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SHORT COMMUNICATION

Morphological and molecular evidence of the occurrence of *Artibeus amplus* (Chiroptera: Phyllostomidae) in Brazil

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ABSTRACT. *Artibeus amplus* Handley, 1987 is a little-known bat species endemic to northern South America. There are confirmed records of the species for Colombia, Venezuela, Guyana, and Suriname. In this study, we report the occurrence of *A. amplus* in Brazil based on the collection of two specimens captured in the municipalities of Cantá and Caracaraí in the state of Roraima. We also found a museum specimen from the state of Amazonas. The specimens were identified based on morphology and mitochondrial Cytochrome b gene analysis. After this contribution, the number of bat species in Brazil is 182. Surveys in other areas in the north of the country, such as the state of Pará, in addition to a comprehensive review of museum specimens, is needed to investigate the distribution of the species in areas where it has not been found yet.

KEY WORDS. Amazonia, biodiversity, distribution extension, new record, taxonomy.

Brazil is one of the countries with the greatest diversity of bats in the world, with 181 valid species before this contribution (Garbino et al. 2022). Of the bat families that occur in Brazil, Phyllostomidae, has the greatest number of described species. It is divided into 10 subfamilies, of which Stenodermatinae is the most speciose, with 38% of the species recorded from Brazil. *Artibeus* Leach, 1821, a genus within Stenodermatinae, is subdivided into two or three subgenera: *Artibeus* Leach, 1821 (large *Artibeus*), *Koopmania* R.D. Owen, 1991 (*Artibeus concolor*), and *Dermanura* P. Gervais, 1856 (small *Artibeus*). Recognition of the subgenus *Koopmania* is still a matter of discussion (e.g., Wetterer et al. 2000, Redondo et al. 2008).

The phylogenetic relationships of *Artibeus* (sensu lato, Patterson et al. 1992, Marques-Aguiar 1994, Van den Bussche et al. 1998, Guerrero et al. 2003, 2004, Lim et al. 2004, Hoofer et al. 2008, Redondo et al. 2008, Solari et al. 2009, Larsen et al. 2010) have been discussed in the literature, and needs to be further clarified. One species-complex that needs elucidation is the *Artibeus jamaicensis* Leach, which contains several subspecies that have been recently elevated to species (e.g., Guerrero et al. 2004, Larsen et al. 2010). This complex displays considerable heterogeneity in the Amazon region (Ferreira et al. 2014). Redondo et al. (2008), using a combination of molecular analyses, investigated the phylogeny and the systematics of the included species, suggested that there are four additional species that reveal a significant cryptic diversity within the genus.

Artibeus (*Artibeus*) *amplus* was described by Handley (1987) and is one of the least known large *Artibeus* Leach, 1821 species with a distribution in northern South America, including Colombia, Venezuela, Guyana, and Suriname (Lim et al. 2003, Ramoni-Perazzi et al. 2012). Long-term studies carried out in the French Guiana have not recorded this species (Brosset and Charles-Dominique 1990, Simmons and Voss 1998). Although its occurrence in Brazil was expected, it had not been confirmed, and for that reason, it had not been included in the list compiled by the Brazilian Bat Research Society (Garbino et al. 2022). In this study, we update the distribution of *A.* (*Ar.*) *amplus* to include the Guiana shield of the Amazon basin, based on an integrative analysis of morphometrics and molecular data.

We analyzed two specimens of large *Artibeus* from Roraima, a state in the extreme north of Brazil (Fig. 1). The specimens were deposited in the zoology collection of the Animal Biodiversity Laboratory of the Universidade Federal de Jataí (CJ 1176 and CJ 1206, both males). The animals are preserved in alcohol and had their skulls extracted for morphometric analysis. Specimen CJ 1176 was captured in October 7, 2019 with a mist net, in an area of seasonal semideciduous forest, on the left bank of the Branco river, municipality of Cantá (2°27'20.23"N; 60°49'28.52"W). Specimen CJ 1206 was captured in February 21, 2020 also on the left bank of the Branco River in an area of dense rainforest in the municipality of Caracaraí (1°52'26.08"N; 60°58'50.33"W). A map with the species distribution points was constructed with the information provided by Ramoni-Perazzi et al. (2012), adding data from Redondo et al. (2008) and the records of the present study (Fig. 1).

