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A new species of *Eptesicus* (Mammalia: Chiroptera: Vespertilionidae), from the sub-Andean Forest of Santa Cruz, Bolivia

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Bats of genus *Eptesicus* are represented in South America by nine species of short-eared taxa (subgenus *Eptesicus*), and 10 species of long-eared species (subgenus *Histiotus*). Here we describe a new species of short-eared *Eptesicus* based on 19 specimens collected in the sub-Andean Bolivian-Tucumanian forest of Santa Cruz, between 1800-2020 masl. For this, we include morphological, morphometric, and molecular comparisons; we use principal component, discriminant function and mitochondrial genes (cytochrome-*b*, cytochrome c oxidase subunit I, and nicotinamide adenine dinucleotide dehydrogenase) to compare the new species with other taxa of the subgenus *Eptesicus* from South America. The new species is distinguished from its congeners by cranial shape, body measurements, and genetic distances. Furthermore, the new species is similar in cranial morphology to *Eptesicus andinus* but presents a highly developed frontal preorbital process, poorly developed in other related species (*i. e.*, *E. andinus, E. furinalis,* and *E. brasiliensis*). All males were consistently darker than females in the new species. This taxon increases to 10 the number of species of bats of the subgenus *Eptesicus* in South America.

Los murciélagos del género *Eptesicus* en Sudamérica están representados por nueve especies de murciélagos de orejas cortas (subgénero *Eptesicus*) y 10 especies de orejas largas (subgénero *Histiotus*). Describimos una nueva especie de *Eptesicus* de orejas cortas, con base en 19 especímenes, que fueron colectados en el Bosque Boliviano-Tucumano del subandino de Santa Cruz, entre los 1800-2020 msnm. Para esto, incluimos comparaciones morfológicas, morfométricas y moleculares; utilizamos los análisis de componente principal, función discriminante y genes mitocondriales (citocromo-b, citocromo c oxidasa subunidad I y nicotinamida adenina dinucleótido deshidrogenasa) para comparar la nueva especie con otros taxones del subgénero *Eptesicus* de Sudamérica. La nueva especie se distingue de sus congéneres por su forma craneal, medidas corporales, dicromatismo sexual notable y las distancias genéticas. Además, la nueva especie es similar en la morfología craneal con *Eptesicus andinus*, pero esta presenta un proceso preorbital frontal muy desarrollado el cual es pobremente desarrollado en las especies relacionadas (*i. e.*, *E. andinus, E. furinalis* y *E. brasiliensis*). Todos los machos en la nueva especie fueron evidentemente más oscuros que las hembras. Este nuevo taxón aumenta a 10 el número de las especies del subgénero *Eptesicus* en Sudamérica.

Keywords: Andes; cryptic diversity; cyt-*b*; COI; *Histiotus*; morphology; ND1.

Introduction

The genus *Eptesicus* Rafinesque, 1820 in South America comprises 19 species. These 19 species are grouped into two subgenera. The subgenus *Eptesicus* includes nine species and several subspecies (Miranda *et al.* 2006; Davis and Gardner 2008; Díaz *et al.* 2016; Sánchez *et al.* 2019): *Eptesicus andinus* Allen, 1914 (monotypic), *Eptesicus brasiliensis* (Desmarest, 1819; with four subspecies), *Eptesicus chiriquinus* Thomas, 1920 (monotypic), *Eptesicus diminutus* Osgood, 1915 (with two subspecies), *Eptesicus furinalis* (d'Orbigny and Gervais, 1847; with two subspecies), *Eptesicus fuscus* (Palisot de Beauvois, 1796; one subspecies), *Eptesicus innoxius* (P. Gervais, 1841; monotypic), *Eptesicus taddeii* Miranda, Bernardi and Passos 2006 (monotypic), and *Eptesicus ulapesensis* Sánchez, Montani, Tomasco, Díaz and Barquez 2019 (monotypic). The subgenus *Histiotus* endemic to South America includes the other 10 species (Rodríguez-Posada *et al.* 2021). *Histiotus* has been considered by some authors as a subgenus of *Eptesicus* (Hoofer and Van Den Bussche 2003; Giménez *et al.* 2019; Simmons and Cirranello 2020), but this suggestion has not been followed by other authors (see Burgin *et al.* 2018; Moratelli *et al.* 2019; Barquez and Díaz 2020). Historically, Neotropical bats of the subgenus *Eptesicus* have been defined as species complexes based on fur variability. For example, Davis (1966) identified three long-haired species: *E. andinus* with two subspecies (*E. inca* and *E. chiriquinus*), *E. montosus* with two subspecies (*E. montosus* and *E. chiralensis*), and *E. fuscus* (monotypic). However, Simmons and Voss (1998) reviewed the holotypes of *E. andinus* and *E. chiralensis* and topotypic specimens of *E. inca* and *E. chiriquinus*, concluding that *E. chiralensis* and *E. andinus* are conspecifics and similarly, *E. inca* and *E. chiriquinus* are also conspecific with *E. chiriquinus* having priority over *E. inca*. Simmons and Voss (1998) suggested that *E. andinus* and *E. chiriquinus* can be differentiated by skull shape and the arrangement of the sagittal and nuchal ridges, as well as differences in size (*E. chiriquinus* tends to be larger than *E. andinus*).

Five species of the subgenus *Eptesicus* currently occur in Bolivia: *E. andinus*, *E. brasiliensis*, *E. chiriquinus*, *E. diminutus*, and *E. furinalis* (Anderson 1997; Siles 2007; Vargas-Espinoza 2007; Aguirre *et al.* 2010, 2019; Poma-Urey *et al.* 2019); being one of the countries with the highest diversity of the subgenus in South America. Despite that, there are several, information gaps related to the distribution and richness of the genus remain (Poma-Urey *et al.* 2019). Here, we describe a new species of the subgenus *Eptesicus* based on 19 specimens collected in the sub-Andean Bolivian-Tucumanian forest of Santa Cruz, and by comparisons with specimens from other localities of South America. The new species is morphologically similar to *E. andinus* but can be differentiated from *E. andinus* and its congeners by genetic distances, discrete morphological and morphometric traits.

Material and Methods

A total of 19 specimens of bats of subgenus *Eptesicus* were collected during field trips between 2007 and 2013 in two localities in the Province of Florida, Santa Cruz Department, Bolivia (Figure 1). To assess their specific identity, 131 specimens belonging to five species of *Eptesicus* with confirmed presence in Bolivia were also examined*:* *E. andinus* (*n* = 39), *E. brasiliensis* (*n* = 5), *E. chiriquinus* (*n* = 21), *E. diminutus* (*n* = 10), and *Eptesicus furinalis* (*n* = 56). The specimens reviewed are housed at the following institutions: American Museum of Natural History, New York, USA (AMNH); British Museum Natural History, London, England (BMNH); Colección de Mamíferos Lillo, Tucumán, Argentina (CML); The Field Museum of Natural History, Chicago, USA (FMNH); the Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia (ICN); Noel Kempff Mercado Natural History Museum, Santa Cruz, Bolivia (MHNNKM); Museo de Historia Natural Universidad del Cauca, Popayán, Colombia (MHNUC); Museo de Historia Natural, Universidad de Caldas, Manizales, Colombia (MHN-UCa); Museo Universidad Distrital Francisco José de Caldas, Bogotá, Colombia (MUD); Colección Teriológica Universidad de Antioquia, Medellín, Colombia (CTUA); Colección Mastozoológica Universidad del Valle, Cali, Colombia (UV), and the National Museum of Natural History, Washington, DC., USA (USNM; Appendix 1).

Several external, and cranial characters described in the literature (Simmons and Voss 1998; Davis and Gardner 2008; Díaz *et al.* 2016) were analysed in all the specimens reviewed, including the development of the sagittal and lambdoidal ridges, and the length and colour of the coat hairs (according to Ridgway 1912), and compared with the information provided in the literature (*e. g.*, Davis 1966).

Morphometric analysis. Nineteen cranio-dental measurements from 149 specimens were explored. These measurements included: greatest length of the skull (GLS), condylo-canine length (CCL), basicraneal length (BL), palatal length (PAL), postorbital constriction (POC), braincase height (BCH), braincase breadth (BCB), mastoid breadth (MB), zygomatic breadth (ZB), interorbital breadth (IOB), breadth across canines (C-C), breadth across upper molars (M3-M3), maxillary toothrow length (C-M3), incisive length-M3 (I-M3), upper molariforms length (P-M3), lower canine-m3 length (c-m3), lower molariforms length (p-m3), mandibular length (MAL), mandibular height (MH; Simmons and Voss 1998; Ramírez-Chaves 2008). We also took seven external measurements (the first two from labels) including: total length (LT), length of tail (TL), length of hind foot (LF), length of ear (EL), length of forearm (FA), and two from the hairs: length of dorsal hair (PD) and length of ventral hair (PV). The cranio-dental measurements were taken with a 0.01 precision digital calliper, and then Log transformed for further normalization and analyses. To show the main measurements that separate the species into different groups, the data of the first two principal main components (PCA) were selected. To define the variability between the groups provided by the first two main components, a Discriminant Function Analysis (DFA; Brown and Wicker 2000) was performed, considering the first two DFAs. For both the PCA and DFA analyses, due to completeness only 14 cranio-dental measurements were used (GLS, BCH, BL, PAL, P-M3, MB, M3-M3, C-C, I-M3, C-M3, CCL, IOB, POC, BCB), and the analyses were calculated using the software PAST ver-

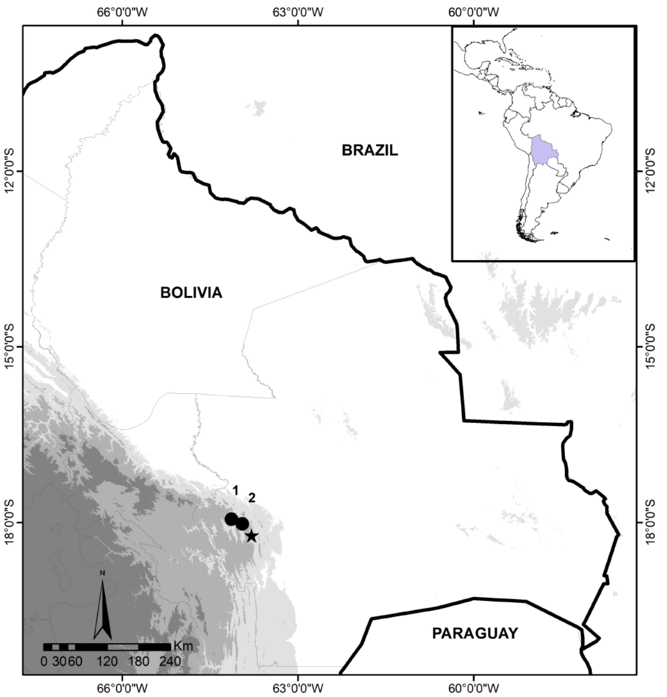

Figure 1. Collecting sites for *Eptesicus langeri* sp. nov. in South America. Municipalities of Samaipata and Pampagrande, Province of Florida, Department of Santa Cruz, Bolivia. Agua Rica Type locality (star), Reserve Municipal El Chape (circle 1) and Agua Clarita (circle 2).

sion 2.17c for Windows platform (Hammer *et al.* 2001). The PCA and DFA included four similar-sized species of *Eptesicus* (*E. andinus*, *E. brasiliensis*, *E. chiriquinus*, and *E. furinalis*), the holotypes of *E. montosus* Thomas, 1920, and *E. chiralensis* Anthony, 1926 (considered both junior synonyms of *E. andinus*), one smaller species (*E. diminutus*), and the 19 specimens of the Province of Florida in Bolivia. We also calculated the cranial index (CRI = (((POC + BCB) × GLS)/2) and the maxillary index (MXI = (((C-C + M3-M3) × C-M3)/2) (Baud and Menu 1993).

