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Reassessment of the evolutionary relationships within the dog-faced bats, genus *Cynomops* (Chiroptera: Molossidae)

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The low representativeness of the dog-faced bats (genus *Cynomops* Thomas, 1920) in collections has constrained the study of the diversity and the evolutionary relationships within this genus. Taxonomic revisions of some taxa, in particular the large-sized *Cynomops abrasus* (Temminck, 1827), are crucial for understanding the phylogeny of *Cynomops*. A total of four subspecies of *C. abrasus* have been described to date, all widespread in South America: *C. a. mastivus* (Thomas, 1911), *C. a. brachymeles* (Peters, 1865), *C. a. cerastes* (Thomas, 1901) and *C. a. abrasus* (Temminck, 1827). Here, we evaluated the phylogenetic relationships within *Cynomops*, and the status of the four *C. abrasus* subspecies using complete sequences of two mitochondrial genes (Cyt *b* and COI) and 39 morphological characters. Maximum-parsimony, maximum-likelihood and Bayesian phylogenetic reconstructions recovered a novel hypothesis for *Cynomops*, supported the recognition of *C. a. mastivus* as a distinct species, separated from *C. abrasus*, and two hypotheses of lineages previously unrecognized for *Cynomops*. The use of mitochondrial genes combined with morphological characters revealed again to be a powerful tool to recover the phylogenetic relationships within *Cynomops* and demonstrated that the genus is more diverse than previously thought.

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Introduction

The neotropical dog-faced bats from the genus *Cynomops* Thomas, 1920 (Chiroptera: Molossidae) are fast-flying aerial insectivores that hunt in open spaces, usually above the canopy level in forested habitats (Kalko *et al.* 1996), and form small colonies of less than 15 individuals (Vizotto & Taddei 1976; Esbérard & Bergallo 2005). Due to their foraging behaviour and low local abundance, *Cynomops* is rarely captured in ground level mist-nets and consequently little represented in collections (Peters *et al.* 2002).

According to Simmons (2005) and Eger (2008), *Cynomops* is composed of six species including *Cynomops abrasus* (Temminck, 1827), *Cynomops greenhalli* Goodwin, 1958; *Cynomops mexicanus* (Jones and Genoways, 1967), *Cynomops milleri* (Osgood, 1914), *Cynomops paranus* (Thomas, 1901) and *Cynomops planirostris* (Peters, 1865). The geographical range of *Cynomops* extends from southern Mexico to Paraguay and northern Argentina, including Trinidad and Tobago (Goodwin & Greenhall 1961; Koopman 1982; Alvarez-Castañeda & Alvarez 1991; Eger 2008).

The taxonomic history of *Cynomops* is complex as the genus itself has previously been considered a subgenus of *Molossops* (Cabrera 1958; Goodwin & Greenhall 1961; Freeman 1981; Koopman 1993, 1994; Simmons & Voss

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1998). *Cynomops* contained only three species up to the 1990s (Koopman 1993), *C. planirostris*, *C. abrasus* and *C. greenhalli* and several described subspecies (Simmons 2005). More recently, based on parsimony analyses of restriction enzyme data, Peters *et al.* (2002) recognized *Cynomops* as a separate genus from *Molossops*, and provided further evidence to the recognition of *C. paranus* (previously suggested by Simmons & Voss 1998) and *C. mexicanus* as distinct species, from, respectively, *C. planirostris* and *C. greenhalli*.

The status of some forms of *Cynomops* has also been subject of debate. Simmons & Voss (1998) recognized *C. paranus* as a distinct species from *C. planirostris*, and Eger (2008) provided evidence to the recognition of *C. milleri*, a taxon with validity in dispute by some other authors (see Koopman 1978, 1993, 1994; Simmons & Voss 1998). Historically, no other *Cynomops* has been more controversial than *Cynomops abrasus* (Sanborn 1932; Cabrera 1958; Husson 1962; Carter & Dolan 1978; Simmons 2005; Eger 2008). The diversity included within *C. abrasus* is currently described into four subspecies distributed throughout South America: *Cynomops abrasus mastivus* (Thomas, 1911) from the Guiana Shield, *Cynomops abrasus brachymeles* (Peters, 1865) from eastern Peru and Bolivia, *Cynomops abrasus cerastes* (Thomas, 1901) from southern Brazil, Paraguay and northern Argentina, and the nominal form restricted to eastern Brazil (Cabrera 1958; Simmons 2005). The distinction of the *C. abrasus* subspecies has been, however, based primarily on body size (Cabrera 1958; Koopman 1994).

The monophyly of *Cynomops* has been recurrently recovered in recent studies conferring taxonomic stability for the genus (Peters *et al.* 2002; Ammerman *et al.* 2012; Gregorin & Cirranello 2015) but only a single analysis of the interrelationships within the genus remains available (Peters *et al.* 2002) based on the incomplete representativeness of the *C. abrasus* subspecies complex.

Herein, we propose a new framework for the understanding of the evolutionary relationships within *Cynomops* using complete sequences of Cytochrome *b*, Cytochrome oxidase subunit I and morphological data and testing the monophyly of *Cynomops abrasus*.

Materials and methods

Taxon sampling

We analysed specimens and obtained DNA sequence data from representative individuals of all currently recognized *Cynomops* species, and all subspecies described for *C. abrasus*. Outgroup taxa included *Eumops auripendulus*, *Molossops temminckii* and *Mormopterus kalinowskii*. The choice of the outgroups was based on recent and conflicting relationships placing *Cynomops* as sister group of *Molossops* (Ammerman *et al.* 2012) or not (Gregorin & Cirranello 2015), although both studies place *Eumops* and *Mormopterus* more distantly. A complete list of the specimens studied is available in the Supporting Information.

The material studied is deposited in the following collections: American Museum of Natural History, New York, USA (AMNH); Biodiversity Institute, University of Kansas, Lawrence, USA (KU); Instituto Nacional de Pesquisas na Amazônia, Manaus, Brazil (INPA); The Field Museum, Chicago, USA (FMNH); Mammal collection of Universidade Federal de Lavras, Lavras, Brazil (CMUFLA); Mammal collection of the Centro de Coleções Taxonômicas of Universidade Federal de Minas Gerais, Belo Horizonte, Brazil (CCT-UFMG); Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZUSP); Natural History Museum, London, UK (BMNH); Royal Ontario Museum, Toronto, Canada (ROM); Museum of Texas Tech University, Lubbock, Texas, USA (TTU/TK); Universidade Federal Rural do Rio de Janeiro, Seropédica, Brazil (ALP); Laboratório de Diversidade de Morcegos da Universidade Federal Rural do Rio de Janeiro, Seropédica, Brazil (LDM), Universidade Estadual Paulista, São José do Rio Preto, Brazil (DZSJRP); and United States National Museum of Natural History, Washington DC, USA (USNM). Tissue samples are housed in the frozen tissue collection of the ALP; AMNH; Au Institut des Sciences de l'Évolution, Montpellier, France (ISEM); CMUFLA; CCT-UFMG; Colección Regional Durango, Instituto Politécnico Nacional, Durango, Mexico (CRD); FMNH; Museo de Historia Natural de la Universidad Nacional San Agustín, Arequipa, Peru (MUSA); Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador (QCAZ); ROM; Smithsonian Tropical Research Institute, Balboa, Panama (STRI – PN); TTU; Universidade Federal da Paraíba, Paraíba, Brazil (UFPB) and USNM.

Morphological data

We used 39 discrete morphological characters, including 30 modified from Velazco (2005), Giannini & Simmons (2007a), Tavares (2008), Tavares *et al.* (2014), Gregorin (2009) and Gregorin & Cirranello (2015). A matrix containing all morphological characters used and a character description list is provided in the Supporting Information. Anatomical nomenclature follows Freeman (1981) and Giannini *et al.* (2006), and the dental homology follows Giannini & Simmons (2007b) as we assumed that the second lower premolar is lost in Chiroptera, and follows Gregorin & Cirranello (2015) as we considered the premolar arrangement of p1, p4 and p5 for molossids.

The glands used to score the penis characters were prepared for scanning electron microscopy by removing the prepuce (outer sheath and inner prepuce) (Ryan 1991a),

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rinsing overnight in water and followed by dehydration through a graded series of alcohol to 100% (50, 70, 80, 90 and 100). The material was kept in each bath for 15 min, except for the last step (100% alcohol), which was conducted twice. Then, specimens were dried to the critical point in CO2, mounted on a metal stub, coated with gold and photographed in a LEICA Stereoscan 440 (Scanning Electron Microscopy Laboratory, Smithsonian Institution – Washington, DC) and JEOL JSM-6360L (Microscope Center of Universidade Federal de Minas Gerais – Brazil). The nomenclature for the *glans penis* and its structures follows Ryan (1991a,b). We recorded external and craniodental measurements in millimetres (mm) using digital calipers accurate to 0.01 mm; body mass is in grams (g). Standard external measurements (TL, total length; HF, hind foot length; E, ear length; and body mass) were taken from skin labels or database records. Measurements are defined as follows:

Forearm length (FA), distance from the tip of the olecranon process to wrist (including carpals), and taken with the wing partially folded.

Greatest length of the skull (GLS), distance from the posteriormost point at the occipital bone to the most anterior point on the rostral-most bone.

Braincase breadth (BB), greatest breadth of braincase.

Mastoid breadth (MB), greatest breadth across mastoid region.

Rostral width (ROS), greatest breadth across the lacrimal ridges.

Condyloincisive length (CIL), distance from the posteriormost margins of occipital condyles to the anterior face of upper incisor(s).

Zygomatic breadth (ZB), greatest breadth across zygomatic arches.

Postorbital breath (POB), least breadth measured in the postorbital region, always posterior to the postorbital process when present.

Maxillary tooth row length (MTRL), distance from the anterior face of the upper canine to the most posterior edge of the last upper molar.

Breadth across upper molars (BM), least breadth across the last upper molars.

