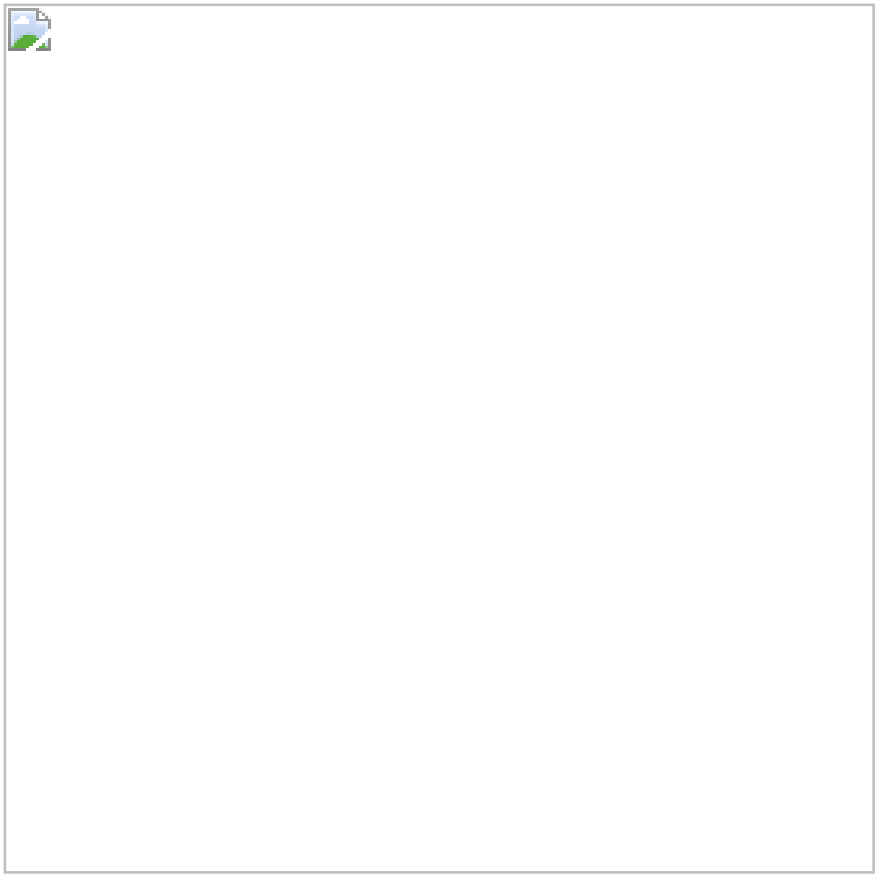

Molecular Differentiation of Large Species of Fruit-Eating Bats (Artibeus) and Phylogenetic Relationships Based on the Cytochrome b Gene

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Molecular differentiation of large species of fruit-eating bats (*Artibeus*) and phylogenetic relationships based on the cytochrome *b* gene

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We analyzed the phylogenetic relationships of all eleven currently recognized species of large *Artibeus* using the cytochrome *b* mitochondrial gene. The topology from a maximum parsimony analysis included: (1) *A. obscurus* and *A. planirostris* as sister species with successively basal lineages of (2) *A. amplus*, (3) a clade with *A. lituratus* and *A. intermedius*, (4) *A. jamaicensis*, (5) a clade of *A. inopinatus* sister to *A. hirsutus* and *A. fraterculus*, (6) *A. fimbriatus*, and (7) the most basal lineage of *A. concolor*. The individual species were monophyletic and well supported by bootstrap and decay values. The monophyletic clade of ((((*obscurus* + *planirostris*) + *amplus*) + (*lituratus* + *intermedius*)) + *jamaicensis*) was also highly supported, although some of the interspecific relationships were less so. Contrary to previous hypotheses of species limits based on a presumed intergradation in body size, *A. jamaicensis* and *A. planirostris* do not form a monophyletic group, refuting their conspecificity and supporting an earlier study concluding that these two taxa represent separate morphological populations. An analysis with *A. jamaicensis* and *A. planirostris* constrained as sister-taxa resulted in a tree 8 steps longer. In addition, the low genetic pair-wise difference between *A. lituratus* and *A. intermedius* (2.8% with Kimura-2 parameters) warrants closer examination of their species limits.

Key words: *Artibeus*, cytochrome *b*, Kimura-2, Llanos, parsimony, Venezuela

INTRODUCTION

Biogeographically, the Llanos savannah of central Venezuela is important as intervening habitat between Amazonian rainforest to the south and coastal mesophytic forest to the north. For *Artibeus*, it also represents the distributional limits of several species including the northern *A. jamaicensis*, and southern *A. obscurus* and *A. planirostris*. The systematic relationship between *A. jamaicensis* and *A. planirostris* has long been disputed and taxonomically confused with other sympatric species of large *Artibeus* including *A. amplus*, and *A. obscurus*, and to a lesser degree *A. lituratus*. Subtle morphological differences of the skull, dentition, and external features often have not been unambiguously diagnostic, especially for widely distributed species exhibiting geographic variation. Four of these large-sized species of *Artibeus* (*A. amplus*, *A. lituratus*, *A. obscurus*, and *A. planirostris*) have been previously reported from the forested areas

of La Serrania de los Pijiguaos near savannah south of the Orinoco River in Venezuela (Ochoa *et al*., 1988).