External and cranial dimensions were taken with a digital caliper accurate to 0.01 mm, with measurements taken on the right side of each specimen: (1) total length (TL), the distance from the tip of the snout to the end of the body where the interfemoral membrane is inserted; (2) forearm length (FA), the distance between the elbow and the wrist when the wing is folded; (3) metacarpal length of the 3rd digit (Me3); (4) first phalange length of the 3rd digit (1p3); (5) second phalange length of the 3rd digit (2p3); (6) the greatest length of skull (GLS), from the posteriormost point on the occiput to the anteriormost point on the premaxillae, excluding the incisors; (7) greatest mastoid breadth (GMB), the greatest cranial breadth across the mastoid region; (8) braincase width (BW), the greatest breadth of the globular part of the braincase; (9) greatest zygomatic width (ZB), the greatest breadth across the zygomatic arches; (10) postorbital constriction (PO), the least breadth across the frontals posterior to the postorbital processes; (11) width across canines (C-C), the distance between the outer margins of the upper canines; (12) width across molars (M-M), the distance between the outer margins of the upper molars; (13) length of mandible (LM), the distance from the anteriormost point, excluding the incisors, to the posteriormost point of the articular process; (14) condyloincisive length (CiL), from the posteriormost point on the occipital condyles to the anteriormost point on the–upper incisors; (15) condylocanine length (CcL), from the posteriormost point on the occipital condyles to the anteriormost point on the upper canines; (16) palatal length (PL), the distance from the posterior margin of the cingulum of the incisors

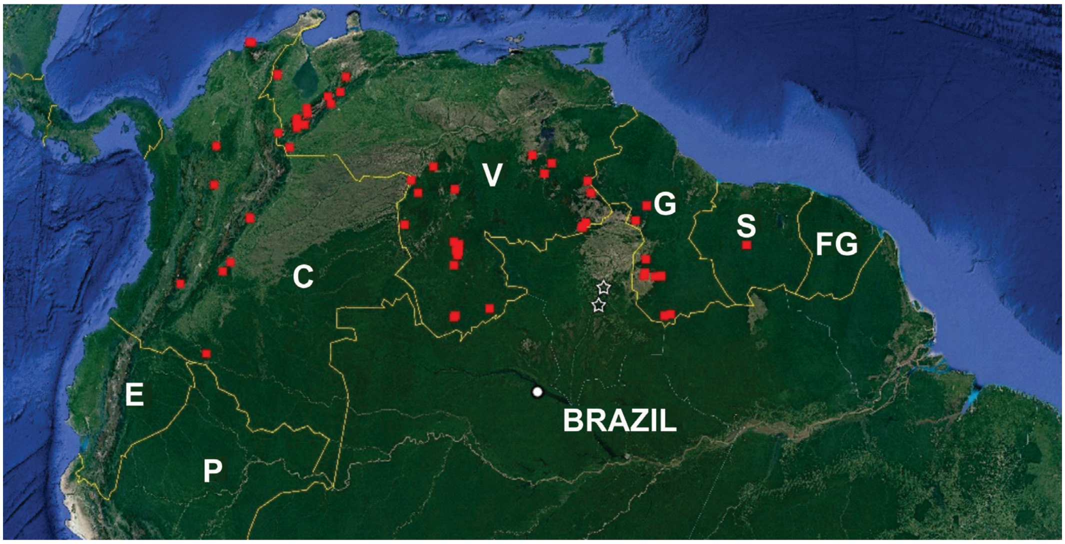

Figure 1. Collecting localities of *Artibeus amplus* in South America. The letters are the initials of the countries. Red squares are previous records compiled by Ramoni-Perazzi et al. (2012), white circle is a record cited by Redondo et al. (2008), and white stars are the two new records in the state of Roraima, Brazil. Image Landsat/Copernicus – 12/13/2015. Google Earth – Data SIO, NOAA, U.S. Navy, NGA, GEBCO.