Width across upper canines (C-C), least width across the upper canines.

Mandible length (ML), from the mandibular symphysis to the condyloid process.

DNA extraction

The genomic DNA was isolated from the liver, muscle, brain or patagium tissue samples, and the DNA extractions were performed with a standard phenol–chloroform–isoamyl alcohol protocol (Sambrook *et al.* 2001) or with DNeasy® extraction kits (Qiagen®, Hilden, Germany). For the specimens USNM 387744 and USNM 319084, the DNA was extracted from dried skins according to the methods described in Wisely *et al.* (2004) and all pre-PCR protocols were conducted in an isolated ancient DNA laboratory facility located in a separate building from the one containing the primary DNA laboratory at the Center for Conservation and Evolutionary Genetics at the Smithsonian Institution, Washington, DC.

DNA sequencing

Cytochrome b (Cyt b–1140 bp). For preserved tissue samples, PCR amplifications were carried out in 15 μL reactions containing 40–60 ng of DNA, 1.5 U of Platinum Taq (Invitrogen®, Carlsbad, CA, USA), 1× Platinum Taq PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs set (Invitrogen®) and 0.3 μM of each primer. The primers used for amplifications were L14121 and H15318, and two additional internal primers – MVZ4 and L14881 – were used for sequencing reactions (Table 1). We used the following cycling scheme for PCR: 5 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 40 s at 50 °C for primer annealing

Table 1 Primers used in this study for amplification and/or sequencing.

Gene	Primers	Sequence (5′–3′)	Length (bp)	Reference
Cyt *b*	L14121	GACTAATGACATGAAAAATCA	1140	Redondo *et al.* (2008)
	H15318	TATTCCCTTTGCCGGTTTACAAGACC		
Cyt *b*	MVZ4	GCAGCCCCTCAGAATGATATTTGTCCTC	610	Smith & Patton 1993;
	L14881	GACATAATTCCATTCCACCCCTAC		Redondo *et al.* (2008)
Cyt *b*	CYNH1	GTATCRGATGTRTARTGTATTGCTAGG	176[1]	This study
Cyt *b*	CYNL2	GAAAYTTCGGCTCYCTYTTAGG	223	This study
	CYNH2	CCATARTAGAGYCCGCGTCC		
Cyt *b*	CYNL3	CCAAYGGRGCYTCAATATTC	262	This study
	CYNH3	TCTACTGAGAAGCCYCCTCAG		
Cyt *b*	CYNL4	CTGCAATYCCCTAYATYGGAAC	301	This study
	CYNH4	CCTAGRAGGTCRGGRGARAAT		
Cyt *b*	CYNL5	CTGAYATAATCCCYTTYCAYCC	226	This study
	CYNH5	CCTCCTARTTTRTTRGGGATTG		
Cyt *b*	CYNL6	CCTYCTAGGAGACCCYGACAA	235	This study
	CYNH6	TCARAATAGGCAYTGGCTTAG		
Cyt *b*	CYNL7	CACACYTCHAAACAACGAAG	224[2]	This study
COI	COX-L2	TGTCTTTAGATTTACAGTCTAATGC	1300	Lara-Ruiz *et al.* (2008)
	H8121	GGGCAGCCRTGRATTCAYTC		Sorenson 2003;
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	657	Folmer *et al.* 1994;
	COXIH	ACTTCAGGGTGTCCGAAGAATCA		Lara-Ruiz *et al.* (2008)

[1]Combined with L14121.

[2]Combined with H15318.

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and 85 s at 72 °C for extension, and a final 10 min extension at 72 °C after the last cycle. To amplify DNA from dried skin samples, PCR amplifications were carried out in 25 μL reactions containing 3 μL of genomic DNA, 1.5 U of TaqGold (Applied Biosystems, Foster City, CA, USA), 1× Ampli Taq Buffer (Applied Biosystems), 2 mM $MgCl_2$, 200 μM dNTPs, 0.4 μM of each primer and 4 μL bovine serum albumin (0.01 mg/μL). To minimize the risk of spurious results and contamination or failure of the reaction, each PCR was conducted with negative and positive controls. For PCR and sequencing reactions, we used internal primers designed for this study based on the sequences generated from the preserved tissues samples (Table 1). We used the following cycling scheme for PCR: 10 min at 95 °C followed by 55 cycles of 1 min s at 94 °C, 1 min at 50 °C for primer annealing and 1 min at 72 °C for extension, and a final 10 min extension at 72 °C after the last cycle.

Cytochrome oxidase (COI–1357 bp). The reagents used and their concentrations were the same as above for preserved tissue samples. The primers used for amplifications were COX-L2 and H8121, and two additional internal primers – LCO1490 and COXIH – were used for sequencing reactions (Table 1). We used the following cycling scheme for PCR: 5 min at 95 °C followed by 35 cycles of 1 min at 95 °C, 1 min at 50 °C for primer annealing and 1 min and 30 s at 72 °C for extension, and a final 10 min extension at 72 °C after the last cycle. We were unable to sequence COI from dried skin samples.

PCR products of tissue samples were sequenced using a 10 μL reaction mixture including 4 μL of PCR product, 2 μL of primer (2.5 μM), 1.5 μL Big Dye 5 × Buffer (Applied Biosystems), 1 μL Big Dye version 3 (Applied Biosystems). The reaction was run using a thermal cycler with denaturation at 96 °C for 10 s, annealing at 50 °C for 5 s and extension at 60 °C for 4 min, repeated for 26 cycles.

The mixture for the dried skin PCR products contained 1 μL of PCR product, 0.5 μL primer (10 μM), 1.75 μL Big Dye 5× Buffer (Applied Biosystems), 0.5 μL Big Dye version 3.1 (Applied Biosystems) and 6.25 μL sterile water. The reaction was run using a thermal cycler with denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s and extension at 60 °C for 4 min, repeated for 30 cycles.

Cycle-sequencing products of tissue samples were purified through an EtOH–EDTA precipitation protocol and the dried skin samples were cleaned using sephadex centrifugation protocol. The sequences of both strands were carried out in an ABI 3130 (Applied Biosystems®) automated sequencer using Big Dye Terminator Cycle Sequencing methodology (Applied Biosystems®).

The sequences produced in this study were deposited on GenBank under accession numbers: KR608122–KR608180 (COI) and KR608181–KR608256, KT724054 (Cyt *b*).

Phylogenetic analyses

Sequences were assembled and checked for their quality using the DNA Baser Sequence Assembler v4 (Heracle BioSoft 2013), aligned using the Muscle algorithm (Edgar 2004) as implemented in MEGA 5.05 (Tamura *et al.* 2011) and checked for coherent codon translation. MEGA 5.05 was also used to calculate intraspecific and interspecific genetic distances for Cyt *b* with a Kimura 2-parameter (K2P) model (Kimura 1980).

We conducted maximum-parsimony (MP) analyses for the combined molecular and morphological data using the heuristic search algorithm implemented in an intel-based version of PAUP* 4.0B10 (Swofford 2002). Each search was conducted with 1000 iterations of the heuristic search algorithm with random taxon addition and TBR branch swapping. All characters were equally weighted in all analyses. Bootstrap values (Felsenstein 1985) were calculated as a measurement of support with 1000 pseudoreplicates using a heuristic search with 100 random additions.

Partition Finder 1.0.1 (Lanfear *et al.* 2012) was used to select the best partitions and models of sequence evolution using the Akaike information criterion (AIC; Akaike 1974). We defined separate data blocks for the three codon positions for both genes.

Maximum-likelihood (ML) analyses were performed for the molecular data alone, and for the combined molecular and morphological data (total evidence analysis) using RAxML 7.2.8 through the CIPRES portal (Stamatakis 2006; Miller *et al.* 2010). The ML was implemented using independent general time-reversible models, with among site rate variations estimated by discrete gamma categories (GTR+G) (Stamatakis 2006) assigned to each codon position and morphological data partition in the total evidence analyses. Bootstrap resampling was used to assess support for the tree nodes with 1000 replicates.

We conducted Bayesian molecular and total evidence analyses using MrBayes 3.2.3 (Huelsenbeck & Ronquist 2001) also through the CIPRES portal, and the best-fit partitioning schemes and models for each data set as retrieved by the partition finder runs, except for the morphological data partition in the combined Bayesian analyses, for which a standard stochastic model (Mkv) was employed (Lewis 2001). The number of generations needed to be run and the burn-in were determined by examining the log-likelihood (lnL) plots as provided by the software Tracer 1.6 (Rambaut *et al.* 2014). Four simultaneous Markov chains (one cold and three heated) were run for 20 million generations, with trees sampled every 200

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generations, and the first 10% of the generations were discarded as 'burn-in'.

Hypothesis testing for the phylogeny of Cynomops

We assessed significance of differences between the phylogenetic hypotheses available for relationships within the genus *Cynomops* (ours and that of Peters *et al.* 2002) using the approximately unbiased test (AU) as implemented in the software *Consel* (Shimodaira 2002). The input trees to be compared were calculated in RAxML using the *constraint* command.

Results

Best-fitting models of sequence evolution for each gene and partition are summarized in Table 2. All nodes were recurrently supported in all analyses with virtually no incongruences. Sequence data alone (Supporting information, Fig. S1) produced the same results as the combination of molecular and morphological data (Fig. 1, Supporting information, Fig. S2), with the exception that morphological data provide stronger support values for the node containing the clades C (*C. milleri* and *Cynomops* sp. 2) and D (*C. abrasus*, *C. a. mastivus*, and *C. greenhalli*) (Fig. 1; BPP = 0.99, MP = 69 Supporting information, Fig. 2, ML = 67; Supporting information, Fig. 1; BPP = 0.83 and ML = 59). The *approximately unbiased* (AU) tests comparing our tree topology and the hypothesis proposed by Peters *et al.* (2002) did not rule out their topological arrangements among taxa (δLnl = 2.3; AU = 0.279).

Table 2 Data set characteristics and best-fitting models of nucleotide substitution.