Molecular analyses. We extracted genomic DNA from muscle tissues preserved in 96 % ethanol from five specimens of *Eptesicus* collected in the sub-Andean Bolivian-Tucumanian forest of Bolivia, and two *E. andinus*, one *E. chiriquinus*, and one *E. furinalis* from Colombia. DNA were extracted using Wizard® Genomic DNA Purification kit (Promega Corporation) following the manufacturer´s protocol. Amplification of three mitochondrial genes was done as follows: the amplification of cytochrome-b (cyt-*b*) gene was performed using two pairs of primers. First pair of primers L14816: 5′-CCATCCAACATCTCAGCATGATGAAA-3′ and H15173: 5′-CCCCTCAGAATGATATTTGTCCTCA-3′ which amplifies a ≈358 bp fragment (Parson *et al.* 2000), and the other pair of primers LGL765F: 5´-GAAAAACCAYCGTTGTWATTCAACT-3´ (Bickham *et al.* 1995) and LGL766R 5´-GTTTAATTAGAATYTYAGCTTTGGG-3´, targeting a ≈1140 bp fragment (Bickham *et al.* 2004). Amplification of nicotinamide adenine dinucleotide dehydrogenase (ND1) gene using primers ND1-Forward 5´-CGCCATTATATGATCAGGATGAGCC-3´ and ND1-Reverse 5´-GTWGAGATRAATCATATTAT-3´ which amplifies a ≈293-295 bp fragment (Hamilton *et al.* 2015). The cytochrome c oxidase subunit I (COI) gene was amplified using the primer pair MCOIF 5′-CTGTACTTAGATTTACAGTCTAATGCC-3′ and MCOIR 5′-CCAAAGCCAGGCAAAATTAAAATATA-3′, which amplify a fragment of approximately 657 bp (Sánchez *et al.* 2019). The final amplification reaction volume was 30 µL, which contained 16.84 µL ultrapure water, 6 µL 5X buffer, 1.8 µL $MgCl_2$ (25 mM), 2.4 µL dNTP mix (10 mM), 0.36 µL of each primer (25 µM), 1.2 U of GoTaq Flexi DNA Polymerase (Promega), and 2 µL DNA (approximately 100–150 ng of DNA).

The amplifications were performed on a Techne TCPLUS thermocycler, according to the following conditions for the cyt-*b* and ND1 genes: initial denaturation of 3 min at 94 °C, followed by 35 cycles of 30 s of denaturation at 94 °C, 30 s of annealing at variable temperature depending on markers (between 46 °C and 50 °C) and 30 s of extension at 72 °C, and a final extension of 5 min at 72 °C. Initial denaturation at 95 °C for 5 min, followed by 5 cycles at 94 °C for 5 min, 46 °C for 1 min 30 s, and 72 °C for 1 min 30 s, followed by 35 cycles at 94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min, completing the reaction with a final extension cycle at 72 °C for 5 min, for the COI gene. The PCR products were quantified by fluorometry using a Quantus Fluorometer™ (Promega®) with the QuantiFluor® dsDNA System (Promega®), according to the manufacturer's protocol. PCR products were sent to Macrogen Inc. (South Korea) for purification and DNA sequencing. The sequenced fragments were evaluated and edited using Geneious Trial v8.14 (Drummond *et al.* 2009). To further compare sequence divergence, we downloaded sequences of closely related taxa of the subgenera *Eptesicus* and *Histiotus* available in GenBank (Appendix 1 and 7). As outgroup, we used *Myotis riparius* and *Neoromicia guineensis* (Appendix 1 A and B).

The sequences for each gene were aligned using ClustalW (Thompson *et al.* 1997), included in the program MEGA X (Kumar *et al.* 2018). Intraspecific and interspecific nucleotide divergences were estimated with the program MEGA X, using the Kimura 2-Parameter distance model (K2P; Kimura 1980) and 1,000 bootstrap replications. For *single and concatenated* sets of mitochondrial genes we selected the best-fitting models of sequence evolution, using the Akaike Information Criterion (AIC) calculated with ModelFinder (Kalyaanamoorthy *et al.* 2017) in PhyloSuite (Zhang *et al.* 2020). For the concatenated analysis, and the cyt-*b* gene (Appendix 1) we selected the GTR+F+I+G4 substitution model. Bayesian Inference (BI) analysis was conducted with MrBayes 3.2.6 (Ronquist *et al.* 2012), with four parallel runs, 2,000,000 generations, in which the initial 25 % of sampled data were discarded as burn-in.

Phylogenetic analyses for the ND1 and COI genes were inferred by using the Maximum Likelihood (ML) method with different evolutionary models (GTR+F+G4 for ND1, and TVM+F+R2 for COI). The ML analysis was conducted with IQ-TREE (Nguyen *et al.* 2015), 5,000 ultrafast bootstraps (Minh *et al.* 2013); as well as Shimodaira–Hasegawa–like approximate likelihood-ratio test (SH-like aLRT) for branches with 1,000 replicates (Guindon *et al.* 2010), all included in PhyloSuite platform (Zhang *et al.* 2020). Finally, we used the graphical viewer of phylogenetic trees FigTree v.1.4.3 (Rambaut 2007).

Results

Based on the review of 131 specimens of *Eptesicus andinus*, *E. brasiliensis*, *E. chiriquinus*, *E. diminutus* and *E. furinalis*, the 19 individuals from the Province of Florida, were assigned to the *andinus* group based on cranial morphology, *E. andinus* being the most morphological similar taxon. However, the specimens from Florida (Bolivia) showed a combination of discrete morphological characteristics that were not observed in any other *Eptesicus* known in South America. Among these, the presence of a well-developed frontal preorbital process allowed us to separate the specimens from Florida from specimens of *E. andinus*. The first two components of the PCA and DFA (Figure 2) account for 84.9 % and 84.7 % of the variation in the dataset, respectively (Table 1). PCA 1 accounted for 78.9 % of the variation, with all positive values, the highest values were given by the variables: GLS, CCL, I-M3 and C-M3. The second PCA explained 6.1 % of the variation; POC, BCB were among the variables with higher positive values, while the negative values were obtained for IOB, MB and PAL (Table 1).

The DFA shows that specimens from Province of Florida are part of the medium-sized *Eptesicus* along with *E. furinalis* and *E. andinus* (Figure 2). Although these species tend to overlap in cranial measurements, the variation observed in DFA 1 is positively influenced by the CCL, I-M3 and C-M3, while those that intervene negatively are BCB and C-C. For the DFA 2, the highest positive values are provided by MB, GLS, and I-M3, while the negative variables were IOB and M3-M3 (Table 1).

We obtained four cyt-*b* (GenBank accession numbers MW488942-MW488945), two COI (MW490595; MW490596], and four ND1 [MW488933-MW488936) individual sequences of specimens from the Bolivian-Tucumanian forest of Bolivia. A BLAST (Basic Local Alignment Search Tool) search of these cyt-*b* and ND1 sequences showed a range between 94.5 % and 92.9 % of identity with *E. furinalis* and *E. diminutus*. The specimens from Province of Florida, Bolivia, showed high cyt-*b* distances (Table 2) when compared with the cyt-*b* sequences of *E. chiriquinus* from Colombia (11 %), *E. andinus* (9.4 to 9.8 %), and *E. furinalis* (7.5 to 7.8 %). For the ND1 gene, the distances from *Eptesicus* from Bolivia were 8.7 to 8.8 % with *E. chiriquinus* (MHN-UCa 1951), 11.2 % with *E. andinus*, and 6.3 to 6.5 % with *E. furinalis* (Table 2). Similarly, the COI distances were between 9.7 to 12.7 % with *E. andinus*, and over 7.0 % with *E. chiriquinus* and *E. furinalis* (see Appendix 2). In addition, the Bayesian Inference and Maximum Likelihood consensus trees for the *single and concatenated* sets of mitochondrial genes show a monophyletic group conformed by the sequences of the Bolivian-Tucumanian forest of Bolivia specimens (Figure 3 and Appendix 1 to 3). Based on the results of the molecular and morphological analyses we described the Bolivian-Tucumanian forest specimens as a new species of *Eptesicus*.

Discussion

Our results show that, morphologically the Bolivian-Tucumanian forest specimens described here as *Eptesicus langeri* sp. nov. are part of the long-haired species of the subgenus *Eptesicus* that include high elevation Andean species such as *E. andinus* and *E. chiriquinus*, and cranially is similar to *E. andinus* rather to any other *Eptesicus* taxa. Most of the Neotropical *Eptesicus* have connected sagittal and nuchal ridges (in *E. brasiliensis*, *E. chiriquinus*, *E. furinalis*, *E. innoxius*, and *E. taddeii*), being *E. andinus* the exception, as this presents poorly developed ridges, creating a flattened and triangular space in dorsal view (Simmons and Voss 1998; Miranda *et al.* 2006; Tirira 2007; Ramírez-Chaves 2008). In addition, Neotropical *Eptesicus* have been grouped by hair length (Davis and Gardner 2008; Díaz *et al.* 2016; Sánchez *et al.* 2019), in short (less than 8.0 mm: *E. brasiliensis*, *E. furinalis*, *E. taddeii* and *E. ulapesensis*) and long-haired species (larger than 8.0 mm: *E. andinus* and *E. chiriquinus*). In contrast, some authors (*e. g.*, García-García *et al.* 2007; Gregorin and Loureiro 2011), provided longer hair measurements for *E. brasiliensis* (9 to 10 mm), overlapping mainly with *E. chiriquinus* (Simmons and Voss 1998).

