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Original investigation

A new species of mastiff bat (Chiroptera, Molossidae, *Molossus*) from Guyana and Ecuador

Livia O. Loureiro [a,*], Burton K. Lim [b], Mark D. Engstrom [a,b]

[a] Department of Ecology and Evolutionany Biology, University of Toronto, Toronto, ON M5S 3B2, Canada
[b] Department of Natural History, Royal Ontario Museum, Toronto, ON M5S 2C6, Canada

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ABSTRACT

We describe a new species of mastiff bat in the genus *Molossus* (Molossidae), which was previously confused with the common and widely distributed *M. molossus*, from Guyana and Ecuador based on morphological and molecular differences. It is diagnosed by the following set of morphological characteristics: bicolored dorsal pelage, rounded anterior arch of the atlas, triangular occipital bone, and smaller body and skull size. In a molecular phylogenetic analysis of mitochondrial and nuclear DNA, maximum likelihood and parsimony trees recovered eight clades in the genus and a polyphyletic relationship for the *M. molossus* species complex. The new species was recovered in a well-supported clade that can be genetically distinguished from other species in the genus by its high level of sequence divergence based on the mitochondrial CO1 gene (8.0–10.1%) and on the nuclear gene beta fibrinogen (1.0–3.1%). It is broadly sympatric with *M. molossus sensu stricto* in northern South America, but morphologically distinct and genetically divergent.

Introduction

Molossus is one of the most diverse genera of free tailed bats in the pantropical family Molossidae. The genus is mainly Neotropical, occurring from the southeastern United States to southern Argentina, and throughout the Caribbean islands (López-González and Presley, 2001). The following anatomical features characterize the genus: slightly triangular, medium size ears; incisors in line with canines; smooth upper lips; a well-developed sagittal crest (more developed in males); and an obtuse-angled rostrum (Fabián and Gregorin, 2007). However, morphological, ecological, and physiological characteristics vary geographically (Dolan, 1989; Jung et al., 2014).

Molossus molossus was the first molossid to be described (*Vespertilio molossus* Pallas, 1766). Since then, many names have been assigned to the genus and the taxonomy of the group has been unstable (Table 1). Dolan's (1989) revision of *Molossus* recognized 7 species (*M. molossus*, *M. aztecus*, *M. rufus*, *M. pretiosus*, *M. coibensis*, *M. bondae* and *M. sinaloae*) based on morphology and karyotypes, however, she relied almost exclusively on samples from Central America. Subsequently, other studies recognized *M. barnesi* from French Guiana as valid (Simmons and Voss, 1998) and placed *M. bondae* as a junior synonym of *M. currentium* based on morphological characters (López-González and Presley, 2001). Jennings et al. (2000) recognized only five species (*M. molossus*, *M. ater*, *M. pretiosus*, *M. bondae* and *M. sinaloae*), and Eger (2008) supported a different assemblage of seven species for the genus (*M. molossus*, *M. rufus*, *M. pretiosus*, *M. coibensis*, *M. bondae*, *M. currentium*, and *M. sinaloae*). More recently, a new species (*M. alvarezi*) was described from the Yucatan Peninsula of Mexico as distinct from *M. sinaloae* on the Pacific slope of Mexico into Central America and northern South America (González-Ruiz et al., 2011). Part of the variation in number of species and the precarious diagnoses of recognized taxa can be attributed to subtle morphological differentiation at the species level, pronounced sexual dimorphism, and different interpretations of inter and intra-specific variation (Eger, 2008; González-Ruiz et al., 2011; Simmons and Voss, 1998). Herein, we follow the taxonomy proposed by Dolan (1989), except that we regard *M. bondae* as a junior synonym of *M. currentium* following González-Ruiz et al. (2011).

Most authors recognize *Molossus* as monophyletic, and its sister group relationship to *Promops* is well established (Ammerman

* Corresponding author at: Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, ON M5S 3B2, Canada.
E-mail address: livia.loureiro@mail.utoronto.ca (L.O. Loureiro).

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Table 1
Comparison of different taxonomic arrangements within *Molossus* according to different authors. In addition, López-González and Presley (2001) considered *M. bondae* as a junior synonym of *M. currentium*, and González-Ruiz et al. (2011) described *M. alvarezi* as a new species.

Dolan (1989)	Simmons and Voss (1998)	Jennings et al. (2000)	Simmons (2005)	Eger (2008)
M. molossus	*M. molossus*	*M. molossus*	*M. molossus*	*M. molossus*
M. aztecus	*M. aztecus*	*M. ater*	*M. aztecus*	*M. rufus*
M. rufus	*M. rufus*	*M. pretiosus*	*M. rufus*	*M. pretiosus*
M. pretiosus	*M. pretiosus*	*M. bondae*	*M. pretiosus*	*M. coibensis*
M. coibensis	*M. coibensis*	*M. sinaloae*	*M. coibensis*	*M. bondae*
M. bondae	*M. bondae*		*M. currentium*	*M. currentium*
M. sinaloae	*M. sinaloae*		*M. sinaloae*	*M. sinaloae*
	M. barnesi		*M. barnesi*	

et al., 2012; Gregorin and Cirranello, 2016). However, phylogenetic relationships within the genus remain undefined, and until recently there have been few molecular studies of *Molossus*. Clare et al. (2007) and Borisenko et al. (2008) examined sequences of the mitochondrial barcoding gene cytochrome c oxidase subunit 1 (CO1) in species of bats from Guyana and Suriname, respectively. Both studies reported relatively high intraspecific variation within *M. molossus* (up to 1.8%) and low interspecific variation of less than 2.2% average sequence divergence from the morphologically distinct and larger *M. rufus*. However, more recent studies incorporating CO1 samples from Jamaica (Lim and Arcila Hernandez, 2016) and the Dominican Republic (Lim et al., 2017) have identified an even more complex polyphyletic relationship among populations assigned to *M. molossus* and several other species of the genus. Lindsey and Ammerman (2016) analyzed part of the cytochrome b mitochondrial gene in several species of *Molossus* and reported *M. m. tropidorhynchus* from Cuba as a divergent monophyletic clade separated from a monophyletic lineage comprising the remaining samples of *M. molossus*, *M. coibensis*, and *M. rufus.*

Herein, in addition to the mitochondrial CO1 gene, we present the first nuclear gene tree for *Molossus* based on beta fibrinogen and additional data on genetic variation in the genus. Based on molecular, morphological, and morphometric evidence and the genetic (Baker and Bradley, 2006) and the evolutionary species concept (Wiley, 1981), we also describe a new species of *Molossus*, morphologically similar to *M. sinaloae* and *M. molossus* that was previously reported from Guyana as *Molossus* sp. (Lim and Engstrom, 2001a) and more recently found in Ecuador.