to the median posterior border of the palate; (17) length of maxillary toothrow (LMxT), the distance from the anterior margin of the cingulum of the canine to the posterior margin of the last molar; (18) length of mandibular toothrow (LMdT), the distance from the anterior margin of the cingulum of the canine to the posterior margin of the last molar; (19) body mass (mass), with the individuals weighed in the field with a digital scale accurate to 0.01 g, with empty stomach.

We isolated DNA from thigh tissue samples stored in absolute ethanol following the phenol-chloroform protocol (Sambrook et al. 1989). We amplified fragments containing the full-length cytochrome b gene (mt-Cytb; genes' acronyms following *Mus musculus* nomenclature of Eppig et al. 2015) for two individuals (CJ 1176 and CJ 1206) by PCR with the primers L14724 modified (Irwin et al. 1991) and Cytb Rev (Casado et al. 2010). The cycling conditions for the mt-Cytb PCR reaction were initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation to 94 °C for 30 s, annealing at 54 °C for 30 seg and extension at 72 °C for 90 seg, and final extension at 72 °C for 5 min. mt-Cytb PCR products were purified (Wizard® SV Gel and PCR Clean-Up System Kit; Promega) and sequenced with the two additional internal primers MEU1 (Gonçalves et al. 2005) and MVZ16 (Smith and Patton 1993). Sequencing reactions were run in an ABI3130xl (Applied Biosystems) platform and electropherograms were checked with Chromas Pro 1.41 (McCarthy et al. 1998) and aligned using in the MEGA 11 (Tamura et al. 2021) with MUSCLE algorithm (Edgar 2004). To compose the analysis additional GenBank sequences were used for *A. amplus* (GenBank AY642924/, field number ROM 107904, AY642923/ ROM 107847, EU160947/ RZ 044, EU160946/ RZ 019, AY684755/ROM 106721), *A. hartii* (GenBank EU16092, EU160971), *A. glaucus* (EU160963, EU160962), *A. anderseni* (EU160966, EU160965), *A. cinereus* (EU160987, EU160685), *A. aztecus* (FJ179238, FJ179237), *A. phaeotis* (DQ869387, U66514), *A. toltecus* (FJ179256, FJ179257), *A. concolor* (EU160951, EU160950), *A. fraterculus* (DQ869388, EU160950), *A. hirsutus* (AY684777, AY684766), *A. inopinatus* (U66501, FJ179229), *A. fimbriatus* (DQ869391, KT149211), *A. jamaicensis* (DQ869420, DQ869419), *A. schwartzi* (DQ869530, DQ869529), *A. obscurus* (DQ869392, AY642922), *A. lituratus* (DQ869393, EU160833), *A. intermedius* (AY144339, AY144338), and *A. planirostris* (DQ869438, DQ869437). Sequences of *Platyrrhinus masu* (FJ154162) and *Chiroderma villosum* (MN823725) were considered outgroups.

The genetic distance was estimated with the p-distance algorithm on MEGA 11 (Tamura et al. 2021). The nucleotide substitution model (TIM2+F+I+G4) was selected as the best model by the Akaike information criterion (AIC) in the MrModelTest (Nylander 2004). Maximum likelihood (ML) phylogenies were obtained with IQ-TREE 2.2.0 (Minh et al. 2020) and nodal bootstrap (bs) values were calculated with 1,000 repetitions. The Bayesian inference (BI) analysis was performed with the method Markov chain Monte Carlo (MCMC) in MrBayes 3.2 (Ronquist et al. 2012) to estimate posterior probability (pp) values. Three hot and one cold chains were run for 1,000,000 generations, and a tree for 500 generations was sampled. The convergence of the chains was evaluated in Tracer 1.7 (Rambaut et al. 2018) (effective sample size [ESS] > 200). The topology was verified in FigTree 1.4.4 (Rambaut 2018).

