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A cryptic new species of *Miniopterus* from south-eastern Africa based on molecular and morphological characters

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Abstract

Resolving species limits within the genus *Miniopterus* has traditionally been complicated by the presence of cryptic species with overlapping morphological features. We use molecular techniques, cranio-dental characters and tragus shape to describe a new species of *Miniopterus* from Mozambique, *M. mossambicus*. *Miniopterus mossambicus* shows > 12% divergence in cytochrome-b sequence from its nearest congeners (the Malagasy *M. gleni* and *M. griveaudi*) and > 15% divergence from the morphologically similar *M. natalensis*, *M. fraterculus* and *M. minor* (all of which occur in southern and eastern Africa). There is considerable overlap in cranio-dental characters of the southern African species, particularly *M. natalensis* and *M. mossambicus*. However, tragus shape and multivariate comparisons of skull measurements can be used to separate these species. Based on morphological comparisons of museum specimens, this species may also be present in neighbouring Malawi and Zimbabwe, suggesting that it is probably distributed widely in south-central Africa.

Key words: taxonomy, morphometrics, molecular genetics, *Miniopterus*, Mozambique

Résumé

Résoudre les délimitations des espèces au sein du genre *Miniopterus* a toujours été compliqué à cause de la présence d'espèces cryptiques dont les caractéristiques morphologiques se chevauchent. Nous utilisons des techniques moléculaires, des caractères cranio-dentaires et la forme des tragus pour décrire une nouvelle espèce de *Miniopterus* venant du Mozambique, *M. mossambicus*. Les séquences de cytochrome-b de *M. mossambicus* montrent une divergence supérieure à 13% par rapport à ses plus proches congénères (les formes malgache *M. gleni* et *M. griveaudi*), et une divergence supérieure à 15% par rapport à *M. natalensis*, *M. minor* et *M. fraterculus* qui lui sont morphologiquement semblables (ces dernières sont distribuées dans le sud et l'est de l'Afrique). Il y a un chevauchement considérable des caractères cranio-dentaires des espèces du sud du continent africain, en particulier *M. natalensis* et *M. mossambicus*. Cependant, la forme du tragus et les comparaisons multivariées des mesures crâniennes peuvent être utilisées pour séparer ces espèces. Sur la base de la comparaison morphologique des spécimens muséologiques, cette espèce pourrait également être présente dans les pays voisins du Malawi et du Zimbabwe, ce qui suggère qu'elle est probablement largement distribuée en Afrique sud-centrale.

Introduction

In the past decade, numerous species of bats new to science have been described from sub-Saharan Africa (Fahr *et al.* 2002; Stanley 2008; Taylor *et al.* 2012; Monadjem *et al.* 2013), underscoring the ever increasing chiropteran

species richness of the continent (Fahr & Kalko 2011; African Chiroptera Report 2012) and the need for more field and associated taxonomic studies, including identification of cryptic taxa. For example, in their review of the bats of Madagascar, Peterson *et al.* (1995) recorded 27 species on the island. Less than two decades later, 44 species are listed from Madagascar, an increase of 61%, which was achieved through a dedicated programme of extensive field surveys and associated molecular and morphological examination of collected specimens (Goodman 2011; Goodman *et al.*, 2012a). Studies of similar intensity have yet to be conducted for sub-Saharan Africa.

It has proved particularly difficult to resolve species limits within the African members of the genus *Miniopterus* (Miller-Butterworth *et al.* 2005). This widespread genus, occurring throughout the Old World (Simmons 2005), has recently been placed in its own family, Miniopteridae (Miller-Butterworth *et al.* 2007). Eleven species are now known to occur on Madagascar, seven of which have been recently described (Goodman *et al.* 2007, 2008, 2009a, 2009b, 2010, 2011). The situation in Madagascar clearly shows the high diversity of cryptic species within this genus, with up to four species occurring sympatrically (Goodman *et al.* 2009a), and similar patterns are expected for the African continent and neighbouring islands.

On the basis of current taxonomy, four species of *Miniopterus* occur in southern Africa: *M. inflatus* Thomas, 1903, *M. natalensis* (A. Smith, 1834), *M. fraterculus* Thomas & Schwann, 1906 and *M. minor* Peters, 1866 (Monadjem *et al.* 2010a), although numerous proposed names have been placed in synonymy (e.g., Peterson *et al.* 1995; Simmons 2005). Of the currently recognized taxa, *M. inflatus* is easily distinguished by its large body size (Monadjem *et al.* 2010a), whereas *M. natalensis*, *M. fraterculus* and *M. minor* overlap in external measurements (Monadjem *et al.* 2010a). Monadjem *et al.* (2010a,b) noted the possible existence of an undescribed species of small *Miniopterus* from Mozambique, which closely resembled *M. fraterculus*. During recent fieldwork to that country, we were able to obtain additional material to address the taxonomic status of this animal. This paper aims in: 1) elucidate the relationships of the small *Miniopterus* bats from south-eastern Africa; and 2) describe a new species to science.

