

SUPPLEMENTARY MATERIAL

METHODS

Morphometric analysis

Permission for field surveys in Sichuan Province was provided by the Forestry Department and National Reserves of China.

For morphometric analysis, we examined 224 specimens of *Murina* deposited in the collections of College of Life Sciences, Guangzhou University, Guangzhou (GZHU), Hungarian Natural History Museum, Budapest, Hungary (HNHM), Harrison Institution, Sevenoaks, United Kingdom (HZM), Institute of Ecology and Biological Resources, Hanoi, Vietnam (IEBR), Natural History Museum of Geneva, Switzerland (MHNG), Museo Civico di Storia Naturale "Giacomo Doria", Genova, Italy (MSNG), Kim Hy Nature Reserve Collection, Vietnam (NF), Prince of Songkla University Zoological Collection, Hat Yai, Thailand (PSUZC), Senckenberg Forschungsinstitut und NaturMuseum, Frankfurt, Germany (SMF), National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM), Natural History Museum, London, UK (BMNH), Field Museum of Natural History, Chicago, USA (FMNH) (see the list of specimens in Appendix 1). External measurements to the nearest 0.1 mm were recorded with a MITUTOYO digital caliper and body mass was recorded to the nearest 0.1 g using a TANITA spring scale (Model: 1476, Japan). Cranial and dental measurements were recorded to the nearest 0.01 mm using MITUTOYO digital caliper.

External measurements include: HB: total length – from the tip of the face/chin to the anus; T: tail vertebrae length – from the tip of the tail to the beginning of the tail vertebrae; E: ear length – from the notch at the base of the ear conch to the tip of the pinna; HF: hind foot length – from the heel to the tip of the longest toe, including the claw; TIB: length of tibia – from the knee to the ankle; FA: forearm length – from the elbow to the wrist with both joints folded. Cranial and dental measurements included: GTL: greatest length of skull – from the posterior edge of the skull to the front of the incisors; CCL: condylocanine length – from the exoccipital condyle to the most anterior part of the canine; CBL: condylobasal length – from the exoccipital condyle to the posterior rim of alveolus of the first upper incisor; BBW: braincase width – greatest width across the braincase; BCH: braincase height – from the basisphenoid at the level of the hamular processes to the highest part of the skull, including the sagittal crest (if present); ZYW: zygomatic width – the greatest width of the skull across the zygomatic arches; MAW: mastoid width – the greatest distance across the mastoid region; PL: palatal length – from the anterior palatal emargination to the midpoint of the posterior palatal emargination; IOW: interorbital width – least width of the interorbital constriction; CM³L: length of maxillary toothrow – from the front of the canine to the posterior edge of the 3rd upper molar; CCW: greatest breadth across the upper canines; M³M³W: width across upper molars – greatest width measured across the outer edges of the second upper molars; RCM: ratio of CCW to M³M³W; CM₃L: length of mandibular toothrow – from the front of the canine to the posterior edge of the 3rd lower molar; LM: greatest length of mandible – greatest length measured from the posterior edge of the mandibular condyles to the front of the lower incisors; CPH: coronoid process height – measured from the inferior surface of the angular process of the ramus to the tip of the coronoid process. Absolute height was used in all height comparisons for individual teeth (e.g., C versus P⁴).

We performed two multivariate statistical analyses for species discrimination based on external and craniodental measurements: principal component analysis (PCA) and discriminant analysis of principal components (DAPC) (Jombart, 2008; Jombart et al., 2010). Both the multivariate methods are dimension–reduction algorithm constitutionally, although DAPC is expected to be better than PCA in discriminating groups because it finds functions maximizing inter–group variation (Jombart, 2008; Jombart et al., 2010). In both PCA and DAPC, the sexes were analysed separately because sexual dimorphisms were noted in several *Murina* species (Kuo et al., 2009; Son et al., 2016) and the new species (Fig. 6; Table 2). Moreover, we also replicated our multivariate statistical analyses in the monophyletic clade formed by *M. leucogaster*, *M. shuipuensis*, *M. rongjiangensis* and the new species. With similarities in size and skull proportions, *M. shuipuensis*, *M. rongjiangensis* and the new species were also compared using Analysis of Variance (ANOVA). All analyses were performed

using packages “phych” and “adegenet” in R environment (Jombart, 2008; R Development Core Team, Vienna, www.R-project.org).

