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Second record of *Lasiurus ebenus* (Chiroptera, Vespertilionidae), with comments on its taxonomic status

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Abstract

Lasiurus ebenus was known only from the holotype, which was collected in 1991, in an Atlantic Forest remnant of Ilha do Cardoso State Park, southeastern Brazil. The species was described based on qualitative and quantitative morphological features. Since its original description, based on a single individual, the taxonomic status of *Lasiurus ebenus* has been questioned. Here we report a second record for the species that comes from Carlos Botelho State Park, São Paulo, ca. 100 km north from the type locality. This new record allowed us to confirm the validity of the species, by presenting additional data that fits in the distinction from sympatric congeners proposed on the original description of *L. ebenus*.

Key words: Atlantic Forest, Lasiurini, morphology, taxonomy

Introduction

Lasiurus Gray, 1831 has been traditionally recognized as the only genus of the tribe Lasiurini, family Vespertilionidae (Simmons 2005). There are three species groups recognized based on morphology: hoary, red, and yellow bats (Baker *et al.* 1988). Molecular phylogenies have corroborated the monophyly of the genus, and also of the three morphology-based species groups (Hoofer & Van Den Bussche 2003; Roehrs *et al.* 2010). These groups were tentatively arranged into two subgenera (*Dasypterus* [yellow bats], *Lasiurus* [hoary and red bats]; Simmons 2005), or three genera (*Lasiurus* [red bats], *Dasypterus* [yellow bats], and *Aeoreastes* [hoary bats]; Baird *et al.* 2015; 2017), but the debate is still open (see Ziegler *et al.* 2016; Novaes *et al.* 2018). Here we follow Gardner & Handley (2008) in recognizing *Lasiurus* as the only genus of Lasiurini.

The genus comprises 17 species of aerial insectivorous bats (Simmons 2005), and is widely distributed in the New World, occurring from Canada southward to Chile and southern Argentina, and also in the remote islands of Galapagos and Hawaii (Simmons 2005). Nine South American species are currently recognized (Gardner & Handley 2008), 7 of which occur in Brazil (Nogueira *et al.* 2014): *L. cinereus* (Palisot de Beauvois), *L. blossevillii* ([Lesson]), *L. ega* (Gervais), *L. egregius* (Peters), *L. salinae* Thomas, *L. castaneus* Handley and *L. ebenus* Fazzolari-Corrêa.

Lasiurus ebenus was described based on a single individual collected in 1991 in the Atlantic Forest of Ilha do Cardoso, southeastern Brazil (Fazzolari-Corrêa 1994). *Lasiurus ebenus* is known only from the holotype (Gregorin 2008), the species is absent in the latest Brazilian Red List (ICMBIO 2014), and classified as data deficient in the IUCN Red List of Threatened Species and in the last assessment of the threatened mammals of São Paulo state (Sampaio *et al.* 2016). The species is morphologically closer to *L. blossevillii* (see Fazzolari-Corrêa 1994), but differs from this and other Lasiurini by the face, muzzle, ears, lips, and membranes almost entirely black; bone-

brown hairs on the uropatagium and ventral wing membranes; and two upper premolars in each side, with the anterior premolar minute and medially displaced (Fazzolari-Corrêa 1994; Gardner & Handley 2008). *Lasiurus ebenus* has not been phylogenetically positioned yet, and although morphologically distinct from other congeners, its taxonomic status has been questioned due to it being known only from the holotype and also because the distinction between *L. ebenus* and *L. blossevillii* is mainly based on the coloration, which is highly variable (Moratelli 2016).

Based in field research conducted in an Atlantic Forest remnant close to the type locality of the species, we report the second specimen of *L. ebenus*. After examination of its holotype and vouchers from other congeners that occur in sympatry, we confirm its taxonomic status as a species distinct from other Lasiurini.

