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The French Guianan endemic *Molossus barnesi* (Chiroptera: Molossidae) is a junior synonym for *M. coibensis*

François Catzeflis^a, Yann Gager^{b,c}, Manuel Ruedi^d, and Benoit de Thoisy^{e,f}

^aInstitut des Sciences de l'Evolution, Case Courrier 064, CNRS UMR-5554, Université Montpellier-2, Place E. Bataillon, F-34095 Montpellier, France, francois.catzeflis@univ-montp2.fr

^bDepartment of Migration and Immuno-Ecology, Max Planck Institute for Ornithology, 78315 Radolfzell, Germany, yann.gager@gmail.com

^cDepartment of Biology, University of Konstanz, 78464 Konstanz, Germany

^dDepartment of Mammalogy and Ornithology, Natural History Museum of Geneva, BP 6434, 1211 Geneva 6, Switzerland, Manuel.Ruedi@ville-ge.ch

^eLaboratoire des Interactions Virus-Hôtes, Institut Pasteur de la Guyane, 23 avenue Pasteur, BP 6010, 97306 Cayenne cedex, French Guiana, bdehoisy@pasteur-cayenne.fr

^fAssociation Kwata, 16 avenue Pasteur, 97300 Cayenne, French Guiana

Corresponding author: François Catzeflis

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1 **Abstract**

2 The taxonomy of the small Neotropical *Molossus* species has been notoriously difficult due to a lack of
3 adequate comparative material. One taxon in particular, *M. barnesi* Thomas, 1905 was believed to be
4 restricted to a narrow stretch of coastal areas around Cayenne, in French Guiana and was so far
5 represented only by three female specimens. It was variously considered as a species on its own, or
6 synonymized with *M. molossus* or *M. coibensis*. Thanks to the discovery of several mixed colonies of
7 these small molossids in two localities in French Guiana, we could obtain and measure a large sample
8 (nearly 200 specimens) of adult individuals to better assess their morphological variation. Owing to
9 largely bimodal and non-overlapping distributions of external measurements such as forearm length,
10 we could demonstrate the existence of two sympatric morphotypes, the smaller one corresponding to
11 *M. barnesi* and the larger one to *M. molossus*. Univariate and multivariate comparisons of cranio-dental
12 and external characters further suggest that the new series of *barnesi* from French Guiana do not differ
13 notably from specimens assigned to *M. coibensis* from elsewhere. Molecular reconstruction based on
14 the barcode gene (CO1) confirmed their genetic distinctness, but also the overall close relationships
15 (mean divergence of 1.7 %) of all assayed taxa in this group. Although none of the haplotypes are
16 shared across taxa, haplotypes of *M. coibensis* from Panama and *M. barnesi* from French Guiana are
17 mixed in a single, poorly supported cluster, suggesting that these animals could represent a single
18 biological species. Based on all evidences, we thus recommend treating *barnesi* as a junior synonym of
19 *M. coibensis*, a species now widely and continuously distributed from Central America to Middle South
20 America.

21

22 **Introduction**

23 The Order Chiroptera represents one fifth of all extant Mammals with well over 1116 recognized
24 species (Simmons, 2005), but the true diversity still remains underestimated in many places and
25 taxonomic issues are continuously revised. Molecular data and phylogenetic reconstructions coupled
26 with careful morphological comparisons provide an integrative framework that helps to better
27 understand the evolution of this biodiversity and has been applied successfully in bat taxonomy
28 (Goodman et al., 2009). We apply here such an integrative approach to resolve the taxonomic status of
29 small Neotropical molossid bats of the genus *Molossus*.

30

31 Thomas (1905) described *Molossus barnesi* (Molossidae) from a single female specimen collected at
32 Cayenne, French Guiana. Since then, this taxon was variously considered as a species on its own, or
33 synonymized with other small Neotropical molossids. For instance, *M. barnesi* was synonymized with
34 *M. molossus* by Freeman (1981) or Brosset and Charles-Dominique (1990), while Dolan (1989) and

35 Eger (2008) rather classified it within *M. coibensis* (another small *Molossus* species originally
36 described by Allen (1904) from the Island of Coiba, Panama). Although the first authors did not justify
37 their taxonomic decision, Dolan compared the holotypes and considered that *barnesi* fell within the
38 morphological variation of a series of Central American *M. coibensis*. More recently, Simmons and
39 Voss (1998) caught two small *Molossus* during a large survey of mammals conducted at Paracou (ca.
40 80 km north-west from Cayenne), French Guiana. They also compared this new material with the
41 holotype of *barnesi* and with several other small-sized Neotropical *Molossus* and showed that these
42 new specimens corresponded well to *barnesi*. However, they concluded that *M. barnesi* was a taxon on
43 its own and clearly distinct (generally smaller-sized and with different pelage and dental patterns) from
44 any other recognized species, including *M. coibensis* or *M. molossus*. They also confirmed that *M.*
45 *barnesi* was so far only known from these 2 localities in French Guiana.

46

47 Gregorin et al. (2011) challenged this taxonomic view arguing that the morphological characters
48 distinguishing *M. barnesi* and *M. coibensis* were too variable in a broader geographic context or were
49 overlapping between the few known (n=3) individuals of *barnesi* compared to the more numerous
50 specimens assigned to *M. coibensis*. Gregorin et al. (2011) further mentioned the biogeographical issue
51 regarding the much localized occurrence of *barnesi* (i.e. restricted to a small coastal area of French
52 Guiana) versus the widespread distribution of *M. coibensis*, supposed to live in a region comprised
53 between southern Mexico and the Brazilian Mato Grosso (Correa da Costa et al., 2013). More recently,
54 the distribution of *M. coibensis* was extended even farther towards the south-east of Brazil, with a new
55 locality in the Atlantic Forest biome (Pimental et al., 2014).

56

57 As the source of most of these taxonomic controversies appears to be a lack of appropriate comparative
58 material of *M. barnesi*, and because no DNA characters have been examined so far in this context, we
59 report here the comparative morphological and molecular analyses of a series of new specimens of *M.*
60 *barnesi* collected in two localities of French Guiana where this taxon lives in sympatry with typical
61 populations of *M. molossus*. We also use DNA sequences of extralimital material of other small
62 Neotropical *Molossus* species, including a series of *M. coibensis*, to reassess their taxonomic status.

63

64 **Material and methods**

65 Over 200 small molossid bats living under several tin roofs of traditional houses were caught at
66 Remire-Montjoly between November and December 2007 (collectors Maël Dewynter and Julien
67 Jemin) and at Cacao in July 2012 (collectors Francois Catzeflis and Manuel Ruedi), both locations
68 being set along the coastal region of north-east French Guiana. The locality of Remire-Montjoly
69 (04°52'30" N; 52°16'30" W) lies in the eastern suburbs adjacent to the city of Cayenne, with
70 surrounding habitats highly anthropized and consisting of a patchwork of private houses, gardens, and

71 small forest fragments. The locality of Cacao (04°34'30'' N; 52°27'10'' W) lays ca. 45 km to the south
72 of Cayenne and is set in an agricultural landscape comprised of various orchards and small plots of
73 organic vegetables, with some secondary forest remains in its immediate vicinity.