Material and methods

Molecular analyses

DNA barcodes of 657 basepairs of COI were sequenced from 346 specimens of *Molossus* and its sister taxon *Promops* from across the Neotropics including Bonaire, Dominican Republic, Ecuador, El Salvador, French Guiana, Guyana, Jamaica, Martinique, Mexico, Panama, Peru, Suriname, and Venezuela. Of these specimens, 50 are new sequences and 296 were obtained from GenBank (Appendix A, Supplementary material). The dataset included specimens of 15 *M. coibensis*, 10 *M.m. daulensis*, three specimens of the new species of *Molossus* reported herein, 221 *M. m. molossus*, 11 *M. m. milleri*, one *M. m. pygmaeus*, eight *M. m. verrilli*, 60 *M. rufus*, four *M. sinaloae*, four specimens of an undescribed taxon of *Molossus* from the savannahs of Venezuela and Guyana, seven *Promops centralis*, and two *Eumops auripendulus*. Based on genetic divergence in CO1, a subset of 35 specimens spanning the breadth of variation were sequenced for 764 basepairs of the nuclear gene beta fibrinogen, including two *M. coibensis*, two specimens of the new species of *Molossus* reported herein, 12 *M. m. molossus*, two *M. m. milleri*, two *M. m. verrilli*, nine *M. rufus*, one *M. sinaloae*, two specimens of an undescribed taxon of *Molossus* from the savannahs of Venezuela and Guyana, one *Promops centralis*, and two *Eumops auripendulus*. The new sequences were deposited on GenBank and on BOLD (The Barcode of Life Data Systems) (Appendix A, Supplementry material). Molecular protocols for the COI gene followed the methods outlined by Clare et al. (2007) and Borisenko et al. (2008) and protocols for the beta fibrinogen gene followed Reeder and Bradley (2007). Phylogenetic relationships were reconstructed using maximum parsimony and likelihood analyses with 1000 bootstrap replications as implemented in MEGA 6.06 (Tamura et al., 2013).

Morphological analyses

Morphological and morphometric comparisons included 654 specimens from 12 species and subspecies of *Molossus*, including 39 *M. coibensis* (3 previously identified as *M. barnesi*), 324 *M. m. molossus*, 64 *M. aztecus,* 23 *M. currentium*, 43 *M. pretiosus, 113M. rufus*, 21 *M. sinaloae,* eight *M. alvarezi,* four *M. m. milleri*, eight *M. m. verrilli,* two *M. m. pygmaeus*, five *Molossus* sp. nov and two undescribed taxon from the savannahs of Venezuela and Guyana (Appendix B, Supplementary material). Specimens are deposited in the following institutions: American Museum of Natural History (AMNH, New York, USA); National Museum of Natural History (USNM, Washington, DC, USA); Royal Ontario Museum (ROM, Toronto, Canada); Universidade Estadual Paulista (DZSJRP, São José do Rio Preto, Brazil); Universidade Federal Rural do Rio de Janeiro (ALP, Seropédica, Brazil); Museu de Zoologia, Universidade de São Paulo (MZUSP, São Paulo, Brazil); Universidade Federal de Lavras (UFLA, Lavras, Brazil); Universidade Federal de Minas Gerais (UFMG, Belo Horizonte, Brazil); Museu Nacional, Universidade Federal do Rio de Janeiro (MN, Rio de Janeiro, Brazil); Universidade Federal do Mato Grosso do Sul (UFMS, Campo Grande, Brazil); Centro de Zoonoses da Cidade de São Paulo (CCZSP, São Paulo, Brazil); and Museu de História Natural da Universidade Pontifícia Católica de Minas Gerais (MCN, Belo Horizonte, Brazil). This study followed animal use guidelines of the American Society of Mammalogists (Sikes, 2016) and the Royal Ontario Museum Animal Use Protocol 2013-17.

We also examined the holotypes of *M. coibensis* (including *M. barnesi*), *M. bondae*, *M. sinaloae*, *M. m. verrilli*, and *M. pretiosus*, and photographs of the holotype of *M. rufus*. In addition, topotypes of *M. alvarezi, M. m. milleri, M. m. molossus*, and *M. m. pygmaeus* were also included in our study. Only adults (defined as having closed cranial sutures and complete epiphyseal ossification of metacarpal and phalanx joints) of both sexes were examined.

Morphometric analyses

All measurements defined below were taken with digital calipers accurate to 0.01 mm: forearm length (FA); greatest length of skull, including incisors (GSLI); greatest length of skull without incisors (GSL); interorbital width (IOW); braincase width (BCW); condyloincisive length (CIL); zygomatic breadth (ZB); palatal length (PAL); width across upper molars (M-M); width across upper

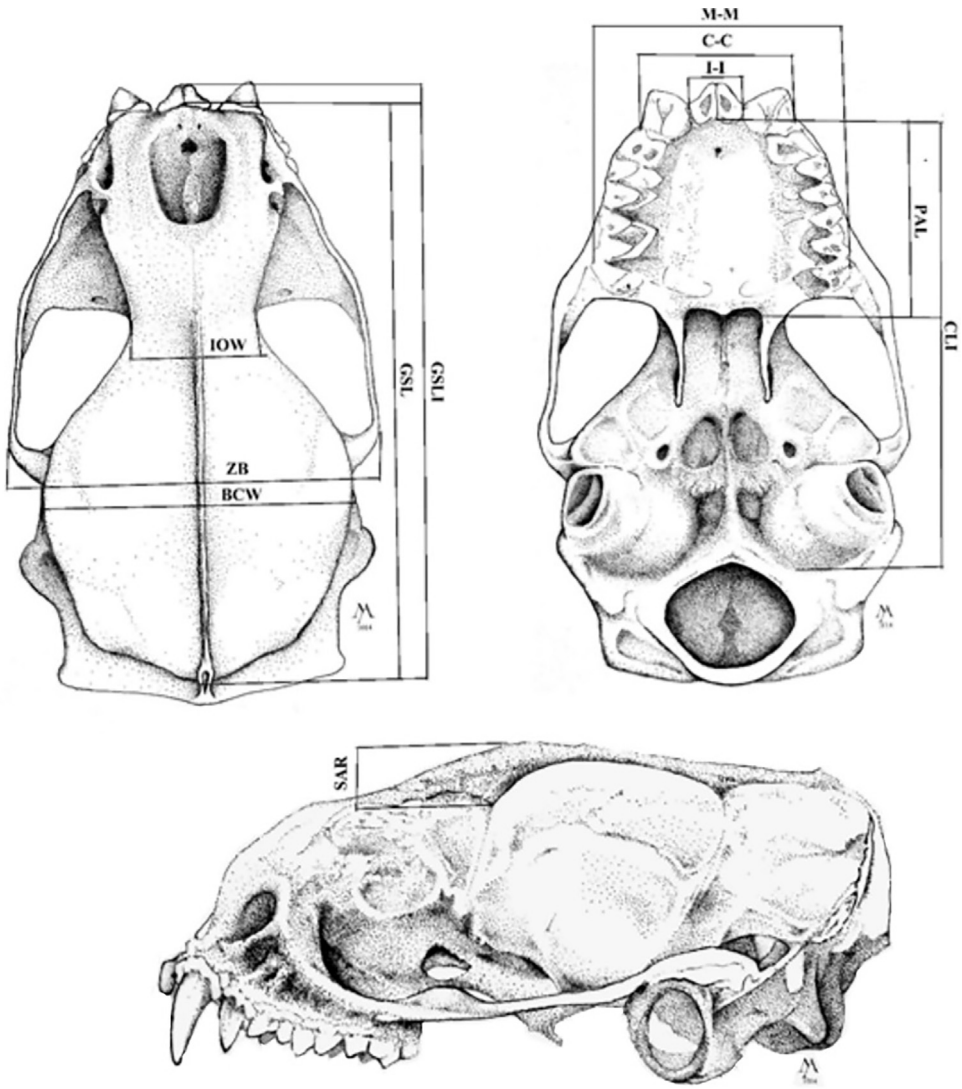

Fig. 1. Skull measurements used in this study of *Molossus*. See Material and Methods for variable abbreviations.

canines (C–C); width of upper incisors (I–I); length of maxillary toothrow (C–M); and height of the sagittal crest (SAR) (Fig. 1).