Materials and methods

Our specimen was compared with representatives of *Lasiurus blossevillii*, *L. cinereus*, and *L. ega*, which are deposited in the following institutions: Coleção Adriano Lucio Peracchi, Universidade Federal Rural do Rio de Janeiro (ALP; Seropédica, RJ, Brazil); Museu Nacional, Universidade Federal do Rio de Janeiro (MN; Rio de Janeiro, RJ, Brazil); and Museu de Zoologia da Universidade de São Paulo (MZUSP; São Paulo, SP, Brazil).

The second specimen of *L. ebenus* is an adult male, collected on February 28 2017, in the Carlos Botelho State Park, São Paulo, Brazil, under the permits SISBIO/ICMBIO 54.381-1/2016 and COTEC/SMA-IF 260108-006.479/2016. This locality is a large remnant in the Atlantic Forest of Southeastern Brazil (São Paulo 2008). The specimen was captured in a mist net set across a stream ($24^{\circ}10'S$, $47^{\circ}58'W$, elev. 630 m) in the central area of the park. It was preserved in fluid (alcohol 70°GL) with the skull removed. Body and skull are deposited in the Museu Nacional (MN 83982).

Qualitative description and comparison follow characters used by Fazzolari-Corrêa (1994) and Gardner & Handley (2008). For morphometric description and comparisons, measurements (from adults only) were taken with a digital caliper. External measurements are based on Fazzolari-Corrêa (1994): total length (TL), tail length (TA), hind foot length (HF), ear height (EA), tragus length (TR), forearm length (FA), tibia length (TB), calcar length (CL), length of phalanx I of the third finger (TP1), length of phalanx II of the third finger (TP2), length of phalanx III of the third finger (TP3), length of dorsal fur (LDF). Skull measurements follow Fazzolari-Corrêa (1994), Simmons & Voss (1998), and Velazco *et al.* (2010): greatest length of skull (GLS), condylo-incisive length (CIL), condylo-canine length (CCL), postpalatal length (PPL), maxillary toothrow length (MTL), palatal breadth (PB), mastoid breadth (MAB), zygomatic breadth (ZB), interorbital breadth (IOB), braincase breadth (BCB), palatal breadth across outside borders of M3 (PM3), palatal width at canines (PAW), mandibular toothrow length (MANDL), dentary length (DENL), and coronoid height (COH).

Principal Component analysis (PCA) was used to summarize patterns of size and shape variation and to compare skull morphology. For this analysis a subset of the skull dimensions was selected (GLS, CIL, MTL, PB, MAB, IOB, BCB, PM3, PAW, MANDL, DENL, COH) to represent different axes of length and width of the skull and mandible. This analysis included the two known specimens of *L. ebenus* and representatives of the other three species of *Lasiurus* that occur in southeastern Brazil (*L. blossevillii*, *L. cinereus* and *L. ega*; see Appendix 1). The PCA was performed in R software using MASS and Lattice packages (R Development Core Team 2012). As this analysis require complete datasets, missing values (<5% of total data set) were estimated from the existing raw data using the Amelia II package (Honaker *et al.* 2011) implemented in R software. Measurements were log-transformed and covariance matrices were computed considering all variables.

Results

Our specimen of *Lasiurus ebenus* (MN 83982) has wing membranes black, pelage almost entirely black, and two upper premolars on each side (Figs. 1, 2). This set of traits allows us to identify it as *L. ebenus* according to the diagnosis proposed by Fazzolari-Corrêa (1994) and the Gardner & Handley's (2008) identification key.

Linear measurements of MN 83982 are close to those of the holotype of *L. ebenus* (Table 1). Additionally, the results of the PCA comparing MN 83982 with representatives of other species that occur in sympatry reveal that this specimen and the holotype of *L. ebenus* are morphologically and morphometrically closer to each other than to

any other representative of congeners that occur in southeastern Brazil, corroborating Fazzolari-Corrêa (1994) proposition that it is a valid species (Fig. 3A). In the PCA, the first principal component (PC1) represents skull size according to the positive loadings of all variables (Fig. 3B). This axis accounted for 94% of the total variation (Fig. 3B, Table 2), and reveals that *L. ebenus* is larger than *L. blossevillii* and smaller than *L. cinereus* and *L. ega*. The two representatives of *L. ebenus* are fully distinct from representatives of all species in sympatry across the two axes (Fig. 3B).



