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

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

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Abstract

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

Introduction

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

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

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

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

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

Material and methods

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

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

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

Morphology

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

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

Molecular analyses

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

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

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

Results

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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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 ± 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.

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

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

	M. barnesi	*M. molossus*	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 Concepcíon), 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 ± 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 *M. coibensis*	36.0 ± 0.6 (34.8 – 36.8) n=20	4.6 ± 1.7 (31.0 – 37.0) n=18
Males *M. barnesi*	36.0 ± 1.0 (33.9 – 37.3) n=11	3.7 ± 1.2 (32.0 – 35.5) n=9
Holotype *M. coibensis*	35.5	NA
Females *M. coibensis*	34.7 ± 0.5 (33.6 – 35.6) n=23	1.8 ± 1.8 (28.0 – 34.0) n=16
Females *M. barnesi*	35.0 ± 0.6 (33.8 – 36.1) n=19	2.0 ± 1.4 (30.0 – 35.0) n=17
Holotype *M. barnesi*	33.8	31.0
	GLS	MTL
Males *M. coibensis*	17.7 ± 0.3 (17.2 – 18.0) n=19	6.2 ± 0.1 (5.9 – 6.4) n=19
Males *M. barnesi*	16.7 ± 0.5 (16.0 – 17.5) n=11	6.0 ± 0.1 (5.8 – 6.3) n=11
Holotype *M. coibensis*	15.9	6.0
Females *M. coibensis*	16.7 ± 0.2 ((16.4 – 17.1) n=16	5.9 ± 0.1 (5.7 – 6.1) n=16
Females *M. barnesi*	15.7 ± 0.2 (15.4 – 16.3) n=19	5.8 ± 0.1 (5.5 – 6.0) n=19
Holotype *M. barnesi*	16.6	5.9
	BB	BaM
Males *M. coibensis*	9.1 ± 0.2 (8.8 – 9.5) n=19	8.0 ± 0.2 (7.7 - 8.2) n=18
Males *M. barnesi*	9.0 ± 0.1 (8.8 – 9.2) n=11	7.7 ± 0.2 (7.4 – 8.1) n=11
Holotype *M. coibensis*	8.4	8.0
Females *M. coibensis*	8.9 ± 0.1 (8.7 – 9.1) n=16	7.7 ± 0.2 (7.3 – 7.9) n=16
Females *M. barnesi*	8.8 ± 0.2 (8.5 – 9.2) n=19	7.5 ± 0.2 (7.2 – 7.8) n=19
Holotype *M. barnesi*	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	Hapl-otype
Eumops auripendulus	ROM-103160	EF080347	Guyana: Upper Takutu-Upper Essequibo	-
Eumops auripendulus	MHNG-1939.069	KU737546 §	French Guiana: Régina: Kaw	-
Eumops hansae	ROM-109153	EF080356	Guyana: Potaro-Siparuni	-
Eumops hansae	ROM-109310	EF080357	Guyana: Potaro-Siparuni	-
Molossus barnesi	MHNG-1894.004	KU737547 §	French Guiana: Régina: Kaw	6
Molossus barnesi	MHNG-1983.020	KU737549 §	French Guiana: Roura: Cacao	7
Molossus coibensis	ROM-105638	JF448088	Ecuador: Napo, Parque Nacional Yasuni	5
Molossus coibensis	ROM-105303	JF448947	Ecuador: Napo, Parque Nacional Yasuni	5
Molossus coibensis	Not preserved	KT721383	Panama: Gamboa	1
Molossus coibensis	Not preserved	KT721396	Panama: Gamboa	2
Molossus molossus	ROM-109045	EF080477	Guyana: Potaro-Siparuni	8
Molossus molossus	ROM-104435	ABECA137-06	Ecuador: Napo, Parque Nacional Yasuni	10
Molossus molossus	ROM-105514	ABECA491-06	Ecuador: Napo, Parque Nacional Yasuni	9
Molossus molossus	ROM-113900	BCBNT729-06	Suriname: Brokopondo, Brownsberg Nature Park	10
Molossus molossus	MHNG-1983.019	KU737548 §	French Guiana: Roura: Cacao	4
Molossus molossus	MHNG-1984.008	KU737550 §	French Guiana: Roura: Cacao	11
Molossus molossus	Not preserved	KT721407	Panama: Gamboa	3
Molossus molossus	Not preserved	KT721409	Panama: Gamboa	4
Molossus rufus	ROM-108420	EF080481	Guyana: Potaro-Siparuni	13
Molossus rufus	MHNG-1880.046	KU737551 §	French Guiana: Régina:Nouragues	12
Molossus sp.	ROM-109176	EF080483	Guyana: Potaro-Siparuni	14