The two specimens match the original diagnosis provided by Handley (1987), Lim and Wilson (1993) and Lim et al. (2003) in most internal and external features. *Artibeus amplus* can be diagnosed by the following combination of traits: large size (FA > 68 mm); the base of the noseleaf merges continuously with the upper lip; the rostral shield is broad and robust, with the lateral edges nearly parallel from the rostrum posteriorly towards the postorbital processes; brownish wing tips; and facial stripes are evident (Fig. 2). Table 1 compares the cranial and external measurements of specimens from Roraima with those from other localities.

Table 1. Selected measures of *Artibeus amplus*. 1) Present study; 2) Range of measurements provided by Lim et al. (2003) which includes data from Handley (1987) and Lim and Wilson (1993); 3) Means of measurements provided by Guerrero et al. (2003).

	CJ 1176[1]	CJ 1206[1]	Lim et al. 2003[2]	Guerrero et al. 2003[3]
TL	86.00	81.20	80.0–100.0	–
FL	71.90	69.10	65.0–75.3	67.61
Me3	70.36	66.75	–	62.87
1p3	21.24	21.84	–	20.07
2p3	37.00	34.74	–	35.16
GLS	31.46	31.75	30.2–33.2	32.59
GMB	16.18	17.55	15.4–17.1	16.52
BW	13.30	13.77	–	14.25
ZB	18.67	19.75	17.3–19.1	18.69
PO	8.26	7.68	7.3–8.4	8.45
C–C	9.20	9.19	8.2–9.6	7.97
M–M	13.25	12.83	12.6–13.9	14.06
LM	21.87	22.16	–	22.47
CiL	28.63	28.70	–	–
CcL	27.52	27.19	–	–
PL	13.94	13.84	11.8–13.4	–
LMxT	11.43	10.90	10.7–11.9	–
LMdT	12.26	12.03	–	
Mass	57.20	61.00	46.0–60.0	–

Figure 2. (A) Male *Artibeus amplus* (CJ 1176) from Roraima state, Brazil; (B) wingtip of the same specimen of *Artibeus amplus*; (B) the wingtip of *Artibeus planirostris* (CJ 989). Photo by Felipe Zenha.

The ML and BI trees retrieved the genus *Artibeus* divided into two main clades, one with *A. hartii* and the other with the remaining species. This latter clade is divided into two subclades: the first with the species of the subgenus *Dermanura*, and the second with species of the subgenus *Artibeus* as the sister group of the subgenus *Koopmania*. In the *Dermanura* subgenus clade, *A. toltecus* is the sister species of *A. phaeotis* and both are sister clades of *A. aztecus* [*A. cinereus* (*A. anderseni*, *A. glaucus*)]. The *Koopmania* subgenus clade is composed of a single species, *A. concolor*. The species of the *Artibeus* subgenus clade, grouped with high support (95%), are divided into two subclades, one with [*A. inopinatus* (*A. hirsutus*, *A. fraterculus*)] and the other with the remaining species. In this latter subclade, *A. fimbriatus* is the first offshoot, followed by *A. jamaicensis*, *A. schwartzi*, *A. obscurus*, and [(*A. lituratus*, *A. intermedius*) (*A. planirostris*, *A. amplus*)].

Haplotypes of all species were grouped with high support (bs = 100% and pp = 1, Fig. 3). All seven *A. amplus* sequences belong to a different haplotype, and the genetic distance estimated between them ranged from 0.20 to 1.08%. *Artibeus amplus* maintains an average genetic distance of 3.6% with the closest species, *A. planirostris*, and 14.0% with *A. hartii*, which is the phylogenetically most distant clade (Table 2).