FIGURE 1. Ventral (A) and dorsal (B) views of the adult male of *Lasiurus ebenus* (MN 83982) from Carlos Botelho State Park, São Paulo, Brazil.

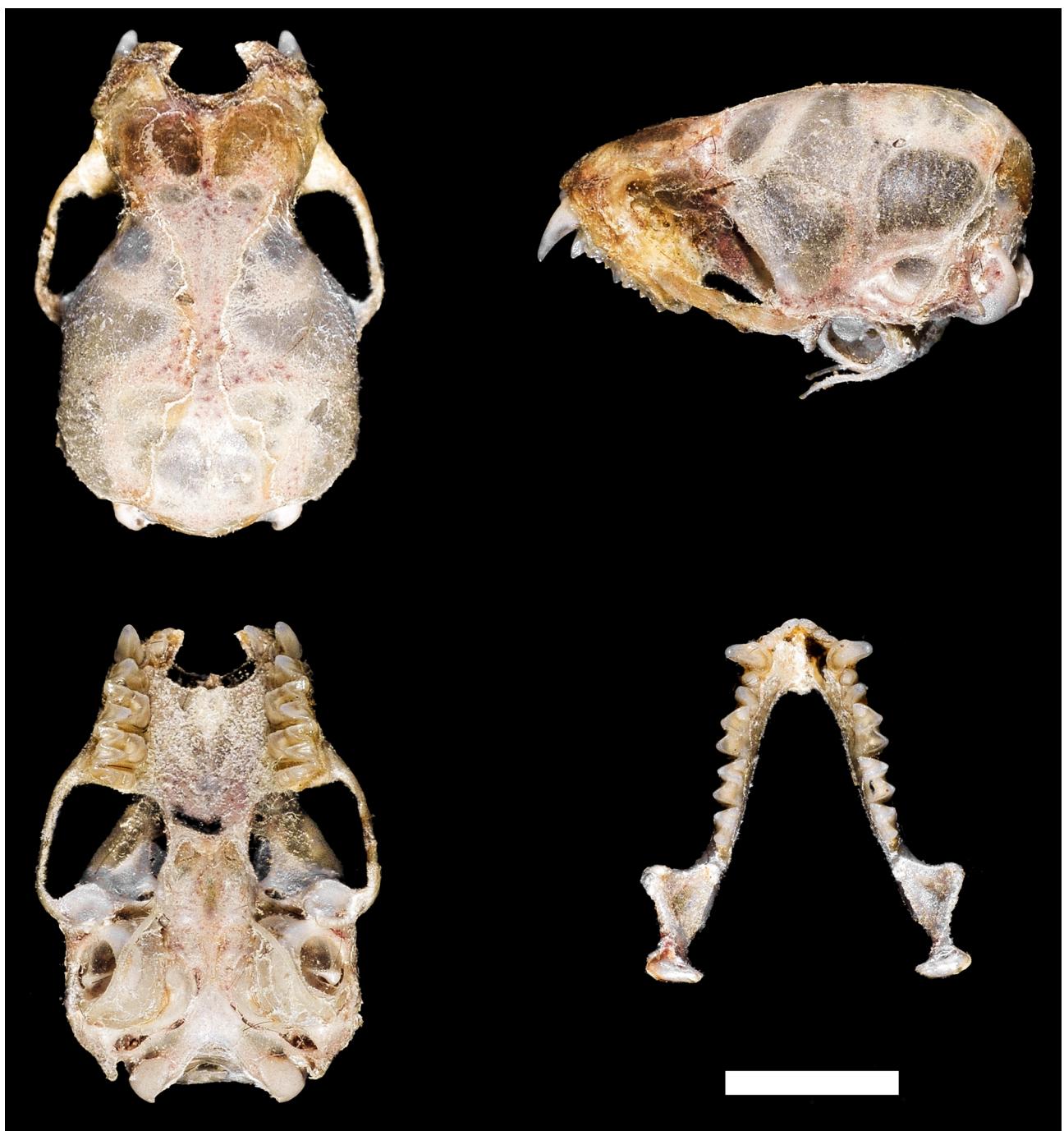


FIGURE 2. Dorsal, ventral and lateral views of the skull, and dorsal view of the mandible of the *Lasiurus ebenus* from Carlos Botelho State Park, São Paulo, Brazil (MN 83982). Scale bar = 5 mm. See Table 1 for measurements.