To examine overall patterns of morphometric similarity among samples, including body size, we conducted a Principal Components Analysis (PCA) on representatives of different species of *Molossus* using the correlation matrix among 11 cranial and body variables. Measurements were log-transformed prior to analysis. An analysis of variance (ANOVA) for each character and a multivariate analysis of variance (MANOVA) were performed to examine the significance of morphometric differentiation between representatives of *Molossus* sp. nov. and other species of *Molossus*, based on the same set of morphological characters used in the PCA. Males and females were separated in the analysis due to sexual dimorphism reported for the genus (Dolan, 1989). The level of significance was $p = 0.05\%$ for all statistical tests. Statistical analyses were performed using R 3.1.0 (R Core Team, 2015) and the software Past 2:17 (Hammer et al., 2001).

Results

Molecular analyses

For the COI and beta fibrinogen sequence data, the best fit maximum likelihood substitution model was HKY (Hasegawa et al., 1985) with a gamma distribution of non-uniformity of evolutionary rates and GTR with gamma distribution (Tavaré and Miura, 1986), respectively, as implemented in MEGA6 (Tamura et al., 2013). The maximum likelihood tree for the combined (Fig. 2) and individual datasets (Loureiro et al., submitted) recovered the genus *Molossus* as monophyletic and the sister group of *Promops*. All data sets recovered eight reciprocally monophyletic terminal clades that are well-supported (bootstrap ≥ 90%) except for the *M. m. molossus* clade (Fig. 2). *M. molossus*, as delimited by Dolan (1989), is non-monophyletic in relation to the morphologically distinct *M. coibensis* and *M. rufus*. Scientific names assignable to the eight clades in the combined data set tree (with countries of specimens used in the molecular study) are: (1) *Molossus* sp. nov. described herein from Guyana and Ecuador; (2) *M. sinaloae* from Honduras and French Guiana; (3) *M. m. milleri* from Jamaica; (4) *M. m. verrilli* from Dominican Republic; (5) *M. rufus* from Mexico, El Salvador, Ecuador, Peru, Guyana, and Suriname; (6) *M. coibensis* from Peru, Ecuador, and French Guiana; (7) an undescribed taxon from the savannahs of Venezuela and Guyana; and (8) *M. m. molossus* from Panama, Peru, Ecuador, Guyana, Suriname, Bonaire and Martinique. Husson (1962) selected a specimen from Martinique as the lectotype for *M. molossus*. The taxonomic assignment of *M. m. molossus* is tentative because specimens from the western slope of the Andes in Ecuador and Peru form a well-supported monophyletic clade refer-

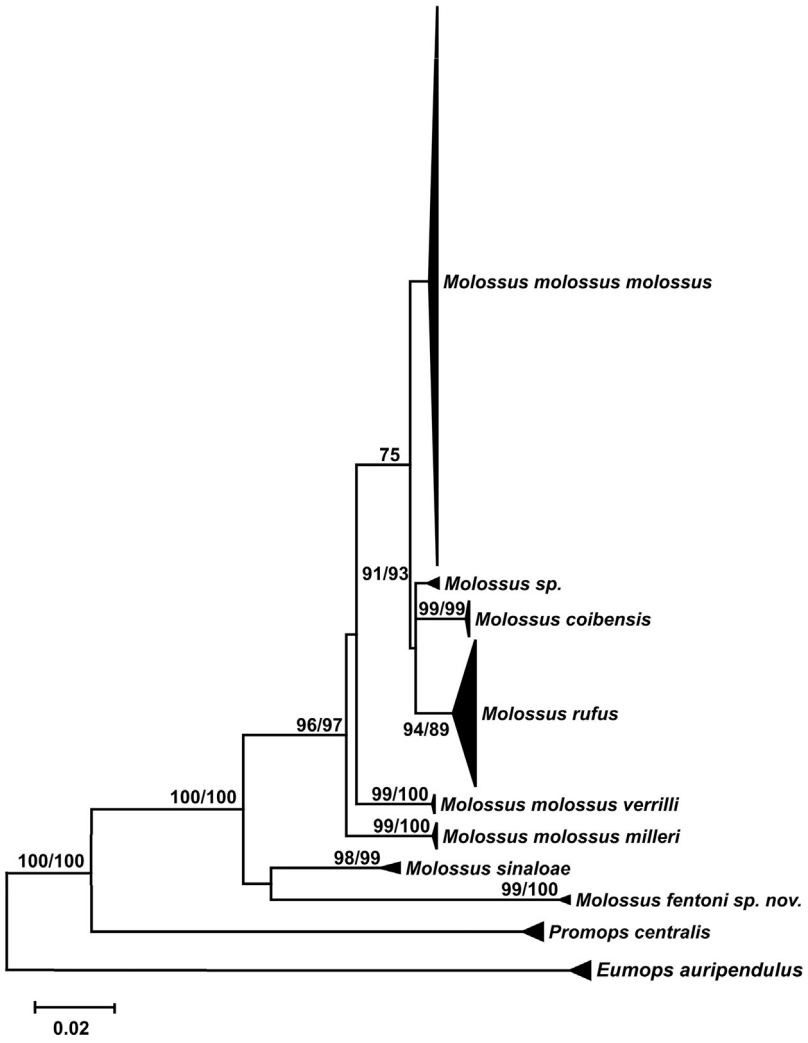

Fig. 2. Maximum likelihood tree of COI and beta fibrinogen combined sequences of *Molossus*. Bootstrap support values (maximum likelihood/maximum parsimony) > 70% are reported for well-supported nodes. *M. m. daulensis* and *M. m. pygmaeus* were recovered inside the *M. molossus molossus* clade.

able to *M. m. daulensis*, and this taxon appears within a paraphyletic cluster of the remaining specimens of *M. m. molossus*. More study and more samples are needed before resolving relationships of the *M. m. molossus* + *M. m. daulensis* clade.

The maximum parsimony trees recovered the same eight reciprocally monophyletic terminal clades as the maximum likelihood trees, and all were relatively well supported (≥90%) with the exception of the *M. m. molossus* clade (Fig. 2). There were two higher level relationships that were congruent among all trees and both have more than 70% support: *Molossus* without *M. sinaloae* and the new species; and this clade without *M. molossus milleri* and *M. molossus verrilli* from the Greater Antilles of the western Caribbean. Other higher level relationships were not congruent and were poorly supported, and *M. molossus* (*sensu lato*) appeared as non-monophyletic in all trees.

The COI sequence divergence among the eight terminal clades ranged from 1.5% to 10.1% (Table 2). However, the highest divergences among clades were between *Molossus* sp. nov. and the all other species of *Molossus* (8.0%–10.1%), and the lowest divergence level, 1.5%, was between *M. m. molossus* (*sensu stricto*) and the undescribed taxon from the savannahs of Venezuela and Guyana. Sequence divergence within the eight terminal clades ranged from 0.01% for *M. molossus verrilli* to 0.8% for *M. rufus* (Table 2). The more conservative intron 7 gene of beta fibrinogen showed lower divergence within *Molossus*, varying from 0.7% to 3.1%. However the highest divergence was also found between *Molossus* sp. nov. and all other species of the genus (1.0–3.1%) (Table 3).

Morphological analyses

In the PCA of the six small-bodied taxa of *Molossus* (*Molossus* sp. nov., *M. aztecus*, *M. coibensis, M. molossus milleri*, *M. m. molossus*, and *M. m. verrilli*), the first two principal components (PC1 and PC2) explained 89% of the total variation in females, and 87% in males. PC1 shows high positive loadings for all measurements, reflecting variation in general size (Table 4). PC2 has positive loading for all variables with the exception of I–I, resulting in a contrast between upper incisor width and general size. The PCA graph shows that there is size overlap among many species of *Molossus*, including *Molossus* sp. nov., which could have been causing confusion regarding the taxonomy of the group, especially considering cryptic species (Eger, 2008; Jennings et al., 2000; López-González and Presley, 2001; Simmons, 2005). In particular, convergence in body size among phylogenetically distinct lineages has resulted in the non-monophyletic relationships of some populations and taxa currently included in *M. molossus* (*sensu lato*). Two main groups of species were identified for both females and males (Fig. 3). The first group is formed by the larger taxa, containing species with wide and narrow upper incisors (*M. aztecus*, *M. m. milleri*, *M. m. verrilli*, and *M. m. molossus*), and the second by the smaller species with

Table 2
Sequence divergence of COI between species (lower matrix) and within species (diagonal) of *Molossus* and the outgroups *Promops centralis* and *Eumops auripendulus*.