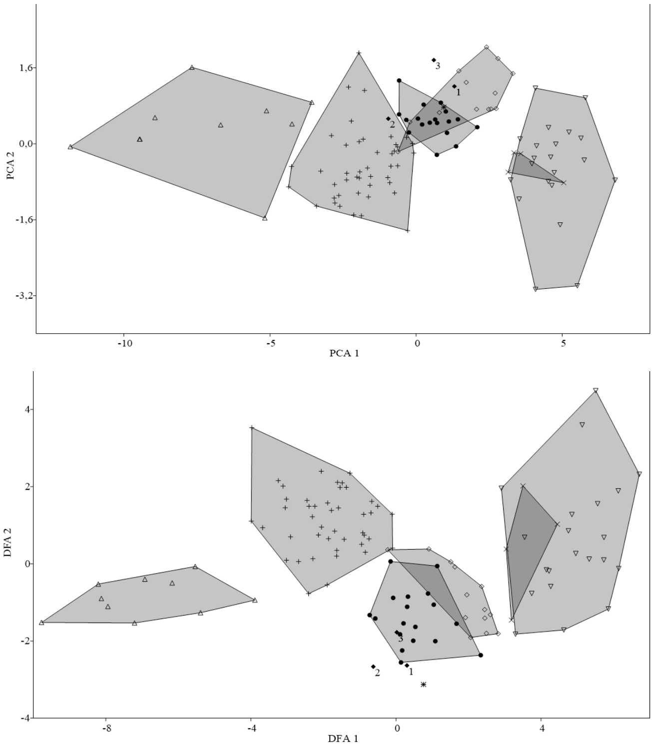

Figure 2. PCA (top) and DFA (bottom) graphs of 14 cranial and external measurements of six *Eptesicus* species: triangle *E. diminutus* (*n* = 10); cross, *E. furinalis* (*n* = 44); circle full, *E. langeri* (*n* = 19); diamond, *E. andinus* (*n* = 13); triangle invert, *E. chiriquinus* (*n* = 21); X, *E. brasiliensis* (*n* = 4). Holotype of *E. langeri* (asterisk); holotype of *E. andinus* (filled diamond 1); *E. chiralensis* (filled diamond 2); *E. montosus* (filled diamond 3).

Table 1. PCA and DFA results for 14 cranial variables of 138 specimens belonging to five *Eptesicus* species.

Variable	**Principal Component Analysis**		**Discriminant Function Analysis**	
	PCA 1	**PCA 2**	**DFA 1**	**DFA 2**
GLS	0.966	-0.094	-28.789	47.078
BCH	0.824	0.081	3.874	-33.086
BL	0.940	-0.173	-14.950	3.470
PAL	0.931	-0.041	8.328	17.541
P-M3	0.932	-0.127	12.880	5.316
MB	0.896	-0.001	8.694	60.880
M3-M3	0.926	-0.050	5.516	-4.856
C-C	0.866	-0.060	-6.571	-15.323
I-M3	0.955	-0.147	25.194	29.397
C-M3	0.955	-0.138	23.414	-26.821
CCL	0.964	-0.075	45.083	-32.240
IOB	0.855	-0.001	6.330	-1.828
POC	0.608	0.711	8.475	-24.852
BCB	0.740	0.479	-1.940	-26.743
Eigenvalue	11.039	0.850	9.330	11.490
% variance	78.9	6.1	73.0	11.7
% variance accumulated	78.9	84.9	73.0	84.7

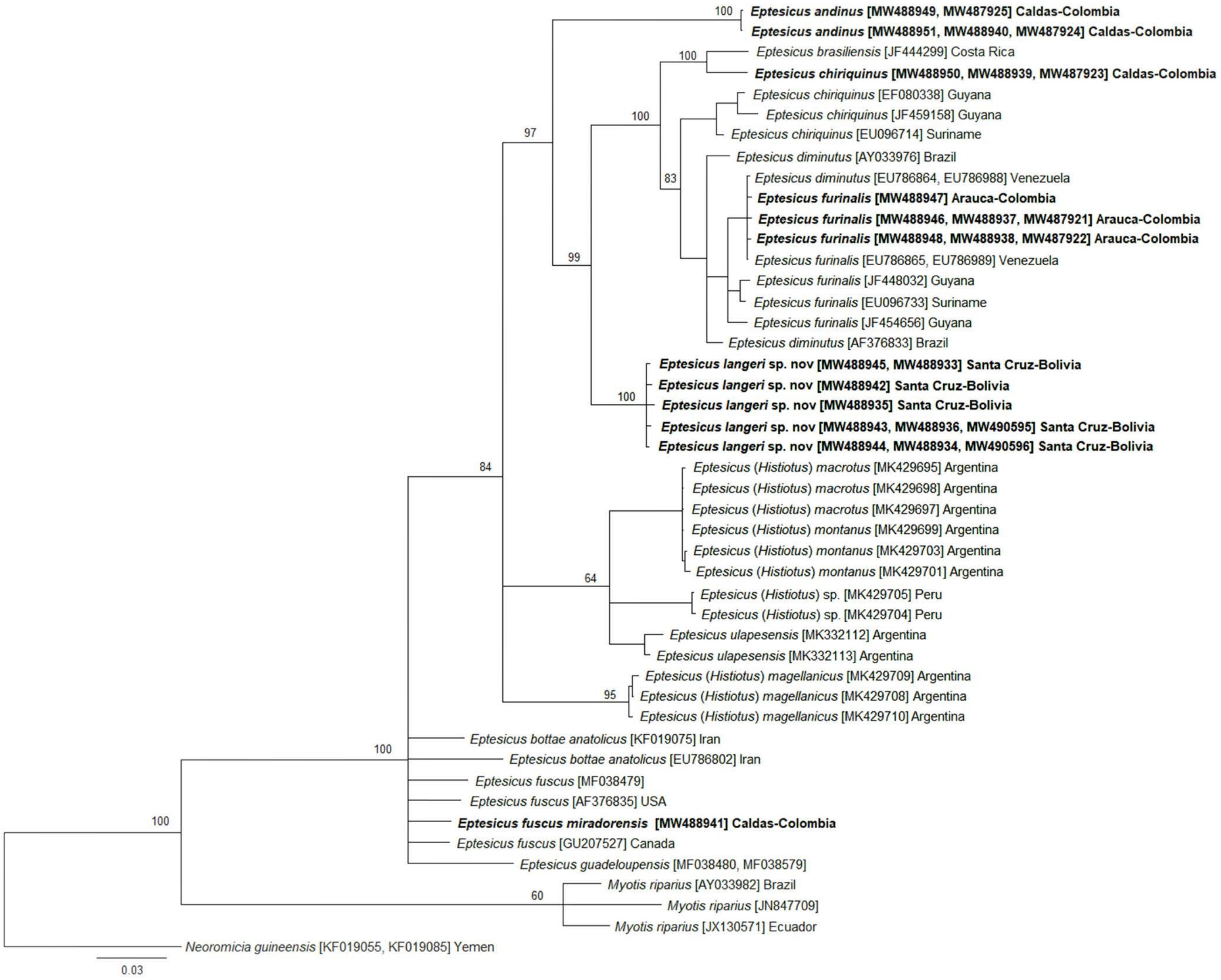

Figure 3. Phylogenetic tree inferred from the concatenation of cyt-*b*, ND1 and COI genes partial sequences of bats specimens collected in the present study (in bold) and sequences from GenBank accession numbers in brackets (cyt-*b*, ND1 and COI respectively) using Bayesian inference (BI) with the GTR+F+I+G4 evolutionary model, and *Neoromicia guineensis* and *Myotis riparius* were used as outgroup. Bayesian posterior probabilities (%) are indicated in the nodes.

Despite the suggested morphological groups, our phylogenetic analyses show that these characters are not related to monophyletic clades, as shown by the position of *E. langeri*, *E. chiriquinus* and *E. andinus* in the phylogenetic trees (Figure 3 and Appendix 1 to 2).

The lack of genetic data for *E. andinus* in previous phylogenetic analyses or species descriptions (Giménez *et al.* 2019; Sánchez *et al.* 2019), limited the assessment of the morphological and phylogenetic association within the Neotropical *Eptesicus*. In this way, our work is also filling these gaps by including and analysing the phylogenetic position of *E. andinus* for first time. In addition, our work showed that the diversity of Neotropical *Eptesicus* has been underestimated as suggested by newly proposed species (Sánchez *et al.* 2019). Finally, the systematics of *Eptesicus* and *Histiotus* is not deeply understood, and integral revisions of both taxa are needed (Giménez *et al.* 2019; Sánchez *et al.* 2019), therefore, the information that we provide here can be useful for additional integrative analyses at continental scale.

Taxonomy

Family Vespertilionidae Gray 1821

Genus *Eptesicus* Rafinesque, 1820

Subgenus *Eptesicus* Rafinesque, 1820

Eptesicus langeri sp. nov.

Holotype: The holotype is an adult lactating female, preserved as skin and skull, and deposited in the mammal collection of the Museo de Historia Natural Noel Kempff Mercado, Universidad Autónoma Gabriel René Moreno, Santa Cruz, Bolivia, with catalogue number MNKM-5584. Collected on December 01, 2013 by Luis H. Acosta, field number L. Acosta 732, at an elevation of 2,020 masl, in a fern grove of the Bolivian-Tucumanian forest surveyed during a biological diagnosis of the locality El Cedral-Agua Rica.

Type locality: El Cedral-Agua Rica, 15 km from the Municipality of Samaipata, Province of Florida, Department of Santa Cruz, Bolivia (-18° 13’ 10.55” S, -63° 47’ 49.74” W; 2,020 masl (Figure 1).

Table 2. Distances based on Kimura two parameters for the mtDNA Cyt-*b* gene (intraspecific on the diagonal and interspecific below the diagonal), and for the mtDNA nicotinamide adenine dinucleotide dehydrogenase (ND1) gene (in bold, above the diagonal).