Material and methods

Specimens. The specimens on which this study is based are deposited in the following museums: The American Museum of Natural History (AMNH); The Natural History Museum (formerly The British Museum of Natural History), London (BMNH); Durban Natural Science Museum (DM); Field Museum of Natural History, Chicago (FMNH); Muséum national d'Histoire Naturelle, Paris (MNHN), and The United States National Museum, Smithsonian Institution, Washington, D.C. (USNM). Measurements of only adult specimens, identified by fully erupted adult dentition and the fusion of the basisphenoid-basioccipital suture, were included in this study.

Comparison to type specimens. We examined type specimens in the BMNH, which included: *Miniopterus natalensis* (BMNH 1848.6.12.19, holotype), *M. fraterculus* (BMNH 1905.5.7.18, holotype), *M. fraterculus* (BMNH 1905.5.7.22, topotype) and *M. inflatus* (BMNH 1903.2.4.8, holotype).

Molecular analyses. The cytochrome-b gene was selected for molecular analysis due to its recent usage in many Afro-Malagasy studies of the genus *Miniopterus*, and resulting utility for direct comparison to genetically diagnosed species. Where possible, the full length of the gene was analyzed (1140 bp). To understand the phylogenetic relationships among morphologically similar species, the dataset includes sequences downloaded from GenBank from previously described small- to medium-sized *Miniopterus* species, along with new sequences from specimens originating from geographic areas that are relevant to the current study (Table 1). A sequence from the Asian *M. fuliginosus* (Hodgson, 1835) (GenBank AB085735) was included as an outgroup to the Afro-Malagasy species.

Genomic DNA was extracted using a lithium chloride and chloroform extraction method as described by Gemmel & Akiyama (1996). The cytochrome-b gene was amplified and sequenced using the primers L14724 and H15915 (Irwin *et al.* 1991). Template DNA was amplified by PCR in 25µL reaction volume containing the following: 1X reaction buffer (Promega, Madison, Wisconsin), 2.5 mM MgCl₂, 0.2 mM of each dNTP, 0.28 mM of each primer, 1 unit of Taq polymerase (Promega) and approximately 100 ng of template DNA. Cycling consisted of an initial denaturation at 94°C for 3 min, followed by 30 cycles at 94°C for 30 s, 45°C for 40 s and 72°C for 60 s and a final extension of 72°C for 3 min.

TABLE 1. Bat specimens of the genus *Miniopterus* used in this study associated with the molecular analyses. Museum acronyms include: BMNH: the Natural History Museum; DM: Durban Natural Science Museum; FMNH: Field Museum of Natural History, Chicago; ROM: Royal Ontario Museum; TM: Ditsong Museum of Natural History (formerly, Transvaal Museum); UADBA: Université d'Antananarivo, Département de Biologie Animale. Field number acronyms include: AM: Ara Monadjem; DSJZM: David S. Jacobs; ECJS: Ernest C.J. Seamark; SKK: Steenkampskraal, specimen from The University of Cape Town; SMG: Steven M. Goodman; WTS : William T. Stanley.

| Species | Museum number | Field number | Locality | GenBank No. | Reference |
|-----------------------|---------------|--------------|----------------------|-------------|---|
| <i>M. manavi</i> | FMNH 5650 | -- | Madagascar | FJ383128 | Goodman <i>et al.</i> (2009a) |
| <i>M. manavi</i> | FMNH 187662 | SMG-14753 | Madagascar | FJ383129 | Goodman <i>et al.</i> (2009a) |
| <i>M. manavi</i> | FMNH 194074 | SMG-15397 | Madagascar | FJ383130 | Goodman <i>et al.</i> (2009a) |
| <i>M. manavi</i> | UADBA 43171 | SMG-16288 | Madagascar | HQ619934 | Goodman <i>et al.</i> (2011) |
| <i>M. manavi</i> | UADBA 43172 | SMG-16294 | Madagascar | HQ619935 | Goodman <i>et al.</i> (2011) |
| <i>M. griveaudi</i> | FMNH 173101 | SMG-12902 | Madagascar | FJ383136 | Goodman <i>et al.</i> (2009a) |
| <i>M. griveaudi</i> | FMNH 179234 | SMG-14261 | Madagascar | FJ383143 | Goodman <i>et al.</i> (2009a) |
| <i>M. griveaudi</i> | FMNH 172817 | SMG-12402 | Madagascar | FJ383144 | Goodman <i>et al.</i> (2009a) |
| <i>M. griveaudi</i> | FMNH 179233 | SMG-14260 | Madagascar | FJ383145 | Goodman <i>et al.</i> (2009a) |
| <i>M. minor</i> | FMNH 198034 | WTS-8368 | Tanzania (Tanga) | FJ232806 | Weyeneth <i>et al.</i> (2008) |
| <i>M. minor</i> | FMNH 198010 | WTS-8400 | Tanzania (Tanga) | FJ232804 | Weyeneth <i>et al.</i> (2008) |
| <i>M. minor</i> | FMNH 198098 | WTS-8430 | Tanzania (Unguja) | FJ232805 | Weyeneth <i>et al.</i> (2008) |
| <i>M. minor</i> | FMNH 198164 | WTS-8511 | Tanzania (Unguja) | FJ232803 | Weyeneth <i>et al.</i> (2008) |
| <i>M. fraterculus</i> | | | South Africa | AJ841975 | Stadelmann <i>et al.</i> (2004) |
| <i>M. fraterculus</i> | | | South Africa | AY614750 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. fraterculus</i> | | | South Africa | AY614751 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. fraterculus</i> | | | South Africa | AY614752 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. fraterculus</i> | | | South Africa | AY614753 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. fraterculus</i> | | | South Africa | AY614754 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. fraterculus</i> | | | South Africa | AY614755 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. fraterculus</i> | TM 42120 | | South Africa | DQ899760 | Goodman <i>et al.</i> (2007) |
| <i>M. fraterculus</i> | TM 41638 | | South Africa | DQ899761 | Goodman <i>et al.</i> (2007) |
| <i>M. mossambicus</i> | FMNH 213651 | SMG-16875 | Mozambique | KF709538 | This study |
| <i>M. mossambicus</i> | FMNH 213652 | SMG-16876 | Mozambique | KF709539 | This study |
| <i>M. mossambicus</i> | | DSJZM4 | Zambia | AY614739 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. mossambicus</i> | | DSJZM1 | Zambia | AY614738 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. natalensis</i> | | SKK-4 | South Africa | DQ899765 | Goodman <i>et al.</i> (2007) |
| <i>M. natalensis</i> | TM 46484 | | South Africa | DQ899762 | Goodman <i>et al.</i> (2007) |
| <i>M. natalensis</i> | TM 46881 | | South Africa | DQ899764 | Goodman <i>et al.</i> (2007) |
| <i>M. natalensis</i> | TM 47622 | | South Africa | DQ899763 | Goodman <i>et al.</i> (2007) |
| <i>M. natalensis</i> | TM 47713 | | Swaziland | KF709548 | This study |
| <i>M. natalensis</i> | DM 8433 | AM2047 | Swaziland | KF723607 | This study |
| <i>M. natalensis</i> | DM 8434 | AM2051 | Swaziland | KF723608 | This study |
| <i>M. natalensis</i> | | ECJS-85/2008 | Namibia | KF709540 | This study |
| <i>M. natalensis</i> | | ECJS-86/2008 | Namibia | KF709541 | This study |