Data set	Terminals	Base pairs/ characters	Invariant sites	Parsimony informative sites	Selected models/ partition
Cyt *b*	77	1140	750	308	GTR + I + G for 1st position, GTR + I for 2nd, GTR + I + G for 3rd
COI	59	1357	965	336	GTR + I + G for 1st position, HKY for 2nd, GTR + G for 3rd
mtDNA	84	2497	1715	644	GTR + I + G for 1st_Cyt *b*, GTR + I for 2nd _ Cyt *b*, GTR + I + G for 3rd_ Cyt *b*; GTR + I + G for 1st_COI, HKY for 2nd COI, GTR + G for 3rd_COI
Morphology	85	39	22	17	Mkv

The monophyly of *Cynomops* was fully supported by our combined data (Fig. 1) that also recovered full support for most of the internal clades (Fig. 1). A total of seven morphological characters support the clade of *Molossops* and *Cynomops*, and four synapomorphies support the monophyly of *Cynomops* (Supplementary material characters 16, 31, 32, and 39). The clade A (Fig. 1) consists of a sister relationship between *C. mexicanus* and a clade containing individuals from Colón and Pacora (Panama), which were previously identified as *C. paranus* and *C. greenhalli* that we provisionally assigned as '*Cynomops* sp. 1′ (Fig. 1). The clade B is composed of specimens of *C. planirostris* from several localities (from Upper Takutu-Upper Essequibo, Guyana to Ñeembucu, Paraguay) and also includes the holotype of *C. paranus*; the clade C consists of *C. milleri* from the Guiana Shield as sister group to a series of *Cynomops* from the eastern Andes of Ecuador, identified as '*Cynomops* sp. 2′ (Fig. 1); and the clade D splits into two main clades, recovering a paraphyly of *C. abrasus*.

A branch of the clade D is composed of individuals of *C. abrasus mastivus* and *C. greenhalli*, and another one contains *C. abrasus* from several localities (from Madre de Dios, Peru to Itapúa, Paraguay). *Cynomops abrasus mastivus* (from the Guianan Shield, Ecuador, and north-western Brazil) is clearly separated from the large clade containing representatives from other subspecies of the *C. abrasus*, including *C. a. abrasus* (south-eastern Brazil), *C. a. brachymeles* (south-eastern Peru) and *C. a. cerastes* (Paraguay) (Fig. 1).

Intraspecific genetic distances for the Cyt *b* gene varied from 0.5% to 3.7% (Table 3). Within *C. greenhalli*, we found individual differences with *p*-distance of 3.7% between individuals from Panama and from the western Andes of Ecuador. A mean *p*-distance of 3.0% was found between two haplogroups of *C. planirostris*: one from Guiana Shield + Peru, and an individual from Ñeembucu, Paraguay and another group composed of individuals from eastern Brazil + Paraguay. Additionally, two individuals of *C. abrasus* from São Paulo, south-eastern Brazil (R3234 and R3627), formed a clade sister to a large *C. abrasus* clade (Fig. 1, clade D), and both groups were 3.3% genetically divergent (Fig. 1 and appendix S2). Values for interespecific variation ranged from 4.6% (*C. greenhalli* vs. *C. a. mastivus* and *C. milleri* vs. *Cynomops* sp. 2) to 12.5% (*Cynomops* sp. 1 vs. *Cynomops* sp. 2).

The paraphyly of *C. abrasus* (*lato sensu*) reinforced by a set of morphological characters that distinguish a lineage formed with individuals of *mastivus* only and the remaining *abrasus* indicate that *C. mastivus* is a distinct species that merits recognition apart from *C. abrasus*. We therefore provide herein a redescription, and an emended diagnosis

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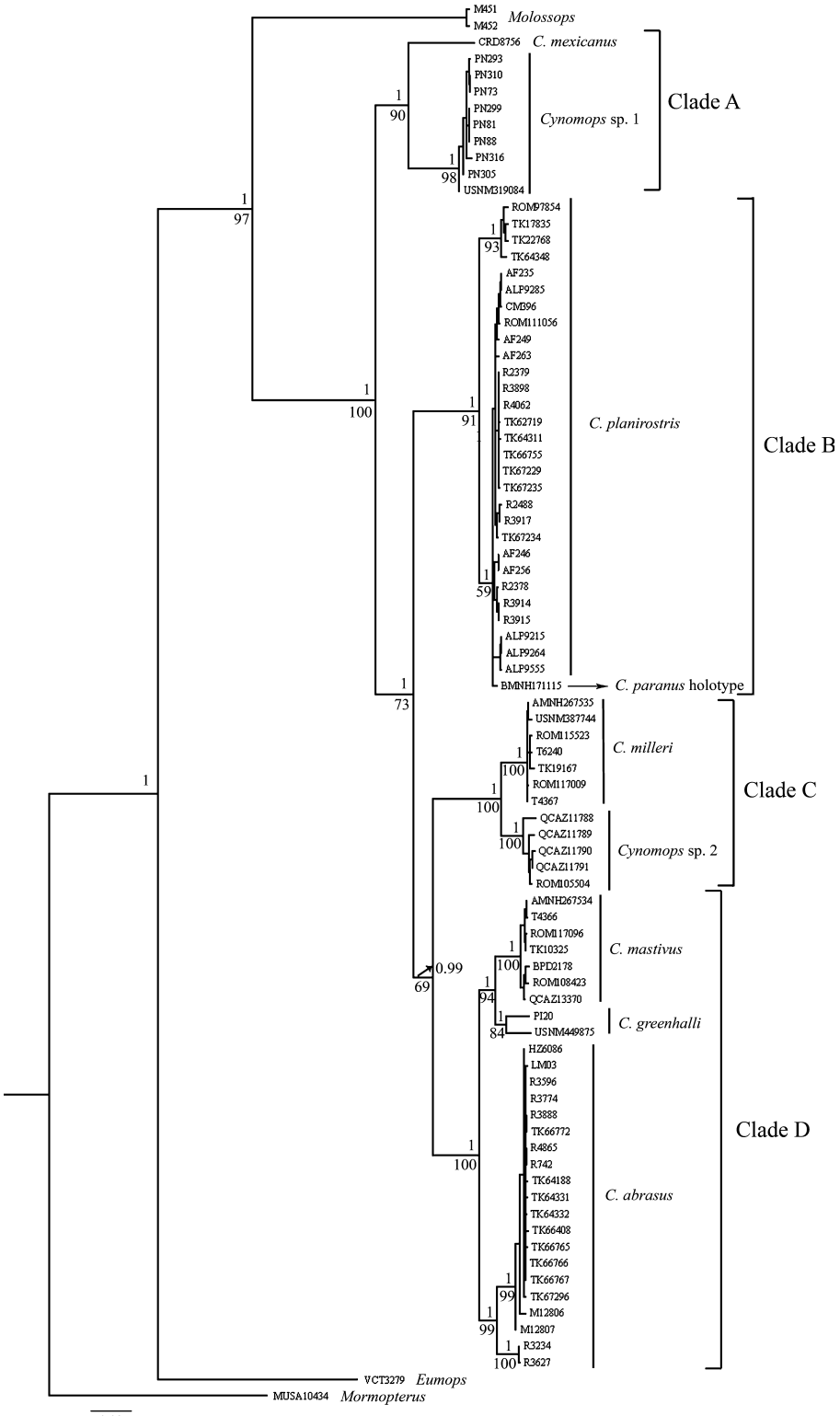

Fig. 1 Bayesian tree for *Cynomops* species generated with 2497 base pairs of the mtDNA (COI + Cyt *b*) and 39 morphological characters. Values above branches represent Bayesian posterior probabilities (BPP) and below branches, the maximum-parsimony (MP) bootstrap. See Supporting information for museum acronyms and collection sites.

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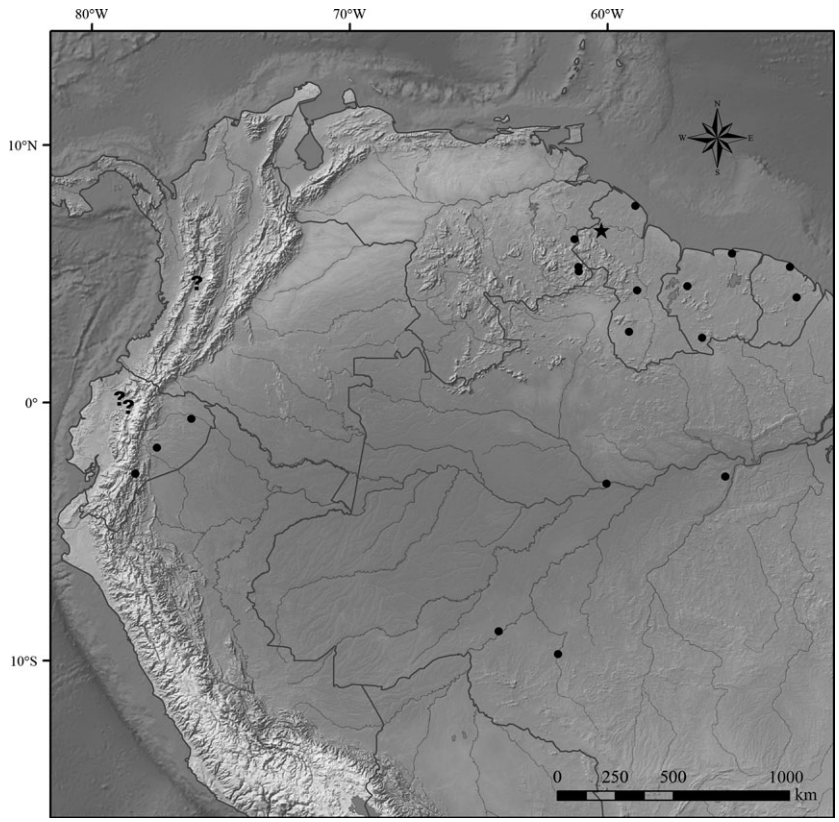

Fig. 2 Distribution of *Cynomops mastivus* in South America, including the type locality (star). The question marks represent the dubious localities on western side of the Andes.