	Species	1	2	3	4	5	6	7	8	9	10	11	12
1	*Eptesicus* sp. nov (Bolivia)	0.000/0.000	**0.112**	**0.068-0.072**	**0.082-0.084**	**0.205-0.213**		0.167-0.170	0.061-0.072				
2	*E. andinus*	0.094-0.098	0.00	**0.093**	**0.101**	**0.187**		0.137	0.093-0.101				
3	*E. furinalis*	0.076-0.079	0.116-0.135	0.000	**0.053-0.056**	**0.191-0.219**		0.165-0.176	0.000-0.048				
4	*E. chiriquinus*	0.111-0.117	0.107-0.111	0.078-0.093	-	**0.236**		0.152	0.053-0.060				
5	*E. bottae anatolicus*	0.202-0.208	0.194-0.205	0.205-0.218	0.232	-		0.182	0.183-0.193				
6	*E. guadeloupensis*	0.118-0.140	0.191-0.203	0.135-0.176	0.180	0.202	-						
7	*E. fuscus*	0.114-0.136	0.164-0.183	0.120-0.149	0.149-0.152	0.170-0.191	0.078-0.093	0.080	**0.160-0.169**				
8	*E. diminutus*	0.072-0.079	0.124-0.134	0.000-0.031	0.063-0.078	0.208-0.214	0.163-0.176	0.137-0.149	0.027				
9	*E.* (*Histiotus*) *montanus*	0.128-0.143	0.131-0.142	0.133-0.161	0.144-0.145	0.196-0.199	0.171-0.172	0.151.0.159	0.128-0.134	0.001-0.003			
10	*E.* (*Histiotus*) *magellanicus*	0.093-0.103	0.124-0.136	0.120-0.132	0.139-0.143	0.181-0.183	0.159-0.160	0.134-0.154	0.132-0.145	0.132-0.137	0.002-0.004		
11	*E.* (*Histiotus*) sp.	0.128-0.134	0.138-0.147	0.155-0.172	0.149-0.152	0.195-0.196	0.176-0.178	0.158-0.176	0.139-0.155	0.072-0.073	0.125-0.130	0.001	
12	*E.* (*Histiotus*) *macrotus*	0.128-0.138	0.131-0.143	0.133-0.156	0.144-0.145	0.196	0.171-0.172	0.151-0.159	0.127-0.136	0.000-0.004	0.132-0.135	0.072-0.074	0.000-0.001

Paratypes: 18 individuals, six females (MNKM 4436, 5088, 5117, 5585, 5587, 5588) and seven males (MNKM 4678, 4679, 5126, 5590, 5591, 5592, 5692) from the type locality; four females (MNKM 5586, 5589, 5636, 5676) from Agua Clarita (-17° 56′47.71″S, -64° 08′0.28″W, 1,578 masl); and one female (MNKM 5697) from Reserva Municipal El Chape (-18° 01′ 21.46″ S, -63° 56′ 50.23″ W, 2,054 masl). External and cranial measurements are provided in Appendix 3 to 5.

Distribution: *Eptesicus langeri* sp. nov. is known from three localities: i) El Cedral-Agua Rica, ii) Agua Clarita, and iii) the Reserva Municipal El Chape, all three in the Province of Florida, Department of Santa Cruz, Bolivia (Figure 1).

Nomenclatural statement: A life science identifier (LSID) number was obtained for new species described herein: urn:lsid:zoobank.org:act:3723D032-6800-401F-ACEE-0326F1AE72B1.

Etymology: The epithet *langeri* is in honour of Fray Andrés Ma Langer o.p., a Dominican parish priest who made important contributions to the mammalogy of the inter-Andean valleys of Bolivia, especially in the Province of Florida of the Department of Santa Cruz. Several specimens collected by Fray Andrés Ma Langer are deposited and catalogued at the Museo de Historia Natural Noel Kempff Mercado.

Diagnosis: *Eptesicus langeri* is a medium-sized bat (forearm length 40 to 44 mm), with dorsal fur that is long (~ 8 mm), dark brown or orange-brown in females and dark brown in males (Figure 4). The skull has developed sagittal and lambdoidal ridges, that do not reach the posterior region of the skull (Figure 5). A developed preorbital process between the smallest width of the interorbital and postorbital (Figure 6). Coronoid process tall (Figure 5).

Description: *Eptesicus langeri* is a medium-sized bat, similar in size to *E. furinalis* and *E. andinus* (Table 3). The dorsal and ventral fur is bicoloured with dark bases and light tips (dark brown to dark orange, near one quarter hair length; Figure 4). Both sexes present the dorsal fur longer between the shoulders (7 to 9 mm) and shorter in the middle part of the body (~ 6 mm), the ventral region hairs differ from the back by being lighter. Males have a longer dark brown dorsal fur between the shoulders (~ 9 mm) and shorter in the middle of the back (~ 7 mm). Pregnant and lactating females have dorsal fur coloured "orange-brown".

The skull is long, with developed sagittal and lambdoidal ridges. The sagittal crest gives the skull an elevated appearance that is more evident in the middle part of the cranial vault, while the lambdoidal or nuchal crest is developed at the edges of the occipitoparietal suture. Both ridges do not come into contact in the posterior region of the skull, leaving a gap between them (Figure 5).

Figure 4. Dorsal and ventral view of the coat colour pattern. From left to right: A. female (MNKM-5584 holotype) and B. male (MNKM- 5592 paratype) of *E. langeri* sp. nov. and C. *E. andinus* (MNKM-5598).

Figure 5. Dorsal (A), ventral (B) and lateral (C) views of the skull of the holotype of *Eptesicus langeri* sp. nov (MNKM-5584).

Eptesicus langeri presents a well-developed frontal pre-orbital processes (Figure 6). In adult females these processes are more developed than in adult males. The upper internal incisors are larger than the external incisors. There is a small diastema between the upper external incisors and canine. The canine is attached to the premolar. First and second upper molars are similar, and the occlusal surfaces are W-shaped. The third upper molar is smaller than the rest of the molars and posteriorly extends beyond the anterior insertion of the zygomatic arches. The lower external incisors are in contact with the canines. The lower canine is slightly inclined towards the back, and in contact with first lower premolar which is approximately half the height of the canine; second lower premolar is higher than the first and in contact with first lower molar.

The mandible has a triangular and slightly curved coronoid process. The anterior part of the dentary has a straight oblique mandibular line; the mandibular process is slightly curved with a rounded end. A semi-circular mandibular incisure is located between the coronoid process and the anterior part of the dentary; the angular process is dorsally curved, and the mandibular ramus has a central depression (Figure 5).

Comparisons: *Eptesicus langeri* is a medium-sized bat (FA: 40.09 to 44.1 mm; GLS: 14.75 to 16.15 mm) with a dorsal and ventral fur length between 6.0 to 10.0 mm. It can be easily distinguished of other South American *Eptesicus* based on the forearm length, *E. diminutus* < 37.0 mm, *E. innoxius* < 39.0 mm, *E. furinalis* < 41.0 mm, *E. taddeii* > 44.1 mm and *E. fuscus miradorensis* > 49.0 mm. *E. langeri* is smaller than *E.*

Figure 6. Top: Frontal-dorsal view of the crania of the frontal pre-orbital process: A, B. *Eptesicus langeri* (MNKM-5564, Holotype female, MNKM-5592, Paratype male). C. *E. andinus* (AMNH 33807, Holotype). D. *E. montosus* (BMNH 2.1.1.1, Holotype). E. *E. chiralensis* (AMNH 47219, Holotype). Bottom: Space between sagittal and lambdoidal crest; notch and base of lambdoidal crest (red arrows); space between lambdoidal ridges and the parietal (white line).

brasiliensis, E. chiriquinus, *E. taddeii* and *E. ulapesensis*, especially in the GLS (Table 3), and discrete characters of the skull and skin (Appendix 9). *Eptesicus langeri* is characterized by a highly developed preorbital process, which is poorly developed in *E. andinus*, *E. furinalis* (Figure 6), *E. brasiliensis*, *E. fuscus miradorensis*, *E. innoxius*, and *E. taddeii*, whereas in *E. chiriquinus* this process is evident but not well-developed. Based on dorsal fur length, *E. langeri* can be differentiated of *E. furinalis* (< 7.0 mm) and *E. ulapesensis* (~ 6.0 mm). In *E. taddeii* the coat is reddish and 7.0 mm long (Miranda *et al.* 2006).

Comparison with holotypes of *Eptesicus andinus* (AMNH 33807), *E. chiralensis* (AMNH 47219), and *E. montosus* (BMNH 2.1.1.1): *Eptesicus langeri* can be differentiated from *E. andinus* and two of its junior synonyms (*E. chiralensis* and *E. montosus*) by cranial characteristics including: a) lambdoidal crest developed in the lateral region of the interparietal in *E. langeri, vs.* poorly developed in *E. andinus, E. chiralensis*, and *E. montosus* (Figures 6 to 8). B) base of the lambdoidal crest, broad in posterior view and with a smooth notch in the middle region in *E. langeri*, *vs.* lacking a notch and almost straight in *E. andinus*, *E. chiralensis*, and *E. montosus* (Figure 6). C) Frontal preorbital process present in *E. langeri vs.* poorly developed in *E. andinus, E. chiralensis*, and *E. montosus* (Figures 6 to 7). D) circular appearance in the nasal-premaxillary region in *E. langeri, vs.* rectangular in *E. andinus, E. chiralensis* and *E. montosus* (Figure 8). E) angular process of the mandible with a robust tip in *E. langeri, vs.* delicate in *E. andinus, E. chiralensis* and *E. montosus* (Figure 8). F) ascending ramus of the coronoid process lacking a steep slope in *E. langeri* (31°), *E. andinus* (30°) and *E. chiralensis* (35°), *vs.* with steep slope in *E. montosus* (40°; Figure 8). G) ramus of ventral mandible curved in *E. langeri, vs.* smoothly curve in *E. andinus* and *E. montosus*, and straight in *E. chiralensis* (Figure 8). *Eptesicus langeri* overlaps in some external and cranial measurements with *Eptesicus andinus*, *E. chiralensis*, and *E. montosus* although *E. langeri* can be separated when the postorbital width and cranial index are plotted (Appendix 6).

Figure 7. Dorsal, and ventral, view of skulls (left to right) of A. *Eptesicus langeri* sp. nov (MNKM-5592, Paratype). B. *E. andinus* (AMNH 33807, Holotype). C. *E. montosus* (BMNH 2.1.1.1, Holotype). D. *E. chiralensis* (AMNH 47219, Holotype).

Ecology: The type locality is part of the Bolivian-Tucumanian forest, characterized by Chari (*Parapiptadenia excelsa*) and Tipa (*Tipuana tipu*) trees, and connected to the Andean vegetation of the Peruvian-Bolivian Yungas (Navarro 2011). Specimens of the type locality were collected from 19:24 to 23:30 h. Most specimens were collected using mist nets installed at a height of 5 to 8 m above ground in forest clearings (newly opened road) of a secondary road. Lactating females and males with testicles in scrotal position have been recorded from November to January. Specimens from Agua Clarita were collected inside a hollow of a standing tree. Other bat species reported at the type locality are *Anoura geoffroyi*, *Sturnira lilium*, *S. oporaphilum*, *Chrotopterus auritus*, *Desmodus rotundus*, *Platyrrhinus masu*, *Myotis nigricans*, and *M. keaysi*.