74

75 Capture methods included mist nets (2.6 × 6 m and 2.6 x 9 m; mesh size = 16 mm) set close to the
76 edges of roofs from where the molossids were leaving their roost at dusk. Upon capture, bats were held
77 temporarily in individual cotton bags. Prior to release, each animal was aged (only adults with
78 completely fused phalangeal epiphyses were considered), sexed and measured for the following three
79 external measurements (with a dial calliper to the nearest 0.1 mm): forearm length (FA; taken from the
80 tip of the elbow to the wrist with the wing held closed), length of metacarpal of third (MC3) and of
81 fourth digit (MC4; measured on the dorsal side of the wing held flat on a solid surface, from the basis
82 of the wrist to the tip of the metacarpal). A selection of 50 specimens (see list in Appendix A) were
83 kept and euthanized following the guidelines of the American Society of Mammalogists for the use of
84 wild mammals in research (Sikes and Gannon, 2011). These specimens were preserved as scientific
85 vouchers for further morphological and genetic analyses. A fragment of chest muscle was kept in 95%
86 ethanol and specimens were fixed for one day in 10% buffered formalin, and stored in 70% ethanol. As
87 no specific decree conserving bats outside protected areas exist in French Guiana, no specific legal
88 authorization was required for captures and handling of bats.

89

90 *Morphology*

91 Reproductive status was acquired from external characteristics (e.g. enlarged nipples or testis) or from
92 gross examination of dissected specimens (Racey, 2009). In addition to the three external characters
93 taken on all bats (FA, MC3 and MC4), tibia length (TI), tail length (TL), wingspan (WS), weight
94 (expressed in grams) and length of mid-dorsal fur (DF) were also recorded on each vouchered
95 specimen. Nine cranio-dental measurements were taken on the cleaned skulls with a dial calliper
96 (accurate to 0.05 mm) following the methods detailed in Simmons and Voss (1998) except when noted:
97 greatest length of skull (bone-to-bone: GLS), maxillary toothrow length (MTL), condylo-incisive
98 length (CIL), breadth across canines (BaC), zygomatic breadth (ZB), mastoid breadth (MB), braincase
99 breadth (BB), post-orbital breadth (PB) and outer breadth across molars (BaM). Because sexual
100 dimorphism is common in Molossidae, including in the genus *Molossus* (Freeman, 1981; Willig and
101 Hollander, 1995), we determined its significance with Mann-Whitney tests (as implemented in the
102 software PAleontological STatistics: Hammer et al., 2001). As most historic specimens are females and
103 also to avoid the confounding factor of sexual dimorphism, the following global morphological
104 comparisons were based only on a subset of 48 female molossids. This subset included 21 *M. barnesi*
105 from French Guiana (including the two females studied by Simmons and Voss, 1998), 19 *M. molossus*
106 from French Guiana (including nine specimens studied by Simmons and Voss, 1998), the holotype of

107 *M. barnesi* (BMNH-5.1.8.7, at the London Natural History Museum) and a series of seven females of
108 *M. coibensis* from Brazil (Universidade Federal do Pará Campus de Bragança: vouchers numbers
109 LJCC-13, LJCC-14, LJCC-16 to -20) assigned to this taxon by Correa da Costa et al. (2013).
110 Morphological shape variation, determined for six cranial (GLS, CIL, MTL, BaM, BB, and PB) and
111 one external (FA) measurements, was analysed using a Principal Component Analysis Biplot on scaled
112 data (PCA Biplot, R Core Team, 2015).

113

114 *Molecular analyses*

115 DNA was extracted with the NucliSENS EasyMag robot (Biomérieux, Craponne, France) following
116 manufacturer's recommendations for tissue extraction. The barcoding fragment of the mitochondrial
117 gene Cytochrome oxidase 1 (CO1) was amplified as recommended by Borisenko et al. (2008). After
118 amplification, PCR products were sent for purification and sequencing at Cogenics (Takeley, UK),
119 using the same primers as for amplifications. Of the ca. one hundred sequences of small *Molossus*
120 already available in GenBank, we selected a subset of 15 distinct haplotypes to represent 1 or 2
121 individuals each of the taxa *coibensis*, *molossus* and *rufus* living in the areas of sympatry (Panama,
122 Ecuador and the Guiana Shield). Together with these 15 sequences retrieved from GenBank (Accession
123 numbers in Appendix B), the six sequences generated here were aligned and checked manually with
124 MEGA 6.0 (Tamura et al., 2013) for absence of gaps or stop codons, to ensure that these were not
125 pseudogenes.

126

127 Phylogenetic relationships between samples were evaluated with neighbour-joining (NJ), maximum
128 likelihood (ML) and Bayesian procedures, and using two *Eumops* species (two exemplars each of *E.*
129 *hansae* and *E. auripendulus* - accession numbers in Appendix B) as outgroups. The Tamura-Nei model
130 (TN93) with gamma (G) rate parameter and a proportion of invariant sites (I) was identified with
131 MEGA 6.0 as the best-fitting nucleotide substitution model. Ten thousand replicates were used for
132 maximum likelihood and neighbour-joining analyses, yielding support values as Bootstrap Percentage
133 (BP). The Bayesian approach was carried out with MrBAYES 3.2 (Ronquist et al., 2012). Markov
134 Chain Monte Carlo (MCMC) simulations were run twice independently for 10 million generations with
135 four simultaneous chains, using a sample frequency of one every 1,000 and a burn-in of 3 million trees.
136 Support values are indicated as Posterior Probabilities (PP) and were calculated from the remaining
137 trees.

138 Together with the phylogenetic trees, we estimated the haplotype network of *Molossus* spp. sequences
139 using Network 4.5.0. and the Median Joining (MJ) network algorithm (Bandelt et al., 1999).

140

141

142 **Results**

143 Capture sessions at Remire-Montjoly and Cacao yielded 196 adult *Molossus* bats. We plotted the
144 distribution of FA lengths measured in these colonies for each sex separately. Measurements of the 128
145 females (53 from Remire-Montjoly and 75 from Cacao) and 68 males (27 and 41, respectively) show
146 clear bimodal distributions, with little or no overlap between the two morphotypes found in syntopy
147 (Figure 1). According to these clear differences, all females with a forearm smaller than 37.1 mm and
148 all males with a FA smaller than 37.4 mm were assigned to the *M. barnesi* morphotype, whereas the
149 larger specimens were assigned to *M. molossus*, as suggested by Simmons and Voss (1998). As
150 expected, all other external measurements correlated with size differed significantly between those two
151 species identified by their forearm size (Table 1). Intraspecific sexual dimorphism, whereby males are
152 larger than females, was also significant in the digit measurements (MC3 and MC4) of *M. barnesi* but
153 not in those of *M. molossus*, as shown in Table 2.

154

155 Cranio-dental measurements also revealed the existence of a significant overall sexual dimorphism ($p <$
156 0.01), with males being larger than female at most variables (GLS, CIL, ZB, MB, BB and BaC).
157 Significant intraspecific sexual dimorphism was also evident in the skulls of both species ($p < 0.01$ for
158 all variables except BaM in *M. barnesi*, and except MTL and PB in *M. molossus*). Regardless of the
159 sex, skulls of *M. molossus* were longer than in *M. barnesi*, with all antero-posterior measurements
160 (GLS, CIL, MTL, PB) being significantly different ($p < 0.01$), whereas these two taxa had similar skull
161 breadth (as measured by ZB, MB, BaM, BB, BaC; $p > 0.05$; Table 3).