During studies on the biodiversity of mammals in northern Colombia, Hershkovitz (1949) concluded that the absence or presence of a small third upper molar was an unreliable character for separating *A. jamaicensis* from *A. planirostris* so the latter should be synonymized with the former. Handley (1987) refined the distributional limits by referring to those without a small third upper molar from northwestern Peru to western Colombia as *A. j. equatorialis*, which intergrades in northern Colombia with the slightly smaller *A. j. trinitatis* from western Venezuela that has a third upper molar. Furthermore, he referred to the larger *A. planirostris*, which also has three molars, from southern Venezuela and the Guianas into eastern Amazonia as another subspecies, *A. j. fallax* said to intergrade with *A. j. trinitatis* in the Llanos of central Venezuela. Two other large-sized subspecies with three molars recognized in South America include *A. j. hercules* in western Amazonia and *A. j. planirostris* from southeastern South America. Therefore, *A. jamaicensis* was considered a widely distributed but highly variable species from Mexico to northern Argentina and essentially sympatric with the also widely distributed *A. lituratus*. Conspecificity of *A. planirostris* and *A. jamaicensis* was based wholly on intergradation in the Venezuelan Llanos (Handley, 1987). Using phenetic techniques, Lim (1997) found bimodal morphometric distributions of populations in Venezuela with no evidence of intergradation between large and small taxa in the Llanos, and recommended the taxonomic separation of *A. planirostris* (occurring south of the Orinoco River and east of the Andes in South America) from *A. jamaicensis* (occurring north of the Orinoco River and west of the Andes in South America into Central America).

A molecular study of the complete cytochrome *b* gene for most species of *Artibeus* by Van Den Bussche *et al*. (1998) was a major step in elucidating the phylogenetic relationships within the genus. However, *A. jamaicensis* was represented by only two specimens from Suriname and French Guiana, which are referable to the taxon *A. planirostris* as was also recently noted by Guerrero *et al*. (2003). In addition, specimens of *A. amplus* were not included in previous molecular analyses. Our fieldwork in the Llanos of Venezuela resulted in the acquisition of *A. amplus*, which is one of the least known species of *Artibeus* and was only recently reported from Suriname (Lim *et al*., 2003). Two other sympatric species of *Artibeus* (*A. obscurus* and *A. planirostris*) were also obtained from the critical biogeographic Llanos region that will further our understanding of the systematics of this genus. Taxonomic sampling for the currently recognized species of large *Artibeus* was completed with the sequencing of the mitochondrial genome for *A. jamaicensis* from Puerto Rico (Pumo *et al*., 1998). We sequenced cytochrome *b* for our specimens from Venezuela and combined them with previously published sequences deposited in GenBank to investigate the molecular phylogenetics of the large species of *Artibeus*, the systematic relationship of *A. amplus*, and the species status of *A. planirostris* with respect to *A. jamaicensis*.

Materials and Methods

A small mammal biodiversity survey was conducted during July and August 1997 at the savannah-forest interface of the Llanos region just south of the Orinoco River in Venezuela. Noteworthy records and habitat descriptions were reported in Lee *et al*. (2000). The voucher specimens were deposited at the Museo de Historia Natural La Salle (MHNLS), Caracas, Venezuela; Abilene Christian University Natural History Collection (ACUHNC), Abilene, Texas, United States; and Royal Ontario Museum (ROM), Toronto, Canada. Three species of *Artibeus*

were captured at Pozon, 50 km northeast of Puerto Ayacucho, Amazonas (06°03'N, 67°25'N) in savannah with patches of forest. These included two specimens of *A. amplus* (ROM 107847, 107904), one *A. obscurus* (ROM 107846), and eight *A. planirostris* (ACUHNC 290, 291, 292, 357; ROM 107848, 107888, 107893, 107905) used in this study. In addition, two individuals of *A. obscurus* (ACUHNC 358; ROM 107937) were caught approximately 300 km to the northeast at 3 km east of Puerto Cabello del Caura, Bolivar (07°10'N, 64°59'N) in disturbed forest adjacent to farmland.

For molecular study, DNA was extracted from frozen liver from our 12 specimens following a similar procedure to Maniatis *et al*. (1988). The complete cytochrome *b* and threonine tRNA genes were amplified by PCR with primers LGL 765 and LGL 766 located in the flanking tRNA's. The 1,254 base pair amplification product was decontaminated of impurities using the QIAquick PCR Purification Kit (Qiagen Inc.). Sequencing reactions used ¼ volumes of ABI Prism BigDye Terminator (Applied Biosystems) with each of the primers and run on an ABI Prism 377 automated sequencer. The resultant sequences were aligned by eye using Sequencher 4.1 (Gene Codes Corporation) with base confirmation in the overlapping mid-section. These 12 sequences of the complete cytochrome *b* gene were deposited in GenBank under accession numbers AY642913–AY642924 (Appendix).