Based on the results of our qualitative and quantitative analyses, we confirm MN 83982 as a representative of *L. ebenus*. We provide below a short description of the species based on the two specimens available (the holotype and MN 83982), and provide a comparison with congeners that occur in sympatry.

***Lasiurus ebenus* Fazzolari-Corrêa, 1994**

Black hairy-tailed bat

Holotype. Adult male collected at Parque Estadual da Ilha do Cardoso ($25^{\circ}05'S$, $47^{\circ}59'W$, elev. 40 m), São Paulo, Brazil (Fazzolari-Corrêa 1995). Skin, partial skeleton and skull are deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP 28125).

Other specimen. Adult male (MN 83982) composed by whole body (in alcohol) with the skull removed, collected at Parque Estadual Carlos Botelho (24°10'S, 47°58'W, elev. 630 m), São Paulo, Brazil.

Diagnosis. Black wing membranes; dorsal and ventral pelage almost entirely black; first upper premolar present; and medium sized within the genus, with forearm length close to 45.5 mm.

TABLE 1. External and skull measurements (in millimeters) of the two known specimens of *Lasiurus ebenus* and the three sympatric species. See Material and Methods for character abbreviations.

Measurements	<i>Lasiurus ebenus</i>		<i>Lasiurus blossevillii</i>	<i>Lasiurus cinereus</i>	<i>Lasiurus ega</i>
	MZUSP 28125 (holotype)	MN 83982 (new record)			
TL	115.5	112.2	—	—	—
TA	58.1	50.1	—	—	—
HF	8.0	8.2	—	—	—
EA	12.4	15.3	—	—	—
TR	6.0	7.5	—	—	—
FA	45.7	45.6	39.0 (36.8–42.2) 11	51.6 (50.3–52.55) 3	47.2 (43.9–49.4) 9
TB	21.2	21.4	—	—	—
CL	15.3	14.1	—	—	—
TP1	48.2	45.0	—	—	—
TP2	18.2	20.0	—	—	—
TP3	24.6	21.1	—	—	—
LDF	6.5	6.5	—	—	—
Body mass	14.0	12.5	—	—	—
GLS	13.52	13.90	12.06 (11.66–12.83) 11	15.49 (14.92–16.06) 2	16.24 (14.82–17.15) 9
CIL	13.56	13.93	11.73 (11.27–12.40) 11	14.80 (14.72–14.85) 3	14.96 (14.29–15.92) 9
CCL	13.72	14.12	11.53 (10.83–12.11) 11	13.94 (13.21–14.80) 3	14.64 (13.25–15.63) 9
PPL	5.80	6.38	—	—	—
MTL	4.81	4.70	3.93 (3.67–4.16) 11	5.55 (5.48–5.61) 3	5.60 (5.35–5.92) 9
PB	2.68	2.80	2.19 (1.95–2.44) 11	3.12 (2.74–3.36) 3	2.64 (2.37–2.98) 9
MAB	8.54	8.38	7.46 (7.12–7.83) 11	9.48 (9.42–9.54) 2	8.85 (8.51–9.31) 8
ZB	10.30	9.91	8.62 (8.41–8.75) 6	11.18 (11.13–11.23) 2	11.25 (10.58–11.95) 7
IOB	4.56	4.64	4.36 (4.22–4.49) 11	5.25 (5.12–5.36) 3	4.66 (4.32–4.86) 9
BCB	8.18	8.28	7.23 (6.96–7.48) 11	8.77 (8.56–8.92) 3	8.38 (7.92–8.63) 9
PM3	6.82	6.94	5.51 (5.14–5.95) 11	7.82 (7.78–7.90) 3	7.49 (7.11–8.08) 9
PAW	5.59	5.52	4.33 (4.10–4.73) 11	6.12 (5.75–6.45) 3	6.19 (5.90–6.62) 9
MANDL	5.57	5.53	4.64 (4.47–4.81) 9	6.38 (6.35–6.42) 2	6.43 (6.24–6.74) 8
DENL	10.06	9.58	8.58 (8.32–8.89) 9	11.65 (11.53–11.77) 2	11.69 (10.91–12.31) 8
COH	3.77	3.28	3.12 (2.99–3.20) 8	3.91 (3.82–4.00) 2	4.87 (4.67–5.36) 7