162

163 An analysis of variance (ANOVA) for size variation at one external (FA) and six cranio-dental
164 measurements (GLS, CIL, MTL, BaM, BB, and PB) of the 48 female reference specimens indicates
165 that *M. molossus* is clearly larger than *M. barnesi* and *M. coibensis* (post-hoc Tuckey test: $p = 0.0001$
166 and $p = 0.0323$, respectively), while females *M. coibensis* and *M. barnesi* do not differ significantly (p
167 $= 0.7998$). The PCA Biplot based on those same variables explained a high percentage of the total
168 variation, 45.5% being associated to the first and 26.0% to the second component. This PCA Biplot
169 indicates that the seven *M. coibensis* from Brazilian Para and all specimens of *M. barnesi* from French
170 Guiana (including the holotype of *barnesi*) form completely overlapping groups, while individuals of
171 *M. molossus* are set in a distinct cluster (Figure 2).

172 Table 4 compares our French Guianan sample consisting of 11 males and 20 females (including the
173 holotype) *M. barnesi* with a sample of 21 males (including the type of *coibensis*) and 23 females *M.*
174 *coibensis* taken in Panama (sample #19 from Chiriquí, La Concepción, in Dolan, 1989). As already
175 mentioned by Dolan (1989), measurements of the female holotype of *barnesi* (BMNH-5.1.8.7) fall
176 within the range of female specimens of *M. coibensis* from Central America. Similarly, the male
177 holotype of *coibensis* (AMNH-18731) conforms well to the variation of males *M. coibensis* from
178 Panama or from those of *M. barnesi* from French Guiana.

179 The phylogenetic reconstructions (Figure 3 – left panel) based on CO1 sequences indicate that small
180 *Molossus* assayed form various monophyletic clades which appear very closely related to each other,
181 differing by an average of 1.7 % substitutions (range 0.0 - 2.0 %). In particular, sequences of *M.*
182 *coibensis* from Ecuador and Panama and those of *M. barnesi* from French Guiana differ by less than
183 1.5% substitutions. Sequences of *M. molossus* from French Guiana, Ecuador, Panama, Suriname and
184 Guyana and those of *M. rufus* from French Guiana and Guyana each form monophyletic taxa (BP
185 support 71% and 95%, respectively), whereas sequences of *M. coibensis* and *M. barnesi* are
186 intertwined. The MJ network which shows in more details relationships of the various haplotypes
187 (Figure 3 – right panel) further indicates that the COI haplotypes of *M. barnesi* and *M. coibensis* derive
188 from the same haplogroup, whereas those of *M. molossus* and *M. rufus* are set further apart.
189
190

191 **Discussion**

192 The newly collected material of *M. barnesi* sampled close to the type-locality in French Guiana adds to
193 the only three historical specimens (all females) reported so far by Simmons and Voss (1998). The now
194 enlarged samples provide an appropriate series for assessing morphological variation and sexual
195 dimorphism in this highly localized taxon. In two different localities (Cacao and Rémire-Montjoly),
196 these small molossids were captured in strict sympatry (i.e. under the same tin roof) with another,
197 larger species (*M. molossus*). It is, however, unclear whether individuals live really in intermixed
198 clusters or whether they form species-specific social groups occupying different portions of the same
199 building. According to the principle of competitive exclusion verified in other sibling species of bats
200 living in sympatry (Arlettaz et al., 1997), these two species should even differ at some ecological
201 aspects but this has still to be ascertained with proper evidences. Synanthropic roosts occupied
202 simultaneously by 2 or 3 species of small molossids were also reported in the Brazilian Atlantic Forest
203 (*M. molossus*, *M. coibensis*, *M. rufus*) by Pimenta et al. (2014) and in central Panama (*M. molossus*, *M.*
204 *coibensis*, and *M. bondae*) by Gager et al. (2016), whereas the syntopic occurrence of *M. coibensis* and
205 *M. molossus* in outside roosts is already known from Ecuador (McDonough et al., 2011), from Guyana
206 (Lim and Engstrom, 2001) and from Brazil (Pimenta et al., 2014).

207 Despite more or less pronounced sexual dimorphism exhibited by both species, highly significant
208 morphometric differences exist between the distinctly smaller *M. barnesi* versus the larger *M. molossus*
209 in French Guiana. This clearly supports the taxonomic distinction proposed by Simmons and Voss
210 (1998). In particular, wing measurements (FA, MC3, MC4 and WS) are all very discriminant, with
211 little or no overlap between those two sympatric species. Our large samples of both *Molossus* species in
212 French Guiana further show that the sexual dimorphism is more pronounced in *M. barnesi* than in *M.*

213 *molossus*, as illustrated by external measurements such as forearm or third and fourth metacarpus. Due
214 to an overall significant sexual dimorphism (males being generally larger than females), these external
215 differences are even more obvious when values are sorted by sex, as detailed in Table 2. Another
216 external character mentioned by Simmons and Voss (1998), i.e. the length of dorsal hairs measured in
217 the mid-dorsum (DF), is less useful for the discrimination of both species because several individuals
218 caught in sympatry in French Guiana and in south-east Brazil (Pimenta et al., 2014) had intermediate
219 values (about 3.0 mm).

220 Regarding the cranio-dental variables, we observed that *M. barnesi* and *M. molossus* have similar
221 measurements in the breadth of the skull (expressed by ZB, MB, BaM, BB or BaC) but differ for length
222 measurements (i.e. GLS, CIL, MTL or PB). Thus, the skull of *M. barnesi* is relatively shorter than that
223 of *M. molossus* for similar breadth. This difference again corroborates earlier remarks on skull shape
224 mentioned for those two species in Brazil (Pimenta et al., 2014). Another qualitative discriminant
225 character proposed by Simmons and Voss (1998) and also noted by Pimenta et al. (2014) is the shape
226 of the upper incisors (Figure 3). The six illustrated specimens indeed show that *M. barnesi* have
227 slightly shorter and more convergent (spatulate) upper incisors, whereas those of *M. molossus* are more
228 elongated and tapering (pincer-like), but these qualitative differences are sometimes difficult to
229 evaluate on single specimens.

230 Although various morphological and morphometric characters support the existence of three distinct
231 species of *Molossus* living in sympatry in French Guiana (the small *M. barnesi* and *M. molossus* and
232 the much larger *M. rufus*, Simmons and Voss, 1998), their CO1 sequences are very similar (differing
233 by about 1.7% nucleotides substitutions), indicating that these haplotypes derive from a recent common
234 mitochondrial ancestor. None of the sequenced bats shared the same haplotype (Figure 3 - right panel)
235 but the number of assayed individuals here is not enough to establish firmly if time since their
236 separation was long enough to lead to reciprocal monophyly of lineages in each species. When other
237 extralimital sequences of other small *Molossus* are included in the molecular analyses, notably those of
238 *M. coibensis* from Ecuador and Panama, the separation of taxa does not improve, as sequences are
239 globally all very closely related (Figure 3). This indicates that most mitochondrial lineages in this
240 group diverged recently from each others. Based on a much larger data set, Gager et al. (2016) also
241 found very closely related CO1 sequences between morphologically distinct *M. coibensis* and *M.*
242 *molossus* from Panama, Ecuador, Guyana and Suriname. Whereas the usefulness of DNA barcoding
243 has proven its effectiveness in several other studies of bat identification (e.g. Clare et al., 2007, 2011;
244 Lim, 2012), this example of morphologically recognizable taxa which do not show necessarily
245 appreciable genetic differentiation indicates that more rapidly evolving genes (such as some fast-
246 evolving nuclear introns or the mitochondrial control region) might be necessary to reach a better
247 phylogenetic resolution.