All 15 cytochrome *b* sequences of large species of *Artibeus* previously deposited in GenBank were included in our phylogenetic analysis (Appendix). With the addition of *A. amplus*, this represents all currently recognized species of large *Artibeus* including *A. amplus*, *A. concolor*, *A. fimbriatus*, *A. fraterculus*, *A. hirsutus*, *A. inopinatus*, *A. jamaicensis*, *A. lituratus* and *A. obscurus* (Simmons, In press) in addition to *A. intermedius* (Davis, 1984) and *A. planirostris* (Lim, 1997). Four outgroup taxa were used including the small-bodied *Artibeus* species *A. cinereus*, *A. glaucus*, and *A. gnomus*, which have been considered as *Dermanura* at the subgeneric (Wetterer *et al*., 2000; Simmons, In press) or generic (Owen, 1987; Van Den Bussche *et al*., 1998) rank, and *Enchisthenes hartii.*

The 31 aligned sequences were analyzed phylogenetically using a heuristic parsimony search algorithm as implemented in PAUP* version 4.0b10 for 32-bit Microsoft Windows (Swofford, 2001) with default settings and equally parsimonious trees summarized by strict consensus. All nucleotide characters were treated as unordered and equally weighted. Clade support was assessed by bootstrap analysis using a heuristic search with 1,000 replications and calculating a decay value using a converse constraints approach. In addition, pair wise sequence differences were estimated based on Kimura-2 parameters.

Results

Of the 1,140 nucleotide base pair positions in the large *Artibeus* cytochrome *b* data set, 776 were constant, 93 were variable but parsimony uninformative, and 271 were variable and parsimony informative. The heuristic search found four equally parsimonious trees with a length of 991 steps, consistency index of 0.436, and retention index of 0.601. Phylogenetic relationships among species level taxa were consistent among all four trees and alternative topologies involved rearrangements only within the *A. planirostris* clade. Therefore, one of the four equally parsimonious trees is presented in Fig. 1. There was a strongly supported monophyletic clade of *A. obscurus* with Kimura-2 pair wise differences ranging from 0 to 4.4% with an average of 3.2% (Table 1). The monophyletic clade of *A. planirostris* was not as strongly supported and the genetic distances ranged from 0.3 to 4.5% with an average of 2.5%. *Artibeus obscurus* and *A. planirostris* were united as sister species, however, this relationship was weakly supported. The sequence divergence between individuals of these two species ranged from 5.5 to 7.5% with an average of 6.4%.

The two new sequences for *A. amplus* formed a strongly supported clade with a sequence divergence of 0.8%. *A. amplus* was weakly supported as the sister group to the clade of *A. obscurus* and *A. planirostris*. The genetic distance between individuals of these two lineages ranged from 3.7 to 7.3% with an average of 5.1%. *Artibeus lituratus* and *A. intermedius* were strongly supported as sister taxa and the pair wise difference between the two specimens was 2.8%. This lineage was weakly supported as the sister group to the clade comprising

