytb* data occur at this node, although none are uniquely derived and unreversed. Despite the low support, this topology (Fig. 4) is 12 steps shorter than having *L. mordax* as sister to *Glossophaga* (Fig. 2), and 12 steps shorter than the fully resolved tree recovered when using maximum likelihood analysis (Fig. 3). Although both these latter arrangements seem unlikely, the position of this species remains uncertain.

Support for the sister relationship between *Lionycteris* and *Platalina* comes entirely from the *Cytb* data; no synapomorphies from the morphological data set support this relationship. The morphological data set offers only limited resolution to lonchophylline systematics (Wetterer et al. 2000), and this result could be expected. Thirteen character state changes in the *Cytb* data occur at this node. Three of these changes are uniquely derived and unreversed: a silent C → A transversion

at position 21, a silent C → G transversion at position 66, and a replacement T → C transition at position 917.

The *Cytb* data allow us to resolve the relationships among lonchophylline genera, albeit with low bootstrap and Bremer support values. Several explanations of this result are possible. First, and most likely, the rate of variation of *Cytb* might be too high to resolve relationships in this group, especially because we found evidence of saturation in 3rd position transitions and transversions, once overall sequence divergence reached 15%.

Second, lonchophyllines missing from our analysis, including extinct taxa, might be critical to fully resolving this phylogeny. In the case of *L. mordax*, taxonomic sampling is complicated by the existence of 2 distinct subspecies: the Amazonian *L. m. mordax* and the Central American and Chocoan *L. m. concava* (1 of which is included in this study). Given the divergence between *L. m. concava* and other lonchophyllines, it is possible that more representatives from throughout the range might break up this long branch (although not necessarily provide a more stable resolution; see *L. thomasi*). Our taxonomic sampling includes all the genera and 5 out of 8 *Lonchophylla* species (70% of the tribe), but because *Cytb* divergence among *Lonchophylla* species is almost as high as among the genera (Fig. 1), unsampled species might resolve the polytomies at the base of the lonchophylline radiation.

Third, lonchophylline phylogeny may indeed have short internodes at its base, produced by short time between speciation events. This would obscure phylogenetic signal from the mitochondrial DNA data (Moore 1995). Assuming that rates of evolution are constant throughout the topology, then the similar rates of divergence we observed between lonchophyllines and glossophagines and among lonchophyllines (Fig. 1) could be explained as rapid speciation. If this was true, analyses of other mitochondrial and nuclear genes also would show short basal branch lengths for any one topology (Brower et al. 1996). Although this might be a possible explanation of our results, the fact that we reject the molecular clock precludes an assumption of rate constancy. To critically test this hypothesis of rapid speciation will require additional data from nuclear genes.

RESUMEN

En este artículo presentamos el primer análisis conjunto de caracteres moleculares y morfológicos para resolver las relaciones filogenéticas entre las especies de la tribu filostómida Lonchophyllini. Para tal fin secuenciamos 1,140 pares de base del gen mitocondrial citocromo *b* de *Platalina*, *Lionycteris* y varias especies de *Lonchophylla* (Chiroptera: Phyllostomidae) y añadimos 150 caracteres morfológicos, de cromosomas sexuales y de sitios de restricción. También evaluamos la monofilia del género *Lonchophylla*, particularmente con respecto a *Platalina*. La hipótesis más parsimoniosa para las relaciones entre lonchophyllinos, utilizando todos los caracteres es: (*L. mordax* ((*L. chocoana* (*L. robusta*, *L. handleyi*))(*L. thomasi* (*Lionycteris*, *Platalina*))). *Lonchophylla* parece ser parafilético, pero estas relaciones no están bien respaldadas en nuestro análisis. En nuestros análisis parece poco probable que