248 Even if barcodes are of limited use in this group (Borisenko et al., 2008), haplotypes of *M. barnesi*
249 from French Guiana are intertwined with those of geographically more distant *M. coibensis* from
250 Panama and Ecuador and do not form distinct haplogroups (Figure 3). Notably, the later include
251 representative sequences of *M. coibensis* sampled close to the type-locality of this taxon in Panama and
252 identified with multiple morphological, bioacoustics and molecular characters (Gager et al., 2016).
253 Again, such close genetic relatedness and lack of reciprocal monophyly calls into question the
254 taxonomic distinctness of these two taxa.

255 Based on our new univariate and multivariate morphological comparisons, we further demonstrate that
256 animals assigned to *M. coibensis* from Brazil (Correa da Costa et al., 2013) and to *M. barnesi* from
257 French Guiana are indistinguishable, whereas all *M. molossus* are clearly set apart on this morphospace
258 (Figure 2). Although none of the specimens of *M. coibensis* from near the type-locality in Panama
259 could be added to this multivariate analysis, measurements of the type specimen (Table 4) and direct
260 morphological comparisons made by earlier researchers (Dolan, 1989; Eger, 2008; Gregorin et al.,
261 2011) also confirm that *coibensis* and *barnesi* cannot be distinguished elsewhere. Given all available
262 genetic and morphologic evidences, we thus recommend to consider *M. barnesi* as a junior synonym of
263 *M. coibensis*. This proposed synonymy would also solve the critical issue raised by Gregorin et al.
264 (2011) concerning the apparent lack of *M. coibensis* in some areas of the Guiana Shield (Lim and
265 Tavares, 2012), whereas it is found further south to the Mato Grosso and the Atlantic Forest biome in
266 Brazil (Paglia et al., 2012; Correa da Costa et al., 2013; Pimenta et al., 2014). Given the
267 anthropophilous character of the species in French Guiana (reported so far as *M. barnesi*) and
268 elsewhere, we anticipate that more localities of *M. coibensis* throughout South America will fill gaps
269 between the current scattered occurrences for this species. In conclusion, we concur with Gregorin et
270 al. (2011) that a more global taxonomic review concerning other small taxa of the genus *Molossus*
271 living in tropical South America is needed, as the exact number of distinct biological species contained
272 in this group is still debated. Unusually divergent barcode sequences of a small *Molossus* sp. found in
273 the Kanuku Mountains of Guyana (Lim and Engstrom, 2001) even suggest that additional cryptic
274 species might occur in the region (Clare et al., 2007).

275

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285

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References

- Allen, J.A., 1904. New bats from tropical America, with note on species of *Otopterus*. Bull. Am. Museum Nat. Hist. 20, 227–237.
- Arlettaz, R., Perrin, N., Hausser, J., 1997. Trophic resource partitioning and competition between the two sibling bat species *Myotis myotis* and *Myotis blythii*. J. Anim. Ecol. 66, 897–911.
- Bandelt, H.J., Forster, P., Rohlf, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.
- Borisenko, A. V, Lim, B.K., Ivanova, N. V, Hanner, R.H., Hebert, P.D.N., 2008. DNA barcoding in surveys of small mammal communities: a field study in Suriname. Mol. Ecol. Resour. 8, 471–9. doi:10.1111/j.1471-8286.2007.01998.x
- Brosset, A., Charles-Dominique, P., 1990. The bats of French Guiana: a taxonomic, faunistic and ecological approach. Mammalia 54, 509–560.
- Clare, E.L., Lim, B.K., Engstrom, M.D., Eger, J.L., Hebert, P.D.N., 2007. DNA barcoding of Neotropical bats: species identification and discovery within Guyana. Mol. Ecol. Notes 7, 184–190. doi:10.1111/j.1471-8286.2006.01657.x
- Correa da Costa, L.J., Gonçalves de Andrade, F.A., Uieda, W., Gregorin, R., Barroncas Fernandes, M.E., 2013. First record of *Molossus coibensis* (Chiroptera: Molossidae) in the Brazilian Amazon. Mastozool. Neotrop. 20, 143–147.
- Dolan, P.G., 1989. Systematics of Middle American mastiff bats of the genus *Molossus*. Spec. Publ. Museum Texas Tech Univ. 29, 1–71.
- Eger, J.L., 2008. Family Molossidae, in: Gardner, A.L. (Ed.), Mammals of South America Marsupials, Xenarthrans, Shrews, and Bats, Vol. 1. The University of Chicago Press, Chicago and London, pp. 399–440.
- Freeman, P.W., 1981. A multivariate study of the family Molossidae (Mammalia, Chiroptera) : Morphology, ecology, evolution. Fieldiana Zool. 7, 1–173.
- Gager, Y., Tarland, E., Lieckfeldt, D., Ménage, M., Botero-Castro, F., Rossiter, S. J., Kraus, R. H. S., Ludwig, A., Dechmann, D. K. , 2016. The value of molecular vs morphometric and acoustic information for species identification using sympatric molossid bats. PLoS ONE 11 (3) : e0150780. doi:10.1371/journal.pone.0150780

Goodman, S.M., Maminirina, C.P., Weyeneth, N., Bradman, H.M., Christidis, L., Ruedi, M., Appleton, B., 2009. The use of molecular and morphological characters to resolve the taxonomic identity of cryptic species: the case of *Miniopterus manavi* (Chiroptera, Miniopteridae). *Zool. Scr.* 38, 339–363. doi:10.1111/j.1463-6409.2008.00377.x

Gregorin, R., Tahara, A.S., Buzzato, D.F., 2011. *Molossus aztecus* and other small *Molossus* (Chiroptera: Molossidae) in Brazil. *Acta Chiropterologica* 13, 311–317. doi:10.3161/150811011X624794

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4, 1–9.

Lim, B.K., Engstrom, M.D., 2001. Species diversity of bats (Mammalia: Chiroptera) in Iwokrama Forest, Guyana, and the Guianan subregion: implications for conservation. *Biodivers. Conserv.* 10, 613–657. doi:10.1023/A:1016660123189

Lim, B.K., Tavares, V. da C., 2012. Review of species richness and biogeography of bats (Chiroptera) from the Guiana subregion of South America with comments on conservation. *Ecotropica* 18, 105–118.

Paglia, A.P., Fonseca, G.A.B. da Rylands, A.B., Herrmann, G., Aguiar, L.M.S., Chiarello, A.G., Leite, Y.L.R., Costa, L.P., Siciliano, S., Kierulff, M.C.M., Mendes, S.L., Tavares, V. da C. Mittermeier, R.A., Patton J. L., 2012. Annotated checklist of Brazilian mammals. *Occas. Pap. Conserv. Biol.* 6, 1–76.