We suggest that discreet dental skull characters should be used together with some measurements for an accurate identification of *E. langeri*. Thus, we propose the following identification key for some South American *Eptesicus* (sensu Davis and Gardner 2008), with emphasis on some cranial variables:

Taxonomic key

1. Ears longer than 20.0 mm, extending well beyond muzzle ....subgenus *Histiotus*

1a. Ears normal, less than 20.0 mm, not extending beyond muzzle ....subgenus *Eptesicus* 2

2. Skull longer than 16.3 mm; upper toothrow > 6.3 mm 3

2a. Skull less than 16.3 mm; upper toothrow less than 6.3 mm 7

3. Forearm greater than 49.0 mm ....*Eptesicus fuscus miradorensis*

3a. Forearm less than 49.0 mm 4

4. U-shaped nasal opening ....*Eptesicus brasiliensis*

4a. V-shaped nasal opening 5

5. Dorsal fur longer than 12.0 mm ....*Eptesicus chiriquinus*

5a. Dorsal fur less than 12.0 mm 6

6. Skull length between 17.3 to 18.4 mm .....*Eptesicus taddeii*

6a. Skull length between 15.9 to 17.0 mm ....*Eptesicus ulapesensis*

7. Skull less than 13.6 mm; upper toothrow less than 5.0 mm ....*Eptesicus diminutus*

7a. Skull longer than 13.6 mm; upper toothrow longer than 5.0 mm 8

8. Sagittal and lambdoidal crests connected 9

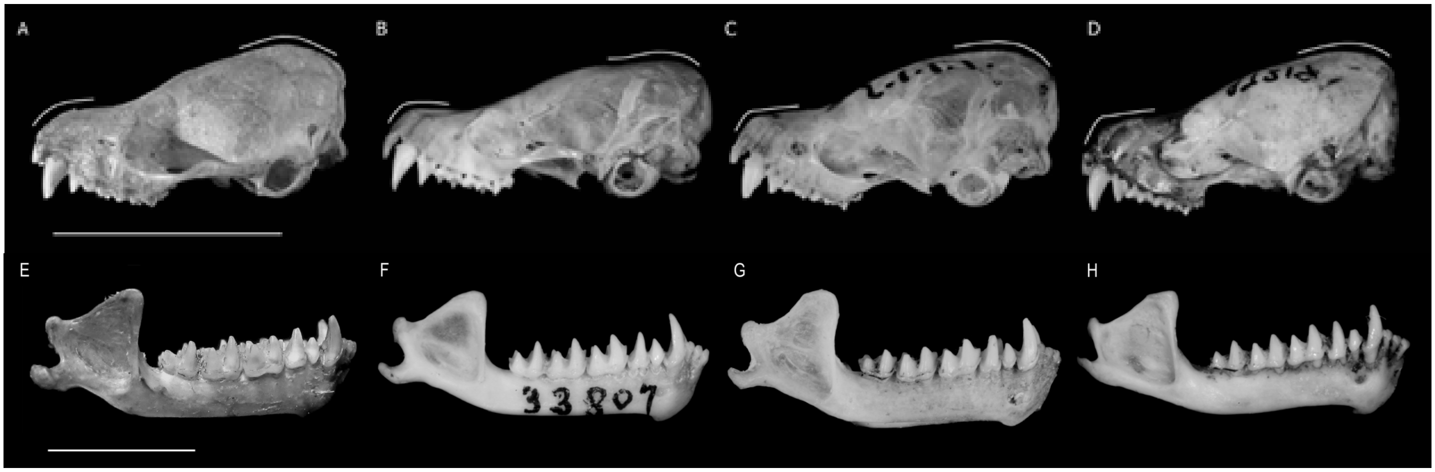

Figure 8. Lateral view of the skull, the white lines show the shape of the rostrum and skull: A. *Eptesicus langeri* sp. Nov (MNKM-5592, Paratype). B. *E. andinus* (AMNH 33807, Holotype). C. *E. montosus* (BMNH 2.1.1.1, Holotype). D. *E. chiralensis* (AMNH 47219, Holotype "inversed"), scale 10mm. Lateral view of the mandible of: E. *Eptesicus langeri* sp. nov (MNKM-5592, Paratype). F. *E. andinus* (AMNH 33807, Holotype). G. *E. montosus* (BMNH 2.1.1.1, Holotype). H. *E. chiralensis* (AMNH 47219, Holotype), scale 5mm.

8a. Skull with separate sagittal and lambdoidal ridges forming a triangular flat bone 10

9. Pale greyish brown hair colour ....*Eptesicus innoxius*

9a. Dark brown to blackish brown hair colour ....*Eptesicus furinalis*

10. Skull with very poorly developed/absent sagittal and lambdoidal ridges, poorly developed preorbital process ....*Eptesicus andinus*

10a. Skull with evident/developed sagittal and lambdoidal crests, preorbital process present ....*Eptesicus langeri*

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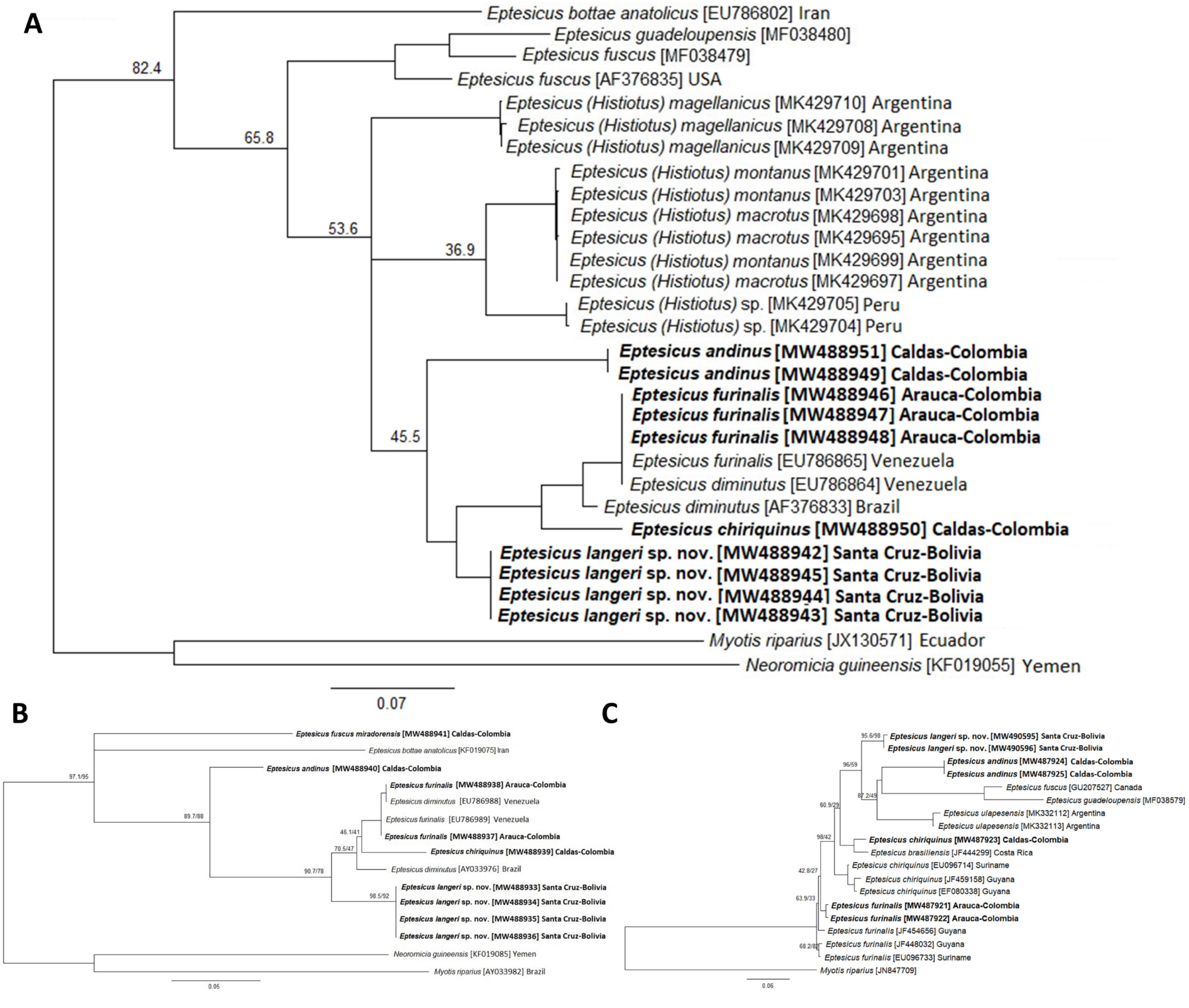
Appendix 1

A) Tree of the mitochondrial cyt-*b* gene partial sequences of bats specimens collected in the present study (in bold) and sequences from GenBank (accession numbers in brackets), obtained by Bayesian inference (BI) by the evolutionary model GTR+F+I+G4, and *Neoromicia guineensis* and *Myotis riparius* were used as outgroup. Bayesian posterior probabilities (%) are indicated in the nodes. B)Tree of the mitochondrial ND1 gene partial sequences of bats specimens collected in the present study (in bold) and sequences from GenBank (accession numbers in brackets), using Maximum Likelihood (ML) method and GTR+F+G4 model. Numbers at nodes are above-selected branch support analysis from left to right: ultrafast bootstraps values, and Shimodaira–Hasegawa–like approximate likelihood-ratio test (SH-like aLRT). The sequence of *Neoromicia guineensis* and *Myotis riparius* were used as outgroup. C) Tree of the mitochondrial COI gene partial sequences of bats specimens collected in the present study (in bold) and sequences from GenBank (accession numbers in brackets), using Maximum Likelihood (ML) method with the TVM+F+R2 evolution model. Numbers at nodes are above-selected branch support analysis from left to right: ultrafast bootstraps values, and Shimodaira–Hasegawa–like approximate likelihood-ratio test (SH-like aLRT). The sequence of *Myotis riparius* was used as outgroup.