Table 3 Average Kimura 2-parameter distances (%) between species of *Cynomops* based on 1140 base pairs of the Cyt *b*. Intraspecific divergence is on the diagonal in bold.

	n	1	2	3	4	5	6	7	8
1. *C. abrasus*	16	**1.1**							
2. *C. greenhalli*	2	5.8	**3.7**						
3. *C. mastivus*	7	4.9	4.6	**1.0**					
4. *C. mexicanus*	1	11.4	11.6	11.9	–				
5. *C. milleri*	7	9.0	9.9	9.7	11.7	**0.5**			
6. *C. planirostris*	24	8.9	9.3	9.6	11.5	9.7	**1.6**		
7. *Cynomops* sp. 1	9	10.6	10.6	10.9	7.9	12.3	11.0	**0.6**	
8. *Cynomops* sp. 2	5	9.8	10.2	9.7	12.0	4.6	10.6	12.5	**1.1**

for the recognition of the species *C. mastivus* and an emended diagnosis for *C. abrasus*.

We were unable to differentiate the types of *C. planirostris* and *C. paranus* based on morphology (Fig. 1, and Supplementary material) and suggest that *C. paranus* is a junior synonym of *C. planirostris*. The darker dorsal and ventral pelage colouration observed by Thomas (1901) and by Simmons & Voss (1998) is also present in series of *C. planirostris* from the Brazilian Amazonia, states of Pará and Amazonas (AMNH 79731, 79733, 92253–55, 92971, 93879–93887, 94630–94653), and may be explained by geographical or individual variation (Fig. 3). Herein, we also provide an emended diagnosis for *C. planirostris*.

Family MOLOSSIDAE

Genus *Cynomops* Thomas, 1920:

***Cynomops mastivus* (Thomas, 1911) (Figs 4–7)**

Molossops mastivus Thomas, 1911: 113; type locality 'Bartica Grove, lower [Río] Essequibo', Cuyuni-Mazaruni, Guyana.

Cynomops mastivus: Thomas 1920:189; first use of current name combination.

Molossops [(*Cynomops*)] *brachymeles mastivus*: Cabrera 1958: 119; name combination.

Cynomops abrasus: Husson 1962: 246; part; not Temminck 1827.

Molossops [(*Cynomops*)] *abrasus mastivus*: Williams & Genoways 1980: 233; name combination.

Cynomops abrasus [*mastivus*]: Simmons 2005; name combination.

Type material. The holotype BMNH 10.11.10.3 is a relatively well-preserved skin and a skull of an adult male from Bartica Grove, lower Essequibo, Cuyuni-Mazaruni, Guyana, collected by Mr. Crozier and brought to the British Museum in 1910 by F. V. McConnell (Lim & Catzeflis 2014). The date of the capture and other information

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are not provided in the label. For material examined see Supporting Information.

Measurements of the holotype. FA 48.00, GLS 23.12, POB 5.73, ROS 10.81, C-C 6.85, ZB 16.20, BB 10.68, MB 15.58, MTRL 8.64, BM 10.24, CIL 22.95 and ML 17.31. Additional measurements (mm) are from Thomas (1911): Third metacarpal length 49, fifth metacarpal 26, first phalanx of third metacarpal 11.5, interorbital breadth 5.5, height of the canine 4.8 and height of mandible below m1 3.5.

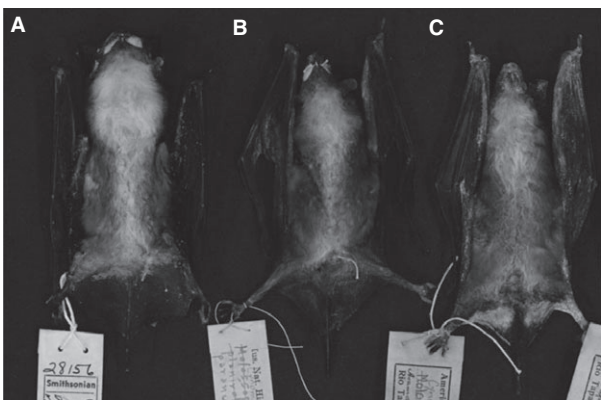

Fig. 3 A–C. *Cynomops planirostris*, female. Variation of the ventral pelage colouration – A. Venezuela (USNM409557). – B. Brazilian Amazon (AMNH 79733). – C. Brazilian Amazon (AMNH 94636). Note that the variation in the width of the bands between the exemplars from A, and B and C. See Supporting information for precise location.

Distribution. As defined here, *Cynomops mastivus* is known from the lowlands (5–534 m a.s.l.) of northern South America, on the eastern slopes of the Andes in Venezuela, Guyana, Surinam, French Guiana, Ecuador and the Brazilian Amazonia (Fig. 2). The only specimen analysed from Colombia, south-western Andes, and labelled as '*abrasus*' (FMNH 89574) is a young male with damaged skull and its identity could not be securely determined.

Emended diagnosis. The largest known *Cynomops* (males: FA 47.00–51.24, $n = 6$; GLS 22.30–24.71, $n = 5$; females: FA 41.77–46 $n = 11$; GLS 19.15–20.96 $n = 10$; Table 4). Dorsal pelage is dark reddish brown, and the ventral colouration is uniformly brown, similar or slightly paler than dorsum; anterior face of lacrimal ridges forms an abrupt angle with the forehead; nasal process of premaxilla are well developed, with the lateral margin of the external

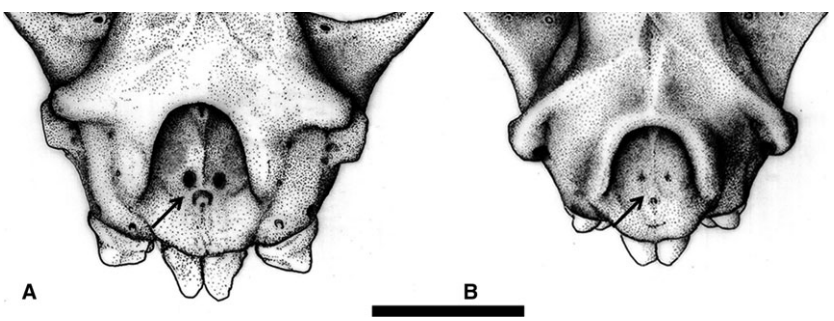

Fig. 4 Ventral views of the skull showing the incisive foramina and accessory foramen (arrows). – A. *Cynomops mastivus* (DZSJRP 11600; male). – B. *Cynomops abrasus* (DZSJRP 2162; male). Note that the accessory foramen is closer to the incisive foramina in *C. mastivus* than it is in *C. abrasus*. Scale bar = 5 mm.

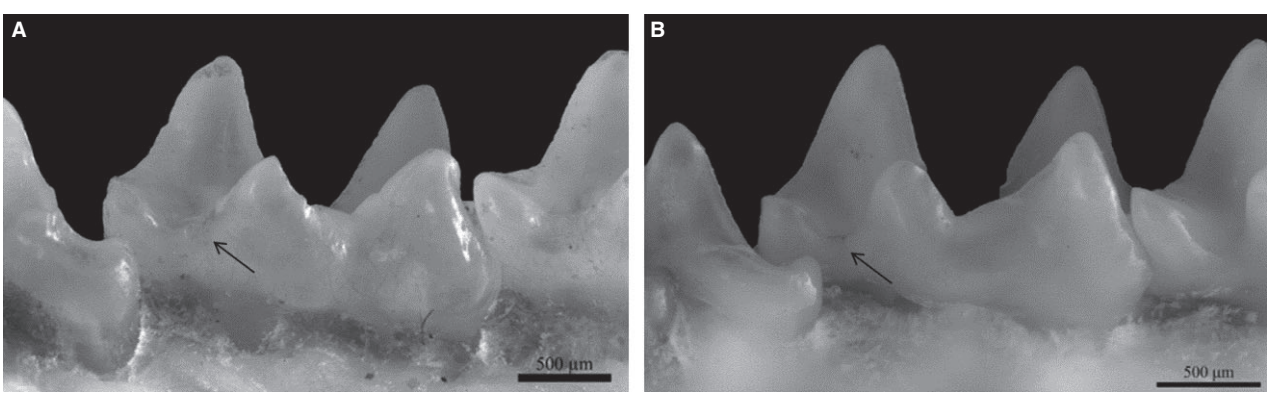

Fig. 5 Lingual view of the first lower molar of species of *Cynomops*. – A. *Cynomops mastivus* (DZSJRP 11600; male) – B. *C. abrasus* (DZSJRP 2162; male). Arrows indicate the trigonid. Note the shallow and wide trigonid in *C. mastivus*, and deep and narrow trigonid in *C. abrasus*.

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nares vertically straight; the incisive foramina are located closer to the accessory foramen, the arrangement of the three foramina forming an equilateral triangle (Fig. 4A); basisphenoid pits are absent; there is a shallow fossa in the posterior squamosal bone, where the zygoma meets the braincase (see Velazco 2005: 12); a large and shallow trigonid occurs on lower M1 (Fig. 5A); a well-developed median ridge on the lingual face of the second lower premolar is present; the lower first premolar measures two-thirds or more of the height of the second lower premolar.

Redescription. The pelage is silky, but the dorsal fur is not very long (4 mm in length, taken on the level of the scapular area), and the individual dorsal hairs are bicoloured, with the basal third of each coloured pale buff. The face is blackish and virtually naked; the upper lip and the dorsal border of the narial region are smooth; the triangular and blackish ears are slightly separated each other at the forehead (space ≤4.0 mm); the patagium, feet and tail are also blackish; the propatagium is narrow, and the posterior plagiopatagium is inserted lateral to the base of the feet. There is dark chocolate brown fur distributed along one-third of the forearm, and along the adjacent propatagium. A second patch of fur extends from the posterodorsal surface of the distal plagiopatagium, next to the wrist, to dactilopatagium IV.