.....continued on the next page

TABLE 1. (Continued)

| Species | Museum number | Field number | Locality | GenBank No. | Reference |
|-----------------------|---------------|--------------|--------------|-------------|---|
| <i>M. natalensis</i> | | ECJS-88/2008 | Namibia | KF709542 | This study |
| <i>M. natalensis</i> | | ECJS-89/2008 | Namibia | KF709543 | This study |
| <i>M. natalensis</i> | | ECJS-90/2008 | Namibia | KF709544 | This study |
| <i>M. natalensis</i> | | ECJS-91/2008 | Namibia | KF709545 | This study |
| <i>M. natalensis</i> | | | South Africa | AY614742 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. natalensis</i> | | | South Africa | AY614743 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. natalensis</i> | | | South Africa | AY614744 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. natalensis</i> | | | South Africa | AY614745 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. natalensis</i> | | | South Africa | AY614746 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. natalensis</i> | | | South Africa | AY614747 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. natalensis</i> | | | South Africa | AY614748 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. natalensis</i> | ROM 70851 | | Zimbabwe | KF709546 | This study |
| <i>M. natalensis</i> | ROM 64656 | | Zimbabwe | KF709547 | This study |
| <i>M. natalensis</i> | | | South Africa | AY614749 | Miller-Butterworth <i>et al.</i> (2005) |
| <i>M. sororculus</i> | FMNH 177259 | SMG-13573 | Madagascar | DQ899771 | Goodman <i>et al.</i> (2007) |
| <i>M. sororculus</i> | FMNH 177264 | SMG-13560 | Madagascar | DQ899773 | Goodman <i>et al.</i> (2007) |
| <i>M. sororculus</i> | FMNH 177267 | SMG-13576 | Madagascar | HQ619937 | Goodman <i>et al.</i> (2011) |
| <i>M. sororculus</i> | FMNH 209183 | SMG-16785 | Madagascar | HQ619938 | Goodman <i>et al.</i> (2011) |
| <i>M. gleni</i> | UADBA 43219 | SMG-16467 | Madagascar | JF440235 | Ramasindrazana <i>et al.</i> (2011) |
| <i>M. gleni</i> | FMNH 209271 | SMG-16468 | Madagascar | JF440236 | Ramasindrazana <i>et al.</i> (2011) |
| <i>M. gleni</i> | FMNH 209250 | SMG-16536 | Madagascar | JF440237 | Ramasindrazana <i>et al.</i> (2011) |
| <i>M. gleni</i> | FMNH 209251 | SMG-16537 | Madagascar | JF440238 | Ramasindrazana <i>et al.</i> (2011) |
| <i>M. majori</i> | UADBA 43264 | SMG-16800 | Madagascar | HQ619953 | Goodman <i>et al.</i> (2011) |
| <i>M. majori</i> | UADBA 43261 | SMG-16797 | Madagascar | HQ619954 | Goodman <i>et al.</i> (2011) |
| <i>M. majori</i> | UADBA 43198 | SMG-16445 | Madagascar | HQ619955 | Goodman <i>et al.</i> (2011) |
| <i>M. majori</i> | FMNH 202518 | SMG-16042 | Madagascar | HQ619939 | Goodman <i>et al.</i> (2011) |
| outgroup | | | | | |
| <i>M. fuliginosus</i> | | | Japan | AB085735 | Sakai <i>et al.</i> (2003) |

The single PCR product was directly sequenced in both directions by a commercial company (Macrogen Inc., [Seoul, Korea]) using the same primers. The sequences were assembled and aligned using Sequencher version 4.6 (Gene Codes Corporation, Ann Arbor, Michigan). Analysis using DNA strider (Marck 1990) showed that sequences did not contain insertions, deletions or stop codons. All new sequences were deposited in GenBank (Accession numbers KF709538-KF709539, KF709540-K709548, KF723607-KF723608).