The presence of *A. amplus* in the Brazilian territory was expected since it occurs along the borders and in neighboring countries in the extreme north of South America. The available data on the distribution of bats in the Brazilian extreme north is a limiting factor to understand bat diversity in Brazil. Similar gaps in the known distribution of bats plague other Brazilian biomes, since many areas are undersampled or have not been sampled at all (Bernard et al. 2011). Few studies and inventories of bats have been carried out in Roraima. Robinson (1998) presented a list of species from

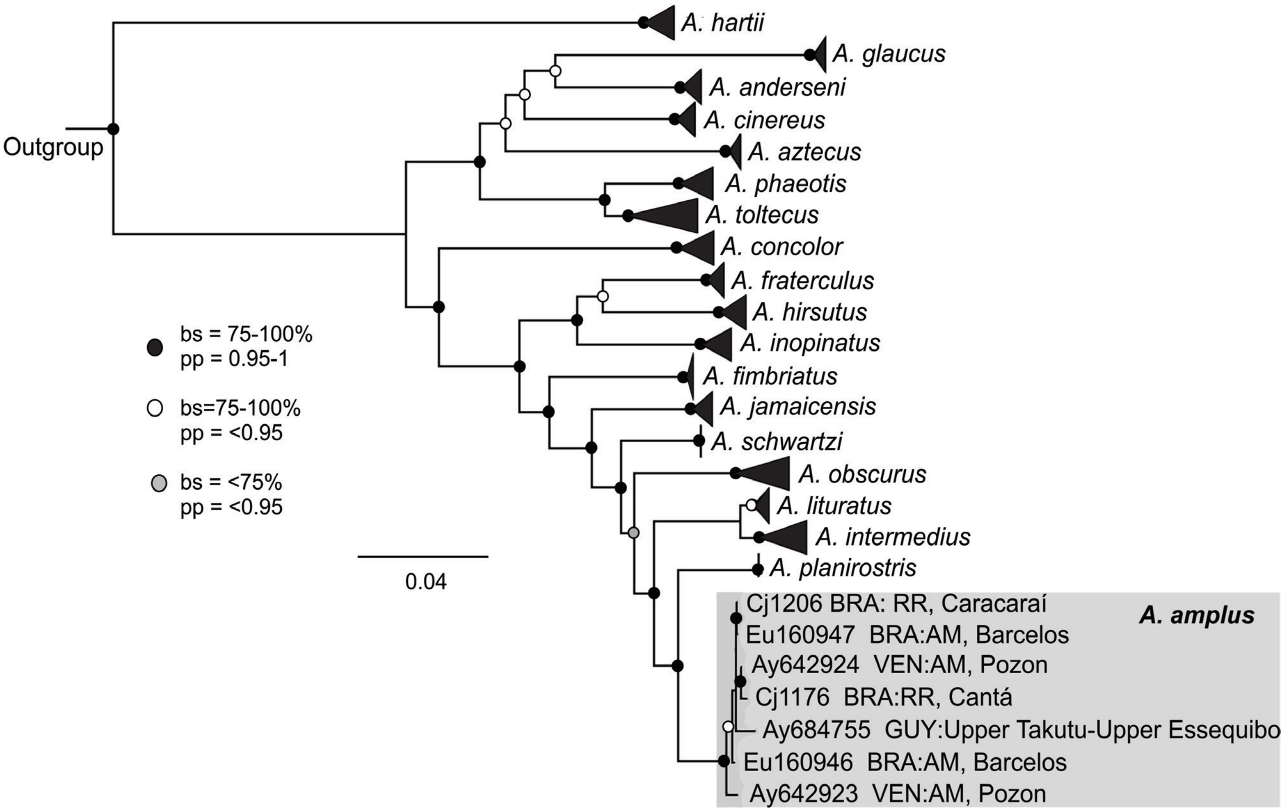

Figure 3. Phylogeny of *Artibeus* based on cytochrome b gene. Symbols near nodes are bootstrap and posterior probability values.