	M. sinaloae	*M. molossus*	*M. rufus*	*M. verrilli*	*M. coibensis*	*M. milleri*	*M. fentoni sp. nov.*	*Molossus* sp.	*P. centralis*	*E. auripendulus*
M. sinaloae	0.005									
M. molossus	0.064	0.006								
M. rufus	0.079	0.024	0.008							
M. verrilli	0.070	0.038	0.040	0.001						
M. coibensis	0.074	0.019	0.025	0.042	0.007					
M. milleri	0.070	0.041	0.048	0.038	0.045	0.001				
M. fentoni sp. nov.	0.080	0.096	0.101	0.093	0.098	0.100	0.003			
Molossus sp.	0.069	0.015	0.020	0.038	0.018	0.041	0.093	0.003		
P. centralis	0.121	0.131	0.133	0.130	0.133	0.139	0.134	0.125	0.007	
E. auripendulus	0.137	0.168	0.166	0.165	0.164	0.165	0.145	0.168	0.135	0.001

Table 3
Sequence divergence of beta fibrinogen between species (lower matrix) and within species (diagonal) of *Molossus* and the outgroups *Promops centralis* and *Eumops auripendulus*. n/c represents species with only one individual and without data for divergence within species.

	M. sinaloae	*M. molossus*	*M. rufus*	*M. verrilli*	*M. coibensis*	*M. milleri*	*M. fentoni sp. nov.*	*Molossus* sp.	*P. centralis*	*E. auripendulus*
M. sinaloae	n/c									
M. molossus	0.021	0.003								
M. rufus	0.024	0.009	0.006							
M. verrilli	0.021	0.019	0.022	0.001						
M. coibensis	0.026	0.011	0.010	0.024	0.001					
M. milleri	0.028	0.024	0.027	0.009	0.029	0.000				
M. fentoni sp. nov.	0.010	0.025	0.028	0.027	0.031	0.028	0.000			
Molossus sp.	0.022	0.007	0.008	0.020	0.010	0.025	0.026	0.000		
P. centralis	0.041	0.039	0.038	0.050	0.045	0.055	0.044	0.041	n/c	
E. auripendulus	0.052	0.051	0.053	0.060	0.057	0.066	0.057	0.052	0.049	0.000

Table 4
Eigenvalue and PCA loading of variables for the first and second components in females and males of *Molossus*. See "Material and methods" for variable abbreviations.

	PC1		PC2	
	Females	Males	Females	Males
Eigenvalue	5.01	4.67	1.09	0.74
IOW	0.31	0.30	0.02	0.2
I-I	0.59	0.58	−0.57	−0.58
C-M	0.20	0.22	0.19	0.24
GSLI	0.25	0.20	0.15	0.21
GSL	0.24	0.21	0.15	0.21
BCW	0.19	0.15	0.12	0.10
CIL	0.20	0.21	0.24	0.21
C-C	0.41	0.32	0.30	0.24
M-M	0.23	0.23	0.10	0.46
ZB	0.26	0.21	0.33	0.14
FA	0.16	0.22	0.22	0.21

Table 5
Measurements (mm) of the holotype and paratype series of *Molossus fentoni* sp. nov. See "Material and methods" for variable abbreviations.

	Holotype	Paratypes ($n = 4$)		
		X	Min.	Max
FA	35.0	35.0	34.0	36.0
GLS	16.8	15.3	15.1	15.5
GLSI	16.5	15.0	14.8	15.2
IOW	3.9	3.5	3.3	3.6
BCW	8.5	8.3	8.1	8.6
CIL	15.2	14.0	13.8	14.2
ZB	10.3	10.2	10.0	10.4
PAL	4.8	4.5	4.4	4.7
M-M	7.3	7.0	6.9	7.1
C-C	4.2	3.8	3.6	4.0
I-I	1.7	1.5	1.4	1.6
C-M	5.7	5.5	5.1	5.6
SAR	1.1	0.8	0.7	1.0

wider incisor width (*M. coibensis*, and *Molossus* sp. nov.). However, one individual of *M. m. molossus* from Curacao (type locality of *M. m. pygmaeus*) clustered with the smaller group.

Molossus sp. nov. is similar in size to *M. coibensis* but distinctly smaller than the other species of the genus. The multivariate analyses of variance (MANOVA) demonstrated that males of the new species were significantly different from *M. aztecus* ($p < 0.01$, $F = 60.2$), *M. molossus milleri* ($p < 0.01$, $F = 54.3$), *M. m. molossus* ($p = 0.02$, $F = 50.7$), and *M. m. verrilli* ($p < 0.01$, $F = 49.2$), but not from *M. coibensis* ($p = 0.13$, $F = 51.6$). The females of the new species followed the same pattern when compared with *M. aztecus*, *M. m. milleri*, *M. m. molossus*, and *M. m. verrilli* ($p < 0.02$, $F > 49.7$) and were not distinct from *M. coibensis* ($p = 0.06$, $F = 45.3$). Despite the shared small body size of *M. coibensis* and the new species, the ANOVA showed that two measurements were significantly different between these two taxa: BCW ($p = 0.04$; $F = 46.1$) and ZB ($p < 0.01$; $F = 70.6$). In addition, although *Molossus* sp nov. overlaps in size with *M. coibensis*, occupying the same PCA morphospace, several qualitative morphological characteristics distinguish both species, including the shape of the upper incisors and the occipital complex, corroborating the results of the molecular analysis.

Our molecular data show that *Molossus* sp. nov. is phylogenetically divergent from other species in the genus and does not form a sister-group relationship with *M. molossus*, the sympatrically-distributed taxon within which it was previously included. In addition, this undescribed species has the highest genetic divergence from other species of the genus in both mitochondrial CO1 (8.0–10.1%) and nuclear beta fibrinogen (1.0–3.1%) sequences. In contrast, *M. molossus* and *M. rufus*, two species very distinct morphologically, only have 2.2% of divergence in the CO1 gene and 0.9% in beta fibrinogen. In combination with morphological distinctions in the shape of the atlas, upper incisors and the occipital complex, we propose that *Molossus* sp nov should be considered a new evolutionary lineage based on the genetic (Baker and Bradley, 2006), and evolutionary species concept (Wiley, 1981).

Molossus fentoni sp. nov. (Figs. 4–7, Tables 5 and 6)
Molossus sp.: Lim and Engstrom, 2001a, p. 625.
Molossus sp.: Lim and Engstrom, 2001b, p. 665.
Molossus sp.: Engstrom and Lim, 2002, p. 367.