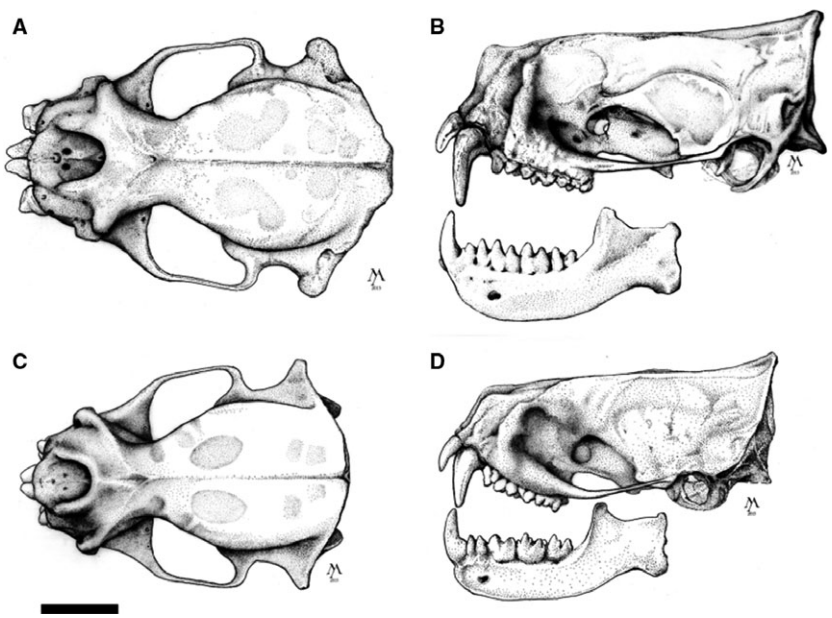

Fig. 6 A–B. *Cynomops mastivus* (DZSJRP 11600; male). – A. Dorsal view. – B. Lateral view. C-D. – *C. abrasus* (DZSJRP 2162; male). C. Dorsal view. – D. Lateral view. Scale bar = 5 mm.

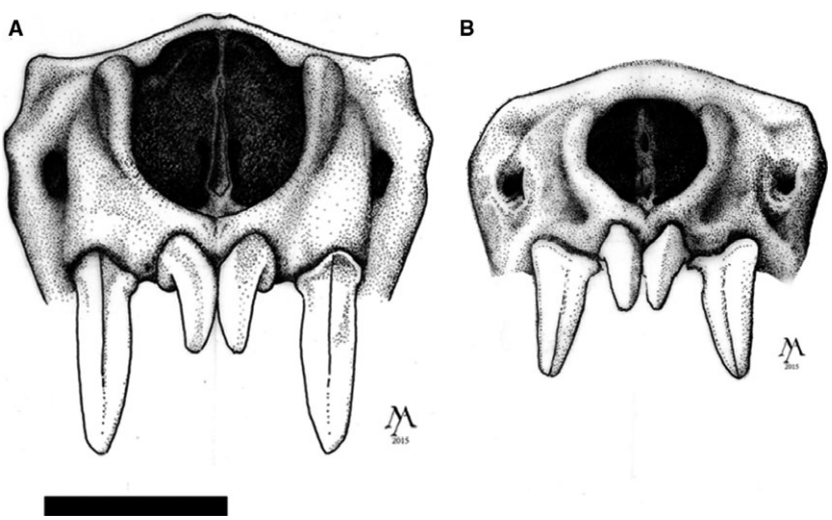

Fig. 7 A. *Cynomops mastivus* (DZSJRP 11600; male), frontal view. – B. *C. abrasus* (DZSJRP 2162; male), frontal view. Note the well-developed nasal process of premaxilla, with the lateral margin of the external nares straight in *C. mastivus*, and the reduced nasal process with lateral margin of the external nares concave in *C. abrasus*. Scale bar = 5 mm.

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Table 4 Measurements (mm) of *Cynomops abrasus* and *C. mastivus* from several localities in South America. Each entry gives mean (range) and sample size.

	Cynomops abrasus		*Cynomops mastivus*	
Number	38 females	20 males	11 females	06 males
Weight	31.50 (30.00, 33.00) 2	31.00	29.57 (27.00–33.00) 3	–
FA	44.51 (40.60–47.50) 35	45.84 (42.20–49.35)18	43.80 (41.77–46.00) 11	49.12 (47.00–51.24) 6
E*	17.83 (17.00–20.00) 6	19.00 (2)	18.40 (16.00–20.00) 5	17.00 (16.00–18.00) 2
TL*	35.46 (34.00–39.00) 14	38.00 (33.00–42.00) 7	35.30 (32.00–42.00) 10	41.50 (39.00–44.00) 2
HF*	11.54 (9.00–13.00) 13	11.80 (10.00–13.00) 5	12.20 (11.00–13.00) 10	10.29 (9.88–11.00) 3
GLS	19.59 (18.40–20.49) 37	21.01 (19.94–22.26) 12	20.08 (19.15–20.96) 10	23.35 (22.30–24.71) 5
POB	5.15 (4.74–5.72) 36	5.24 (4.99–5.56) 12	5.21 (4.97–5.40) 10	5.77 (5.51–6.29) 5
ROS	8.82 (8.05–9.68) 35	9.63 (8.63–10.23) 16	8.80 (8.43–9.20) 9	10.72 (10.16–11.31) 5
C-C	5.68 (5.19–6.24) 35	6.33 (5.94–6.78) 17	5.91 (5.63–6.15) 10	7.13 (6.64–7.88) 5
ZB	14.01 (12.93–14.56) 31	14.95 (14.29–15.85) 14	14.26 (13.85–14.93) 10	16.82 (16.05–17.90) 4
BB	9.99 (9.37–10.44) 37	10.22 (9.59–10.75) 17	10.12 (9.80–10.33) 10	10.98 (10.57–11.42) 5
MB	13.59 (12.44–14.40) 31	14.97 (13.93–16.21) 15	13.83 (12.90–14.48) 9	17.04 (15.58, 17.86) 3
MTRL	7.66 (7.10–8.16) 36	8.12 (7.45–8.61) 17	7.82 (7.36–8.13) 10	8.82 (8.64–9.06) 5
BM	9.60 (9.03–10.22) 37	9.95 (9.45–10.42) 16	9.63 (9.25–9.98) 10	10.46 (10.09–10.99) 5
CIL	19.80 (18.42–20.86) 35	21.19 (19.77–22.48) 17	20.33 (19.38–21.39) 10	23.63 (22.95–24.91) 5
ML	14.88 (13.89–15.64) 37	15.80 (14.71–16.68) 17	15.31 (14.81–15.77) 10	17.52 (16.61–18.23) 5

*Measurements taken from the labels.

The skull is robust, with the sagittal and the occipital crests consistently well developed in males; the anterior face of the lacrimal ridges forms an abrupt angle with the forehead (Fig. 6A, B); the nasal process of the premaxilla is well developed, with the lateral margin of the external nares vertically straight, particularly in males, and less markedly in females (Fig. 7A), and the incisive foramina are located relatively close to the accessory foramen (Fig. 4A).

The basisphenoid pits are absent and there is a shallow fossa on the posterior squamosal bone, where the zygoma meets the braincase (see Velazco 2005: 12). The mandible of males is massive, with a convex corpus along its length (Fig 6B). There is a large and shallow trigonid on the lower M1 (Fig. 5A) and a well-developed median ridge on the lingual face of the second lower premolar, and the first lower premolar is two-thirds or more of the height of the second lower premolar.

Comparisons. *Cynomops mastivus* can be readily distinguished from all the other species of the genus (*C. abrasus*, *C. greenhalli*, *C. milleri* and *C. planirostris*) by its large size (Table 4), and by the consistently well-developed posterior sagittal and occipital crests in males. *Cynomops mastivus* resembles *C. abrasus*, and both can be distinguished from the small-median species of *Cynomops* (*C. greenhalli*, *C. milleri* and *C. planirostris*) by their larger size, with males and females having forearm lengths measuring more than 42 mm and 40 mm, respectively, while males and females of smaller *Cynomops* have forearm lengths measuring less than 40 mm and 38.50 mm, respectively. *Cynomops mastivus* and *C. abrasus* can also be separated from smaller forms of *Cynomops* by patterns of the ventral pelage colouration, which is only slightly lighter than the dorsum in the two larger forms and may be much paler in the smaller species, at least in part of the ventral axis of the body.

Cynomops mastivus can be distinguished from *C. abrasus* by the presence of a shallow and wide trigonid on the lower M1, which is deep and narrow in *C. abrasus* (Fig. 5); the anterior face of lacrimal ridges forms an abrupt angle with the forehead in *C. mastivus*, and slopes smoothly to the forehead in *C. abrasus*; by massive and convex mandible of the males of *mastivus*, as opposed to the gracile and relatively straight, not convex, mandible of *C. abrasus*; and by the larger and more robust skull of *C. mastivus* (Table 4; Fig. 6).

***Cynomops abrasus* (Temminck, 1827) (Figs 4–7)**

Dysopes abrasus Temminck, 1827: 232; type locality 'parties intérieures du Brésil'; restricted to Votuporanga, São Paulo, Brazil by Eger (2008).

M[*olossus (Promops)*]. *abrasus*: Peters, 1865: 574; name combination.

M[*olossus* (*Molossops*)]. *brachymeles* Peters, 1865: 575, footnote; type locality 'Peru'; herewith restricted to Marcapata, Cusco, Peru.

Molossus cerastes Thomas, 1901: 440; type locality 'Villa Rica', Guairá, Paraguay.

[*Molossus* (*Myopterus*)] *cerastes*: Trouessart 1904: 101; name combination.

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Molossops cerastes: Miller 1907: 248; name combination.

Cynomops cerastes: Thomas, 1920: 189; generic description and name combination.