Pimenta, V., Fonseca, B. S., Hoppe, J. P., Ditchfield, A. D., 2014. First occurrence of *Molossus coibensis* Allen, 1904 (Chiroptera, Molossidae) in Atlantic Forest. *Chiroptera Neotropical* 20, 1237–1242.

R Core Team, 2015. R: A language and environment for statistical computing.

Racey, P.A., 2009. Reproductive assessment of bats, in: Kunz, T.H., Parsons, S. (Eds.), *Ecological and Behavioral Methods for the Study of Bats*. Johns Hopkins University Press, Baltimore, USA, pp. 249–264.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61, 539–42. doi:10.1093/sysbio/sys029

Sikes, R.S., Gannon, W.L., 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *J. Mammal.* 92, 235–253. doi:10.1644/10-MAMM-F-355.1

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

Simmons, N.B., Voss, R.S., 1998. The mammals of Paracou, French Guiana, a Neotropical lowland rainforest fauna. Part 1, Bats. *Bull. Am. Museum Nat. Hist.* 237, 219.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* 30, 2725–9. doi:10.1093/molbev/mst197

Thomas, O., 1905. New Neotropical *Molossus*, *Conepatus*, *Nectomys*, *Proechimys*, and *Agouti*, with a note on the genus *Mesomys*. *Ann. Mag. Nat. Hist.* 15, 584–591. doi:10.1080/03745480509442857

Willig, M.R., Hollander, R.R., 1995. Secondary sexual dimorphism and phylogenetic constraints in bats: A multivariate approach. *J. Mammal.* 76, 981–992. doi:10.2307/1382592

Tables

Table 1. External measurements for small molossids caught in syntopy at Remire-Montjoly and Cacao, in French Guiana. All females with a forearm smaller than 37.1 mm and all males with a forearm smaller than 37.4 mm were assigned to the *M. barnesi* morphotype, whereas the larger specimens were assigned to *M. molossus*. Values are mean \pm one standard-deviation (minimum and maximum); n = sample size. See Material and Methods for abbreviations of measurements. W stands for weight, expressed in grams, while all other variables are expressed in mm.

	<i>M. barnesi</i>	n	<i>M. molossus</i>	n
FA	35.1 \pm 0.9 (32.9 – 37.3)	142	39.1 \pm 1.0 (37.3 – 41.6)	54
MC3	34.2 \pm 0.9 (32.0 – 36.0)	127	38.6 \pm 1.2 (36.0 – 41.5)	48
MC4	32.5 \pm 0.9 (30.0 – 34.5)	127	36.6 \pm 1.0 (34.0 – 39.5)	48
TL	32.9 \pm 1.6 (28.0 – 36.5)	60	37.3 \pm 2.0 (33.0 – 40.0)	16
TI	12.6 \pm 0.5 (11.5 – 13.5)	60	13.8 \pm 0.5 (13.0 – 15.0)	17
WS	260.7 \pm 7.39 (248.0 – 280.0)	47	300.5 \pm 11.3 (280.0 – 322.0)	15
DF	2.5 \pm 0.3 (2.0 – 3.0)	12	3.1 \pm 0.2 (3.0 – 3.5)	7
W	11.5 \pm 1.9 (7.8 – 16.3)	53	13.1 \pm 1.6 (9.5 – 16.0)	20

Table 2. Three wing measurements (FA, MC3, MC4) of small *Molossus* spp. caught in syntopy at the localities of Remire-Montjoly and Cacao in French Guiana. Values (in mm) are expressed as the mean \pm one standard deviation (minimum - maximum); n = sample size. The p value of the last column represents the significance of sexual dimorphism investigated with Mann-Whitney tests.

	<i>M. barnesi</i>				p
	females	n	males	n	
FA	34.8 \pm 0.7 (32.9 – 36.3)	95	35.9 \pm 0.7 (33.9 – 37.3)	47	< 0.0001
MC3	34.0 \pm 0.8 (32.0 – 35.5)	83	34.6 \pm 0.8 (32.5 – 36.0)	44	< 0.0001
MC4	32.4 \pm 0.9 (30.0 – 34.5)	83	32.8 \pm 0.9 (31.0 – 34.5)	44	0.0204
	<i>M. molossus</i>				p
	females	n	males	n	
FA	39.0 \pm 0.8 (37.3 – 40.8)	33	39.4 \pm 1.2 (37.4 – 41.6)	21	0.4087
MC3	38.8 \pm 1.2 (36.0 – 41.5)	29	38.4 \pm 1.2 (37.0 – 40.5)	19	0.2479
MC4	36.6 \pm 0.9 (34.0 – 38.0)	29	36.6 \pm 1.2 (35.0 – 39.5)	19	0.3813

Table 3. Mean, standard deviation and ranges of 9 cranio-dental measurements (expressed in mm) taken on skulls of *M. barnesi* (n = 30) and *M. molossus* (n = 20) caught in syntopy in French Guiana. Males and females were not distinguished. Significance of differences was investigated with Mann-Whitney (p values). See Material and Methods for abbreviations of these cranio-dental variables.

	<i>M. barnesi</i>	<i>M. molossus</i>	p
GLS	16.1 ± 0.6 (15.4 – 17.5)	16.6 ± 0.7 (15.5 – 17.9)	0.0078
CIL	14.9 ± 0.5 (14.2 – 15.9)	15.7 ± 0.5 (14.5 – 16.3)	<0.0001
ZB	10.6 ± 0.3 (10.1 – 11.2)	10.5 ± 0.4 (9.8 – 11.2)	0.9420
MB	10.2 ± 0.5 (9.3 – 11.1)	10.1 ± 0.4 (9.4 – 10.9)	0.5438
MTL	5.9 ± 0.2 (5.5 – 6.3)	6.2 ± 0.2 (5.6 – 6.5)	<0.0001
BaM	7.6 ± 0.2 (7.2 – 8.1)	7.6 ± 0.3 (7.0 – 8.1)	0.3233
PB	3.8 ± 0.1 (3.5 – 4.1)	3.6 ± 0.2 (3.3 – 4.0)	0.0011
BB	8.9 ± 0.2 (8.5 – 9.2)	8.8 ± 0.3 (8.4 – 9.3)	0.0721
BaC	4.3 ± 0.2 (4.0 – 4.6)	4.4 ± 0.2 (4.0 – 4.8)	0.0537

Table 4. Selected external and cranio-dental measurements (in mm) indicating that *Molossus barnesi* is morphometrically similar to *M. coibensis*. Values of *M. barnesi* are for 30 French Guianan individuals from Cacao and Remire-Montjoly; values of *M. coibensis* are for 43 Panamanian individuals from Chiriquí (La Concepción), corresponding to population sample-19 in Dolan (1989). The values for the holotypes of *M. barnesi* (BMNH-5.1.8.7) and *M. coibensis* (AMNH-18731) are taken from Table 64 in Simmons and Voss (1998). Values are mean \pm standard-deviation (minimum and maximum); n = sample size. Abbreviations GLS, MTL, BB, BaM: see text in Material and Methods; NA = Not Available.