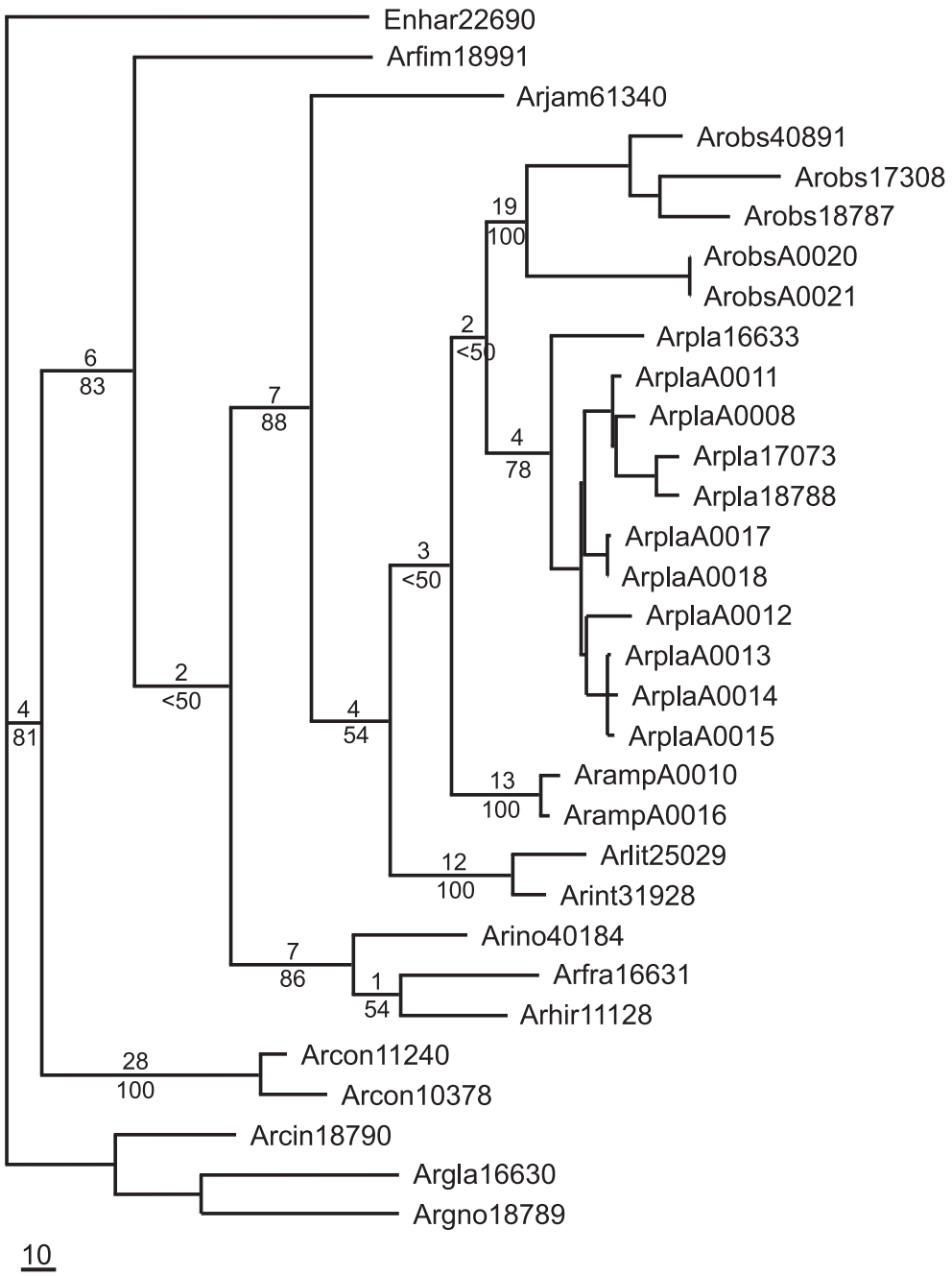

FIG. 1. One of four equally parsimonious trees derived from an heuristic search of cytochrome *b* variation for large species of *Artibeus*. The alternative trees involved rearrangements within the *A. planirostris* clade. The first five letters of the haplotypes identify the species: Aramp, *A. amplus*; Arcin, *A. cinereus*; Arcon, *A. concolor*; Arfim, *A. fimbriatus*; Arfra, *A. fraterculus*; Argla, *A. glaucus*; Argno, *A. gnomus*; Arhir, *A. hirsutus*; Arino, *A. inopinatus*; Arint, *A. intermedius*; Arjam, *A. jamaicensis*; Arlit, *A. lituratus*; Arobs, *A. obscurus*; Arpla, *A. planirostris*; and Enhar, *Enchisthense hartii*. The last five alphanumerics are individual identifiers as listed in the text. Branch lengths are proportional to the number of nucleotide changes with decay values listed above the branches and bootstrap percentages below the branches

A. amplus, *A. obscurus*, and *A. planirostris*. The pair wise difference between individuals of these two lineages ranged from 5.2 to 8.7% with an average of 6.7%. The next basal lineage to this clade was *A. jamaicensis* with the monophyly strongly supported. The genetic distance between *A. jamaicensis* and other species in the sister lineage ranged from 5.7 to 9.1% with an average of 7.1%.

There was a strongly supported monophyletic clade of *A. inopinatus* sister to a weakly supported grouping of *A. fraterculus* and *A. hirsutus*. The sequence divergence range for these three taxa was 6.2 to 7.8% with an average of 7.0%. In turn, this clade is the weakly supported sister to the previously described clade of *A. jamaicensis*, *A. intermedius*, *A. lituratus*, *A. amplus*, *A. planirostris*, and *A. obscurus*. The sequence divergence between individuals within these two lineages ranged from 6.5 to 11.2% with an average of 9.0%. The monophyly of this clade and the next basal lineage, *A. fimbriatus*, was strongly supported and the genetic distance between individuals of these two lineages was 7.9 to 10.3% with an average of 9.0%. The two individuals of *A. concolor* formed a strongly supported clade and were separated by a pair wise difference of 2.4%. This species formed the basal taxon for large *Artibeus* whose monophyly was strongly supported. The pair wise difference between individuals of *A. concolor* and the other species of large *Artibeus* ranged from 9.5 to 12.9% with an average of 11.1%. The average genetic distance between all species of large *Artibeus* was 8.6% with a range from 4.5% for *A. amplus* and *A. planirostris* to 12.6% for *A. concolor* and *A. lituratus*.

Discussion

The three species of large *Artibeus* (*A. amplus*, *A. planirostris* and *A. obscurus*) found sympatrically at Pozon in the Llanos of Venezuela are molecularly well differentiated by long branch lengths and are each grouped into discrete monophyletic species clades (Fig. 1). The relationships amongst these three species are not as strongly supported although *A. planirostris* and *A. obscurus* appear as sister species in the shortest trees. There are two trees that are two steps longer uniting *A. amplus* with *A. planirostris.* The strongly supported clade of *A. lituratus* and *A. intermedius* is the sister lineage to the clade of *A. amplus*, *A. obscurus*, and *A. planirostris* with *A. jamaicensis* appearing as the basal lineage. This larger clade is monophyletic and well supported but interspecific relationships within the clade have relatively less support based on bootstrap and decay values. Nonetheless, it would require a tree eight steps longer for *A. planirostris* and *A. jamaicensis* to be sister species in a monophyletic clade as was suggested by Handley (1987).