FindModel (<http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html>) was used to determine the model of molecular evolution and this model was used in the phylogenetic analyses. The Akaike Information Criterion estimated the model, TrN+G,-AIC = 673.540 and shape parameter of gamma distribution = 0.149.

Minimum Evolution or Neighbour-joining (NJ) trees were conducted using PAUP* 4.0 (Swofford 2003). The NJ method used pairwise sequence distances estimated by the TrN+G model of evolution. Nodal support of MP and NJ trees were estimated with 1,000 bootstrap pseudoreplicates. Maximum Likelihood (ML) trees were produced using the online RAxML black box (Stamatakis *et al.* 2008) (<http://phylobench.vital-it.ch/raxml-bb/index.php>) with gamma model of rate heterogeneity and 100 bootstrap replicates.

Bayesian analysis was conducted using the program MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). The TrN+G model was specified, flat priors were used and starting trees were random. Four chains (3 hot, 1 cold) were run for 2,000,000 generations, sampling trees every 100 generations. Bayesian runs were determined to have

achieved sufficient convergence by ascertaining that the average standard deviation of split frequencies between chains had reached below 0.01 (0.009558) at the end of the run and that the potential scale reduction factor (PSRF) of each parameter was 1.000. Plots of generation versus the log probabilities of observing actual data did not reveal any trends for the last 75% of generations. The first 25% of generations were excluded from the calculation of posterior probabilities.

Genetic divergence was calculated as pairwise TrN with the software PAUP* 4.0 (Swofford 2003). The average within and between clade genetic distance was calculated using Excel 2008. The values obtained were comparable with previous studies reporting taxonomic inferences on miniopterid bat species based on K2P genetic distances (Appleton *et al.* 2004; Goodman *et al.* 2007, 2008; Juste *et al.* 2007; Weyeneth *et al.* 2008).

Cranial and dental morphometrics. The following standard external measurements were taken in the field: total body length, tail length, hindfoot length (without claw), ear length, and forearm length. Forearm length was taken with callipers to the closest 0.1 mm (except for the *M. minor* specimens which were measured to the closest 1 mm); all other measurements were at an accuracy of 1 mm. Body mass was taken with a Pesola spring balance to the closest 1 g.

Eight cranial and seven dental measurements were taken with callipers to the closest 0.01 mm following Goodman *et al.* (2012b). The cranial measurements include: greatest skull length (GSKL), from the posterior-most point of the cranium to the anterior-most point of the incisors; condylo-incisive length (CIL), from the occipital condyles to the anterior-most point of the incisors; greatest zygomatic breadth (ZYGO), the greatest width across the zygomatic arches; postorbital width (POB), narrowest dorsal width posterior to the postorbital constriction of the cranium; greatest mastoid breadth (MAST), greatest breadth of cranium at mastoid processes; greatest braincase width (GBW), lateral braincase width taken posterior to the posterior insertion of the zygomatic arches; lachrymal width (LW), greatest width across rostrum at lachrymal projections; and greatest mandible length (MAND), taken from the posterior-most point of the condylar processes to the anterior-most point of the incisors. The dental measurements included: width across the third molars (M^3 - M^3), taken across the outer-most point of the alveoli of the 3rd molars; complete upper canine-molar tooth row (C - M^3), taken from the anterior-most point of the alveolus of the canine to the posterior-most point of the 3rd molar; complete upper tooth row (I^1 - M^3), taken from the anterior-most point of the alveolus of the first incisor to the posterior-most point of the 3rd molar; complete upper molar tooth row (UPMOLS), taken from the anterior-most point of the alveolus of the anterior premolar to the posterior-most point of the 3rd molar; width across upper canines (C - C), taken across the outer-most points of the alveoli of the canines; complete mandibular molar tooth row (LWMOLS), taken from the anterior-most point of the alveolus of the anterior premolar to the posterior-most point of the 3rd molar; complete lower tooth row (i^1 - m^3), taken from the anterior-most point of the alveolus of the first incisor to the posterior-most point of the 3rd molar; and complete mandibular canine-molar tooth row (c - m^3), taken from the anterior-most point of the alveolus of the canine to the posterior-most point of the 3rd molar. Tooth abbreviations are as follows: I = incisor, C = canine, P = premolar, M = molar; with upper teeth presented in upper case and lower teeth in lower case.

Statistical analyses. A principal components analysis (PCA) of log-transformed values of the above cranial measurements was conducted on the variance-covariance matrix in the program PAST (Hammer *et al.* 2001) to compare the morphology of the various taxa measured in this study.