Description. *Lasiurus ebenus* is a medium-sized species (body mass 12.5 g, 14 g; forearm length 45.6 mm, 45.7mm; other measurements are in Table 1). Membranes, muzzle, lip and ear borders are black. One-third to one-half of the proximal portion of the interfemoral membrane and the ventral region of the humeri and forearms have bone-brown to dark-brown hairs. Ears are broad and rounded; tragus is triangular and measures ca. 50% of the height of the ear; hindfoot small, less than two-thirds of the length of the tibia; the calcar is about twice as long as the hindfoot, and about as long as the free margin of the interfemoral membrane. Pelage color is black in general, with dorsal fur tricolored (black basal band, bone-brown to dark-brown in the middle, black tips), and ventral fur bicolored (ca. 2/3 of the basis is bone-brown, and 1/3 of the tip is black); without stripes, frosting or spots (Fig. 1).

Dental formula is i 1/3 c 1/1 p 2/2 m 3/3 = 32. The second premolar is double-rooted, the rostrum is relatively short, and the braincase is broad, with a low sagittal crest.

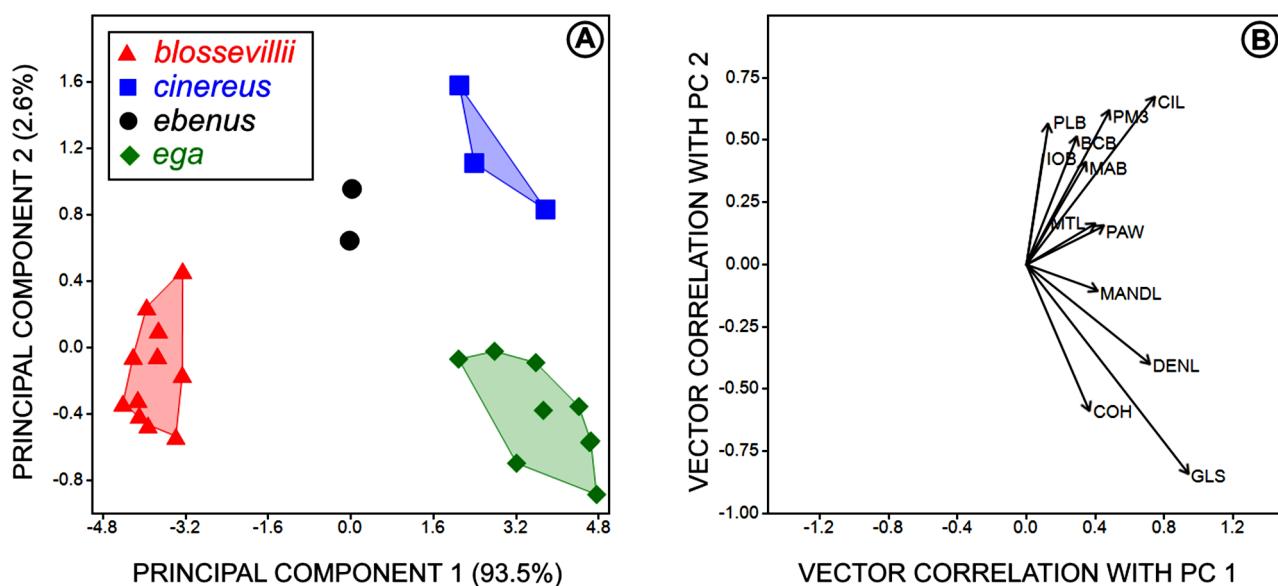


FIGURE 3. Plots of multivariate individual scores of samples of *Lasiurus blossevillii*, *L. cinereus*, *L. ebanus*, and *L. ega* in the first two principal components (A). Corresponding vector correlations of craniometric characters with the first two eigenvectors of the principal components (B). See Table 2 for vector correlation coefficients between original variables and principal components (PC1 and PC2).