	Forearm length	Tail length
Males <i>M. coibensis</i>	36.0 \pm 0.6 (34.8 – 36.8) n=20	4.6 \pm 1.7 (31.0 – 37.0) n=18
Males <i>M. barnesi</i>	36.0 \pm 1.0 (33.9 – 37.3) n=11	3.7 \pm 1.2 (32.0 – 35.5) n=9
Holotype <i>M. coibensis</i>	35.5	NA
Females <i>M. coibensis</i>	34.7 \pm 0.5 (33.6 – 35.6) n=23	1.8 \pm 1.8 (28.0 – 34.0) n=16
Females <i>M. barnesi</i>	35.0 \pm 0.6 (33.8 – 36.1) n=19	2.0 \pm 1.4 (30.0 – 35.0) n=17
Holotype <i>M. barnesi</i>	33.8	31.0
	GLS	MTL
Males <i>M. coibensis</i>	17.7 \pm 0.3 (17.2 – 18.0) n=19	6.2 \pm 0.1 (5.9 – 6.4) n=19
Males <i>M. barnesi</i>	16.7 \pm 0.5 (16.0 – 17.5) n=11	6.0 \pm 0.1 (5.8 – 6.3) n=11
Holotype <i>M. coibensis</i>	15.9	6.0
Females <i>M. coibensis</i>	16.7 \pm 0.2 ((16.4 – 17.1) n=16	5.9 \pm 0.1 (5.7 – 6.1) n=16
Females <i>M. barnesi</i>	15.7 \pm 0.2 (15.4 – 16.3) n=19	5.8 \pm 0.1 (5.5 – 6.0) n=19
Holotype <i>M. barnesi</i>	16.6	5.9
	BB	BaM
Males <i>M. coibensis</i>	9.1 \pm 0.2 (8.8 – 9.5) n=19	8.0 \pm 0.2 (7.7 - 8.2) n=18
Males <i>M. barnesi</i>	9.0 \pm 0.1 (8.8 – 9.2) n=11	7.7 \pm 0.2 (7.4 – 8.1) n=11
Holotype <i>M. coibensis</i>	8.4	8.0
Females <i>M. coibensis</i>	8.9 \pm 0.1 (8.7 – 9.1) n=16	7.7 \pm 0.2 (7.3 – 7.9) n=16
Females <i>M. barnesi</i>	8.8 \pm 0.2 (8.5 – 9.2) n=19	7.5 \pm 0.2 (7.2 – 7.8) n=19
Holotype <i>M. barnesi</i>	8.8	7.3

Appendices

Appendix A. Examined material. Animals sequenced for the CO1 barcoding gene are indicated with hashtag (#). *Molossus barnesi* - French Guiana: Roura: Cacao: MHNG-1983.014, 1983.015, 1983.020#, 1983.022; 1984.007; 1984.011 to 1984.013; Régina: Kaw-Roura: MHNG-1894.004#; Remire-Montjoly: MHNG-1979.023 to 1979.032, 1979.034, 1979.035; 1984.061 to 1984.068, 1984.072, 1984.073. *Molossus molossus* - French Guiana: Roura: Cacao: MHNG-1972.019 to 1972.024; 1983.016, 1983.017, 1983.019#, 1983.023 to 1983.025; 1984.008# to 1984.010, 1984.014 to 1984.016; Remire-Montjoly: MHNG-1979.033. *Molossus rufus* - French Guiana: Régina: Nouragues MHNG-1880.046#.

Appendix B. List of animals with CO1 barcoding fragment: 15 individuals retrieved from GenBank and 6 individuals (indicated with §) sequenced for this study. Abbreviations for specimen numbers: MHNG = Muséum d’histoire naturelle de Genève (Switzerland); ROM = Royal Ontario Museum (Toronto, Canada). The column “Haplotype” provides the haplotype number for animals of the MJ network of right panel in Figure 3.

Taxon	Specimen	GenBank	Locality	Haplotype
<i>Eumops auripendulus</i>	ROM-103160	EF080347	Guyana: Upper Takutu-Upper Essequibo	-
<i>Eumops auripendulus</i>	MHNG-1939.069	KU737546 §	French Guiana: Régina: Kaw	-
<i>Eumops hansae</i>	ROM-109153	EF080356	Guyana: Potaro-Siparuni	-
<i>Eumops hansae</i>	ROM-109310	EF080357	Guyana: Potaro-Siparuni	-
<i>Molossus barnesi</i>	MHNG-1894.004	KU737547 §	French Guiana: Régina: Kaw	6
<i>Molossus barnesi</i>	MHNG-1983.020	KU737549 §	French Guiana: Roura: Cacao	7
<i>Molossus coibensis</i>	ROM-105638	JF448088	Ecuador: Napo, Parque Nacional Yasuni	5
<i>Molossus coibensis</i>	ROM-105303	JF448947	Ecuador: Napo, Parque Nacional Yasuni	5
<i>Molossus coibensis</i>	Not preserved	KT721383	Panama: Gamboa	1
<i>Molossus coibensis</i>	Not preserved	KT721396	Panama: Gamboa	2
<i>Molossus molossus</i>	ROM-109045	EF080477	Guyana: Potaro-Siparuni	8
<i>Molossus molossus</i>	ROM-104435	ABECA137-06	Ecuador: Napo, Parque Nacional Yasuni	10
<i>Molossus molossus</i>	ROM-105514	ABECA491-06	Ecuador: Napo, Parque Nacional Yasuni	9
<i>Molossus molossus</i>	ROM-113900	BCBNT729-06	Suriname: Brokopondo, Brownsberg Nature Park	10
<i>Molossus molossus</i>	MHNG-1983.019	KU737548 §	French Guiana: Roura: Cacao	4
<i>Molossus molossus</i>	MHNG-1984.008	KU737550 §	French Guiana: Roura: Cacao	11
<i>Molossus molossus</i>	Not preserved	KT721407	Panama: Gamboa	3
<i>Molossus molossus</i>	Not preserved	KT721409	Panama: Gamboa	4
<i>Molossus rufus</i>	ROM-108420	EF080481	Guyana: Potaro-Siparuni	13
<i>Molossus rufus</i>	MHNG-1880.046	KU737551 §	French Guiana: Régina:Nouragues	12
<i>Molossus sp.</i>	ROM-109176	EF080483	Guyana: Potaro-Siparuni	14