Phylogenetic analyses

In the phylogenetic analysis, total genomic DNA was isolated from approximately 20 mg muscle tissue using a Mini BEST Universal Genomic DNA Extraction Kit (TaKaRa, Dalian, China). We selected the partial cytochrome oxidase subunit I (*Co1*, 670 bp), one of widely-used mitochondrial barcoding marker in *Murina* (Francis et al., 2010), and the partial nuclear recombination activating protein 2 gene (*Rag2*, 1339 bp), one common nuclear locus in vespertilionid phylogenetic analysis, as our molecular markers (Heaney et al., 2013; Kuo et al., 2017; Lack & Bussche, 2010; Roehrs et al., 2010). *Co1* gene was amplified from all specimens of all species for this study, while *Rag2* genes were sequenced from single individuals of each species confirmed by our phylogenetic and morphological species determination (GenBank accession numbers: MN549027–MN549101). Both loci were amplified by polymerase chain reactions (PCRs). The chromatograms were checked and edited using GENEIOUS 4.8.3 (Drummond et al., 2011). All available *Co1* and *Rag2* sequences of *Murina* collected from NCBI-nt and our specimen were aligned using MUSCLE (Edgar, 2004). Final alignment was partitioned by different codon position and the parameters of best nucleotide substitution models were determined by PartitionFinder2 (Lanfear et al., 2017) using greedy algorithm (Lanfear et al., 2012). Maximum likelihood trees were searched in RAxML7.4.2 (Stamatakis et al., 2008), and the reliability of nodes were evaluated by 500 rapid bootstrap matrixes.

Colour calibration and measurement

To describe the pelage colour digital photographs of freshly euthanized individuals were taken in the field in a standardized way using a Nikon D300s camera with built-in flashlight (to reduce the influence of environmental light) and a Micro-Nikkor 105mm f/2.8G lens in RAW mode. This system was attached to a copy stand, maintaining a standardized distance (~30 cm) from all specimens. Following the method of Davis and Castleberry (2010) a ColorChecker Passport (X-rite, Inc.) chart was used to assist the colour calibration. All images were colour calibrated through an automating Colour Control in RAW Workflows in ADOBE Lightroom. Colour of the pelage were extracted and described in ADOBE Photoshop following the Pantone colour code, adopting the RGB system.

SUPPLEMENTARY REFERENCES

Davis AK, Castleberry SB. 2010. Pelage color of red bats *Lasiurus borealis* varies with body size: An image analysis of museum specimens. *Current Zoology*, **56**(4): 401–405.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32**(5): 1792–1797.

Francis CM, Borisenko AV, Ivanova NV, Eger JL, Lim BK, Guillénservert A, Kruskop SV, Mackie I, Hebert PD. 2010. The role of DNA barcodes in understanding and conservation of mammal diversity in southeast Asia. *Plos One*, **5**: e12575–e12575.

Jombart T. 2008. Adegnet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, **24**: 1403–1405.

Jombart T, Devillard S, Balloux F. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, **11**: 94.

Kuo HC, Fang YP, Csorba G, Lee LL. 2009. Three New Species of *Murina* (Chiroptera: Vespertilionidae) from Taiwan. *Journal of Mammalogy*, **90**(4): 980–991.

Kuo HC, Soisook P, Ho YY, Csorba G, Wang CN, Rossiter SJ. 2017. A Taxonomic Revision of the *Kerivoula hardwickii* Complex (Chiroptera: Vespertilionidae) with the Description of a New Species. *Acta Chiropterologica*, **19**(1): 19–39.

Lack JB, Bussche RA. 2010. Identifying the confounding factors in resolving phylogenetic relationships in Vespertilionidae. *Journal of Mammalogy*, **91**(6): 1435–1448.

Lanfear R, Calcott B, Ho SY, Guindon S. 2012. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, **29**(6): 1695–1701.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses. *Molecular Biology and Evolution*, **34**(3): 772–773.

Roehrs ZP, Lack JB, Van Den Bussche RA. 2010. Tribal phylogenetic relationships within Vespertilioninae (Chiroptera: Vespertilionidae) based on mitochondrial and nuclear sequence data. *Journal of Mammalogy*, **91**(5): 1073–1092.