The systematic relationship between *A. jamaicensis* and *A. planirostris* has often been confounded in the literature because of inconsistent taxonomic usage. The specimens (CM 68950 from Suriname and AMNH 267202 from French Guiana) listed as *A. jamaicensis* and forming the sister lineage to *A. planirostris* (MVZ 170016 from Peru) in Van Den Bussche *et al*. (1998) were a mixed application of the two different taxonomic hypotheses. Based on distribution, all three of these specimens would be assignable to *A. planirostris*, which occurs in Amazonian rainforest east of the Andes in most of South America. Morphologically the identification of the French Guiana specimen as *A. jamaicensis* followed Handley's (1987) recognition of a single widely distributed species (Simmons and Voss, 1998). As reported in Simmons and Voss (1998), their specimens were within the range of size variation for *A. planirostris* (*A. j. fallax* of Handley, 1987) in Guyana as

TABLE 1. Pair wise sequence divergence (in %) estimated by Kimura-2 parameters for cytochrome *b* variation in large species of *Artibeus*. Haplotype abbreviations are described in the text

Haplotype	Arobs 17308	Arobs 18787	Arobs 40891	Arobs A0020	Arobs A0021	Arpla 16633	Arpla 18788	Arpla 17073	Arpla A0013	Arpla A0014	Arpla A0012	Arpla A0015	Arpla A0017	Arpla A0018	Arpla A0008
Arobs 17308															
Arobs18787	3.2														
Arobs40891	3.5	2.4													
ArobsA0020	4.4	4.1	2.9												
ArobsA0021	4.4	4.1	2.9	0.0											
Arpla16633	6.8	6.3	6.4	6.6	6.6										
Arpla18788	6.3	5.5	6.5	6.9	6.9	3.9									
Arpla17073	6.7	6.1	6.7	7.3	7.3	4.5	1.2								
ArplaA0013	7.1	6.1	5.6	5.8	5.8	3.8	2.8	3.1							
ArplaA0014	7.1	6.3	5.8	6.0	6.0	4.0	3.0	3.2	0.4						
ArplaA0012	7.2	6.4	6.2	6.2	6.2	4.3	3.5	3.6	1.8	2.0					
ArplaA0015	7.0	6.2	5.7	5.9	5.9	3.9	2.9	3.1	0.3	0.4	1.7				
ArplaA0017	7.0	6.2	6.0	6.2	6.2	3.5	3.0	3.1	1.6	1.8	2.1	1.7			
ArplaA0018	6.9	6.1	5.9	6.1	6.1	3.4	2.9	3.0	1.5	1.7	2.1	1.6	0.1		
ArplaA0008	7.4	6.5	6.1	6.5	6.5	4.4	2.1	2.0	2.0	2.0	2.2	2.1	2.0	1.9	
ArplaA0011	7.5	6.6	6.2	6.8	6.8	4.0	2.0	1.9	1.8	2.0	2.2	1.9	1.7	1.6	0.9
ArampA0010	7.3	6.9	6.1	6.1	6.1	5.8	5.5	5.6	3.8	4.0	4.4	3.9	4.2	4.1	4.4
ArampA0016	7.2	6.9	6.0	6.2	6.2	5.5	5.4	5.5	3.7	3.9	4.3	3.8	3.9	3.8	4.5
Arlit25029	8.7	7.8	7.7	8.4	8.4	7.1	7.1	7.0	5.9	6.1	6.7	6.0	6.1	6.0	7.1
Arint31928	7.9	7.1	7.2	7.5	7.5	6.0	6.7	6.6	5.2	5.4	6.2	5.3	5.8	5.7	6.8
Arjam61340	9.1	8.6	7.8	8.1	8.1	7.4	7.5	7.6	6.2	6.4	6.8	6.5	6.4	6.3	6.6
Arfra16631	9.8	9.1	8.9	8.8	8.8	6.5	9.3	9.5	8.3	8.3	8.9	8.4	8.5	8.4	9.0
Arhir11128	9.8	9.0	9.2	9.1	9.1	8.7	9.6	9.6	8.3	8.3	9.5	8.3	9.0	9.1	9.3
Arino40184	9.2	8.8	8.5	8.5	8.5	9.6	10.6	11.2	9.0	9.0	9.7	9.1	9.7	9.6	10.3
Arfim18991	10.1	9.0	8.5	9.0	9.0	9.3	9.1	9.7	8.1	7.9	9.0	8.2	9.2	9.1	9.5
Arcon11240	10.8	10.8	10.3	9.8	9.8	11.0	12.0	12.3	11.3	11.1	11.5	11.4	11.4	11.3	11.6
Arcon10378	10.6	10.1	10.1	9.5	9.5	10.4	11.5	11.9	11.2	11.2	11.4	11.3	11.4	11.3	11.6
Arcin18790	13.0	12.9	11.9	11.6	11.6	12.4	14.1	14.9	12.5	12.5	12.8	12.6	12.6	12.7	13.9
Argla16630	13.7	13.5	13.2	13.3	13.3	13.4	14.0	14.3	13.6	13.2	13.6	13.7	12.7	12.9	13.6
Argno18789	14.0	14.1	14.1	14.0	14.0	12.7	14.1	14.4	13.1	12.8	13.3	13.2	13.0	13.2	13.6
Enhar22690	15.7	16.4	16.3	15.6	15.6	15.2	17.3	17.5	15.9	16.1	16.3	16.2	16.3	16.4	16.9