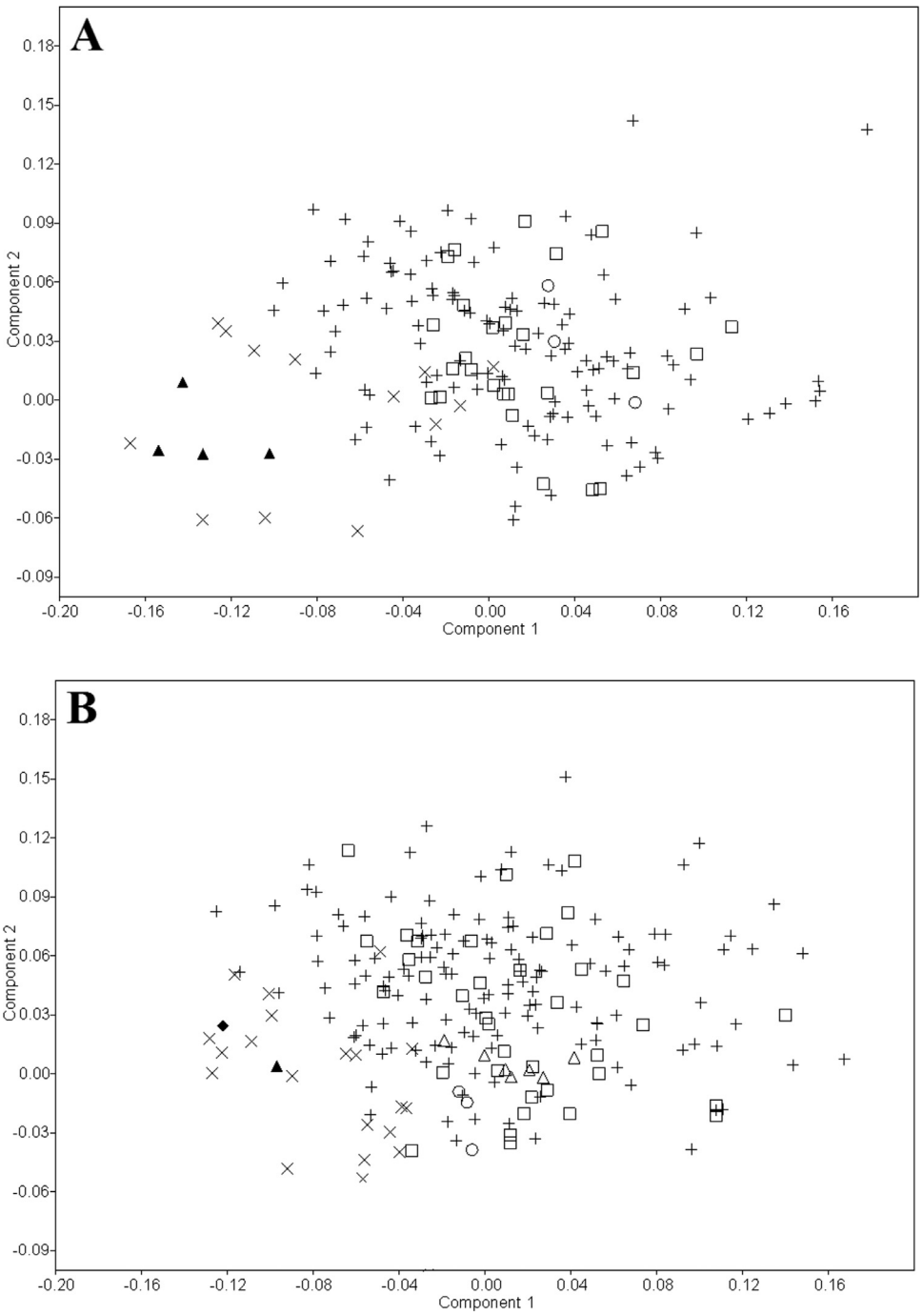

Fig. 3. Principal component analysis (PCA) carried out using the correlation matrix of 11 measurements for females (A) and males (B) of *Molossus*. *Molossus fentoni sp. nov.* (▲), *M. m. molossus* (+), *M. aztecus* (□), *M. coibensis* (X), and *M. molossus verrilli* (◯), *M. molossus milleri* (△), and *M. molossus pygmaeus* (♦).

Molossus sp.: Lim and Engstrom, 2005, p. 79.
Molossus sp.: Lim et al., 2005, p. 89.
Molossus sp.: Clare et al., 2007, p. 185.
Molossus sp.: Clare et al., 2011, p. 4.
Molossus sp.: Lim, 2012, p. 255.
Molossus sp.: Lim and Tavares, 2012, p. 117.
Molossus sp.: Lim, 2016, p. 155.
Molossus sp. nov: Lim et al., 2017, p. 889.

Holotype

ROM 122583 (field number F59101), adult male with skin, skull and postcranial skeleton (Figs. 4–7). Collected on 1 October 2013 by B. K. Lim, C. Osborne, and A. Ignance. External and craniodental measurements for the type series are presented in Table 5.

Type locality

Bototo Wau, near the village of Parabara, Upper Takutu-Upper Essequibo, Guyana (2.18201°, −59.33706°, elevation 245 m).

Paratypes

ROM 31781 and ROM 31782, adult females, body mass 7.2 g and 8.1 g, respectively, collected on 3 March 1961 at Nappi Creek, Upper Takutu-Upper Essequibo, Guyana (3.38333°, −59.437994°); ROM 109176, adult female, body mass 10 g, collected on 14 November 1997 at S Falls, Siparuni River, Potaro-Siparuni, Guyana (4.533°,

Fig. 4. Dorsal, ventral, posterior, and lateral views of the skull of the holotype of *Molossus fentoni sp. nov.*

Fig. 5. Holotype of *Molossus fentoni sp. nov.* (ROM 122583). Adult male with a medium brown dorsal pelage.

−59.083°); and ROM 118821, adult female, body mass 11 g, collected on 18 May 2006 at 42 km S and 9.5 km E of Pompeya Sur, Orellana, Ecuador (−0.68°, −76.382°).

Diagnosis

A set of traits distinguishes *Molossus fentoni* from other *Molossus*. In *M. fentoni* the infra-orbital foramen is laterally directed; the basioccipital pits are rounded and of moderate depth; the occipital is triangular in posterior view; the upper incisors are thin and long with parallel tips and project forward in an oblique plane relative to the anterior face of the canines (Fig. 4); and the anterior arch of the atlas is rounded (Fig. 6).

Description

External measurements for the new species include: total length, 88–93 mm; length of tail, 30–34 mm; length of ear, 12–14 mm; length of hind foot, 8–11 mm; and length of forearm, 34–36 mm. Table 5 summarizes craniodental measurements. Overall, the dorsal pelage is dark to medium brown with a pale brown band at the base (1/4–1/2 of the fur length; Figs. 5 and 7). Ventral hairs are markedly bicolored with dark brown tips and pale yellow bases (Fig. 7). The hairs between the shoulders are long (4.3–4.6 mm). The uropatagium is dark brown. The skull has a rounded braincase with a moderate sagittal crest (0.7–1.0 mm), which is most pronounced in males. There is a medium-size infra-orbital foramen (0.5–0.8 mm), which is laterally directed. Basal occipital pits are rounded and of moderate depth. The mastoid process is directed ventrally in relation to the foramen magnum (Fig. 4). The occipital is triangular in posterior view. The upper incisors are thin and long with parallel tips and project forward in an oblique plane relative to the anterior face of the canines (Fig. 4). The lower incisors, upper and lower premolars, and upper and lower molars are similar and indistinguishable in morphology from other species of *Molossus*. The anterior arch of the atlas is rounded (Fig. 6).