Figure captions

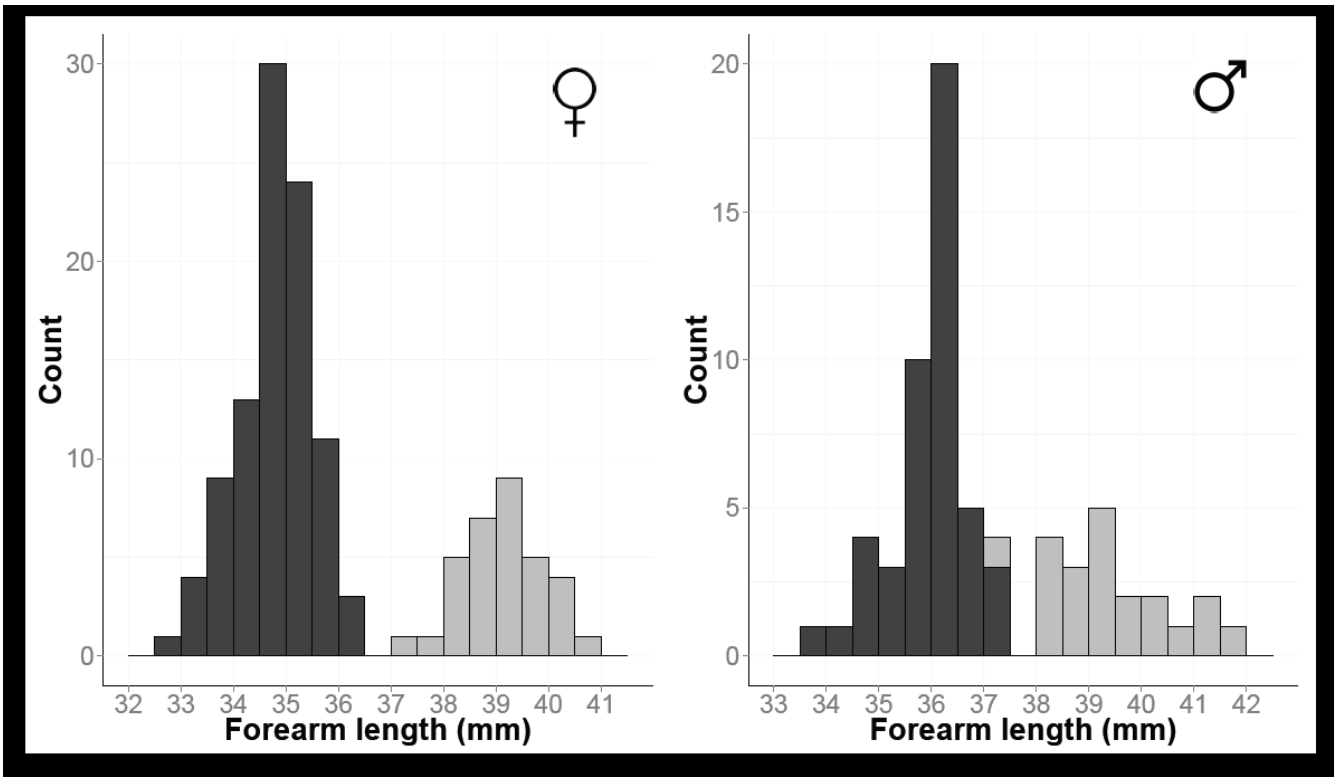

Figure 1. Distribution of forearm lengths for 128 females (left) and 68 males (right) of *Molossus* spp. caught in syntopy at two localities in French Guiana. The smaller animals correspond to the *M. barnesi* morphotype (represented in dark gray, FA smaller than 37.1 or 37.4 mm for females and males,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