Table 2. p-distance genetic distance estimates (%) between *Artibeus* species with mt-Cytb data.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*A. amplus*																		
2	*A. planirostris*	3.6																	
3	*A. jamaicensis*	6.0	6.0																
4	*A. lituratus*	4.7	5.0	6.5															
5	*A. schwartzi*	4.7	5.2	5.4	5.1														
6	*A. obscurus*	5.5	5.5	6.4	6.2	5.3													
7	*A. fimbriatus*	7.0	6.4	6.3	7.7	6.4	6.6												
8	*A. glaucus*	11.9	11.4	12.0	11.8	11.9	12.1	11.5											
9	*A. cinereus*	10.0	10.5	11.0	10.9	10.6	11.2	8.8	9.1										
10	*A. phaeotis*	10.1	10.0	10.1	11.0	9.6	11.0	9.4	9.6	8.1									
11	*A. intermedius*	5.5	5.8	7.1	2.2	5.6	7.1	8.1	12.6	11.4	11.1								
12	*A. fraterculus*	8.5	8.6	7.9	7.4	7.5	7.8	7.8	12.0	9.3	10.2	7.9							
13	*A. hirsutus*	8.9	8.2	8.4	7.7	7.8	8.4	8.7	11.3	9.8	10.9	8.5	6.0						
14	*A. concolor*	9.6	10.0	9.7	10.0	10.0	8.9	9.1	10.9	9.2	10.2	10.7	9.8	10.7					
15	*A. aztecus*	10.0	10.6	11.1	11.0	10.0	10.3	10.0	9.3	8.8	9.5	11.5	10.2	9.7	11.6				
16	*A. toltecus*	10.3	10.5	10.1	10.7	10.1	10.5	9.0	9.3	8.2	4.0	11.0	9.9	10.4	10.2	9.3			
17	*A. hartii*	14.0	14.1	14.0	13.7	13.0	13.7	13.7	15.0	13.4	14.0	14.1	13.5	14.3	13.7	14.3	13.8		
18	*A. inopinatus*	8.3	8.7	8.3	8.4	7.8	7.7	8.0	11.5	10.2	10.9	8.6	6.9	7.3	10.3	10.2	10.3	13.8	
19	*A. anderseni*	10.5	10.6	10.5	10.4	10.3	10.6	10.0	8.5	7.5	7.7	10.9	9.7	10.7	9.4	8.5	8.0	13.0	10.9

the Maracá Island with three species of large *Artibeus* (*A. lituratus*, *A. jamaicensis* = *A. planirostris* [?], and *A. fuliginosus* = *A. obscurus*). Nevertheless, most records are unvouchered and cannot be reliably confirmed (Lim and Engstrom 2001). In another study carried out in Boa Vista, Roraima, but restricted for the urban region of the municipality, three large *Artibeus* (*A. lituratus*, *A. obscurus*, and *A. planirostris*) were recorded (Capaverde-Jr et al. 2014). Redondo et al. (2008) gave insufficient information on two individuals of *A. amplus* from municipality of Barcelos, state of Amazonas, near the border with Venezuela. The municipality of Barcelos has an area of 122,476 km^2 (almost the size of Suriname, at 163,820 km^2) and its urban center is located 300 km in a straight-line northwest of the border with Venezuela. On the map, we indicate the collection sites based on the coordinates of the municipality (Fig. 1). We were also unable to identify the collection referenced in the appendix of the voucher specimens (RZ019, RZ044). The reported presence of *A. amplus* in Barcelos had not been previously considered for Brazil (see Garbino et al. 2022 for an updated list of bats in Brazil).