Comparisons

Molossus fentoni can be distinguished from *M. aztecus* by several characters. *M. fentoni* has long and bicolored dorsal pelage with

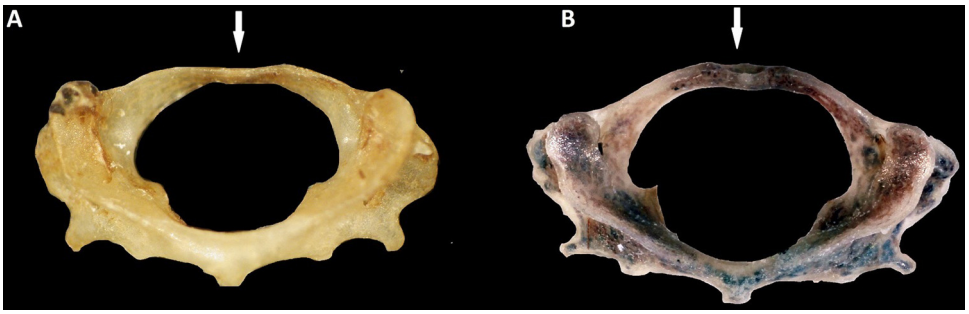

Fig. 6. Atlas of *Molossus molossus* (A) and *Molossus fentoni* sp. *nov*. (B). Note the anterior arch of the atlas is rounded in *M. fentoni sp. nov*., and flattened in *M. molossus*.

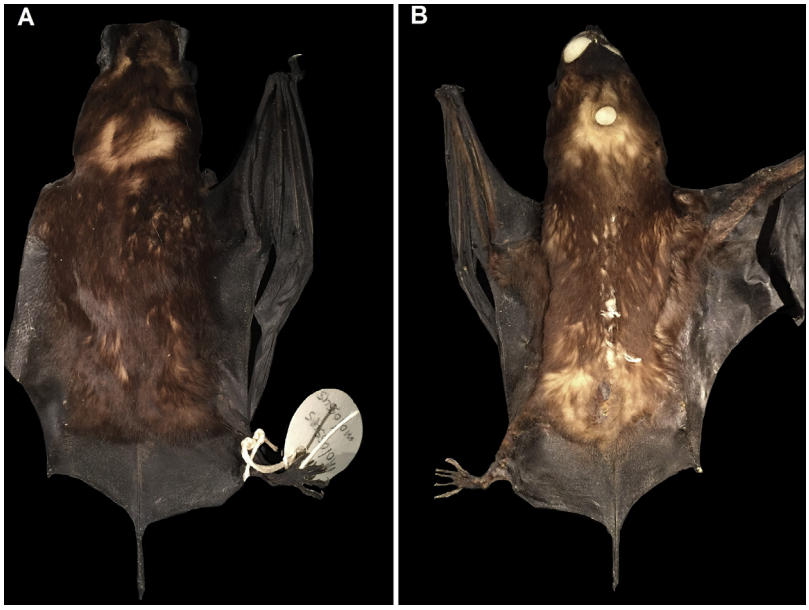

Fig. 7. Dorsal (A) and ventral (B) views of the skin of the holotype of *Molossus fentoni sp. nov*. (ROM 122583).

dark tips and pale bases, whereas *M. aztecus* has short, dark brown and unicolored or faintly bicolored dorsal fur. The nasal foramen is directed laterally in *M. fentoni* (Fig. 8C), and positioned ventrally in *M. aztecus* (Fig. 8D); and the occipital complex is triangular in *M. fentoni* (Fig. 8A) and quadrangular in *M. aztecus* (Fig. 8B). The sagittal and lambdoidal crests are moderately developed in *M. fentoni* (Fig. 8A), and well developed in *M. aztecus* (Fig. 9B). Based on examination of several individuals of different age groups, this sagittal crest does not show any discernible variation with age. *M. fentoni* has thinner and longer upper incisors with parallel tips (Fig. 8C), and *M. aztecus* has short and spatulate upper incisors, with convergent tips (Fig. 8D). In addition, *M. fentoni* is smaller than *M. aztecus* in almost all cranial and external dimensions (Table 6). Although the holotype of *M. aztecus* has not been studied, several specimens from the distributional range of this species matching with the original description (Saussure, 1980) were examined.

External and craniodental dimensions of *M. coibensis* (including its junior synonym *M. barnesi*; see Dolan, 1989; Eger, 2008; Catzeflis et al., 2016), are similar to those of *M. fentoni* (Fig. 5; Table 6). Nevertheless, several characters distinguish the two species. The basal occipital pits are very shallow in *M. coibensis* (Fig. 8F), and deep in *M. fentoni* (Fig. 8E). The brain case appears broader in *M. coibensis* than in *M. fentoni*, corroborated by the ANOVA, and as in *M. aztecus*, *M. coibensis* has an unicolored or faintly bicolored pelage and a quadrangular occiptal (Fig. 8B). In addition, *M. fentoni* has thin and long upper incisors with parallel tips, whereas the upper incisors are short and spatulate in *M. coibensis* (Fig. 8D). However, both species clustered together in the PCA, which indicates that this clear morphological distinction in the form of the upper incisors was not captured by the measurement I–I in the morphometric analysis.

Molossus fentoni is morphologically similar to *M. m. molossus*, *M. m. milleri* and *M. m. verrilli*. All these taxa have long and bicolored dorsal hairs with a broad, pale band at the base covering up to 1/2 of the total hair length; the occipital shape is triangular (Fig. 8A), the upper incisors are long and thin with parallel tips (Fig. 8C), and the basiocciptal pits have moderate depth (Fig. 8E). However, *M. fentoni* has smaller cranial and body measurements than these three taxa (Fig. 3; Table 6); the infra-orbital foramen opens laterally in *M. fentoni* (Fig. 8C) and anteriorly in the other subspecies (Fig. 8D); and the anterior arch of the atlas is rounded in *M. fentoni* and flattened in *M. m. molossus*, *M. m. verrilli* and *M. m. milleri* (Fig. 6). In addition, *M. fentoni* is sympatric with *M. m. molossus*, but *M. m. milleri* is restricted to Jamaica and Cayman Islands, and *M. m. verrilli* is endemic to Hispaniola, allopatric to the South American distribution of *M. fentoni*.

Molossus sinaloae, M. alvarezi and *M. fentoni* share several morphological characters such as bicolored dorsal pelage, thin and long upper incisors, and triangular occipital bone. In many characters *M. fentoni* appears similar to a very small version of *M. sinaloae*, as also suggested by its position in the molecular analysis (Fig. 2). However, *M. sinaloae* and *M. alvarezi* are much larger than *M. fentoni* and their sagittal crests are not well developed in males proportional to the skull size.

Table 6
Cranial and body measurements of eight recognized species of *Molossus* (*sensu* Dolan, 1989), and *M. fentoni sp. nov*. We used the name *M. currentium* instead of *M. bondae* as suggested by González-Ruiz et al. (2011). See "Material and methods" for variable abbreviations.