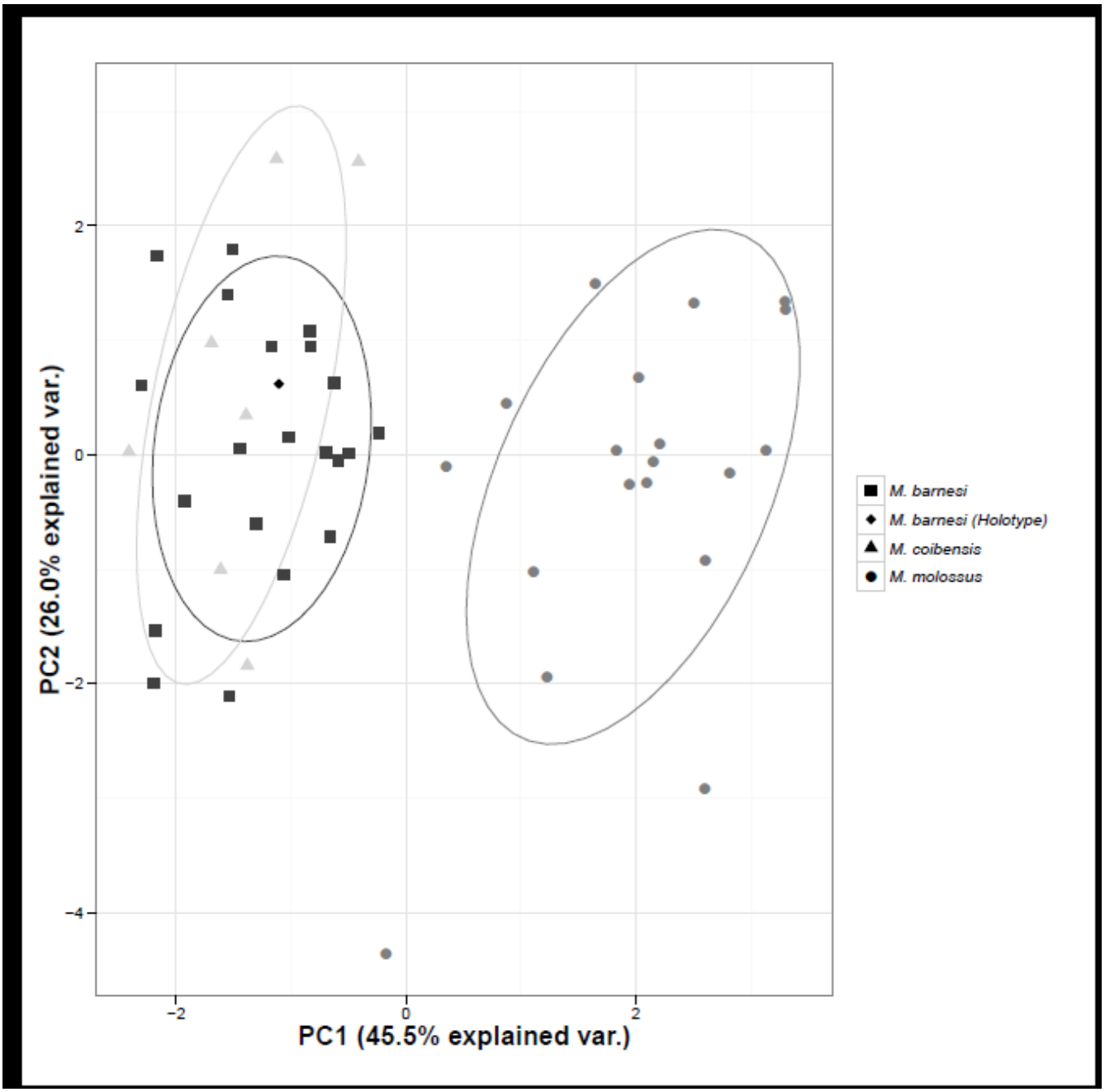

Figure 2. Principal Component Analysis Biplot with confidence ellipses based on six cranio-dental (GLS, CIL, MTL, BaM, BB, and PB) and one external (FA) measurements measured in 48 female specimens of *Molossus* spp. The