Molossops [(*Cynomops*)] *brachymeles brachymeles*: Cabrera 1958: 118; name combination. *Cynomops abrasus*: Husson 1962: 246; first use of current name combination.

Molossops [(*Cynomops*)] *brachymeles cerastes*: Cabrera 1958: 118; name combination.

Molossops (*Cynomops*) *abrasus*: Carter & Dolan 1978: 84; name combination.

Cynomops abrasus cerastes: Barquez *et al.* 1999: 170; name combination.

Type material. The holotype RNH17374 is an adult female alcohol preserved with skull removed from the interior of Brazil (Husson 1962), collected by Maximilian, Prinz zu Wied-Neuwied. The date and exact location of capture are not provided, and we are following Eger (2008) assuming Votuporanga, São Paulo, Brazil, as the type locality. For material examined, see Supporting Information.

Measurements of the holotype. As we could not examine the type at the Leiden Museum, all measurements (mm) are from Husson (1962), who extracted the skull and made a detailed description: forearm 42, length of third metacarpal 42.5, first phalanx 18.5, second phalanx 15, third phalanx 4, length of fourth metacarpal 41, first phalanx 15.5, second phalanx 6, length of fifth metacarpal 26.5, first phalanx 10, second phalanx 4, length of ear 14, width across molars 9.2, width across cingula canines 5.3, upper tooth row, c-m^3 7.4, lower tooth row, c-m^3 8.3 and length of mandible 14.3.

Distribution. *Cynomops abrasus* is distributed on the eastern slopes of the Andes, known from low to medium lands (5–811 m a.s.l.) of Peru, Bolivia, Brazil, Paraguay and northern Argentina (Eger 2008; Acosta & Paca 2010; Supporting information).

Emended Diagnosis. A big-sized *Cynomop*s (males: FA 42.20–49.35, $n = 18$; GLS 19.94–22.26, $n = 12$; females: FA 40.60–47.50, $n = 35$; GLS 18.40–20.49, $n = 37$) with dorsal pelage varying from lighter reddish brown to dark brown and ventral colouration slightly paler than dorsum. The individual dorsal hairs are bicoloured, with the basal third of each coloured pale buff; the anterior face of the lacrimal ridge slopes smoothly to the forehead (Fig. 6D); the incisive foramina are located much posterior to the accessory foramen, and the arrangement of the three foramina (incisive and accessory) forms an isosceles triangle (Fig. 4B); the basisphenoid pits are shallow; the trigonid on the lower M1 is deep and narrow (Fig. 5B); the mandible is gracile and relatively straight, not convex, along its length (Fig. 6D); the nasal process of the premaxilla is reduced, with the lateral margin of the external nare concave (Fig. 7B).

***Cynomops planirostris* (Peters, 1865)**

M[*olossus* (*Molossops*)]. *planirostris* Peters, 1865: 575, footnote; type localities Guyana, Barra do Rio Negro [Brazil] and Buenos Aires [Argentina]; restricted by Cabrera (1958: 119) to Guyana; later corrected by D. C. Carter & Dolan (1978: 86) to Cayenne, French Guiana, based on the lectotype.

[*Molossus* (*Myopterus*)] *planirostris*: Trouessart 1897: 142; name combination.

Molossus planirostris paranus Thomas 1901: 190; type locality 'Para,' Pará, Brazil. [*Molossus* (*Myopterus*) *planirostris*] *paranus*: Trouessart 1904: 101; name combination.

Molossops planirostris: Miller 1907: 248; name combination.

Molossops paranus: Miller 1907: 248; name combination.

Cynomops planirostris: Thomas 1920: 189; generic description and first use of name combination.

Cynomops paranus: Thomas 1920: 189; name combination.

Molossops planirostris paranus: Vieira 1942: 432; name combination.

Molossops [(*Cynomops*)] *planirostris planirostris*: Cabrera 1958: 119; name combination.

Molossops [(*Cynomops*)] *planirostris paranus*: Cabrera 1958: 119; name combination.

Cynomops planirostris paranus: Goodwin, 1958: 5; name combination.

Molossops greenhalli: Mares *et al.* 1981; : 112; not Goodwin 1958.

Type material. The lectotype ZMB 2513 is an adult male alcohol preserved with skull removed from Cayenne, French Guiana, collected by Sir Robert Hermann Schomburgk among 1835–1844. The exact date of the capture and other information are not provided in the label. For material examined, see Supporting Information.

Measurements of the Lectotype. FA 33.61, GLS 16.38, POB 4.22, ROS 7.15, C-C 4.80, BB 8.48, MB 10.87, MTRL 6.43, BM 7.37, CIL 16.47 and ML 11.65.

Distribution. *Cynomops planirostris* is widely distributed in South America on the eastern slopes of the Andes, known from low and highlands (4–2500 m a.s.l.) of French Guiana, Guyana, Suriname, Venezuela, Colombia, Peru, Bolivia, Brazil, Paraguay and Argentina (Supporting information).

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Emended Diagnosis. A small *Cynomop*s (males: FA 31.36–36.56, $n = 50$; GLS 15.21–17.29, $n = 46$; females: FA 29.00–34.92, $n = 83$; GLS 14.11–16.20, $n = 93$) with dorsal pelage varying from chocolate brown to grayish brown and ventral colouration paler than dorsum with gular and mid-ventral region usually whitish or pale buff coloured. The individual dorsal hairs are bicoloured, with the basal half of each hair pale buff; the anterior face of the lacrimal ridge slopes smoothly to the forehead, as in *C. abrasus* (Fig. 6D); the incisive foramina are located closer to the accessory foramen, and the arrangement of the three foramina (incisive and accessory) forms an equilateral triangle, as in *C. mastivus* (Fig. 4A); the basisphenoid pits are shallow; a well-developed median ridge is present in the lingual face of the second lower premolar (p5).

Discussion

The monophyly of *Cynomops* and its sister relationship with *Molossops* recovered by our analysis is consistent with the phylogenetic hypothesis of Peters *et al.* (2002) based on restriction enzymes of mtDNA and of Ammerman *et al.* (2012) based on their multiloci analyses. On the other hand, the morphological data of Gregorin & Cirranello (2015) suggest a basal position for *Cynomops* in a clade composed of *Molossops*, *Neoplatymops*, *Platymops*, *Sauromys* and *Mormopterus*, or a sister group relationship between *Cynomops* and a clade that contains *Myopterus*, *Cheiromeles*, *Molossus* and *Promops*. As we have not included all the content of molossids as did Ammerman *et al.* (2012) and Gregorin & Cirranello (2015), this question remains in debate.

According to our analysis, a total of seven morphological characters support the clade of *Molossops* and *Cynomops*, and four synapomorphies support the monophyly of *Cynomops*. *Cynomops* can be differentiated from *Molossops* by several characters, including the distance separating the insertion of the inner ear margins over the head (>4.0 mm in *Molossops*), the presence of two lower incisors in each maxillary ramus (one in *Molossops*), the pattern of shallow basisphenoid pits (deep pits in *Molossops*) and the absence of a premetacrista on the M3 (medially developed in *Molossops*) (Thomas 1920; Williams & Genoways 1980; Eger 2008; Supporting information). Karyotype data for diploid and fundamental numbers, and chromosome morphology differentiating *Cynomops* and *Molossops*, have also been reported (Gardner 1977; Morielle-Versute *et al.* 1996).

Our phylogeny overall agreed with that of Peters *et al.* (2002) in recovering four main clades within the *Cynomops* tree. Furthermore, we uncovered three additional lineages: *C. abrasus* (Brazil, Paraguay and Peru), *Cynomops* sp. 1, consists of a series from Panama and *Cynomops* sp. 2, consists of a series from the eastern of Ecuador. The position of *C. planirostris* within the *Cynomops* tree is still conflicting. While Peters *et al.* (2002) recovered moderate support for sister relationships between *C. planirostris* and *C. milleri* (referred there as *C. paranus*), our results suggest a basal position for *C. planirostris* (clade B) to all the other species of *Cynomops*, with the exception of *C. mexicanus* and *Cynomops* sp. 1.

Cynomops mastivus is closer to *C. greenhalli* than to *C. abrasus*, which in turn is sister to the *greenhalli* + *mastivus* clade. Some karyological data (Warner *et al.* 1974) indicated differences between the karyotypes of *C. abrasus* from south-eastern Peru and *C. greenhalli* based on the examination of a single individual of *C. abrasus*. Unfortunately, there are no karyological data available for *C. mastivus* (Santos *et al.* 2001; Leite-Silva *et al.* 2003). More recently, techniques such as banding and fluorescent *in situ* hybridization have shown that there are differences in number of NOR-bearing chromosomes in *Cynomops* as *C. abrasus* have NORs in five (11, 12, 14, 15 and 16) pairs of chromosomes (Morielle-Versute *et al.* 1996) while *C. planirostris* have NORs located only in two pairs (9 and 10) (Leite-Silva *et al.* 2003).

The mean *p*-distances observed among allopatric clades of *C. planirostris*, *C. abrasus*, and *C. greenhalli* were high, and above the threshold suggested by Bradley & Baker (2001) for the intraspecific variation of mammals but the morphological data indicate little intraspecific variation. More independent data sets, and phylogeographical analyses may be used in the future to clarify whether these taxa internally represent conspecific populations or cryptic taxa (e.g. Clare 2011).