Stamatakis A, Hoover P, Rougemont J. 2008. A Rapid Bootstrap Algorithm for the RAxML Web Servers. *Systematic Biology*, **57**(5): 758–771.

SUPPLEMENTARY APPENDIX I

List of specimens used in morphological and/or molecular analyses. GenBank accession numbers for *Col* and *Rag2* sequences are listed within brackets when available. Star mark indicates the specimens only used in molecular analysis.

***Harpiocephalus harpia*: Dongyang, Zhejiang:** GZHU 17323* (–, MN549097)

***Harpiola isodon*: Ailaoshan, Yunnan:** GZHU 14197* (–, MN549099)

***Murina aurata*: Wolong, Sichuan, China:** GZHU 87052 (–, –)

***Murina chrysochaetes*: Nanling, Guangdong, China:** GZHU 12448 (–, –), GZHU 13508 (–, –), GZHU 13527 (–, –), GZHU 13531 (–, MN549096), GZHU 13523 (–, –)

***Murina cyclotis*: Darong Mountain, Guangxi, China:** GZHU 13030 (–, –), GZHU 13029 (–, –); **Heishiding, Guangdong, China:** GZHU 14128 (MN549027, –), GZHU 14153 (MN549028, –), GZHU 14163 (MN549029, –), GZHU 14169 (MN549030, –), GZHU 14127 (MN549031, –), GZHU 14152 (MN549032, –), GZHU 14154 (MN549033, –), GZHU 14161 (MN549034, –), GZHU 14165 (MN549035, –), Jingxi, Guangxi: GZHU 16335* (–, MN549100)

***Murina eleryi*: Nanling, Guangdong, China:** GZHU 12450 (–, –), GZHU 13128 (–, –), GZHU 12446 (–, –), GZHU 13129 (–, –); **Jinggang Mountain, Jiangxi, China:** GZHU 13334 (–, –), GZHU 13413 (–, –), GZHU 13294 (–, –), GZHU 13319 (–, –), GZHU 13400 (–, –), GZHU 13411 (–, –), GZHU 13412 (–, –), GZHU 13430 (–, –), GZHU 13431 (–, –), GZHU 13471 (–, –); **Wolong, Sichuan, China:** GZHU 14465 (–, MN549088); **Libo, Guizhou, China:** GZHU 16417 (–, MN549089)

***Murina fanjingshanensis*: Libo, Guizhou, China:** GZHU 15618 (–, MN549093)

***Murina beelzebub*: Huong Hoa, Quang Tri, Vietnam:** HNHM 2007.50.24. (–, –), HNHM 2007.50.6. (–, –), HNHM 2007.50.7. (–, –), **Kon Ka Kinh, Gia Lai, Vietnam:** HZM 3.32053 (–, –), **Ba To, Quang Nai, Vietnam:** IEBR 3636 (–, –), IEBR 3904 (–, –), **Ngoc Linh, Kon Tum, Vietnam:** IEBR 4149 (–, –), IEBR VN11–1208 (–, –), IEBR VN11–1244 (–, –)