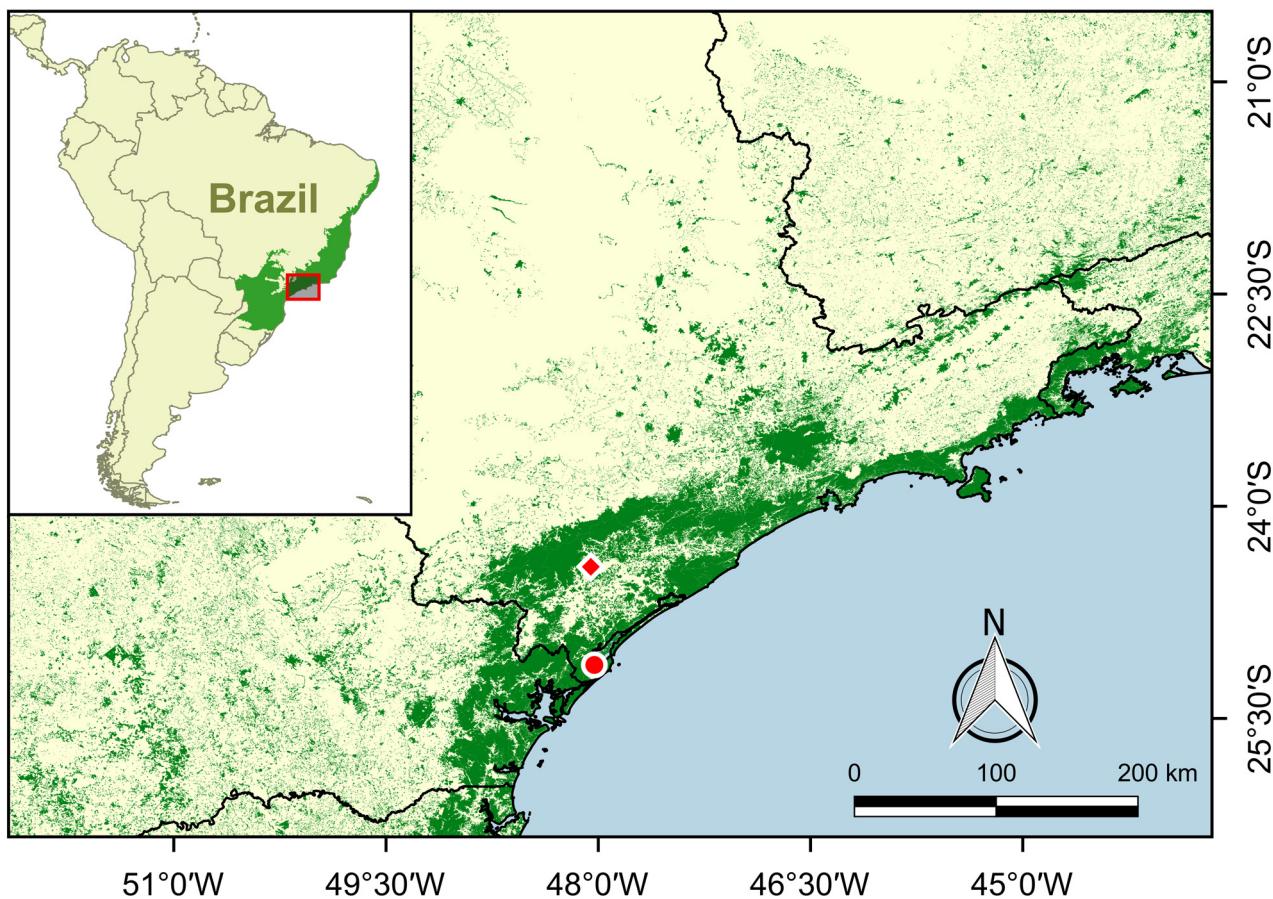


FIGURE 4. Occurrence localities for *L. ebanus* in São Paulo, Brazil: type locality in the Ilha do Cardoso State Park (circle); new record (MN 83982) for the Carlos Botelho State Park (diamond). The green area represents the Atlantic Forest remnants.