Platalina sea simplemente un *Lonchophylla* grande, como había sido sugerido en previos estudios morfológicos. Los valores de soporte bajos encontrados para los nodos basales en este estudio probablemente son producto de la saturación del gen citocromo *b* en las terceras posiciones de nuestros caracteres mitocondriales. Además dos hipótesis alternas también son viables, aunque poco probables: algunos lonchofilinos ausentes en nuestro muestreo son necesarios para resolver las relaciones filogenéticas basales de este grupo o la falta de resolución se debe a la especiación rápida tras la separación entre lonchofilinos y glosófaginos. En el futuro, el trabajo para resolver la filogenia de los lonchofilinos debe concentrarse en describir nuevas especies, obtener muestras de tejido de las especies y subespecies que no se han representado en este estudio y añadir caracteres genéticos nucleares a nuestros datos mitocondriales.

ACKNOWLEDGMENTS

This paper is a contribution from the Monell Molecular Laboratory and the Cullman Research Facility in the Department of Ornithology, American Museum of Natural History, and has received generous support from the Lewis B. and Dorothy Cullman Program for Molecular Systematics Studies, a joint initiative of The New York Botanical Garden and The American Museum of Natural History. This material is based upon work supported by the National Aeronautics and Space Administration under grant NAG5-8543 and the Center for Biodiversity and Conservation at the American Museum of Natural History. We thank N. B. Simmons for her support, advice, and tissue samples; R. Baker, M. Engstrom, J. Patton, and J. Wible for loan of tissues; J. L. Cracraft and J. Feinstein for guidance and assistance in the laboratory; and C. Iudica for his extraction protocol for formalin-fixed, ethanol-preserved DNA. J. A. Guerrero assisted in the 1999 field expedition to Tambito during which all Colombian tissues of *L. robusta* were obtained. These and other tissues deposited at the AMNH were collected by following the guidelines of the American Association of Mammalogists (Animal Care and Use Committee 1998). The Tambito expedition was funded by Bat Conservation International, the Royal Geographic Society (London), The Explorers Club (New York), and the Institute of Latin American Studies and the Center for Environmental Research and Conservation at Columbia University. We thank E. Gaitán, J. D. Palacio, and J. Tohme at the molecular laboratory of Instituto de Investigación de Recursos Biológicos Alejandro von Humboldt and the Biotechnology Research Unit of the Centro Internacional de Agricultura Tropical for providing laboratory facilities in Colombia. L. M. Dávalos is supported by an international graduate student grant from the American Museum of Natural History and Columbia University.