***Murina feae*: Phong Saly, Laos:** AMNH 32199 (–, –), **Ba Be, Vietnam:** HNHM 2000.84.4. (–, –), HNHM 2000.84.7. (–, –), **Mondol Kiri, Cambodia:** HNHM 2005.81.35. (–, –), HNHM 2005.81.36. (–, –), HNHM 2005.81.49. (–, –), HNHM 2005.81.50. (–, –), HNHM 2005.81.52. (–, –), HNHM 2005.81.53. (–, –), HNHM 2006.34.40. (–, –), **Na Hang, Tuyen Quang, Vietnam:** HNHM 22823 (–, –), **Pu Mat, Vietnam:** HZM 1.31524 (–, –), HZM 1.31780 (–, –), IEBR 3068 (–, –), **Pu Huong, Nghe An, Vietnam:** IEBR 1360 (–, –), IEBR 1363 (–, –), IEBR 1364 (–, –), **Kim Hy, Bac Kan, Vietnam:** IEBR 3154 (–, –), **Duc Xuan, Ha Giang, Vietnam:** IEBR 3264 (–, –), **Pu Hu, Thanh Hoa, Vietnam:** IEBR 3718 (–, –), IEBR 3728 (–, –), IEBR QHB74 (–, –), IEBR QHB005 (–, –), **Phong Nha–Ke Bang, Vietnam:** IEBR 3869 (–, –), IEBR 3871 (–, –), **Bac Huong Hoa, Vietnam:** IEBR 4116 (–, –), **Pu Luong, Thanh Hoa, Vietnam:** IEBR 4121 (–, –), IEBR 4123 (–, –), IEBR 4125 (–, –), IEBR 4127 (–, –), **Na Hang, Tuyen Quang, Vietnam:** IEBR 495 / HNHM 22826 (–, –), **Cat Tien, Vietnam:** IEBR NTS267 (–, –), **Vu Quang, Ha Tinh, Vietnam:** IEBR VN11–0007 (–, –), **Ba Thuoc, Thanh Hoa, Vietnam:** IEBR VN11–0495 (–, –), **Vu Quang, Ha Tinh, Vietnam:** IEBR VN11–1331 (–, –), **Phong Saly, Laos:** MHNG 1926.035 (–, –), **Karen, Myanmar:** MSNG 44307 (–, –), **Kim Hy, Bac Kan, Vietnam:** NF 071206.2 (–, –), **Than Sa, Vietnam:** HNHM 23558 (–, –), **Doi Ang Khang, Vietnam:** SMF 75355 (–, –), **Than Sa, Vietnam:** IEBR Thong Coll.12 (–, –), **Chu Mom Rai, Kon Tum, Vietnam:** IEBR Thong Coll.32 (–, –), **Phuong Vien, Bac Kan, Vietnam:** IEBR T.210708.1 (–, –), IEBR T.250607.1 (–, –), **Son La, Vietnam:** IEBR T.251107.5 (–, –), **Xuan Son NP, Vietnam:**

IEBR T.290708.8 (–, –), **Bach Ma NP, Thua Thien–Hue, Vietnam:** IEBR Thong Coll.T112 (–, –), **Kim Hy, Bac Kan, Vietnam:** IEBR Thong Coll. VN013–H8 (–, –), **Huong Son, Ha Tinh, Vietnam:** IEBR VN01–C4 (–, –), **Chantaburi, Vietnam:** USNM 528305 (–, –)

***Murina harrisoni*: Jinggang Mountain, Jiangxi, China:** GZHU 13479 (–, –); **Shaoguan, Guangdong, China:** GZHU 14287 (MN549036, –), GZHU 14290 (MN549037, –), GZHU 14292(MN549038, –), GZHU 14305 (MN549039, –), GZHU 14307 (MN549040, –), GZHU 14308 (MN549041, –), GZHU 14309 (MN549042, –), GZHU 14310 (MN549043, –), GZHU 14291 (MN549046, –), GZHU 14311 (MN549047, –); **Qixiling, Jiangxi, China:** GZHU 14377 (MN549044, –), GZHU 14344 (MN549048, –); **Julianshan, Jiangxi, China:** GZHU 15111 (–, –), GZHU 15124 (–, –), GZHU 15125 (–, –), GZHU 15126 (–, –), GZHU 15157 (–, –), GZHU 15161 (–, –), GZHU 15198 (–, –), GZHU 15284 (–, –), GZHU 15291 (–, –), GZHU 15090 (–, –), GZHU 15093 (–, –), GZHU 15109 (–, –), GZHU 15127 (–, –), GZHU 15128 (–, –), GZHU 15129 (–, –), GZHU 15130 (–, –), GZHU 15141 (–, –), GZHU 15142 (–, –), GZHU 15158 (–, MN549090), GZHU 15159 (–, –), GZHU 15160 (–, –), GZHU 15162 (–, –), GZHU 15201 (–, –), GZHU 15283 (–, –), GZHU 15285 (–, –); **Heishiding, Guangdong, China:** GZHU 14155 (MN549045, –)