TABLE 2. Vector correlation coefficients (loadings) between original variables and principal components (PC1 and PC2) for representatives of *Lasiurus blossevillii*, *L. cinereus*, *L. ebenus*, and *L. ega* from southeastern Brazil.

Variables	PC1	PC2
DENL	0.540	-0.482
MANDL	0.425	0.386
COH	0.222	0.091
GLS	0.073	0.325
CIL	0.195	0.231
MAB	0.055	0.256
BCB	0.166	0.292
IOB	0.275	0.353
PAW	0.250	0.086
PM3	0.233	-0.058
MTL	0.407	-0.226
PLB	0.207	-0.333

Comparisons. Rostrum short, sagittal crest weak, first upper premolar (P1) present, and fourth upper premolar (P4) double rooted indicate that *L. ebenus* belongs to the red bats group (see Baird *et al.* 2015), which also includes *L. atratus*, *L. blossevillii*, *L. borealis*, *L. castaneus*, *L. degelidus*, *L. egregius*, *L. minor*, *L. pfeifferi*, *L. seminolus*, and *L. varius* (see Baird *et al.* 2015; Fazzolari-Corrêa 1994). This group is represented in Brazil by *L. blossevillii*, *L. castaneus*, *L. ebenus* and *L. egregius* (see Nogueira *et al.* 2014). *Lasiurus ebenus* can be distinguished from these species by the pelage almost entirely black, and wing membranes black, which are diagnostic for the species. The pelage is reddish above and paler below in *L. blossevillii*; chestnut above and dark-brown below, with buffy-yellow and whitish patches on shoulders in *L. castaneus*; and reddish above and dark brown below, with bright red tips in *L. egregius*. *Lasiurus ebenus* (45.6 mm, 45.7 mm) is larger than *L. blossevillii* (forearm length: ~ 42 mm) and similar in size to *L. castaneus* (~ 45 mm) and *L. egregius* (forearm larger than 48 mm). Beyond fur color, *L. ebenus* can be distinguished from *L. castaneus* in the hindfoot length (11 mm in *ebenus*, 8 mm in *castaneus*), and the braincase breadth (7.6 mm in *ebenus*, 8.2 mm in *castaneus*; see Bianconi & Pedro, 2007; Fazzolari-Corrêa 1994; Handley 1960; Reid 2009).

Lasiurus cinereus and *L. ega* also occur in Brazil (see Nogueira *et al.* 2014). The pelage of *L. ega* varies from pale whitish-buff to yellowish and orange, with ventral fur generally paler. *L. cinereus* has dorsal fur tricolored (basis and tips dark-brown, intermediate band yellowish, with a frosting appearance in general), and ventral fur bicolored (basis dark-brown, tips light-brown). These species are allocated in other morphological groups, and can be also distinguished from *L. ebenus* by a suite of qualitative and quantitative traits (Barquez *et al.* 1999; González 1989; Kurta & Lehr 1995; Vieira 1942).

Distribution. *L. ebenus* is known from two localities in the south portion of the Serra do Mar mountain chain (Fig. 4), which is the largest remnant of Atlantic Forest in southeastern Brazil. Both localities are composed by ombrophilous dense forest. The holotype comes from Ilha do Cardoso State Park, Cananéia, São Paulo, Brazil (25°05'S, 47°59'W, elev. 40 m); and the other specimen (MN 83982) comes from Carlos Botelho State Park, Sete Barras, São Paulo, Brazil, on the eastern slope of the Serra do Mar mountain chain (24°10'S, 47°58'W, elev. 630 m). The second record for the species is 101 kilometers away from the type locality (Fig. 4).