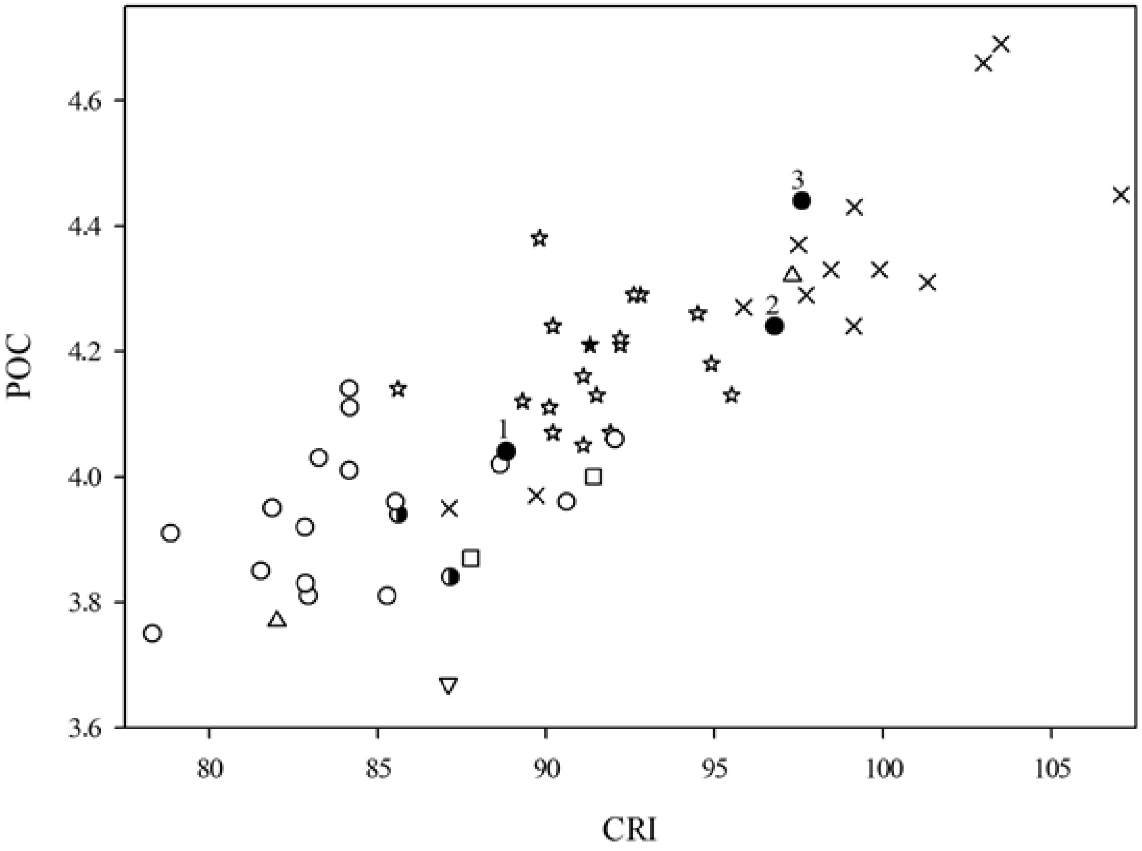
Appendix 2

Average intraspecific (on the diagonal) and interspecific (below the diagonal) distances based on Kimura two parameters for the mtDNA cytochrome c oxidase subunit I (COI) gene.

	Species	1	2	3	4	5	6	7	8	9
1	*Eptesicus* sp. (Bolivia)	0.005								
2	*E. furinalis*	0.085-0.094	0.006-0.025							
3	*E. chiriquinus*	0.077-0.079	0.037-0.067	0.011-0.074						
4	*E. andinus*	0.097-0.127	0.137-0.168	0.119-0.138	0.000					
5	*E. ulapesensis*	0.116-0.129	0.135-0.160	0.129-0.146	0.148-0.154	0.011				
6	*E. brasiliensis*	0.091	0.069-0.071	0.035-0.065	0.124-0.137	0.134-0.140	-			
7	*E. fuscus*	0.170-0.172	0.169-0.191	0.183-0.202	0.206-0.220	0.188-0.194	0.194	-		
8	*E. guadeloupensis*	0.207-0.214	0.178-0.204	0.190-0.215	0.243-0.245	0.212-0.223	0.206	0.101	-	
9	*Myotis riparius*	0.277-0.283	0.258-0.292	0.279-0.304	0.330-0.353	0.304-0.313	0.290	0.312	0.320	-

Appendix 3

Morphometric comparison of two cranial variables of *Eptesicus andinus* from: Bolivia (square), Brazil (inverted triangle), Colombia (X), Ecuador (black and white circle), Peru (triangle) and Venezuela (circle); *E. langeri* (star), holotype (star full); holotypes from *E. andinus* (circle full 2), *E. chiralensis* (circle full 1) and *E. montosus* (circle full 3).



Appendix 4

Comparison of measurements (mm) of *E. langeri* with other Neotropical *Eptesicus* species. Average, minimum and maximum (parentheses), number of specimens examined.

Variable	***E. diminutus***	***E. furinalis***	***E. andinus***	***E. langeri sp. nov.***	***E. ulapesensis*** (Sánchez *et al.* 2019)	***E. brasiliensis***	***E. chiriquinus***	***E. taddeii*** (Miranda *et al.* 2006)
GLS	13.7 (12.5–14.7) 10	15.2 (14.4–16.1) 44	15.4 (14.2–16.7) 38	15.6 (14.8–16.2) 19	16.5 (15.9–17.0) 9	17.0 (16.7–17.4) 5	17.0 (16.5–17.8) 21	17.9 (17.3–18.4) 24
CCL	12.8 (11.9–13.6) 10	14.1 (13.3–14.9) 44	15.3 (14.3–15.9) 13	14.5 (13.7–14.9) 19	15.8 (15.6–16.1) 9	15.8 (15.6–16.4) 5	16.0 (15.6–16.5) 21	16.8 (16–17.4) 24
BL	11.4 (10.2–13.7) 10	12.7 (12.0–13.5) 44	13.4 (12.9–14.1) 13	13.1 (12.6–13.6) 19	–	–	14.3 (13.8–14.8) 21	–
PAL	5.8 (5.1–6.4) 10	6.7 (6.2–7.1) 44	7.3 (6.9–7.5) 13	7.2 (6.5–7.7) 19	6.7 (6.0–7.6) 6	7.6 (7.5–8.0) 5	7.8 (6.9–8.8) 21	–
POC	3.7 (3.4–4.0) 10	3.8 (3.5–4.9) 44	4.1 (3.7–4.7) 38	4.2 (4.0–4.4) 19	3.9 (3.8–4.1) 9	4.0 (3.8–4.1) 5	4.2 (3.8–4.6) 21	4.1 (3.9–4.4) 24
BCH	5.1 (4.7–6.1) 10	5.7 (4.9–6.2) 44	5.9 (5.7–6.3) 13	6.4 (5.9–6.8) 19	–	–	6.5 (5.8–7.6) 21	–
BCB	6.9 (6.5–7.6) 10	7.3 (6.8–7.7) 44	7.6 (7.1–8.4) 38	7.5 (7.4–7.7) 19	8.3 (8.1–8.6) 9	7.7 (7.5–8.1) 5	7.8 (6.3–8.3) 21	8.1 (7.7–8.5) 24
MB	7.4 (6.8–8.2) 10	8.3 (7.7–9.3) 44	8.3 (7.8–8.8) 36	8.4 (8.1–8.6) 19	8.8 (8.2–9.2) 9	9.1 (8.7–9.5) 5	8.9 (8.5–9.3) 21	–
ZB	9.3 (8.6–10.3) 6	10.3 (9.7–10.8) 35	10.7 (10.1–11.2) 12	10.7 (10.4–11.0) 19	11.1 (10.6–11.6) 9	12.0 (12.0–12.1) 2	11.5 (10.7–12.0)	12.2 (11.7–12.9)
IOB	4.6 (4.1–4.9) 10	4.9 (3.8–5.5) 44	5.4 (4.9–5.8) 13	5.2 (4.9–5.4) 19	–	6.4 (5.9–7.2) 5	5.8 (5.4–6.4) 21	–
C–C	4.3 (3.9–4.7) 10	4.8 (4.3–5.1) 44	5.1 (4.6–5.4) 13	4.9 (4.7–5.3) 19	5.1 (4.8–5.4) 9	5.5 (5.3–5.7) 5	5.3 (4.9–5.6) 21	5.7 (5.5–6.1) 24
M3–M3	6.1 (5.5–6.6) 10	6.7 (6.0–6.9) 44	6.7 (6.3–7.4) 38	6.9 (6.4–7.2) 19	6.7 (6.2–7.2) 9	7.3 (7.1–7.6) 5	7.4 (7.0–7.8) 21	7.5 (7.0–7.8) 24
C–M3	4.9 (4.6–5.4) 10	5.7 (5.1–6.0) 44	5.9 (5.5–6.2) 38	6.0 (5.8–6.3) 19	6.2 (5.9–6.5) 9	6.4 (6.2–6.7) 5	6.6 (6.4–6.9) 21	6.8 (6.4–7.1) 24
I–M3	5.8 (5.4–6.2) 10	6.5 (6.2–6.8) 44	6.9 (6.7–7.3) 13	6.8 (6.6–7.0) 19	–	–	7.6 (7.2–8.1) 21	–
c–m3	5.4 (5.0–5.8) 10	6.2 (5.5–6.5) 44	6.6 (6.4–6.8) 13	6.4 (6.3–6.8) 19	6.8 (6.4–7.3) 9	6.8 (6.7–7.0) 5	7.2 (6.9–7.5) 21	7.3 (7.1–7.7) 24
P–M3	3.9 (3.6–4.2) 10	4.5 (4.1–4.9) 44	4.8 (4.6–5.1) 13	4.8 (4.6–5.0) 19	–	–	5.2 (4.3–5.6) 21	–
p–m3	4.7 (4.3–5.2) 7	5.5 (5.2–6.2) 44	5.7 (5.4–5.9) 13	5.6 (5.4–5.9) 19	–	–	6.2 (5.7–6.6) 21	–
MAL	10.1 (9.5–11.0) 10	11.7 (11.0–12.4) 44	12.5 (11.3–13.0) 13	12.1 (11.2–12.6) 19	12.2 (11.8–12.8) 9	13.1 (12.9–13.5) 5	13.7 (13.1–14.4) 21	13.5 (13.0–13.7) 24
MH	3.9 (3.4–4.6) 10	4.6 (4.1–5.2) 44	4.6 (4.2–5.2) 13	4.5 (4.2–4.8) 19	–	–	5.2 (4.9–5.7) 21	–
LT	84.8 (80–91) 8	95.4 (84–107) 38	97.1 (83–116) 35	96.4 (90–106) 19	103.6 (93–115) 9	108.8 (101–117) 6	104.6 (92–114) 17	108.6 (99–117) 24
TL	34.6 (27–39) 8	40.7 (35–48) 38	40.2 (31–52) 35	42.1 (39–46) 19	44.7 (36–49) 9	43.8 (37–49) 6	43.4 (36–48) 21	47.9 (43–53) 24
EL	13.4 (12–15) 7	14.6 (10–19) 38	14.6 (8–19) 30	14.8 (12.5–17) 19	15.3 (13–19) 9	16.8 (16–18) 6	14.2 (10–17) 21	15.6 (14–17) 24
LF	7.8 (7–10) 8	8.6 (7–10) 38	9.7 (7–13) 37	8.6 (7–10) 19	8.6 (7–10) 9	9.1 (8–11) 6	10.3 (8.6–11.24) 21	–
Weight	6.3 (5–7.5) 4	8.0 (5–11.5) 36	9.7 (7–13) 13	9.6 (8–12) 17	10.7 (9–14) 9	5.9 (4.9–6.5) 5	11.6 (10–14) 14	–
PD	6.3 (5–8) 3	6.8 (6–8) 13	9.5 (8–12) 12	7.7 (6–9.5) 16	~ 6	–	9.3 (7–11) 18	~ 7
PV	(7) 1	6.1 (5–7) 13	7.9 (7–9) 12	6.8 (6–8) 14	–	–	7.7 (7–9) 17	–
FA	33.8 (31.2–35.9) 9	40.1 (36.4–43.4) 44	41.4 (37.2–47.0) 38	42.2 (40.1–44.1) 19	42.8 (41.0–44.6) 9	42.0 (40.5–45.0) 5	45.9 (43.6–47.6) 21	46.5 (44.1–48.7) 24
CRI	73.1 (62–83) 10	84.1 (76–92) 44	90.3 (78–107) 38	91.3 (86–96) 19	100.9 (98–106) 9	–	116 (92–108) 21	–
MXI	25.9 (22–29) 10	32.1 (27–36) 44	36.3 (33–39) 13	35.5 (33–39) 19	36.5 (34–41) 9	–	41.9 (40–45) 21	44.8 (41–49) 24