***Murina huttoni*: Nanling, Guangdong, China:** GZHU 09229 (–, –), GZHU 09244 (MN549049, –), GZHU 12193 (–, –), GZHU 12196 (–, –), GZHU 12198 (MN549050, –), GZHU 12199 (–, –), GZHU 12288 (–, –), GZHU 12451 (–, –), GZHU 13094 (–, –), GZHU 13096 (MN549051, –), GZHU 13100 (–, –), GZHU 13102 (–, –), GZHU 13138 (MN549052, –), GZHU 13139 (MN549053, –), GZHU 09230 (MN549058, –), GZHU 09231 (MN549059, –), GZHU 09232 (–, –), GZHU 12192 (MN549060, –), GZHU 12194 (MN549061, –), GZHU 12195 (MN549062, –), GZHU 12197 (MN549063, –), GZHU 12284 (–, –), GZHU 12287 (–, –), GZHU 12289 (–, –), GZHU 12290 (–, –), GZHU 12303 (–, –), GZHU 12305 (MN549064, –), GZHU 12452 (–, –), GZHU 13077 (–, –), GZHU 13095 (–, –), GZHU 13101 –, – (), GZHU 13524 (–, –), GZHU 13529 (–, –); **Jinggang Mountain, Jiangxi, China:** GZHU 13237 (MN549054, –), GZHU 13275 (MN549055, –), GZHU 13300 (MN549056, –), GZHU 13302 (MN549057, –), GZHU 13403 (–, –), GZHU 13423 (–, –), GZHU 13445 (–, –), GZHU 13447 (–, –), GZHU 13448 (–, –), GZHU 13358 (MN549065, –), GZHU 13359 (MN549066, –), GZHU 13402 (–, –), GZHU 13410 (–, –), GZHU 13424 (–, –), GZHU 13432 (–, –), GZHU 13433 (–, –), GZHU 13444 (–, –), GZHU 13446 (–, –), GZHU 13450 (–, –), GZHU 13451 (–, –), GZHU 13453 (–, –), GZHU 13455 (–, –), GZHU 13458 (–, –), GZHU 13478 (–, –), **Chunan, Zhejiang:** GZHU 17303* (–, MN549098)

***Murina hilgendorff*: Heilongjiang, China:** GZHU HLJ–8–3* (–, MN549094)

***Murina jaintiana*: Chin Hills, India:** BM(NH) 16.3.26.5 (–, –), BM(NH) 16.3.26.7 (–, –), BM(NH) 16.3.26.8 (–, –), BM(NH) 16.3.26.88 (–, –), HNHM 2000.20.1. (–, –), **Chungtang, Sikkim, India:** FMNH 35829 (–, –), **Lushai Hills, India:** FMNH 76053 (–, –), **Mawryngueng, Khasi, India:** FMNH 76054 (–, –), **Dening, Mishmi Hills, India:** FMNH 82775 (–, –), FMNH 82776 (–, –), **Jaintia Hills, India:** MHNG M1619 (–, –)

***Murina jinchui* sp nov: Wolong, Sichuan, China:** GZHU 14453 (MN549067, –), GZHU 14454 (MN549068, –), GZHU 14455 (MN549069, –), GZHU 14463 (MN549070, MN549091), GZHU 14462 (MN549071, –), GZHU 14461 (MN549072, –)