Figure captions

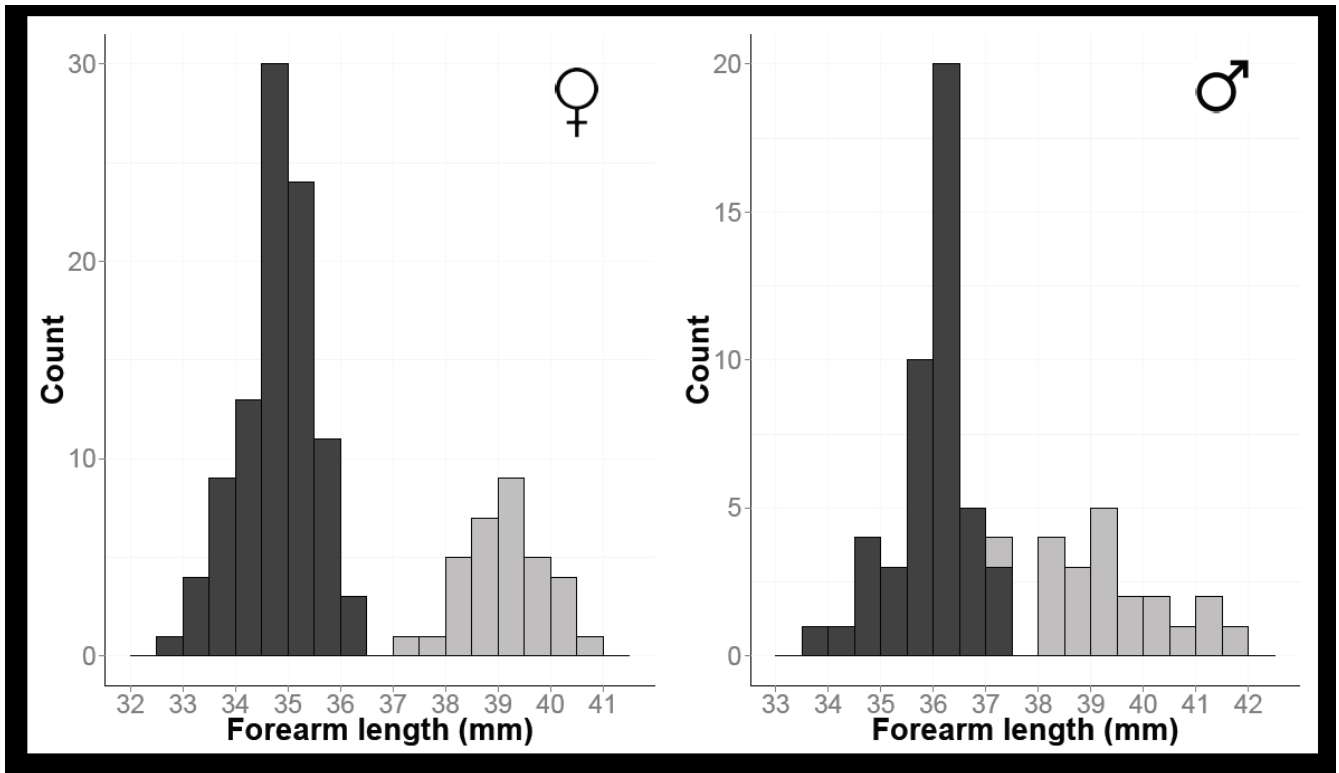


Figure 1. Distribution of forearm lengths for 128 females (left) and 68 males (right) of *Molossus* spp. caught in syntopy at two localities in French Guiana. The smaller animals correspond to the *M. barnesi* morphotype (represented in dark gray, FA smaller than 37.1 or 37.4 mm for females and males, respectively); the larger animals correspond to the *M. molossus* morphotype (represented in light gray, FA larger than 36.5 or 37.3 mm for females and males, respectively).

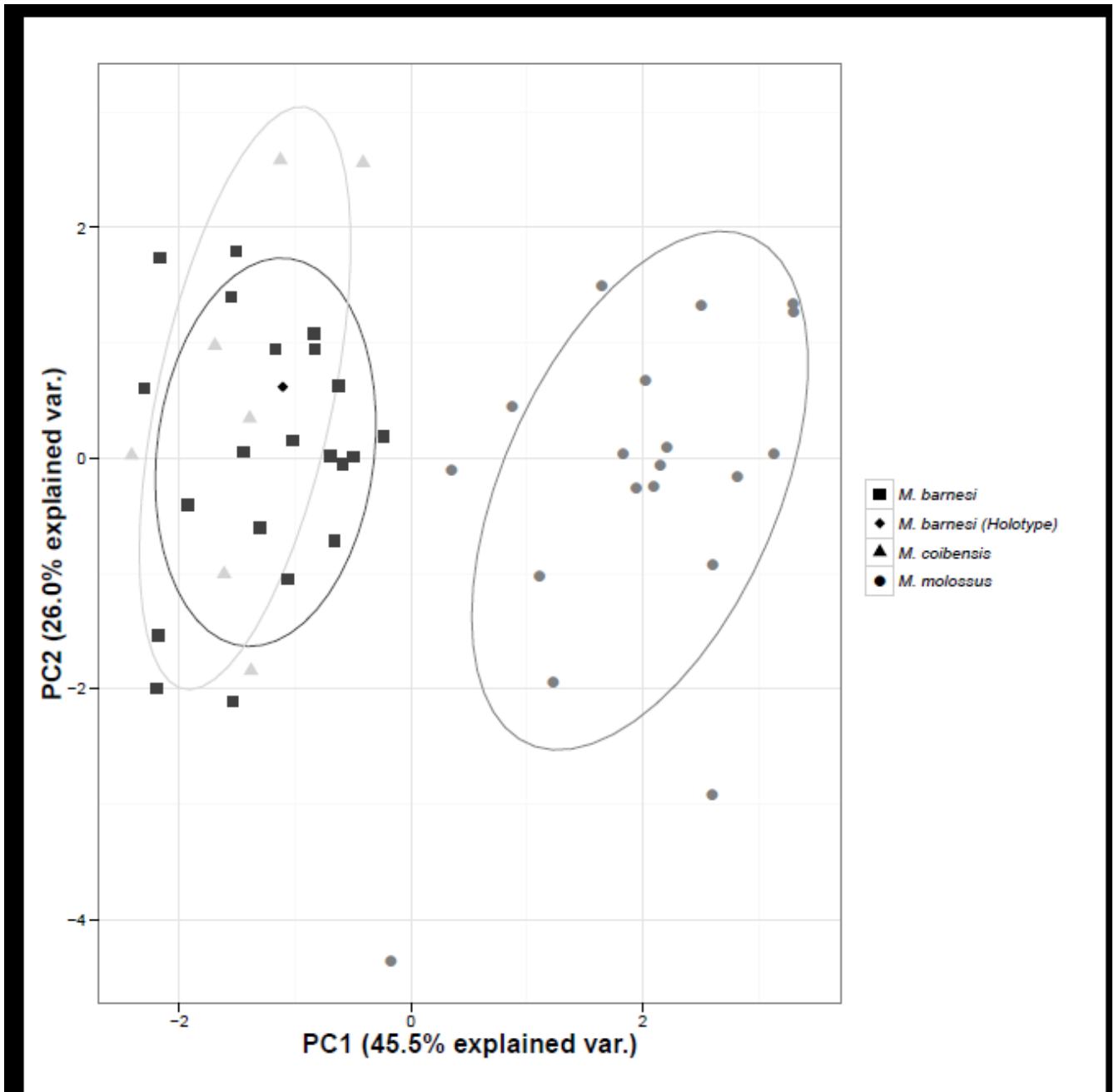


Figure 2. Principal Component Analysis Biplot with confidence ellipses based on six cranio-dental (GLS, CIL, MTL, BaM, BB, and PB) and one external (FA) measurements measured in 48 female specimens of *Molossus* spp. The legend for species is as follows: *M. barnesi* (black squares), *M. coibensis* (light gray point-up triangles) and *M. molossus* (gray circles). Notice the position of the holotype of *M. barnesi* (black diamond), which is placed in the middle of the groups including all *M. barnesi* from French Guiana and those of *M. coibensis* from Brazil. Specimens of *M. molossus* from French Guiana form a distinct cluster. The dataset comprises the holotype of *M. barnesi* (BMNH-5.1.8.7), seven *M. coibensis* from Brazilian Para (Correa da Costa et al., 2013), 21 *M. barnesi* and 19 *M. molossus* from French Guiana (including 11 specimens studied by Simmons and Voss, 1998). Abbreviations: PC = Principal Component; var. = variance.