Appendix 5

Cranio-dental and external measurements of male and female and holotype of *Eptesicus langeri*, compared with the holotypes of *E. montosus* and *E, chiralensis* (junior synonyms of *E. andinus*), and *E. andinus*.

Variables	***montosus***	***chiralensis***	***andinus***	***langeri***	***langeri***	***langeri***
	BMNH 2.1.1.1	**AMNH 47219**	**AMNH 33807**	**MNKM 5584**	**n=7**	**n=12**
	Bolivia	**Ecuador**	**Colombia**	**Bolivia**	**Bolivia**	**Bolivia**
Sex	Male	Male	Male	Female	Male	Female
GLS	15.79	15.22	16.01	15.44	14.75-15.98	15.26-16.15
CCL	14.80	14.68	15.13	14.64	13.77-14.78	14.29-14.93
BL	12.73	12.6	13.47	12.69	12.55-13.27	12.69-13.57
PAL	6.92	6.66	6.87	7.09	6.85-7.37	6.51-7.69
POC	4.44	4.04	4.24	4.21	4.05-4.38	4.02-4.29
BCH	5.97	6.00	5.98	6.43	6.30-6.63	5.95-6.83
BCB	7.92	7.63	7.85	7.62	7.43-7.70	7.43-7.70
MB	8.64	8.10	8.40	8.39	8.06-8.48	8.13-8.64
ZB	10.35	10.34	10.55	10.68	10.35-10.86	10.49-11
IOB	5.23	5.15	5.30	5.24	4.92-5.30	4.97-5.39
C-C	5.14	4.94	5.28	5.33	4.67-5.03	4.83-5.33
M3-M3	6.76	6.69	6.62	7.07	6.37-6.92	6.73-7.15
C-M3	5.94	5.83	6.07	6.08	5.86-6.15	5.83-6.30
I-M3	6.76	6.47	6.74	6.72	6.59-6.93	6.68-7.01
c-m3	6.30	6.26	6.40	6.41	6.25-6.53	6.26-6.75
P-M3	4.50	4.48	4.81	4.76	4.67-4.94	4.6-5.04
p-m3	-	5.05	5.67	5.54	5.42-5.73	5.39-5.92
MAL	10.90	11.49	12.21	12.39	11.16-12.32	11.64-12.6
MH	-	4.44	4.49	4.77	4.22-4.84	4.29-4.77
LT	98.00	97.00	100	94	90.00-106.00	90.00-100.00
TAIL	43.00	38.00	35.00	42.5	38.50-43.00	40.00-46.00
EAR	18.00	-	-	16.5	12.50-15.60	13.00-17.00
HF	10.04	10.00	8.00	9	7.00-9.00	7.00-10.00
FA	42.79	41.10	43.60	43.02	40.09-43.11	40.64-44.10
PD	9.00	7.00	9.00	7	8.00-10.00	6.00-10.00
PV	-	7.00	8.00	5	6.00-8.00	5.00-7.00
CRI	97.60	88.80	96.80	91.3	85.60-94.90	88.60-95.50
MXI	35.30	33.90	36.10	37.7	32.60-36.40	34.20-38.40

Appendix 6

Morphometry of *E. langeri* sp. nov. from Bolivia, * holotype.

	MNKM	MNKM	MNKM	MNKM	MNKM	MNKM	MNKM	MNKM	MNKM	MNKM	MNKM	MNKM	MNKM	MNKM	MNKM	MNKM	MNKM	MNKM	MNKM
	5584 *	5117	5585	4436	5586	5587	5588	5589	5676	5636	5088	5697	4679	5592	5126	4678	5692	5590	5591
Sex	female	female	female	female	female	female	female	female	female	female	female	female	male	male	male	male	male	male	male
GLS	15.44	15.79	15.39	15.48	15.26	15.64	15.45	16.15	15.40	15.74	15.58	16.02	15.09	15.76	14.75	15.80	15.77	15.70	15.98
CCL	14.64	14.88	14.41	14.53	14.29	14.51	14.46	14.93	14.30	14.38	14.60	14.80	13.90	14.78	13.77	14.57	14.50	14.44	14.75
BL	12.69	13.46	12.80	13.27	13.07	12.98	12.83	13.57	13.15	13.33	13.14	13.24	12.55	13.12	13.13	13.27	12.88	13.2	12.73
PAL	7.09	7.69	6.51	7.38	6.99	7.36	7.01	7.43	6.93	7.29	7.06	7.34	6.87	7.22	6.85	7.36	7.16	7.37	7.10
POC	4.21	4.13	4.12	4.02	4.24	4.16	4.07	4.13	4.11	4.21	4.29	4.26	4.38	4.07	4.14	4.29	4.05	4.22	4.18
BCH	6.43	6.44	6.38	6.33	6.30	6.39	6.19	6.83	5.95	6.32	6.20	6.68	6.40	6.63	6.35	6.40	6.44	6.30	6.49
BCB	7.62	7.46	7.49	7.43	7.58	7.49	7.61	7.70	7.59	7.50	7.62	7.54	7.52	7.59	7.47	7.43	7.50	7.52	7.70
MB	8.39	8.22	8.13	8.44	8.43	8.35	8.34	8.64	8.37	8.62	8.60	8.29	8.27	8.48	8.40	8.38	8.17	8.06	8.31
ZB	10.68	10.49	10.69	10.59	10.58	10.69	10.62	11.00	10.73	10.72	10.76	10.69	10.86	10.85	10.53	10.84	10.35	10.39	10.66
IOB	5.24	5.16	5.18	4.97	5.19	5.02	5.23	5.19	5.19	5.05	5.37	5.39	4.93	5.14	5.14	5.25	4.92	5.17	5.30
C-C	5.33	4.85	4.83	4.96	4.93	4.99	5.04	4.94	5.06	4.97	4.88	5.22	4.71	5.03	4.67	4.96	4.75	4.92	5.03
M3-M3	7.07	7.10	6.74	6.74	6.78	6.80	6.90	6.84	6.92	6.73	6.99	7.15	6.75	6.84	6.78	6.87	6.37	6.92	6.87
C-M3	6.08	6.30	5.92	6.09	6.03	6.07	5.83	5.96	5.99	5.99	5.89	6.22	5.90	6.09	5.86	6.12	5.88	6.15	6.03
I-M3	6.72	7.01	6.80	6.98	6.77	6.68	6.71	6.76	6.77	6.83	6.71	6.97	6.63	6.85	6.59	6.93	6.70	6.86	6.84
c-m3	6.41	6.66	6.29	6.63	6.37	6.41	6.26	6.50	6.47	6.44	6.51	6.75	6.38	6.34	6.25	6.53	6.45	6.39	6.42
P-M3	4.76	4.69	4.77	4.83	4.66	4.72	4.63	4.67	4.60	4.66	4.82	5.04	4.67	4.93	4.79	4.82	4.84	4.87	4.94
p-m3	5.54	5.88	5.62	5.64	5.59	5.92	5.39	5.65	5.49	5.58	5.48	5.85	5.46	5.72	5.42	5.73	5.53	5.59	5.73
MAL	12.39	12.60	12.26	12.54	12.45	12.48	12.15	12.13	11.64	11.70	11.97	11.98	11.16	12.32	11.87	11.90	11.44	12.23	12.30
MH	4.77	4.57	4.69	4.75	4.49	4.72	4.40	4.72	4.29	4.34	4.5	4.46	4.38	4.37	4.22	4.84	4.46	4.36	4.47
LT	94	100	98	97	95	96	97	100	90	92	94.5	97	90	106	97	98.5	90	100	100
TL	42.5	40	43	43	46	44.5	44.5	44	38.5	40.5	41	44	38.5	43	39	41	41	40	40
EL	16.5	14	16	13.5	13	17	15	13.5	14	14	15.5	15	15	15	12.5	15.6	15.5	15	14
HF	9	10	8	10	8.5	10	8	9.5	8	9	7	8	9	8	8.5	9	8.5	9	7
FA	43.02	41.52	41.54	42.95	43.2	43.25	43.76	44.1	41.15	43.35	40.64	42.77	41.58	41.85	42.66	43.11	41.15	40.09	40.54
PD	7	-	7	7	6	10	8	-	7	7	8	-	10	8	9	9	10	8	8
PV	5	-	6	6	6	7	6	-	-	-	6	-	7	7	7	8	8	6	7
CRI	91.30	91.50	89.30	88.60	90.20	91.10	90.20	95.50	90.10	92.20	92.80	94.50	89.80	91.90	85.60	92.60	91.10	92.20	94.90
MXI	37.70	37.60	34.20	35.60	35.30	35.70	34.80	35.10	35.90	35.00	34.90	38.40	33.80	36.10	33.50	36.20	32.60	36.40	35.90

Appendix 7

GenBank accession numbers for sequences generated in this study are indicated in **boldface type**; all others were published previously. Cyt-*b* = cytochrome b; ND1 = nicotinamide adenine dinucleotide dehydrogenase; COI = cytochrome oxidase subunit I.