TABLE 1. Continued

Haplotype	Arpla A0011	Aramp A0010	Aramp A0016	Arlit 25029	Arint 31928	Arjam 61340	Arfra 16631	Arhir 11128	Arino 40184	Arfim 18991	Arcon 11240	Arcon 10378	Arcin 18790	Argla 16630	Argno 18789
ArampA0010	4.5														
ArampA0016	4.2	0.8													
Arlit25029	6.8	6.1	5.8												
Arint31928	6.5	5.7	5.6	2.8											
Arjam61340	6.5	6.0	5.7	6.6	7.1										
Arfra16631	9.3	9.1	8.6	8.4	7.2	8.4									
Arhir11128	9.6	9.3	9.0	9.3	8.1	9.5	6.4								
Arino40184	10.6	8.7	8.6	9.7	8.8	9.2	7.8	6.9							
Arfim18991	9.7	9.1	8.5	10.3	9.3	8.6	9.0	8.7	8.8						
Arcon11240	12.3	10.4	10.1	12.4	11.1	11.3	10.8	12.4	10.9	10.6					
Arcon10378	12.0	10.5	10.3	12.9	11.5	11.5	11.0	11.8	11.0	10.7	2.4				
Arcin18790	13.8	12.3	12.0	12.7	12.0	11.8	10.9	11.6	10.9	11.3	11.7	12.5			
Argla16630	14.3	13.2	12.6	13.8	13.3	13.6	12.0	12.4	13.6	12.1	12.8	13.8	10.7		
Argno18789	14.4	14.4	14.1	14.7	12.9	14.3	13.4	12.8	13.3	12.7	13.3	13.4	10.6	10.6	
Enhar22690	17.4	15.8	15.9	17.1	15.9	15.9	15.9	16.9	15.9	15.5	16.1	15.4	15.5	16.3	16.3

summarized by Lim and Wilson (1993). Likewise, we examined the Suriname specimen, which was also identified following the taxonomy of Handley (1987), and it conforms to the diagnosis of *A. planirostris* as described in Lim and Wilson (1993). Our molecular analysis firmly aligned these specimens with *A. planirostris* and not *A. jamaicensis* (Fig. 1).

Within species, the topology of *A. obscurus* suggested geographic structuring from east to west for the Guiana Shield specimens with French Guiana and Suriname as sister lineages, and Guyana and Venezuela as successive basal branches. Sampling in the Amazon basin and Atlantic Forest of Brazil would provide a better understanding of phylogeography within this species. Ditchfield (2000) found similar levels of sequence divergence (3.3%) among 29 specimens of *A. obscurus* from localities in the Guiana Shield and Atlantic Forest but this represented only the first 402 base pairs of cytochrome *b*. For *A. planirostris*, the only consistent geographic structuring was the western Amazonian specimen from Peru being the sister lineage to the Guiana Shield clade. There was no obvious geographic structuring within the Guiana Shield specimens of *A. planirostris* as shown with *A. obscurus* because the Venezuela samples never formed a monophyletic clade to the exclusion of Suriname and French Guiana individuals.

Although not a primary focus of this study, the sequence divergence (Table 1) between *A. lituratus* and *A. intermedius* (2.8%) is on a comparable scale to the average divergence of 2.2% within species of large *Artibeus* for which there are at least two specimens (*A. amplus*, 0.8%; *A. concolor*, 2.4%; *A. obscurus*, 3.2%; and *A. planirostris*, 2.5%). These values are similar to the mean (3.0%) and range (0.1–8.7%) for intraspecific variation of cytochrome *b* sequence divergence for bats estimated by

Kimura-2 parameters (Bradley and Baker, 2001). Furthermore, the 2.8% sequence divergence between *A. lituratus* and *A. intermedius* is less than the 6.4% difference for the sister species hypothesized in our analysis (*A. obscurus* and *A. planirostris*; *A. fraterculus* and *A. hirsutus*). With the removal of the sequence data for *A. lituratus*/*A. intermedius* (because the two might be conspecific; e.g., Marques-Aguiar, 1994) and *A. jamaicensis*/*A. planirostris* (because *A. planirostris* was sampled and not *A. jamaicensis* in the analysis of Van Den Bussche *et al*., 1998), the revised values for divergence between sister species of Bradley and Baker (2001) were slightly higher with a mean value of 7.6% and range of 3.2% to 16.4%. As also noted by Van Den Bussche *et al*. (1998), thorough geographic sampling including individuals in the putative zones of sympatry for *A. lituratus* and *A. intermedius* in Middle America (Davis, 1984), is needed to properly address their taxonomic status.

The only morphological systematic study of all large species of *Artibeus* was done by Marques-Aguiar (1994), although she synonymized *A. planirostris* with *A. jamaicensis* and *A. intermedius* with *A. lituratus*. Her two shortest trees, of which a strict consensus is presented in Fig. 2A, had no fully resolved relationships that were shared with our molecular tree (Figs. 1 and 2C). This inconsistency highlights the lack of genetic congruence with the subtle morphological differences that have historically confounded the taxonomy of *Artibeus*. For example, our cytochrome *b* data suggest that *A. jamaicensis* and *A. planirostris* are distantly related taxa but Marques-Aguiar (1994) could not distinguish them morphologically. In contrast, Lim (1997) found them to represent morphometrically distinct populations based on size; however, this difference has not been translated into discrete qualitative character states that can be scored across the genus. A similar problem occurs with *A. hirsutus* and *A. inopinatus*, which were treated as separate species even though they were scored as morphologically identical by Marques-Aguiar (1994).