	M. fentoni sp. nov. (*n* = 5)	*M. verrilli* (*n* = 5)	*M. milleri* (*n* = 7)	*M. molossus* (*n* = 263)	*M. aztecus* (*n* = 59)	*M. coibensis* (*n* = 33)	*M. rufus* (*n* = 89)	*M. pretiosus* (*n* = 32)	*M. alvarezi* (*n* = 8)	*M. sinaloae* (*n* = 10)	*M. currentium* (*n* = 10)
FA	35.5 (34.0–36.0)	40.1 (39.0–41.0)	39.0 (38.0–40.0)	39.8 (36.7–42.6)	39.3 (37.0–41.5)	36.5 (34.3–37.0)	49.8 (47.7–54.0)	47.2 (45.0–48.5)	46.1 (44.1–47.1)	47.5 (47.0–49.0)	41.6 (39.0–44.7)
GLS	15.0 (14.8–16.5)	17.7 (17.7–17.9)	16.6 (16.0–16.8)	17.3 (15.8–18.9)	17.0 (16.0–18.1)	15.6 (14.5–16.4)	21.4 (19.6–23.3)	20.1 (18.6–20.3)	18.9 (17.8–19.3)	20.5 (18.8–21.8)	17.9 (17.4–18.7)
GLSI	15.3 (15.2–16.8)	17.2 (17.0–17.4)	16.1 (15.8–16.4)	18.0 (16.0–19.5)	17.2 (16.5–18.3)	16.0 (14.9–16.9)	22.1 (19.9–23.8)	20.5 (18.9–22.4)	19.3 (19.0–20.1)	21.0 (19.4–22.4)	18.3 (17.9–19.4)
IOW	3.5 (3.3–3.9)	4.1 (4–4.3)	4.0 (3.9–4.1)	4.3 (3.5–5.0)	3.8 (3.4–4.5)	4.2 (3.2–5.0)	4.5 (3.9–5.0)	4.2 (4.0–4.5)	3.9 (3.9–4.1)	4.0 (3.7–4.4)	4.0 (3.9–4.3)
BCW	8.3 (8.1–8.6)	8.0 (7.9–8.3)	8.5 (8.2–8.7)	8.0 (7.1–9.1)	9.1 (8.4–9.6)	8.5 (8.1–8.7)	10.7 (9.8–11.7)	10.2 (9.7–10.8)	9.5 (9.4–9.7)	9.8 (9.6–10.0)	9.5 (8.6–9.9)
CIL	14.2 (13.8–15.2)	14.5 (14.1–14.8)	14.9 (14.5–15.2)	15.1 (14.0–16.3)	13.7 (12.8–14.6)	13.0 (11.9–13.9)	17.5 (16.0–18.1)	16.4 (15.4–17.5)	15.9 (15.3–16.7)	17.0 (15.3–17.3)	14.6 (14.3–15.2)
ZB	10.2 (10.0–10.4)	11.3 (11.1–11.6)	10.8 (10.7–10.9)	11.1 (9.6–13.0)	10.8 (9.6–12.3)	10.1 (9.5–10.6)	13.8 (12.0–14.3)	12.9 (12.0–13.7)	11.7 (11.1–12.1)	12.4 (11.7–13.1)	11.7 (11.5–12.2)
PAL	4.6 (4.4–4.8)	5.2 (5.0–5.4)	5.1 (4.9–5.2)	5.5 (4.7–5.9)	5.4 (4.8–6.3)	5.0 (4.6–5.3)	6.9 (6.2–7.5)	6.5 (5.9–6.8)	6.3 (5.9–6.7)	6.7 (6.3–7.0)	5.6 (5.3–6.0)
M-M	7.1 (6.9–7.3)	8.0 (7.9–8.3)	7.8 (7.7–7.8)	7.8 (6.2–8.9)	7.8 (7.3–7.9)	7.4 (7.1–7.8)	9.8 (8.5–10.4)	9.4 (8.3–10.1)	8.4 (7.9–8.6)	9.1 (8.4–9.9)	8.3 (8.2–8.5)
C-C	4.0 (3.6–4.2)	4.6 (4.5–4.8)	4.4 (4.2–4.5)	4.5 (3.9–5.7)	4.5 (4.3–5.9)	4.3 (4.1–8.7)	5.8 (5.2–6.5)	5.4 (4.8–6.1)	4.9 (4.6–5.3)	5.3 (5.0–6.2)	4.9 (4.6–5.2)
I-I	1.5 (1.4–1.7)	1.8 (1.7–1.9)	1.7 (1.6–1.9)	1.5 (1.3–2.0)	1.7 (1.5–2.1)	1.7 (1.3–1.9)	2.4 (2.0–3.0)	2.1 (1.7–2.4)	1.8 (1.7–1.9)	2.1 (1.9–2.5)	2.0 (1.8–2.2)
C-M	5.5 (5.1–5.7)	6.3 (6.2–6.4)	6.0 (5.9–6.1)	6.2 (5.5–7.7)	6.1 (5.8–6.6)	5.8 (5.5–6.0)	7.9 (7.0–8.6)	7.5 (7.0–8.1)	7.2 (6.9–7.8)	7.8 (7.3–8.5)	6.7 (6.6–7.0)
SAR	0.9 (0.7–1.1)	1.0 (0.8–1.1)	0.9 (0.7–1.0)	1.0 (0.6–2.0)	1.1 (0.5–1.9)	0.7 (0.3–9.0)	2.2 (1.0–2.9)	1.6 (1.4–2.4)	1.6 (1.3–1.9)	2.2 (2.0–2.8)	1.5 (1.2–2.0)

Molossus fentoni is easily distinguished from *M. currentium*, *M. pretiosus*, and *M. rufus*. These species are much larger than *M. fentoni* in external and craniodental dimensions. The dorsal pelage in these species is dark and unicolored, contrasting with *M. fentoni*, which has bicolored dorsal hairs. Cranial and dental characters of these species are also very different from *M. fentoni*, including shape of occipital complex, incisors, infra-orbital foramen, and basisphenoid pits.

Distribution

Molossus fentoni is currently known from the administrative regions of Potaro-Siparuni and Upper Takutu-Upper Essequibo in Guyana and in Orellana province in Ecuador. Although, it has not been documented in the intervening 2000 km of lowland Amazonian forest, we anticipate that it will be found to have a broader distribution then initially represented in our collections. One individual of *M. fentoni* was collected in syntopy with *M. coibensis*, *M. m. molossus*, and *M. rufus* at 42 km S and 9.5 km E of Pompeya Sur, Orellana, Ecuador on 18 May, 2006.

Etymology

This species is named in honour of M. Brock Fenton, Professor Emeritus, Western University, London, Ontario, and one of the world's foremost researchers in bat ecology and behaviour. He was born in Guyana to Canadian parents and conducted fieldwork in the country in 1970.

Taxonomic remarks

Husson (1962) designated the lectotype of *M. molossus* as the larger of the two bats described by Buffon and Daubenton (1763). Later, Husson (1962) restricted the type locality of *M. molossus* to Martinique, which previously had only been designated as the Americas in the first citation of this specimen (Buffon and Daubenton, 1759). Specimens of *M. molossus* from Martinique were morphologically analyzed in our study and have all the characteristics described above for *M. molossus*, and not for *M. fentoni*. In addition, the DNA sample of *M. molossus* from Martinique clustered with several other samples of *M. molossus* from the mainland in the phylogenetic trees (Fig. 2), such as Guyana, Suriname, and Brazil, corroborating its affiliation with *M. molossus* and the distinction from *M. fentoni*.

Discussion

What is a species? According to the evolutionary species concept, species are ancestor-descendant lineages that evolve separately from other such lineages and have their own evolutionary tendencies and historical fate (Wiley, 1981). The genetic species concept adds an evidentiary character set to the evolutionary species concept that considers a species as a group of genetically compatible interbreeding natural populations that is genetically isolated from other such groups. Under this definition, speciation is the accumulation of genetic changes in two lineages that produce genetic isolation and protection of the integrity of the two respective gene pools that have independent evolutionary fates (Baker and Bradley, 2006). Accepting that species are independent evolutionary lineages that can be recognized using different lines of evidence (e.g. genetic and morphological divergence), *M. fentoni* is clearly distinct from other *Molossus* in morphology, morphometrics and in the parsimony and maximum likelihood analyses of sequences from the mitochondrial COI and nuclear beta fibrinogen gene. *M. fentoni* appears as the sister group of *M. sinaloae*, a very mensurally distinct species, but poorly supported phylogenetically. Likewise, individuals of the new species was recovered in a well-supported clade that can be genetically distinguished from other species in the genus by its high level of interspecific sequence