legend for species is as follows: *M. barnesi* (black squares), *M. coibensis* (light 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.

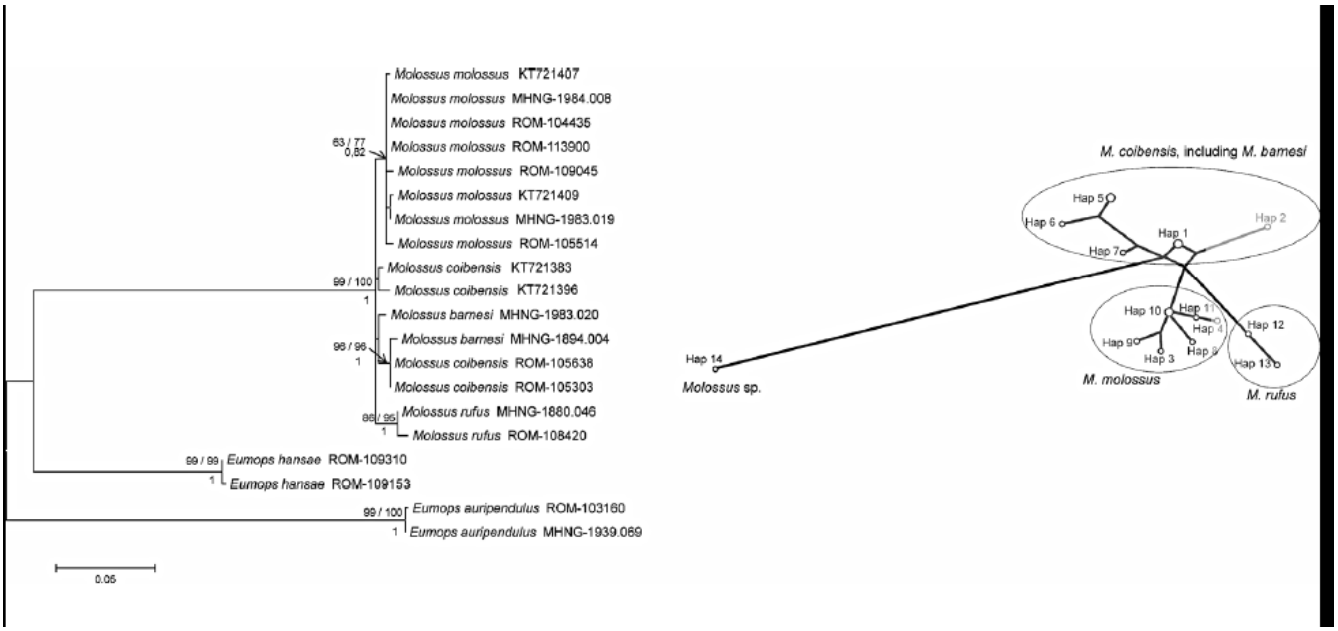

Figure 3.

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).