Recent systematic revisions of Neotropical bats have shown that widespread taxa often correspond to species complexes, and many new species have been described over the last decade (e.g. Velazco & Patterson 2008, 2014; Velazco *et al.* 2010; Tavares *et al.* 2014). The evidence obtained in the present study revealed that *Cynomops* may be a complex of six to eight species. Cabrera (1958) suggested that the morphological variation between the forms of *Cynomops abrasus* was not enough to separate them as distinct species, but instead, they could be recognized as 'geographical variations'. In fact, many authors have commented on the similarities in size of *C. a. abrasus*, *C. a. brachymeles* and *C. a. cerastes*, suggesting that additional material was necessary to confirm their taxonomic status (Sanborn 1932; Husson 1962; Eger 2008). In contrast, the larger size, darker colouration and other cranial characters related to the robustness of the skull were evidence long claimed in supporting the status of *C. a. mastivus* as species (Husson 1962; Uieda & Taddei 1980; Eger 2008). In agreement with those statements, our morphological and molecular evidence allowed us to recognize two species within the pool of individuals previously under the name

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C. abrasus: the type nominal *C. abrasus* from several localities in South America, widely distributed from Peru to Argentina and including the type locality in state of São Paulo, Brazil (Eger 2008), and *C. mastivus* inhabiting Amazonian lowlands in the Guiana Shield, eastern Ecuador and Brazil (Fig. 2).

Alberico & Naranjo (1982) reported three individuals of *C. abrasus* from south-western Colombia (2 males and 1 female), but the measurements of both males did not fit the expected variation for *C. mastivus*. Two additional records from the western Andes in Ecuador were reported by Tirira (2012). A single specimen from the western Andes of Colombia (FMNH 89574) that we were able to analyse has a broken skull, warranting no precise identification. Therefore, a revision of the material of large-sized *Cynomops* from the west side of Andes is recommended.

Mitochondrial markers have been exceptionally useful for testing morphology-based taxonomy and detecting possible cryptic species in bats (e.g., Mayer & von Helversen 2001; McDonough *et al.* 2008; Larsen *et al.* 2011; Siles *et al.* 2013) as we have also shown here. The morphological characters herein employed have concurrently improved the support of the nodes recovered and the understanding of the evolution of the genus, emphasizing the importance of using multiple approaches for phylogenetic studies and integrative taxonomy (Nixon & Carpenter 1996; Nylander *et al.* 2004; Giannini & Simmons 2005; Padial *et al.* 2010).

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References

Acosta, L. S. & Paca, R. C. (2010). Registros del murciélago *Cynomops abrasus* (Temminck, 1827) (Chiroptera, Molossidae), en el Bosque Seco Chiquitano de Bolivia. *Chiroptera Neotropical*, *16*, 528–530.

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, *19*, 716–723.

Alberico, M. S. & Naranjo, L. G. H. (1982). Primer registro de *Molossops brachymeles* (Chiroptera: Molossidae) para Colombia. *Cespedesia*, *11*, 141–143.

Alvarez-Castañeda, S. T. & Alvarez, T. (1991). *Los murciélagos de Chiapas*. México, DF: Instituto Politecnico Nacional.

Ammerman, L. K., Lee, D. N. & Tipps, T. M. (2012). First molecular phylogenetic insights into the evolution of tree-tailed bats in the subfamily Molossinae (Molossidae, Chiroptera). *Journal of Mammalogy*, *93*, 12–28.

Barquez, R. M., Mares, M. A. & Braun, J. K. (1999). *The bats of Argentina Special Publications of the Museum 42*. Lubbock: Texas Tech University Press.

Bradley, R. D. & Baker, R. J. (2001). A test of the genetic species concept: cytochrome-b sequences and mammals. *Journal of Mammalogy*, *82*, 960–973.

Cabrera, A. (1958). Catálogo de los mamíferos de América del Sur. *Revista del Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (Ciencias Zoológicas)*, *4*, 1–308.

Carter, D. C. & Dolan, P. G. (1978). Catalog of type specimens of Neotropical bats in selected European museums. *Special Publications of the Museum*, *15*. Lubbock: Texas Tech University Press.

Clare, E. L. (2011). Cryptic Species? Patterns of maternal and paternal gene flow in eight Neotropical bats. *PLoS ONE*, *6*, 1–13.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*, 1792–1797.

Eger, J. L. (2008). Family Molossidae. In A. L. Gardner (Ed.) *Mammals of South America, Volume 1, Marsupials, Xenarthrans, Shrews, and Bats* (pp. 399–439). Chicago: University of Chicago Press.

Esbérard, C. E. L. & Bergallo, H. (2005). Nota sobre a biologia de *Cinomops abrasus* (Temminck) (Mammalia, Chiroptera, Molossidae) no Rio de Janeiro, Brasil. *Revista Brasileira de Zoologia*, *22*, 514–516.

Felsenstein, J. (1985). Phylogenies and the comparative method. *The American Naturalist*, *125*, 1–15.

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Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular marine biology and biotechnology*, *3*, 294–299.

Freeman, P. W. (1981). A multivariate study of the family Molossidae (Mammalia, Chiroptera): morphology, ecology, evolution. *Fieldiana Zoology*, 7, 1–173.

Gardner, A. L. (1977). Taxonomic implications of the karyotypes of *Molossops* and *Cynomops* (Mammalia: Chiroptera). *Proceedings of the Biological Society of Washington*, *89*, 545–550.

Giannini, N. P. & Simmons, N. B. (2005). Conflict and congruence in a combined DNA–morphology analysis of megachiropteran bat relationships (Mammalia: Chiroptera: Pteropodidae). *Cladistics*, *21*, 411–437.

Giannini, N. P. & Simmons, N. B. (2007a). The chiropteran premaxilla: A reanalysis of morphological variation and its phylogenetic interpretation. *American Museum Novitates*, *3585*, 1–44.

Giannini, N. P. & Simmons, N. B. (2007b). Element homology and the evolution of dental formulae in megachiropteran bats (Mammalia: Chiroptera: Pteropodidae). *American Museum Novitates*, *3559*, 1–27.

Giannini, N. P., Wible, J. R. & Simmons, N. B. (2006). On the cranial osteology of Chiroptera I. Pteropus (Megachiroptera: Pteropodidae). *Bulletin of the American Museum of Natural History*, *295*, 1–134.

Goodwin, G. G. (1958). Three new bats from Trinidad. *American Museum Novitates*, *1877*, 1–6.

Goodwin, G. G. & Greenhall, A. M. (1961). A review of the bats of Trinidad and Tobago. *Bulletin of the American Museum of Natural History*, *122*, 187–302.

Gregorin, R. (2009). Phylogeny of *Eumops* Miller 1906 (Chiroptera: Molossidae) using morphological data. *Acta Chiropterologica*, *11*, 247–258.

Gregorin, R. & Cirranello, A. (2015). Phylogeny of Molossidae Gervais (Mammalia: Chiroptera) inferred by morphological data. *Cladistics*, *31*, 1–34.

Heracle BioSoft SRL Romania(2013). DNA Baser Sequence Assembler v4, [Software]. Available: http://www.DnaBaser.com.

Huelsenbeck, J. P. & Ronquist, F. (2001). MRBAYES: bayesian inference of phylogenetic trees. *Bioinformatics*, *17*, 754–755.

Husson, A. M. (1962). The bats of Suriname. Zoo. *Verhandelingen. Rijksmuseum Natuurlijke Historie Leiden*, *58*, 1–282.

Kalko, E. K. V., Handley, C. O.Jr & Handley, D. (1996). Organization, diversity, and long-term dynamics of a Neotropical bat community. In: M. L. Cody & J. A. Smallwood (Eds.) *Long-Term Studies of Vertebrate Communities*(pp. 503–553), San Diego: Academic Press.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*, 111–120.

Koopman, K. F. (1978). Zoogeography of Peruvian bats with special emphasis on the role of the Andes. *American Museum Novitates*, *2651*, 1–33.

Koopman, K. F. (1982). Biogeography of the bats of South America. In: M. A. Mares & H. H. Genoways (Eds.) *Mammalian biology in South America. Special Publication Series*, *vol. 6*(pp. 273–302) Linesville: Pymatuning Laboratory of Ecology, University of Pittsburgh.

Koopman, K. F. (1993). Order Chiroptera. In: D. E. Wilson & D. M. Reeder (Eds) *Mammal Species of the World: a Taxonomic and Geographic Reference*, 2nd ed. (pp. 137–241), Washington, D.C: Smithsonian Institution Press.

Koopman, K. F. (1994). Chiroptera: systematics. *Handbuch der Zoologie*, vol. *8*. Berlin: Walter de Gruyter.

Lanfear, R., Calcott, B., Ho, S. Y. W. & Guindon, S. (2012). Partition Finder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, *29*, 1695–1701.

Lara-Ruiz, P., Chiarello, A. G. & Santos, F. R. (2008). Extreme population divergence and conservation implications for the rare endangered Atlantic Forest sloth, *Bradypus torquatus* (Pilosa: Bradypodidae). *Biological conservation*, *41*, 1332–1342.

Larsen, P. A., Siles, L., Pedersen, S. C. & Kwiecinski, G. G. (2011). A new species of *Micronycteris* (Chiroptera: Phyllostomidae) from Saint Vincent, Lesser Antilles. *Mammalian Biology*, *76*, 687–700.

Leite-Silva, C., Santos, N., Fagundes, V., Yonenaga-Yassuda, Y. & Souza, M. J. (2003). Karyotypic characterization of the bat species *Molossus ater*, *M. molossus* and *Molossops planirostris* (Chiroptera, Molossidae) using FISH and banding techniques. *Hereditas*, *138*, 94–100.

Lewis, P. O. (2001). A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology*, *50*, 913–925.

Lim, B. & Catzeflis, F. M. (2014). History of mammalogy in the Guianas. In: J. Ortega, J. L. Martínez, D. G. Tirira, (Eds.) *Historia de la mastozoología en Latinoamérica*, *Las Guianas y el Caribe* (pp. 265–292). Quito and México, DF: Editorial Murciélago Blanco y Asociación Ecuatoriana de Mastozoología.

Mares, M. A., Willig, M. R., Streilein, K. E. & Lacher, T. E., Jr (1981). The mammals of northeastern Brazil: A preliminary assessment. *Annals of the Carnegie Museum*, *50*, 81–137.

Mayer, F. & von Helversen, O. (2001). Cryptic diversity in European bats. *Proceedings of the Royal Society of London B: Biological Sciences*, *268*, 1825–1832.