LITERATURE CITED

- ANIMAL CARE AND USE COMMITTEE. 1998. Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. *Journal of Mammalogy* 79:1416–1431.
- BAKER, R. J., C. A. PORTER, J. C. PATTON, AND R. A. VAN DEN BUSSCHE. 2000. Systematics of the family Phyllostomidae based on *RAG2* DNA sequences. *Occasional Papers, The Museum, Texas Tech University* 202:1–16.
- BAKER, R. J., V. A. TADDEI, J. L. HUDGEONS, AND R. A. VAN DEN BUSSCHE. 1994. Systematic relationships within *Chiroderma* (Chiroptera: Phyllostomidae) based on cytochrome *b* sequence variation. *Journal of Mammalogy* 75:321–327.
- BARKER, F. K., AND F. M. LUTZONI. 2002. The utility of the incongruence length difference test. *Systematic Biology* 51:525–637.
- BREMER, K. 1994. Branch support and tree stability. *Cladistics* 10:295–304.
- BROWER, A. V. Z., R. DESALLE, AND A. VOGLER. 1996. Gene trees, species trees, and systematics: a cladistic perspective. *Annual Review of Ecology and Systematics* 27:423–450.
- DÁVALOS, L. M. 2004. A new Chocoan species of *Lonchophylla* (Chiroptera: Phyllostomidae). *American Museum Novitates* 3426:1–14.
- ERIKSSON, T., 1999. AutoDecay ver. 4.0. Bergius Foundation, Royal Swedish Academy of Sciences, Stockholm, Sweden.
- FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1995. Testing significance of incongruence. *Cladistics* 10:315–319.
- FELSENSTEIN, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. *Systematic Zoology* 27:401–410.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791.
- GALAZ, J. L., J. C. TORRES-MURA, AND J. YAÑEZ. 1999. *Platalina genovesium* (Thomas, 1928), un quiróptero nuevo para la fauna de Chile (Phyllostomatidae: Glossophaginae). *Noticiario Mensual Museo de Historia Natural* 337:6–12.
- GIMENEZ, E. 1993. Morfologia lingual comparada, filogenia e evolução dos hábitos alimentares na superfamilia Phyllostomoidea (Mammalia: Chiroptera). M.S. thesis, Universidade Estadual Paulista, São Paulo, Brazil.
- GIMENEZ, E., H. FERRAREZZI, AND V. A. TADDEI. 1996. Lingual morphology and cladistic analysis of the New World nectar-feeding bats (Chiroptera: Phyllostomidae). *Journal of Comparative Biology* 1:41–64.
- GOLDMAN, N. 1993. Statistical tests of models of DNA substitution. *Journal of Molecular Evolution* 36:182–198.
- GRIFFITHS, T. 1982. Systematics of the New World nectar-feeding bats (Mammalia, Phyllostomidae), based on the morphology of the hyoid and lingual regions. *American Museum Novitates* 2742:1–45.
- GRIFFITHS, T. 1983. On the phylogeny of the Glossophaginae and the proper use of outgroup analysis. *Systematic Zoology* 32:283–285.
- HUELSENBECK, J. P. 1995. The performance of phylogenetic methods in simulation. *Systematic Biology* 44:17–48.
- IUDICA, C. A., W. M. WHITTEN, AND N. H. WILLIAMS. 2001. Small bones from dried mammal museum specimens as a reliable source of DNA. *BioTechniques* 30:733–736.
- JANSA, S., S. GOODMAN, AND P. K. TUCKER. 1999. Molecular phylogeny and biogeography of the native rodents of Madagascar (Muridae: Nesomyine): a test of the single-origin hypothesis. *Cladistics* 15:253–270.
- KOOPMAN, K. 1994. Chiroptera: systematics. *Handbuch der Zoologie* 8:1–217.
- MADDISON, W. P., AND D. R. MADDISON. 1992. *MacClade: analysis of phylogeny and character evolution*. Version 3.0. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.
- MICKEVICH, J. E., AND J. S. FARRIS. 1981. The implications of congruence in *Menidia*. *Systematic Zoology* 30:351–370.
- MOORE, W. S. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution* 49:718–726.
- PHILLIPS, C. 1971. The dentition of glossophagine bats: development, morphological characteristics, variation, pathology, and evolution.