A recent study of most species of large *Artibeus* concluded that morphometric data were useful in phylogenetic analyses (Guerrero *et al*., 2003). However, in their morphometric phylogeny (Fig. 2B) there are no fully resolved relationships that are shared with the molecular phylogeny. From a genetic perspective using cytochrome *b*, the obvious successive progression from small to large-bodied taxa in the morphometric tree fails to reflect phylogenetic relationships. The large sized *A. fimbriatus*, which was relatively basal in our molecular analysis but not included in their study, would probably occur in a near terminal position as the sister-taxon to the clade of *A. lituratus* and *A. amplus* in the morphometric-based tree (Fig. 2B) of Guerrero *et al*. (2003). In contrast, there does appear to be a genetic basis for a distinction between small and large *Artibeus* as previously demonstrated by Van Den Buscche *et al*. (1998). However, there is overlap in size as measured by forearm length between the 'small' *A. aztecus* (41–49 mm) and the 'large' *A. concolor* (45–51 mm) and *A. inopinatus* (48–53 mm) indicating that the distinct phylogenetic entities are not absolutely correlated to size. The designation of the smaller *Artibeus* as *Dermanura* at the taxonomic rank of genus is a matter of subjective degree because it is the sister taxon to the larger *Artibeus*, as opposed to an issue of paraphyly as suggested by Owen (1987) who placed *Dermanura* as sister to the white-shouldered bats and not to *Artibeus*. However, traditional generic rank within the subfamily Stenodermatinae (e.g., Simmons, In press) has been based on diagnosable combinations of discrete morphological

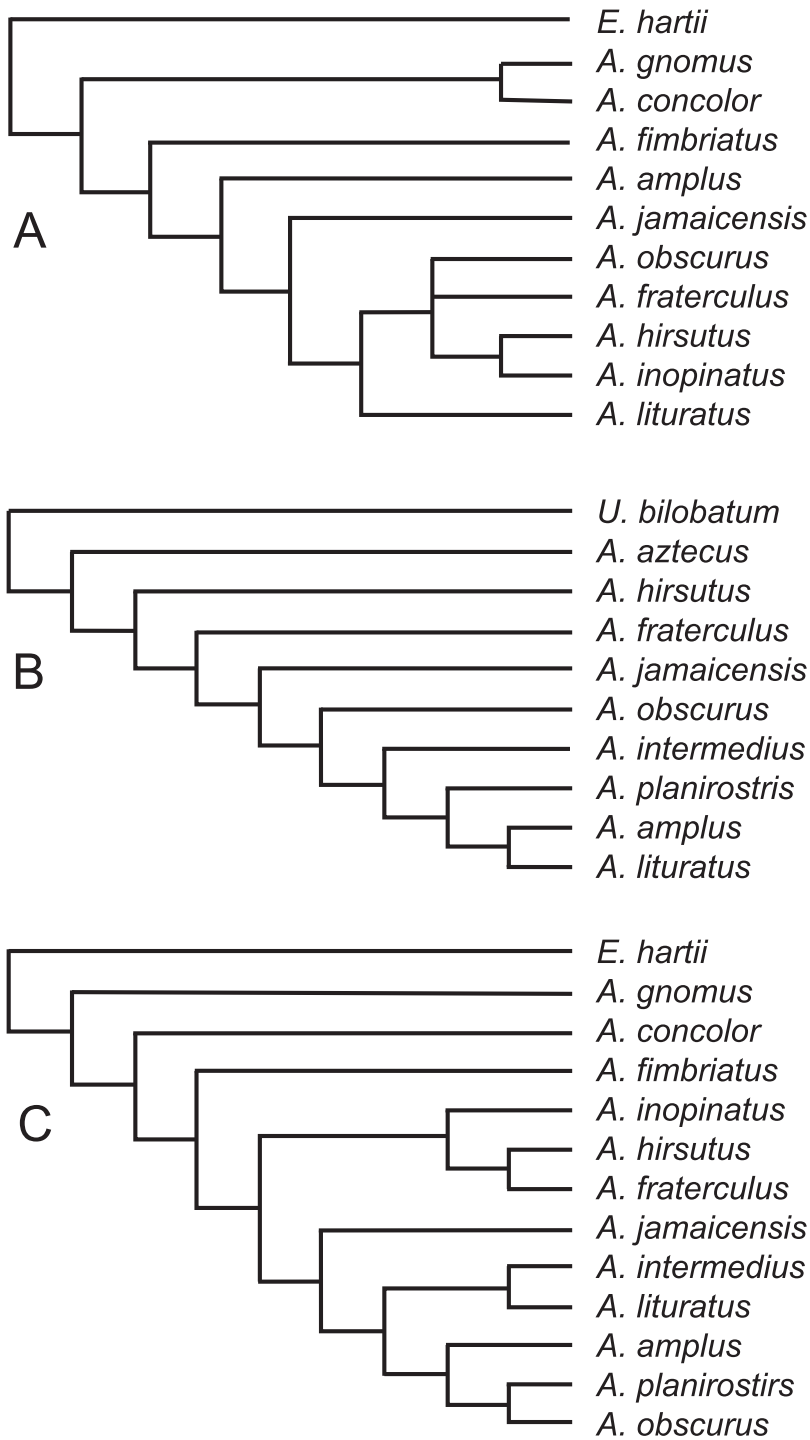

FIG. 2. (A) Morphological phylogeny of Marques-Aguiar (1994). *Artibeus planirostris* was considered conspecific with *A. jamaicensis*, and *A. intermedius* was considered conspecific with *A. lituratus*. (B) Morphometric phylogeny of Guerrero *et al*. (2003). Note ladderized topology from small to large size species (*A. aztecus* to *A. lituratus*). (C) Molecular phylogeny of this study