Results

Molecular analyses

The phylogenetic tree for the African and Malagasy *Miniopterus* species selected for this study is presented in Figure 1. The analysis showed similar tree topologies for all three phylogenetic analyses. The trees agreed on major nodes, while some of the terminal branching differed slightly (data not shown). The nomenclature follows recent publications resolving the taxonomy of Malagasy *Miniopterus* species (e.g., Goodman *et al.* 2008; Ramasindrazana *et al.* 2011), and utilizes many of the sequences from those publications, allowing direct comparison.

For the most part, the basal portion of the tree is not resolved, however all species are clearly distinct from one another with genetic distances of 8–21% (Table 2). The African species *M. minor* and *M. fraterculus* group are sister to one another. The Malagasy species *M. gleni* and *M. majori* are sister to one another with *M. manavi* sensu

TABLE 2. Average pairwise comparisons of TrN genetic distances of *Miniopterus* spp.. Where available intraspecific values are given in bold along the diagonal. Abbreviations are: Msor = *M. sororculus*, Mman = *M. manavi* sensu stricto, Mmaj = *M. majori*, Mmos = *M. mossambicus* sp. nov., Mgle = *M. gleni*, Mfra = *M. fraterculus*, Mmin = *M. minor*, Mgri = *M. griveaudi*, Mnat = *M. natalensis*, Mful = *M. fuliginosus*.

| | Msor | Mman | Mmaj | Mmos | Mgle | Mfra | Mmin | Mgri | Mnat | Mful |
|------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|------|
| Msor | 0.0046 | | | | | | | | | |
| Mman | 0.1380 | 0.0092 | | | | | | | | |
| Mmaj | 0.1376 | 0.1083 | 0.0067 | | | | | | | |
| Mmos | 0.1180 | 0.1231 | 0.1219 | 0.0258 | | | | | | |
| Mgle | 0.1124 | 0.1156 | 0.1026 | 0.1365 | 0.0085 | | | | | |
| Mfra | 0.2187 | 0.1608 | 0.1624 | 0.1547 | 0.1782 | 0.0069 | | | | |
| Mmin | 0.1599 | 0.1723 | 0.1559 | 0.1721 | 0.1444 | 0.0831 | 0.0026 | | | |
| Mgri | 0.1404 | 0.1304 | 0.1437 | 0.1397 | 0.1299 | 0.1626 | 0.1431 | 0.0107 | | |
| Mnat | 0.1552 | 0.1433 | 0.1467 | 0.1771 | 0.1464 | 0.1787 | 0.1857 | 0.1288 | 0.0124 | |
| Mful | 0.2336 | 0.2252 | 0.2291 | 0.2426 | 0.2312 | 0.3326 | 0.2499 | 0.2692 | 0.3899 | - |

stricto also grouping with low support. The samples collected in Mozambique group with two previously published sequences from Zambia identified as *M. schreibersii* (Miller-Butterworth *et al.* 2005). This clade shows a clear distinction from all other described clades composed of bats with comparable body size and geographic distribution to the Mozambique specimens, with 11–17% sequence divergence from all other ingroup taxa. Comparable levels of sequence divergence are found between all of the ingroup taxa and this is indicative of species level divergences reported in previous publications. To further resolve aspects of the phylogeny of African *Miniopterus*, additional markers, including nuclear genes, will need to be added.

On the basis of the molecular results, with a clear separation of the small *M. natalensis*-like specimens from Mozambique and different morphological measurements and characters, there is substantial basis to consider these animals as hitherto unknown to science. Below we describe them as a new species.

Systematics

Family Miniopteridae, Dobson, 1875

Genus *Miniopterus* Bonaparte, 1837

Miniopterus mossambicus sp. nov.

Mozambique long-fingered bat

Holotype. FMNH 213651 (field number SMG-16875) was collected by Steven M. Goodman and Ara Monadjem on 9 October 2010. This is an adult male fixed in formalin and currently preserved in 70% alcohol. Pectoral muscle was preserved in lysis buffer. The skull has been extracted and cleaned. The specimen has a full adult dentition and the basisphenoid–basioccipital sutures are completely fused. External measurements are: total length 103 mm, tail length 50 mm, hind-foot length (without claw) 6 mm, tragus length 6 mm, ear length 10 mm, and forearm length 41.2 mm. The animal weighed 6.7 g (Table 3).

Type locality. The specimen was collected in a mist net set over a swimming pool at the Bamboo Inn (15.10306°S; 39.21748°E), along the road to Malawi and on the outskirts of Nampula (town) in the Nampula Province, northern Mozambique, at an altitude of 420 m above sea level.

Etymology. This species is named after the country from which the type series was collected, Mozambique.

Paratype. A second adult male specimen FMNH 213652 (field number SMG-16876) was collected at the same locality and date as the holotype. Both individuals were captured in the same mist net placement, approximately within 15 minutes of one another. Pectoral muscle was conserved in lysis buffer. The skull was not extracted from the specimen. Measurements are provided in Tables 3.