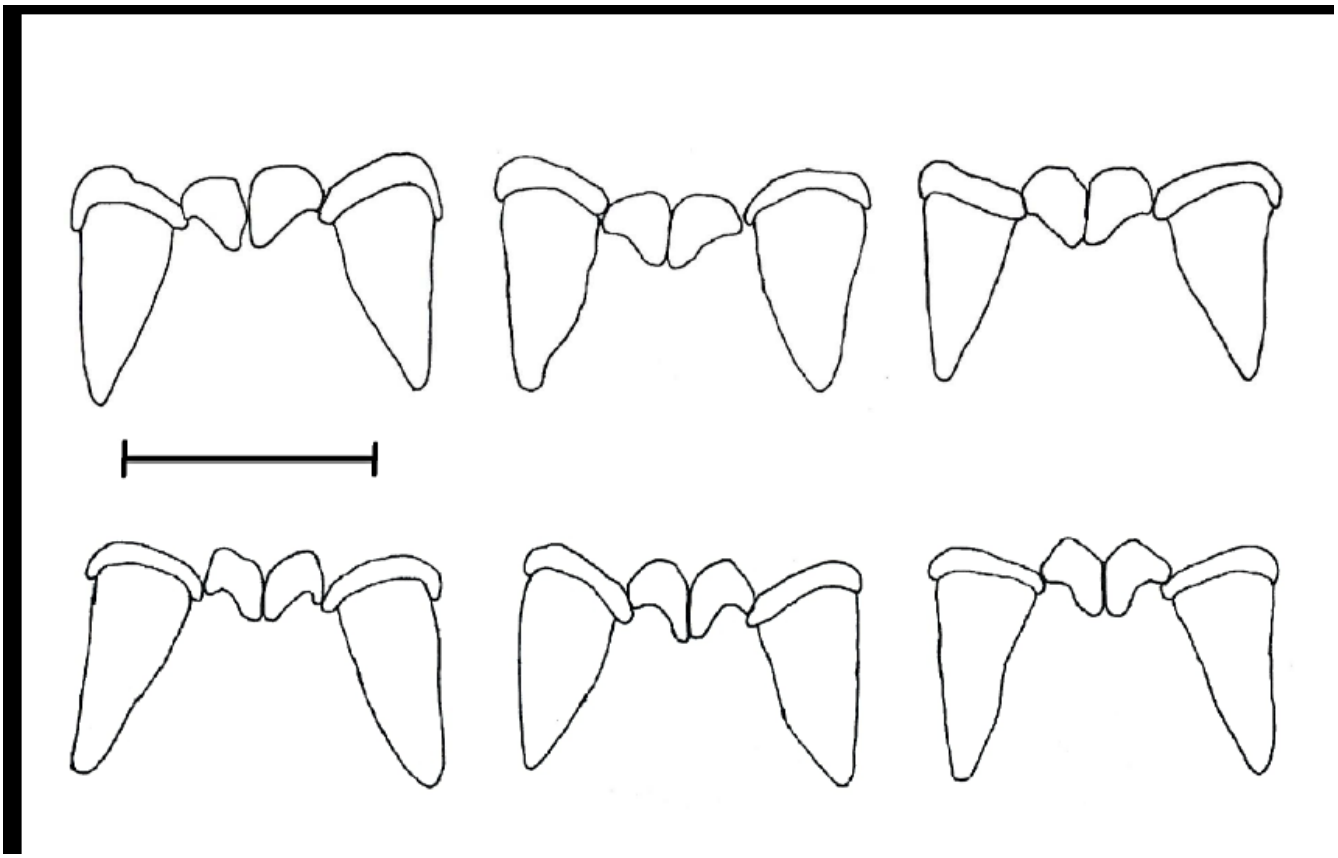

Figure 4. Frontal view of the upper dentition of three *Molossus barnesi* (top row) and three *M. molossus* (bottom). The shape of upper incisors are more or less species–specific, i.e. more spatulate in *M. barnesi* (from left to right: MHNG-1979.023; 1979.026 and 1979.029) versus pincerlike in *M.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