characters. We are not aware of any diagnostic characters that distinguish *Dermanura* from *Artibeus* but recognize their size differences and discrete molecular lineages as subgenera within *Artibeus*. Nonetheless, other genetic markers are needed for a better understanding of the magnitude of differentiation between these two taxa.

A phylogenetic analysis of a 381 base pair region of the mitochondrial genes ATPase 8 and ATPase 6 sequenced for three species of *Artibeus* from the western slope of the Andes in Peru had a topology of ((*A. planirostris*, *A. lituratus*), *A. fraterculus*) rooted with *A. fimbriatus* (Patterson *et al*., 1992). Based on distribution, we assume that the specimens listed as *A. jamaicensis* are *A. planirostris* as suggested as a possibility by Patterson *et al*. (1992). These relationships are consistent with our molecular analysis but not congruent with the results of the morphological analysis of Marques-Aguiar (1994) and the morphometric analysis of Guerrero *et al*. (2003) based on our assumption that the large size *A. fimbriatus* would render it more similar to *A. lituratus* than to *A. planirostris* or *A. fraterculus* in their analysis.

We do not consider *A. jamaicensis* as a widely distributed species throughout the Neotropics as proposed by Hershkovitz (1949), Handley (1987), and Marques-Aguiar (1994). The separation *A. planirostris* from *A. jamaicensis* is based on the paraphyletic relationship as hypothesized by the cytochrome *b* parsimony analysis, the lack of support for a sister-group relationship for these two taxa, sequence divergence suggesting a closer similarity of *A. planirostris* with *A. amplus* and not *A. jamaicensis*, and the morphometric study of Lim (1997) that refuted the size intergradation suggested by Handley (1987). Further tests of our molecular phylogeny with other genes and more comprehensive distributional coverage would greatly facilitate our understanding of the congruence of variation in cytochrome *b* to the evolutionary history of bats and the systematic relationships within *Artibeus*, especially in light of what appears as episodes of rapid radiation in the genus. Because of relatively poor sampling in the Venezuelan Llanos, geographic limits for *A. jamaicensis* and *A. planirostris* are not precisely known in this region with its vast intervening habitats of grassland, which provides marginal roosting sites for bats. As presently known, the two species are allopatric wherein *A. jamaicensis* is restricted from approximately north of the Orinoco River into Central America, and *A. planirostris* is distributed south of the Orinoco River into southeastern South America (Lim, 1997).

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Appendix

Within each species used in the cytochrome *b* analysis of large *Artibeus*, the haplotype is listed first followed by the GenBank accession number and its first publication (or museum number if it represents a new sequence from this study):

Artibeus amplus — ArampA0010, AY642923 (ROM 107847); ArampA0016, AY642924 (ROM 107904);

A. cinereus — Arcin18790, U66511 (Van Den Bussche *et al*., 1998);

A. concolor — Arcon10378, U66518; Arcon11240, U66519 (Van Den Bussche *et al*., 1998);

A. fimbriatus — Arfim18991, U66498 (Van Den Bussche *et al*., 1998);

A. fraterculus — Arfra16631, U66499 (Van Den Bussche *et al*., 1998);

A. glacaus — Argla16630, U66512 (Van Den Bussche *et al*., 1998);

A. gnomus — Argno18789, U66513 (Van Den Bussche *et al*., 1998);

A. hirsutus — Arhir11128, U66500 (Van Den Bussche *et al*., 1998);

A. inopinatus — Arino40184, U66501 (Van Den Bussche *et al*., 1998);

A. intermedius — Arint31928, U66502 (Van Den Bussche *et al*., 1998);

A. jamaicensis — Arjam61340, AF061340 (Pumo *et al*., 1998); *A. lituratus* — Arlit25029, U66505 (Van Den Bussche *et al*., 1998);

A. obscurus — Arobs17308, U66506; Arobs18787, U66507 (Van Den Bussche *et al*., 1998); Arobs40891, AF423079 (Daválos and Jansa, 2004); ArobsA0020, AY642921 (ROM 107937); ArobsA0021, AY642922 (ACUNHC 358);

A. planirostris — Arpla17073, U66503; Arpla18788, U66504; Arpla16633, U66508 (Van Den Bussche *et al*., 1998); ArplaA0008, AY642919 (ACUNHC 357); ArplaA0011, AY642920 (ROM 107848); ArplaA0012, AY642915 (ACUNHC 290); ArplaA0013, AY642913 (ACUHNC 291); ArplaA0014, AY642914 (ROM 107888); ArplaA0015, AY642916 (ROM 107893); ArplaA0017, AY642917 (ACUHNC 292); ArplaA0018, AY642918 (ROM 107905);

Enchisthenes hartii — Enhar22690, U66517 (Van Den Bussche *et al*., 1998).