Natural history. The holotype of *L. ebenus* was captured in a mist net placed over a stream in continuous forest. MN 83982 was captured on the lowest shelf of the mist net, which was about 1 m above the water. The water level was about one foot deep, and the substrate is composed by rocks and sand in a lotic water system. Representatives of other species of *Lasiurus* are often netted in similar conditions, feeding on insects that fly close to watercourses (Handley 1960, 1996; Kurta & Lehr 1995; Villalobos-Chaves & Dick 2014). We speculate that *L. ebenus* forages on watercourses and streams, catching insects in flight, similarly to its congeners. Bat flies collected in the holotype of *L. ebenus* were later described by Graciolli (2003) as a new species, *Basilia insularis*, from which the only reported host is still *L. ebenus* (Graciolli *et al.* 2007).

Discussion

The diagnostic qualitative characters proposed for *L. ebenus* are present in the MN 83982. In addition, our quantitative analyses show that *L. ebenus* is morphometrically distinct from its congeners that occur in the region. Although the species has not been included in any phylogenetic study of the group, *L. ebenus* has been treated as part of the Red Bats group (Baird *et al.* 2015; Fazzolari-Corrêa 1994), and we think it is reasonable to assume the species is indeed part of this group, even without a molecular phylogeny.

This record is 26 years apart from the only other known record of *L. ebenus* that was captured in 1991 (Fazzolari-Corrêa 1994, 1995). Considering that substantial sampling efforts have been conducted in the southern portion of the Serra do Mar mountains and adjacent regions close to the type locality (e.g., Arnone 2008; Kaku-Oliveira 2010; Passos *et al.* 2003; Portfors *et al.* 2000; Taddei & Pedro 1996; de Vivo & Gregorin 2001), we speculate that *L. ebenus* is rarely sampled using the traditional mist nets. Additionally, the genus seems to be uncommon on the state of São Paulo (Fazzolari-Corrêa 1994), the only congener recorded on the region is *L. ega* (Garbino 2016) and only *L. ebenus* was captured on Carlos Botelho State Park.

The collection of the individual representing the second record of the species was necessary to address the taxonomic status of *L. ebenus*. Moreover, the collection of vouchers for alpha taxonomy is well covered for many groups, any previous molecular data is available for the species and only one preserved voucher is available, which is in accordance to the guidelines established by Russo *et al.* (2017). Finally, the impact of the removal of this individual on the population is minimal because if collecting a voucher would push the species to extinction, then the species or population is already lost (Moratelli 2014).

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APPENDIX. Localities of *Lasiurus* specimens from Brazil examined here. Collections acronyms are in Material and methods. Localities are arranged by species and alphabetically by major political unities (State: cities, protected area). All specimens were included in the multivariate analyses.

Lasiurus blossevillii: Ceará: Crato, Floresta Nacional do Araripe (MZUSP 18722–18725); Minas Gerais: Santo Antônio da Vargem Alegre (MZUSP 1319); São Paulo: Piracicaba (MZUSP 287), São Paulo (MZUSP 2448), São Vicente (MZUSP 6555); Southeastern Brazil, locality non-specified (MN 3374, 3594).

Lasiurus cinereus: Rio de Janeiro: Seropédica (ALP 3197); Rio Grande do Sul: Porto Alegre (MN 3466); São Paulo: São Paulo (MZUSP 1325).

Lasiurus ebenus: São Paulo: Sete Barras, Parque Estadual Carlos Botelho (MN 83982), Cananéia, Parque Estadual da Ilha do Cardoso (MZUSP 28125 [holotype]).

Lasiurus ega: Ceará: Crato, Floresta Nacional do Araripe (MZUSP 18728, 18729); Distrito Federal: Brasília (MN 3613); Goiás: Alexânia (MZUSP 18727); Minas Gerais: Lagoa Santa (MN 6529); Pernambuco: Exu (MZUSP 18730); Rio de Janeiro: Parque Nacional da Tijuca (MN 3659), Rio de Janeiro (MN 3548), Teresópolis (MN 3476).