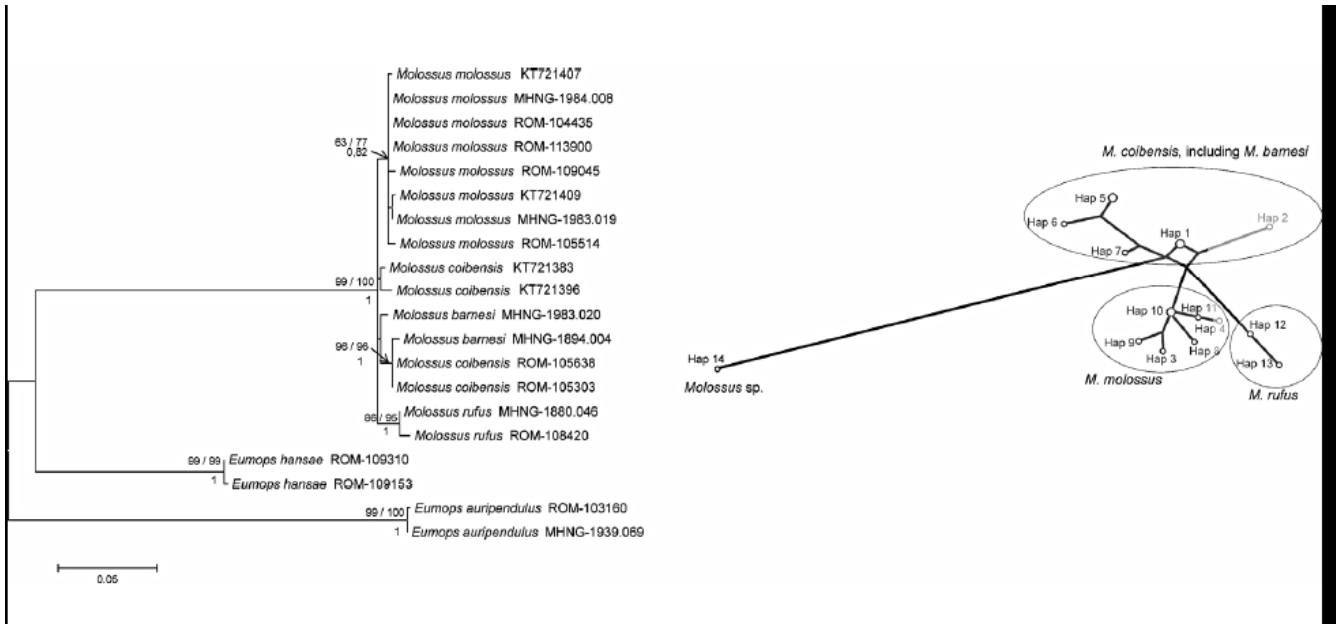


Figure 3.

Left: Maximum likelihood tree showing the phylogenetic relationships among 16 CO1 barcodes of the following *Molossus* spp.: *M. barnesi* (French Guiana), *M. coibensis* (Ecuador, Panama), *M. rufus* (French Guiana; Guyana), and *M. molossus* (Ecuador, French Guiana, Guyana, Panama, Suriname). Each individual is identified with its voucher number (see details in the Appendix B). Bootstrap support from NJ / ML and posterior probabilities (PP) from a Bayesian analysis are shown above and below major nodes, respectively. The tree was rooted with sequences of *Eumops hansae* and *E. auripendulus*.

Right: Median-Joining network for 14 different CO1 haplotypes (Hap) of *Molossus* spp. (see Appendix B for linking haplotype number and specimen details).

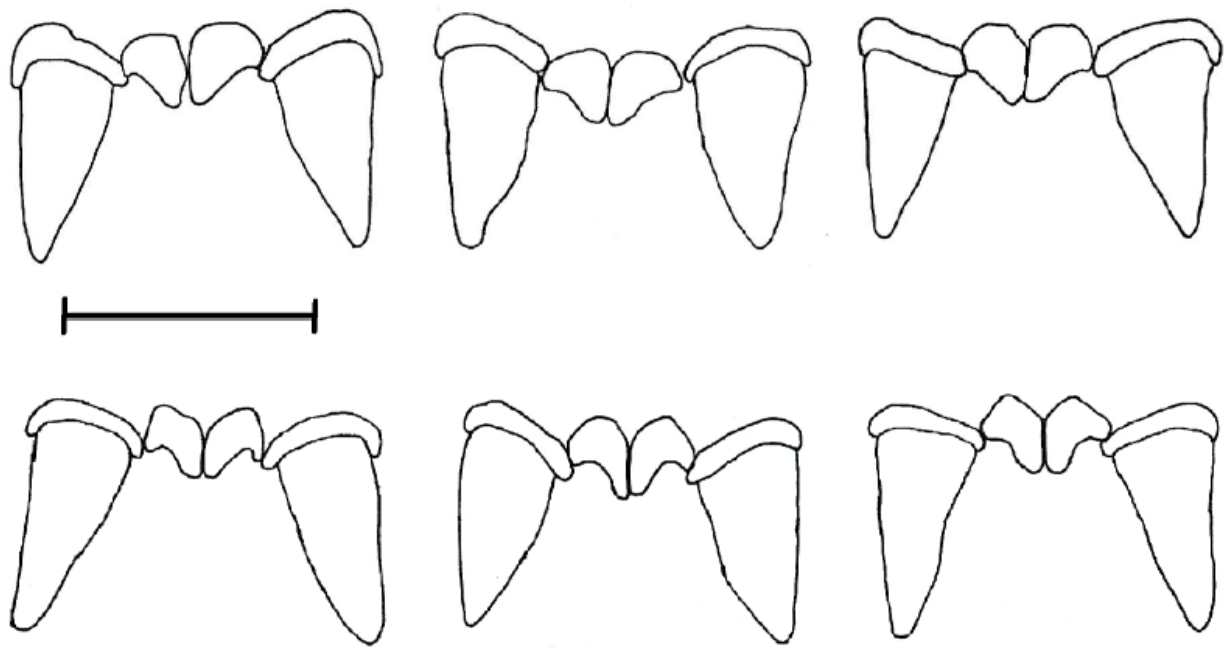


Figure 4. Frontal view of the upper dentition of three *Molossus barnesi* (top row) and three *M. molossus* (bottom). The shape of upper incisors are more or less species-specific, i.e. more spatulate in *M. barnesi* (from left to right: MHNG-1979.023; 1979.026 and 1979.029) versus pincerlike in *M. molossus* (MHNG-1972.020, 1972.022 and 1972.023). The scale bar is 3.0 mm.