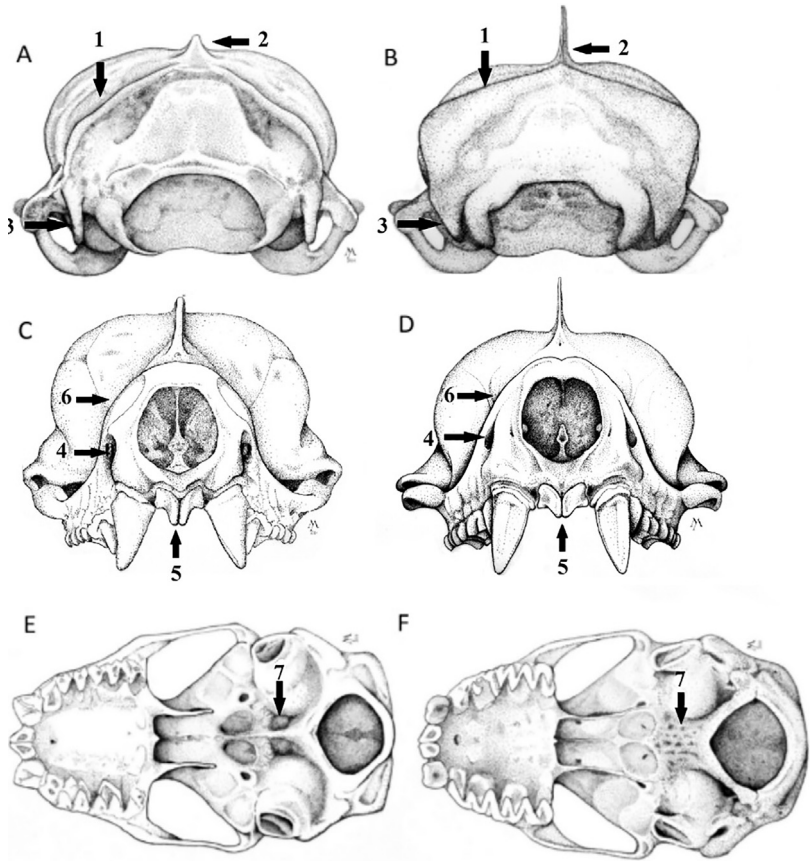

Fig. 8. Schematic comparison of cranial features in *Molossus*. A and B – Posterior view; C and D – frontal view; E and F – Ventral view. Numbers represent characters described in the text. 1 – Lambdoidal crest and occipital complex; 2 – Sagittal crest; 3 – Mastoid process; 4 – Infra-orbital foramen; 5 – Upper incisors; 6 – Rostrum shape; 7 – Basioccipital pits.

divergence in the mitochondrial CO1 (8.0–10.1%) and nuclear beta fibrinogen gene (1.0–3.1%), compared to the low level of genetic intraspecific variation for both genes found in our analyzes (<1%). The high genetic divergence found between *M. fentoni* and all other species of *Molossus* compared to the intraspecific variation in other species of the genus is consistent with Clare et al. (2007) who found 1.8% of divergence in the CO1 gene within *M. molossus*, and Catzeflis et al. (2016) who found and average of 1.7% of divergence in the CO1 sequence within different populations of *M. coibensis* (including its junior synonym *M. barnesi*).

A specimen of *M. fentoni* from Ecuador was collected sympatrically with *M. coibensis*, *M. molossus*, and *M. rufus*, with no sign of introgression. In size, *M. fentoni* is most similar to *M. coibensis* (=*M. barnesi*), however, *M. fentoni* appears as a distinct genetic lineage and differs from *M. coibensis* in shape of the occipital region of the skull, shape of incisors, and colour banding of the dorsal hairs. In addition, *M. fentoni* is morphologically similar to its sister species *M. sinaloae* although *M. sinaloae* is much larger than *M. fentoni*. In the past, many species of *Molossus* (and in particular *M. molossus*) have been characterized, in part, by similarity of body size, which in *M. molossus* (sensu lato) has resulted in a polyphyletic construct based on a non-homologous character (small body size). It is therefore not surprising that this group was singled out by Simmons (2005), who stated "this complex is desperately in need of a revision".

Three species lacking COI and beta fibrinogen sequences—*M. alvarezi*, *M. currentium,* and *M. pretiosus*—are easily distinguished from *M. fentoni* by their larger size and dark, unicolor pelage. We also lack molecular sequences for *M. aztecus*, and this species overlaps with *M. fentoni* in some cranial measurements. However, several characteristics such as dark unicolor or faintly bicolor pelage and quadrangular occipital shape in *M. aztecus*, morphologically distinguish it from *M. fentoni*. Although systematic uncertainty remains within the *M. m. molossus* complex that requires more taxonomic and geographic sampling, especially in the Amazon basin, the new species *M. fentoni* is morphologically and molecularly distinct compared to all the other taxa examined, and is a phylogenetic outlier to *M. molossus*.

Lim and Engstrom (2001b) suggested that the new species might be assignable to *M. pygmaeus* described from Curaçao (Miller, 1900) based on size and color. Although these two taxa are similar in size and cluster together in the PCA plot (Fig. 3), examination of a topotype of *M. pygmaeus* (AMNH 30393) from Curaçao indicates that *M. pygmaeus* groups with *M. molossus* and not with *M. fentoni* (e.g., the infra-orbital foramen opens frontally). In addition, DNA barcoding of a sample from Bonaire, approximately 40 km east of Curaçao, nests within the *M. m. molossus* clade (Fig. 2), indicating that the name *M. pygmaeus* should be considered as a junior synonym of *M. molossus*, as suggested by Dolan (1989).

As currently defined, *M. molossus* is non-monophyletic, in part because it has been recognized based on non-homologous morphological characters such as overall body size. In addition to the new species and a putative distinct savannah taxon identified here (*Molossus* sp.), two other taxa, synonymized by Dolan (1989) under the name *M. molossus*, appear in our analysis as distinct evolutionary lineages. The first clade, *M. milleri* Johnson, 1952, includes our samples from Jamaica and the Cayman Islands; and the second clade, *M. verrilli* Allen, 1908, is confined to Dominican Republic. Both groups are genetically divergent from *M. molossus* (*sensu stricto*), and appear as successive sisters groups to the clade of *M. molossus* + *M. rufus* + *M. coibensis* + *Molossus* sp. (the last taxon

from the savannahs of Venezuela and Guyana). Therefore, we recommend that these two taxa (*M. milleri* and *M. verrilli*) should be treated as distinct and separate species, distinct from *M. molossus*.

Further systematic study of the genus is needed as suggested by the non-monophyletic arrangement of specimens previously assigned to *M. molossus* in relation to *M. coibensis* and *M. rufus*. Likewise, the status of *M. m. daulensis* from the western slope of the Andes in Ecuador and Peru remains unresolved. Interestingly, *M. m. molossus* is morphologically very distinct from the larger and darker *M. rufus*, but there is only 2.4% sequence divergence between these two species in the COI and less than 1% in the beta fibrinogen genes. In contrast, *M. m. molossus* (*sensu stricto*) is more similar in size and morphology to both *M. verrilli* and *M. milleri*, but differs from them in COI sequence by 3.8% and 4.1% respectively, and in the beta fibrinogen sequence by 1.8% and 2.2% respectively, and these latter taxa are phylogenetically distinct from *M. molossus* and from each other.

The genus *Molossus* has a complex taxonomic history and species diversity and geographical distribution are not well understood. This description based on morphology and DNA sequence data of *Molossus fentoni* from Guyana and Ecuador is part of a larger project on the systematics and biogeography of *Molossus*. Morphological study over the past two centuries has not resolved the taxonomy and evolutionary history of the genus. We anticipate that a thorough molecular analysis including both mitochondrial and nuclear datasets, with comprehensive taxonomic and geographic sampling will be required to clarify the poorly known phylogenetic relationships of *Molossus* and within the species complex currently assigned to *M. molossus*.

Conflict of interests

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.mambio.2018.01.008.

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