The external and cranial measurements of the specimens from Roraima are within the known range of the species, except for the metacarpals and phalanges, which were larger than those observed by Guerrero et al. (2003) for specimens from Colombia and Venezuela. The wing membrane of the specimens is almost entirely dark, as shown by Lim et al. (2003). However, our specimens show a small transparent margin, with the third phalanx of the third metacarpal being very clear. This feature had been mentioned by Handley (1987) in the original description: the wing tips are undifferentiated or grayish, never white. Here, we draw attention to the need to consider this detail for a reliable identification in the field.

Artibeus amplus occurs in a variety of habitats, although most records have been in forested areas, as observed in the present study. Ramoni-Perazzi et al. (2012) presented some scenarios with potential distribution sites for the species based on the best habitat conditions (maximum entropy niche modeling). The authors noted that northern Brazil is an area with a great potential for the occurrence of this species, particularly in forested areas with potential roosts (caves). This modeling was based on specimens obtained from a scientific collection and did not include the record from Barcelos (Redondo et al. 2008). In their study, Ramoni-Perazzi et al. (2012) predicted a wide potential distribution area for *A. amplus* in Brazil, including the extreme north and northwest of the state of Roraima and the north of the state of Pará. We agree with these authors that there is a need to review museum specimens from the northern portion of Brazil, which may reveal new areas of distribution for the species. We believe that some specimens previously identified as *A. planirostris* may, in fact, be misidentifications of *A. amplus*.

The holotype of *A. amplus* was collected in a cave in the state of Zulia, Venezuela (Handley 1987). Because caves are the only type of refuge known to date for this species, Ramoni-Perazzi et al. (2012) assumed that the presence of these natural chambers may influence the distribution of the species. Based on the biology of other large *Artibeus*, we disagree with this assumption. Many large *Artibeus* may even occasionally use caves as shelter, although the use of foliage is more common for several species (Zortéa and Chiarello 1994, Ortega and Castro-Arellano 2001, Haynes and Lee 2004, Hollis 2005). The authors presented data from a 30-year investigation conducted in caves in northern Venezuela, with no records of *A. amplus* (Ramoni-Perazzi et al. 2012). There are not many caves in the state of Roraima (Jansen et al. 2012) and we hypothesize that this type of shelter is not important for the species and therefore is unlikely to limit its distribution.

The results of our phylogenetic analysis agree with previous publications in confirming the monophyly of the three subgenera of *Artibeus* (Fig. 3). However, the position of the subgenus *Koopmania*, which appears as a sister clade of the subgenus *Artibeus* in our analysis, conflicts with previous studies that found that the position of *A. concolor* varies according to the phylogenetic method, gene, or number of samples used (Redondo et al. 2008). Other studies using the cytochrome b also showed the subgenus *Koopmania* as the sister group of the subgenus *Artibeus* (Lim et al. 2004). The phylogenetic reconstruction provides support for the monophyly of all *Artibeus* species analyzed, as previously reported (Larsen et al. 2007, Redondo et al. 2008). The position of *A. amplus* as a sister species to *A. planirostris* corroborated the findings of a previous study using the cytochrome b (Redondo et al. 2008), but not the results of another study with the same molecular marker (Lim et al. 2004). Our sequence sample of *A. amplus* includes samples from Venezuela, Guyana, and the Brazilian states of Amazonas (Barcelos municipality) and Roraima (Caracaraí and Cantá municipalities). Despite its proximity to Barcelos and Guyana, the sequence from Cantá is more closely related to one of the two sequences from Venezuelan than to the sequences from Barcelos and Guyana, indicating that the *A. amplus* population is genetically uniform. This result clearly shows that specimens from the Brazilian Roraima belong to *A. amplus*.

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Data Resources

All sequences generated were deposited in the GenBank under the accession number OQ918092 (CJ1206) and OQ918093 (CJ1176).

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