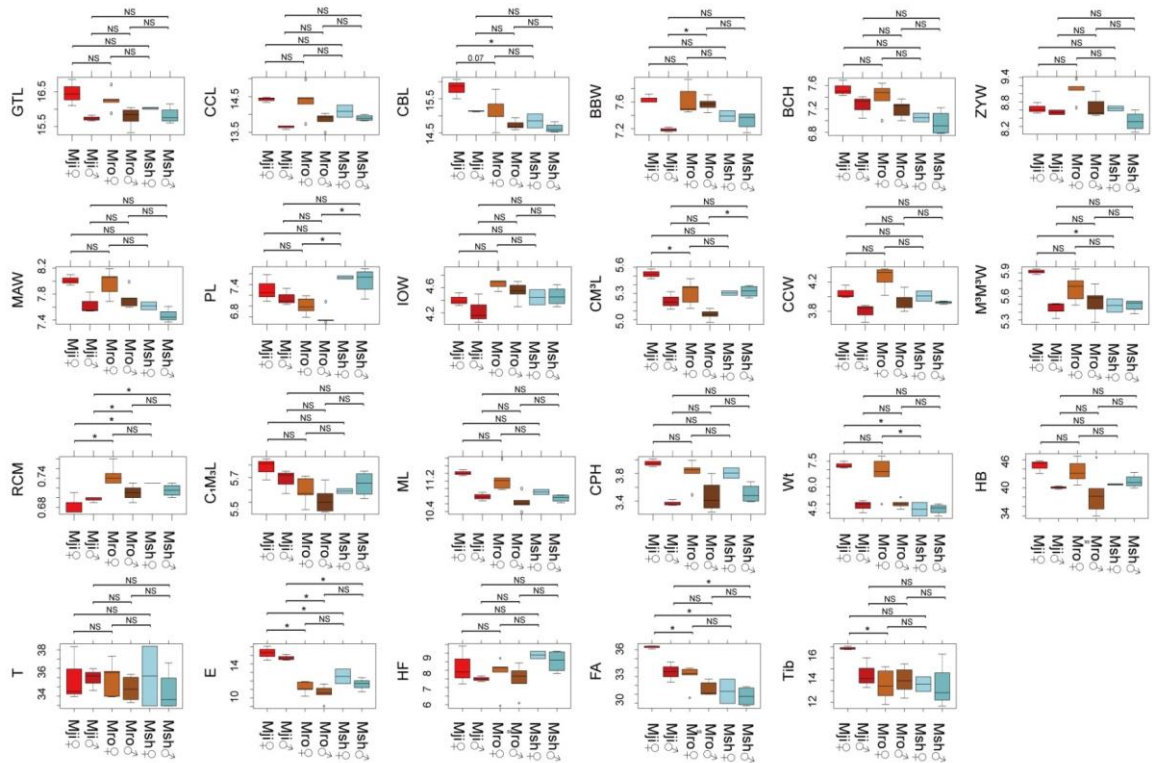
***Murina leucogaster*: Pingwu, Sichuan, China:** GZHU 06002 (–, –), GZHU 06003 (–, –), GZHU 06004 (–, –), GZHU 06005 (–, –); **Yaan, Sichuan, China:** GZHU 10122 (–, –)

***Murina lorelleae*: Ailao mountain, Yunnan:** GZHU 14261* (–, MN549095)

***Murina shuipensis*: Shuipu, Guizhou, China:** GZHU 15423 (MN549073, –), GZHU 15424 (MN549074, –), GZHU 15619 (MN549075, MN549092), GZHU 15403 (MN549076, –), GZHU 15420 (MN549077, –), GZHU 15416 (MN549077, –)

***Murina rongjiangensis*: Rongjiang, Guizhou, China:** GZHU 15116 (MN549078, –), GZHU 15118 (MN549079, –), GZHU 15119 (MN549080, –), GZHU 15120 (MN549081, –), GZHU 15200 (MN549082, –), GZHU 15375 (MN549083, –), GZHU 15100 (MN549084, –), GZHU 15096 (MN549085, –), GZHU 15139 (MN549086, –), GZHU 15240 (MN549087, –), **Julianshan, Jiangxi:** GZHU 15089* (–, MN549101)

Supplementary Figure



Supplementary Figure S1 Standard boxplots for absolute values of external and craniodental measurements of *M. jinchui* **sp. nov.**, *M. shuipuensis* and *M. rongjiangensis*. Abbreviations for taxa include: Mji♀ for females of *M. jinchui*, Mji♂ for males of *M. jinchui*, Mro♀ for females of *M. rongjiangensis*, Mro♂ for males of *M. rongjiangensis*, Msh♀ for females of *M. shuipuensis* and Msh♂ for males of *M. shuipuensis*. Statistical differences between the groups are ticked with different lower case letters, groups with the same letter indicates no significant difference ($P>0.05$).

Supplementary Table

Supplementary Table S1 Selected external and craniodental measurements (mm) of *Murina aurata*, *M. beelzebub*, *M. chrysochaetes*, *M. cyclotis*, *M. eleryi*, *M. fanjingshanensis*, *M. feae*, *M. harrisoni*, *M. huttoni*, *M. jaintiana*, *M. jinchui* **sp. nov.**, *M. leucogaster*, and *M. shuipuensis*

Supplementary Table S1 was listed as a separate file for the table is too big.