TABLE 3. External measurements (mm) and mass (g) of specimens of the small *Miniopterus* species from southern Africa. Measurements presented as mean \pm standard deviation, range and sample size (n). In cases where the sample size was one, the sample size is not presented. The holotype and paratype of *M. mossambicus* were measured by the same field collector (SMG), while the referred specimens by an assortment of different individuals. In the former case, the hindfoot length is without the claw and in the latter case with the claw.

| Specimen or taxon | Total length | Tail length | Hindfoot length | Ear length | Forearm length | Body mass |
|---|--------------------------------------|-----------------------------------|--------------------------------------|---------------------------------------|---|--------------------------------------|
| <i>Miniopterus mossambicus</i> sp. nov. | 103 | 50 | 6 | 10 | 41.2 | 7.6 |
| Holotype FMNH 213651 | | | | | | |
| <i>Miniopterus mossambicus</i> sp. nov. | 100 | 47 | 6 | 10 | 41.0 | 6.7 |
| Paratype FMNH 213652 | | | | | | |
| Other referred specimens | 101.3 \pm 2.16, 99–104, n = 3 | 47 | 8.3, 7.7–9.0, n = 2 | 9.4, 8.8–10.0, n = 2 | 43.9 \pm 1.38, 43.0–44.9, n = 5 45.7 | 8.50 \pm 0.61, 8.0–9.0, n = 3 |
| <i>Miniopterus natalensis</i> | - | - | - | - | - | - |
| Holotype BMNH 1848.6.12.19 | | | | | | |
| Other adults | 109.5 \pm 4.20, 102–116, n = 16 | 49.6 \pm 4.91, 42–57, n = 15 | 10.5 \pm 0.99, 9.0–13.0, n = 15 | 9.6 \pm 1.20, 7.5–12, n = 15 | 45.5 \pm 1.24, 43.4–47.8, n = 16 43.5 | 10.8 \pm 0.97, 8.3–11.8, n = 12 |
| <i>Miniopterus fraterculus</i> | 106 | 52 | - | 9 | - | - |
| Holotype BMNH 1905.5.7.18 | | | | | | |
| Topotype BMNH 1905.5.7.22 | 110 | 53 | - | 12 | 43.5 | - |
| Other adults | 100.4 \pm 3.86, 95–107, n = 10 | 45.6 | 8.4 \pm 0.84, 7.7–10.0, n = 8 | 8.4 \pm 0.81, 7.0–9.5, n = 8 | 43.4 \pm 0.94, 41.4–44.2, n = 13 | 8.1 \pm 0.94, 6.5–9.1, n = 8 |
| <i>Miniopterus minor</i> | 93.5 \pm 3.21, 89–99, n = 11 | 42.7 \pm 2.61, 37–46, n = 11 | 7.91 \pm 0.54, 7.0–9.0, n = 11 | 10.1 \pm 0.30, 10.0–11.0, n = 11 | 38.5 \pm 1.13, 37.0–40.0, n = 11 | 4.9 \pm 0.28, 4.6–5.6, n = 11 |
| Holotype BM 1903.2.4.8 | 113 | 48 | - | 11 | 46.4 | - |
| Other adults | 113.0 \pm 5.45, 105–120, n = 7 | 54.2 \pm 1.92, 52–57, n = 5 | 10.1 \pm 0.74, 9.0–11.0, n = 5 | 11.5 \pm 1.29, 10.0–13.5, n = 7 | 46.9 \pm 0.55, 45.7–47.5, n = 9 | 13.3 \pm 2.54, 9.9–16.0, n = 6 |