Taxon	Cyt-b	ND1	COI
Eptesicus andinus	**MW488951, MW488949**	**MW488940**	**MW487924, MW487925**
Eptesicus brasiliensis			JF444299
Eptesicus bottae anatolicus	EU786802	KF019075	
Eptesicus chiriquinus	**MW488950**	**MW488939**	JF459158, EU096714, EF080338, **MW487923**
Eptesicus diminutus	EU786864, AF376833	EU786988, AY033976	
Eptesicus furinalis	EU786865, **MW488946-MW488948**	EU786989, **MW488937, MW488938**	EU096733, JF448032, JF454656, **MW487921, MW487922**
Eptesicus fuscus	AF376835, MF038479		GU207527
Eptesicus fuscus miradorensis		MW488941	
Eptesicus guadeloupensis	MF038480		MF038579
Eptesicus langeri	**MW488942-MW488945**	**MW488933-MW488936**	**MW490595; MW490596**
Eptesicus ulapesensis			MK332112, MK332113
Eptesicus (*Histiotus*) *montanus*	MK429701, MK429703, MK429699		
Eptesicus (*Histiotus*) *magellanicus*	MK429710, MK429709, MK429708		
Eptesicus (*Histiotus*) *macrotus*	MK429698, MK429697, MK429695		
Eptesicus (*Histiotus*) sp.	MK429705, MK429704		
Myotis riparius	JX130571	AY033982	JN847709
Neoromicia guineensis	KF019055	KF019085	

Appendix 8

Revised Specimens:

Eptesicus andinus (*n* = 39). BOLIVIA. Beni: Lago Largo-Comunidad Maravilla (MNKM 5598). Cochabamba: Corani (AMNH 268653). Choro (BMNH 2.1.1.1 "Holotype *E. montosus*"). La Paz: PN ANMI Madidi (MNKM 5599). BRASIL. Goiás (AMNH 134910). COLOMBIA. Cauca: Município de Páez (Belalcázar); Parque Nacional Natural, (P.N.N.) Nevado del Huila, Irlanda, Estación Inderena, 2800 m (ICN 7637-40). Caldas: Aranzazu, vereda El Diamante, 3420 m (MHN-UCa-M 2633-2634). Huila: Valle de las Papas (AMNH 33807 "Holotype *E. andinus*"). Quindío: municipio de Salento; 3250 m (UV 3358-59). Reserva Natural Cañón Quindío, frente de reforestación "La Montaña", 2900 m (ICN 12449). Frente de reforestación "La Romelia", 2630 m, (ICN 12450). Frente de reforestación "La Picota", 2730 m (ICN 12451-2). El Roble (AMNH 32802). Risaralda: Pereira, vereda La Pastora, Parque Regional Natural Ucumarí, 2470 m (ICN 11270). ECUADOR. El Oro: El Chiral (AMNH 47218, 47219 "Holotype *E. chiralensis*", 47220). PERÚ. Chanchamayo (BMNH 76152). Junín, Tarma, San Ramón (AMNH 23780). Cuzco, Pillahuata (FMNH 123953). VENEZUELA. Caracas: Los Venados (USNM 370935-37, 370943-44, 370949-53, 370955, 370962-63). Maracaibo: Falcon Cerro Sapoco (USNM 441764). Montalbán: La Copa (USNM 441755).

Eptesicus brasiliensis (n = 4*)*. ARGENTINA. Corrientes, Goya (BMNH 98345, 98346). BOLIVIA. Santa Cruz: Comarapa (AMNH 260257). COLOMBIA. Meta (UV 7725). ECUADOR. Oriente, Canelos (BMNH 54373).

Eptesicus chiriquinus (*n* = 21). COLOMBIA. Quindío: municipio Finlandia; vereda El Roble, Reserva Forestal Bremen-La Popa, 1950 m, (ICN 12483). Antioquia: San Luis; San Pablo, quebrada San Antonio, 810 m (ICN 9881-82). Yarumal; El Cedro, Media Luna (CTUA 11083). El Cedro, El Sombrero (CTUA 10965). Cauca (JVS-254, 269, MHNUC 1497); municipio de Cajibío: vereda El Cofre, finca La Herencia 1700 m (UV 13178). Cundinamarca: municipio de Paime, vereda El Carmen, Inspección de Policía Cuatro Caminos, 1400 m (MUD 116, 125, 138, 139). Santander: municipio Los Santos, vereda Mesitas de San Javier, bosque de Los Alpes, 1550 m (ICN 16653). Encino, Vereda Río Negro, sitio Cachalú, finca La Desdichada, 2000 m (ICN 17623). Valle Del Cauca: municipio La Victoria; hacienda El Chaquiral (UV 4055). Pance: río Pance, estación Pueblo Pance 1460 m (UV 3551, 3889, 4363). Municipio El Cairo, Estación Cerro del Inglés 2000 m (UV 13094). Valle Del Cauca: Buenaventura; vía Buenaventura-Zaragoza, 180 m (MHNUC, colector number HERC-502).

Eptesicus diminutus (*n* = 7). ARGENTINA. Río Negro, Balneario Las Cañas (CML 1859). Buenos Aires: Delta, Canal 6 y P. Palmas (CML 1820). Jujuy: Laguna La Brea 25km al W de Palma Sola (CML 3086). Salta Dept. General San Martin; 11 km intersección ruta 34 camino a Acambuco, (CML 6139). Anta Arroyo La Sala Centro Administrativo Parque Nacional El Rey (CML 6050). Corrientes (BMNH 24664). Santa Fe, Esperanza (BMNH 1241). BOLÍVIA. SANTA CRUZ, Ñuflo de Chávez (MNKM 4527). Florida (MNKM 5658). PARAGUAY. Villa Rica (BMNH 1811).

Eptesicus furinalis (*n* = 56). ARGENTINA. Chaco: Almirante Brown (CML 3220, 3221, 3225, 3226, 3850, 3854). Güemes (CML 5397). Córdoba: Cruz del Eje (BMNH 2251). Corrientes: Ituzaingó, San Borgita (BMNH 691246). Formosa: Bermejo (CML 3855-56). Pilcomayo (CML 4670); Rio Bermejo (CML 5311). Estero Poi, Pto. Algarrobo (CML 4572). Jujuy: Laguna La Brea (CML 3085). Doctor Manuel Belgrano (CML 4312-13). Rio Ledesma (CML 5223). Rio de Sora (CML 5224). La Rioja: San Blas de los Sauces (CML5445). Misiones: Guaraní (CML 3857). Salta: Piquirenda Viejo (CML 5220, 522). Rio Itiyuro (CML 5372). Oran: Santa María (CML 5221), Oran (CML 4331, 5142-45). Tucumán: Arroyo Aguas Chiquitas (CML 5225-27), Concepción (BMNH 25311). Rio chico: Reserva Provincial Santa Ana (CML 5430). BOLIVIA. Beni: Reserva de la Paraba Barba Azul (MNKM 4982). Santa Cruz: Parque Kaa Iya, Cerro Cortado (MNKM 3440). Agua Rica (MNKM 4677-5692). Lajas (MNKM 4999). Pampa Grande (MNKM 5594). Buena Vista (BMNH 2612421). Estancia San Miguelito (MNKM 4546-47). San José de Chiquitos TCO Turubó Este (MNKM 4957-58-59). San Miguel (MNKM 4888-47). Parque Noel Kempff Mercado (MNKM 5565-95-96). Tarija: Parque Aguaragüe (MNKM 5597). COLOMBIA. Cundinamarca: Bogotá, Santa Fe De Bogotá (BMNH 711368). Cueva del Ermitaño (BMNH 991142). Tolima: Santana, Near Honda (BMNH 109238). Valle Del Cauca: Villa Carmelo (ICN 6298). Risaralda: Pueblo Rico, camino a la bocatoma (ICN 11519). GUYANA. Georgetown: Demerara (BMNH 51111).

Eptesicus langeri sp. nov. (*n* = 19). BOLIVIA. Santa Cruz: Agua Rica (MNKM 5584 holotype, MNKM 5117, 5585, 4436, 5587, 5588, 5088, 5590, 5591, 4679, 5592, 5126, 4678, 5692 paratypes). Agua Clarita (MNKM 5586, 5589, 5676, 5636 paratypes). Reserva Municipal El Chape (MNKM 5697 paratype).

Appendix 9

Comparison of diagnostic traits among six species of the genus *Eptesicus*.

Characters	**E. furinalis**	**E. andinus**	***E. langeri*** sp. nov.	*E. chiriquinus*	*E. brasiliensis*	*E. ulapesensis*
Sagittal and nuchal crests	Joined	Separated	Separated	Joined	Joined	Joined
Development of sagittal and nuchal crests	Developed	Poorly developed	Developed	Well developed	Well developed	Well developed
Dorsal coloration	Dark or pale brown	Dark brown	Dark brown	Dark brown – oily black	Dark brown	Yellowish-brown
Dorsal fur length	< 7 mm	~ 9 mm	~ 8 mm	> 8 mm	< 9 mm	~ 6 mm
Bands of dorsal hair	Base almost black, tips light Brown, can change geographically	Dark Brown with lighter tips	Slightly bi-colored, dark base with lighter tips	Blackish, homogeneous	Base dark Brown with lighter tips	Base dark brown with tips brownish or goldish-yellowish
Bands of ventral hair	Base dark Brown, almost black, with yellowish tips	Strongly bi-colored, base dark and lighter tips "tanny color"	Base dark with lighter tips	Base dark Brown with lighter tips	Base dark Brown with paler tips (yellowish)	Base dark Brown with whitish tips
Preorbital process	Poorly developed	Poorly developed	Well developed	Moderately developed	Poorly developed	Poorly developed
Braincase	Straight and flattened	Enlarged and rounded	Elevated and rounded	High and developed due to the presence of cranial crests	Straight and flattened	Domed
Rostrum	Flattened and robust	Delicate and slender	Robust and inflated	Delicate and slender	Robust and inflated	Slightly flattened and robust