- Miscellaneous Publications, Museum of Natural History, University of Kansas 54:1–138.
- POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817–818.
- SIDDALL, M. E., AND M. F. WHITING. 1999. Long-branch abstractions. *Cladistics* 15:9–24.
- SOLMSEN, E. 1994. Vergleichende Untersuchungen zur Schädelkonstruktion der neuweltlichen Blütenfledermause sowie zu ihrer systematischen Ordnung unter besonderer Berücksichtigung der Glossophaginae (Phyllostomidae, Chiroptera, Mammalia). Ph.D. dissertation, Universität Hamburg, Hamburg, Germany.
- SOLMSEN, E. 1998. New World nectar-feeding bats: biology, morphology and craniometric approach to systematics. *Bonner Zoologische Monographien* 44:1–118.
- SWOFFORD, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.
- TADDEI, V. A., L. D. VIZOTTO, AND I. SAZIMA. 1983. Uma nova espécie de *Lonchophylla* do Brasil e chave para identificação das espécies do gênero (Chiroptera: Phyllostomidae). *Ciência e Cultura* 35:625–629.
- VAN DEN BUSSCHE, R. A., AND R. J. BAKER. 1993. Molecular phylogenetics of the New World bat genus *Phyllostomus* based on cytochrome *b* DNA sequence variation. *Journal of Mammalogy* 74:793–802.
- VAN DEN BUSSCHE, R. A., R. J. BAKER, H. A. WICHMAN, AND M. J. HAMILTON. 1993. Molecular phylogenetics of Stenodermatini bat genera: congruence of data from nuclear and mitochondrial DNA. *Molecular Biology and Evolution* 10:944–959.
- WARNER, R. 1983. Karyotypic megaevolution and phylogenetic analysis: New World nectar-feeding bats revisited. *Systematic Zoology* 32:279–282.
- WETTERER, A., M. ROCKMAN, AND N. B. SIMMONS. 2000. Phylogeny of Phyllostomid bats (Mammalia: Chiroptera): data from diverse morphological systems, sex chromosomes, and restriction sites. *Bulletin of the American Museum of Natural History* 248:1–200.
- WRIGHT, A. J., R. A. VAN DEN BUSSCHE, B. K. LIM, M. D. ENGSTROM, AND R. J. BAKER. 1999. Systematics of the genera *Carollia* and *Rhinophylla* based on the cytochrome *b* gene. *Journal of Mammalogy* 80:1202–1213.
- YANG, Z. 1994. Statistical properties of the maximum likelihood method of phylogenetic estimation and comparison with distance matrix methods. *Systematic Biology* 43:329–342.

Submitted 31 December 2002. Accepted 30 April 2003.

Associate Editor was Robert D. Bradley.

APPENDIX I

Specimens examined.—The following list is organized by taxon, locality, country of origin, and voucher number of specimen or GenBank accession code. Classification follows Wetterer et al. (2000). Collections are listed with the following abbreviations: AMNH, American Museum of Natural History; CMNH, Carnegie Museum of Natural History; AF and L, GenBank accession code; MHN, Museo de Historia Natural–Popayán (Colombia); MVZ, Museum of Vertebrate Zoology, University of California, Berkeley; ROM, Royal Ontario Museum; and TTU, Museum of Texas Tech University.

Mormoops megalophylla, Grutas de Lanquín, Guatemala, AF338690; *Brachyphylla cavernarum*, Grand Terre, Guadeloupe, CMNH 112372; *Anoura caudifer*, Barinitas, Venezuela, L19506; *Glossophaga soricina*, Paracou, French Guyana, AMNH 267950; *Lionycteris spurrelli*, Darién, Panama, TTU39127, TTU 39912; *L. spurrelli*, Madre de Dios, Peru, MVZ 166628, MVZ 166630, MVZ 166632; *Lonchophylla chocoana*, Esmeraldas, Ecuador, ROM 105786; *Lonchophylla handleyi*, Tingo María, Peru, TTU 46164; *Lonchophylla handleyi*, Junín, Peru, AMNH 230214; *Lonchophylla mordax*, Esmeraldas, Ecuador, ROM 105798; *Lonchophylla robusta*, Altos de Campana, Panama, ROM 104268; *L. robusta*, Tambito, Colombia, MHN 512–515; *Lonchophylla thomasi*, Voltzberg, Surinam, AF187034; *L. thomasi*, Bolívar, Venezuela, ROM 107906; *L. thomasi*, Paracou, French Guyana, AMNH 267943; *L. thomasi*, Beni, Bolivia, AMNH 209358; *L. thomasi*, Napo, Ecuador, ROM 104064, ROM 105527; *Platalina genovesium*, Arequipa, Peru, AMNH 257108; *Phyllonycteris aphylla*, Bluefields, Jamaica, AF187033; *Carollia perspicillata*, locality not given, AF187026; *Artibeus obscurus*, Poataro, Guyana, ROM 109345; *Uroderma bilobatum*, Arima, Trinidad, L28941; and *Sturnira lilium*, Tingo María, Peru, AF187035.