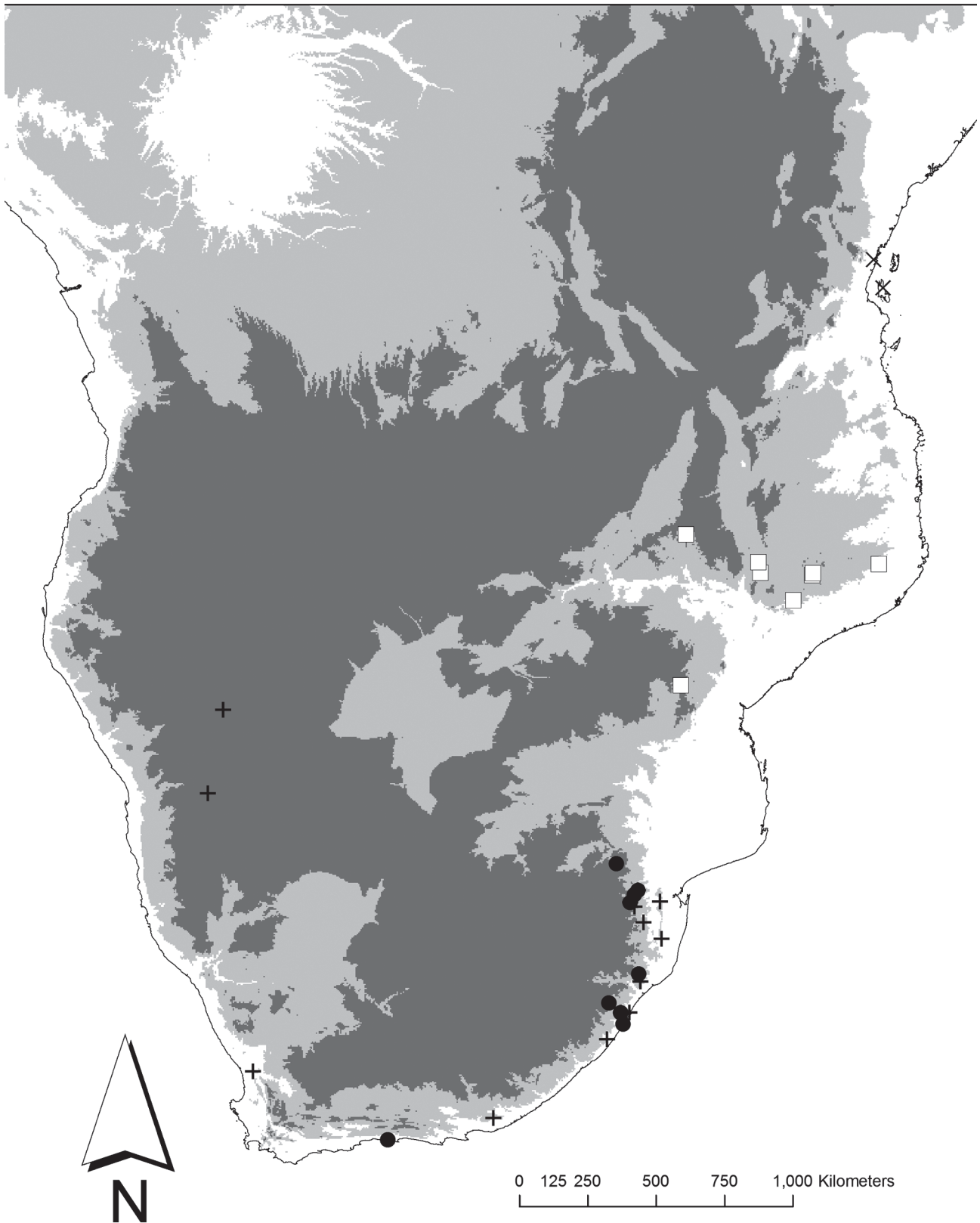


FIGURE 2. Map of southern Africa showing the type locality for *Miniopterus mossambicus* sp. nov., as well as localities for the other mainland African specimens mentioned in this study. *Miniopterus mossambicus*—□; *M. fraterculus*—●; *M. minor*—×; *M. natalensis*—+.

Specimens referable to *Miniopterus mossambicus*. The following specimens have not been sequenced and therefore their assignment to *M. mossambicus* must at present remain tentative; however, morphologically they can

be identified as *M. mossambicus*. All were collected from mid- to high-altitudes in Mozambique and neighbouring Malawi, Zambia and Zimbabwe (Figure 2). Mozambique: DM 8484 and DM 8520, Gurue Tea Plantation (15° 27.733'S; 37° 01.151'E, altitude 700 m), on 19 and 20 June 2010, respectively; DM 10836, Mount Mabu (16° 17.189'S; 36° 24.181'E, altitude 980 m) on 27 November 2008; DM 9840, Manho Forest, Mount Namuli (15° 22.155'S; 37° 03.682'E, altitude 1600 m), date not known. Malawi: BMNH 1987.1178, Liwonde National Park (15° 02'S; 35° 15'E, altitude 700 m), on 5 June 1985; BMNH 1987.1177 and BMNH 1987.1156, Zomba Plateau (15° 20'S; 35° 19'E, altitude 1800 m), on 23 February and 21 March 1985, respectively. Zambia: BMNH 1968.1014-1968.1015, BMNH 1968.1017-1968.1018, Missale Old Mine (14° 07'S; 32° 52'E, altitude 900 m), between 4 February and 1 May 1963. Zimbabwe: DM 3687, Umtali (=Mutare) (19° 05'S; 32° 07'E, altitude 1000 m), 17 October 1981. Some of these specimens had previously been assigned to other species: BMNH 1987.1178 – *M. schreibersii* (Happold *et al.* 1987); BMNH 1968.1014-1968.1018 – *M. schreibersii natalensis* (Ansell 1978); BMNH 1987.1177 and 1987.1156 – *M. fraterculus* (Happold *et al.* 1987).

Diagnosis. A small-sized *Miniopterus* with a forearm length of 41–45 mm. The pelage is grey-brown on the upper and slightly paler on the under parts (Figure 3), with the individual hairs of the under parts having dark bases giving the pelage a bicoloured appearance. Compared with *M. natalensis*, *M. mossambicus* is smaller (Table 3) and has a more gracile skull (Figures 4 and 5). *Miniopterus mossambicus* has a tragus measuring 5–6 mm that is a relatively thick structure, particular towards the tip and slightly constricted towards the base (Figure 6).



FIGURE 3. Portrait of the holotype of *Miniopterus mossambicus* sp. nov. (FMNH 213651). (Photograph by Ara Monadjem.)