McDonough, M. M., Ammerman, L. K., Timm, R. M., Genoways, H. H., Larsen, P. A. & Baker, R. J. (2008). Speciation within bonneted bats (genus *Eumops*): the complexity of morphological, mitochondrial, and nuclear data sets in systematics. *Journal of Mammalogy*, *89*, 1306–1315.

Miller., G. S., Jr (1907). The families and genera of bats. *Bulletin of the United States National Museum*, *57*, *xvii*, *28*, xvii+282 pp., 14 pls..

Miller, M. A., Pfeiffer, W. & Schwartz, T. (2010). Creating the Cipres Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE 2010) (pp. 1–8), Piscataway: Institute of Electrical and Electronics Engineers.

Morielle-Versute, E., Varella-Garcia, M. & Taddei, V. A. (1996). Karyotypic patterns of seven species of molossid bats (Molossidae, Chiroptera). *Cytogenetic and Genome Research*, *72*, 26–33.

Nixon, K. C. & Carpenter, J. M. (1996). On simultaneous analysis. *Cladistics*, *12*, 221–241.

Nylander, J. A. A., Ronquist, F., Huelsenbeck, J. P. & Nieves-Aldrey, J. L. (2004). Bayesian phylogenetic analysis of combined data. *Systematic Biology*, *3*, 47–67.

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Padial, J. M., Miralles, A., Riva, I. & Vences, M. (2010). The integrative future of taxonomy. *Frontiers in Zoology*, 7, 1–16.

Peters, S. L., Lim, B. K. & Engstrom, M. D. (2002). Systematics of dog-faced bats (*Cynomops*) based on molecular and morphometric data. *Journal of Mammalogy*, *83*, 1097–1110.

Rambaut, A., Suchard, M. A., Xie, D. & Drummond, A. J. (2014). Tracer MCMC Trace Analysis Tool, University of Oxford, Oxford, UK. Available via http://tree.bio.ed.ac.uk/software/tracer/.

Redondo, R. A. F., Brina, L. P. S., Silva, R. F., Ditchfield, A. D. & Santos, F. R. (2008). Molecular systematics of the genus *Artibeus* (Chiroptera: Phyllostomidae). *Molecular Phylogenetics and Evolution*, *49*, 44–58.

Ryan, J. M. (1991a). Comparative morphology of the glans penis in *Molossus*, *Promops*, and *Eumops* (Chiroptera: Molossidae). *Bulletin of the American Museum of Natural History*, *206*, 122–137.

Ryan, J. M. (1991b). Morphology of the glans penis in four genera of molossid bats (Chiroptera: Molossidae). *Journal of Mammalogy*, *72*, 658–668.

Sambrook, J., Russell, D. W. & Sambrook, J. (2001). *Molecular Cloning: a Laboratory Manual*, *third ed*. New York: CSHL press.

Sanborn, C. C. (1932). Neotropical bats in the Carnegie Museum. *Annals of the Carnegie Museum*, *21*, 171–183.

Santos, N., Fagundes, V., Yonenaga-Yassuda, Y. & Souza, M. J. (2001). Comparative karyology of Brazilian vampire bats *Desmodus rotundus* and *Diphylla ecaudata* (Phyllostomidae, Chiroptera): banding patterns, base specific fluorochromes and FISH of ribosomal genes. *Hereditas*, *134*, 189–194.

Shimodaira, H. (2002). An approximately unbiased test of phylogenetic tree selection. *Systematic Biology*, *51*, 492–508.

Siles, L., Brooks, D. M., Aranibar, H., Tarifa, T., Vargas, M. R. J., Rojas, J. M. & Baker, R. J. (2013). A new species of *Micronycteris* (Chiroptera: Phyllostomidae) from Bolivia. *Journal of Mammalogy*, *94*, 881–896.

Simmons, N. B. (2005). Ordem Chiroptera. In D. E. Wilson & D. M. Reeder (Eds) *Mammal Species of the World: A Taxonomic and Geographic Reference*, third ed. (pp. 312–529) Baltimore: Johns Hopkins University Press.

Simmons, N. B. & Voss, R. (1998). The mammals of Paracou, French Guiana: a Neotropical lowland rainforest fauna, Part 1 Bats. *Bulletin of the American Museum of Natural History*, *237*, 1–239.

Smith, M. F. & Patton, J. L. (1993). The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the Akodontine tribe. *Biological Journal of the Linnean Society*, *50*, 149–177.

Sorenson, M. D. (2003). *Avian mtDNA Primers*. Boston, Massachusetts: Boston University.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, *22*, 2688–2690.

Swofford, D. L. (2002). . *PAUP** - Phylogenetic Analysis Using Parsimony (* *and Other methods)*. Ver. 1. *[Computer software and manual]*. Sunderland, Massachusetts: Sinauer Associates.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28*, 2731–2739.

Tavares, V. C. (2008). Revisionary systematics and phylogeny of Vampyressa and Sternodermatinae (Chiroptera: Phyllostomidae). *Ph.D. dissertation*. New York: Program in Biology, City University of New York.

Tavares, V. C., Gardner, A. L., Ramírez-Chaves, H. E. & Velazco, P. M. (2014). Systematics of *Vampyressa melissa* Thomas, 1926 (Chiroptera: Phyllostomidae), with descriptions of two new species of *Vampyressa*. *American Museum Novitates*, *3813*, 1–27.

Temminck, C. J. (1827). Sixième monographie. Sur le genre molosse. *Dysopes* (Illig.). In Monographies de mammalogie ou description de quelques genres de mammifères dont les espèces ont été observées dans les différens musées de l'Europe (pp. 205–244, pls. 17-24). Paris: G. Dufour et E. d'Ocogne.

Thomas, O. (1901). A new free-tailed bat from the lower Amazons. *The Annals and Magazine of Natural History*, 7, 190–191.

Thomas, O. (1911). Three new South-American mammals. *The Annals and Magazine of Natural History*, *8*, 113–115.

Thomas, O. (1920). A further collection of mammals from Jujuy. *The Annals and Magazine of Natural History*, *9*, 188–196.

Tirira, D. G. (2012). Comentarios sobre registros notables de murciélagos cola de ratón (Chiroptera, Molossidae) para el Ecuador. In D. G. Tirira & S. F. Burneo (Eds) *Investigación y conservación sobre murciélagos en el Ecuador. Publicación especial sobre los mamíferos del Ecuador 9*. (pp 217–234). Quito: Pontificia Universidad Católica del Ecuador, Fundación Mamíferos y Conservación y Asociación Ecuatoriana de Mastozoología.

Trouessart, E.-L. (1897). Catalogus mammalium tam viventium quam fossilium. Fasciculus I. Primates, Prosimiae, Chiroptera, Insectivora. Berolini: R. Friedländer & Sohn.

Trouessart, E.-L. (1904). Catalogus mammalium tam viventium quam fossilium. Quinquennale supplementium. Berolini: R. Friedländer & Sohn.

Velazco, P. M. (2005). Morphological phylogeny of the bat genus *Platyrrhinus* Saussure, 1860 (Chiroptera: Phyllostomidae) with the description of four new species. *Fieldiana Zoology*, *New Series*, *105*, 1–53.

Velazco, P. M. & Patterson, B. D. (2008). Phylogenetics and biogeography of the broad-nosed bats, genus *Platyrrhinus* (Chiroptera: Phyllostomidae). *Molecular Phylogenetics and Evolution*, *49*, 749–759.

Velazco, P. M. & Patterson, B. D. (2014). Two new species of yellow-shouldered bats, genus *Sturnira* Gray, 1842 (Chiroptera, Phyllostomidae) from Costa Rica, Panama and western Ecuador. *ZooKeys*, *402*, 43–66.

Velazco, P. M., Gardner, A. L. & Patterson, B. D. (2010). Systematics of the *Platyrrhinus helleri* species complex (Chiroptera: Phyllostomidae), with descriptions of two new species. *Zoological Journal of the Linnean Society*, *159*, 785–812.

Vieira, C. O. da. C. (1942). Ensaio monográfico sobre os quirópteros do Brasil. *Arquivos de Zoologia do Estado de São Paulo*, *3*, 219–471.

Vizotto, L. D. & Taddei, V. A. (1976). Notas sobre *Molossops temminckii temminckii* e *Molossops planirostris* (Chiroptera-Molossidae). *Naturalia*, *2*, 47–59.

Warner, J. W., Patton, J. L., Gardner, A. L. & Baker, R. J. (1974). Karyotypic analyses of twenty-one species of molossid bats (Molossidae: Chiroptera). *Canadian Journal of Genetic Cytology*, *16*, 165–176.

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Williams, S. L. & Genoways, H. H. (1980). Results of the Alcoa Foundation-Suriname Expeditions IV. A new species of bat of the genus Molossops (Mammalia: Molossidae). *Annals of Carnegie Museum*, *49*, 487–498.

Wisely, S. M., Maldonado, J. E. & Fleischer, R. C. (2004). A technique for sampling ancient DNA that minimizes damage to museum specimens. *Conservation Genetics*, *5*, 105–107.

Uieda, W. & Taddei, V. A. (1980). Ocorrência de *Molossops brachymeles mastivus* Thomas 1911, no Brasil (Chiroptera, Molossidae). *Acta Amazonica*, *10*, 225–227.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Morphological matrix.

Appendix S2. Material examined with museum registration numbers, locality data and other label information.

Table S1. Locality, catalogue/tissue and GenBank numbers for the sequences used in this study.

Fig. S1. Bayesian tree of *Cynomops* species generated with 2497 base pairs of the mtDNA (COI + Cyt *b*). Values above branches represent Bayesian posterior probabilities (BPP) and below branches, the maximum-likelihood (ML) bootstrap.

Fig. S2. Maximum-likelihood tree of combined mtDNA (COI + Cyt *b*) and morphology. Values above branches represent the maximum-likelihood (ML) bootstrap.

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