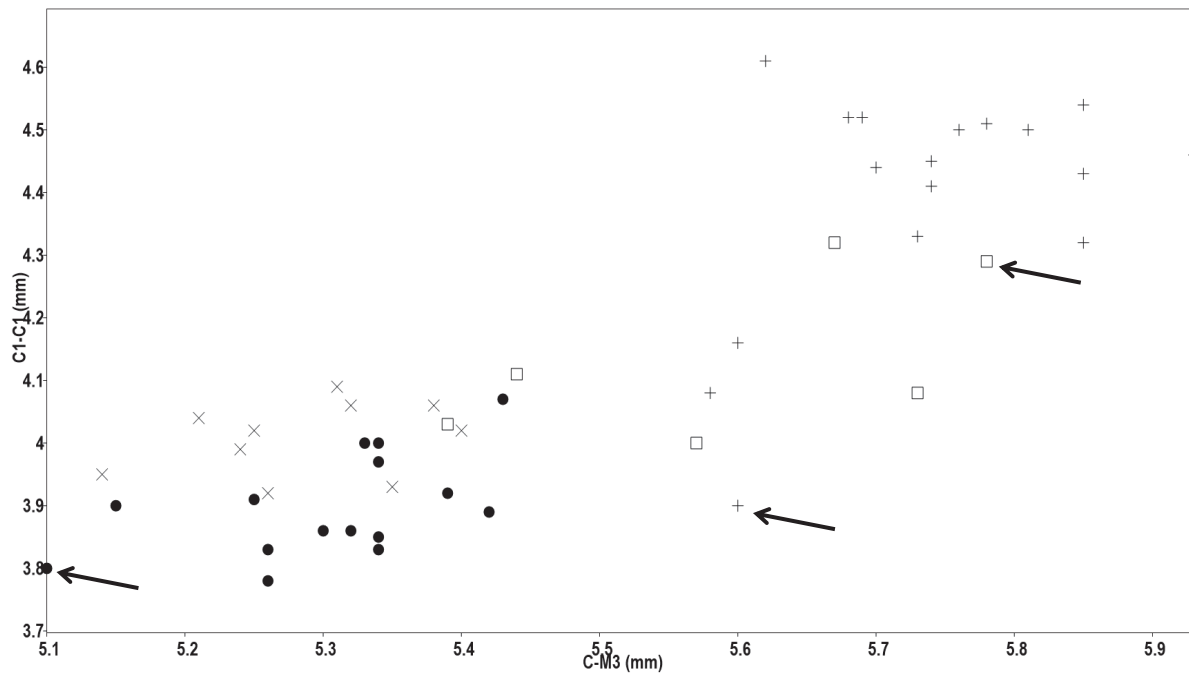


FIGURE 4. Graph showing width across upper canines plotted against complete upper canine-molar tooththrow in different *Miniopterus* spp. (crosses = *M. minor*, filled circles = *M. fraterculus*, open squares = *M. mossambicus* sp. nov.; plus = *M. natalensis*). Arrows point to type specimens.

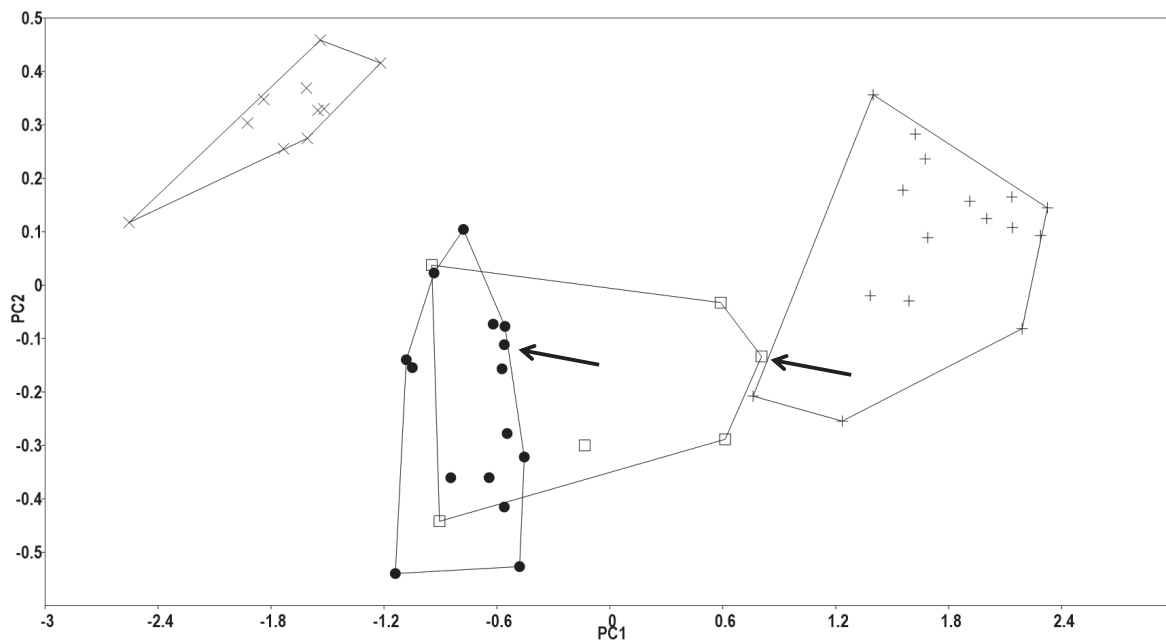


FIGURE 5. A graph plotting the first two components of a principal component (PC) analysis for cranio-dental measurements of the small African *Miniopterus* species from eastern and southern Africa (crosses = *M. minor*, filled circles = *M. fraterculus*, open squares = *M. mossambicus* sp. nov., plus = *M. natalensis*). Arrows point to type specimens. See Table 6 for the variables used in this analysis and the loadings on PC1 and PC2.

Description. *External characters.* A relatively small-sized *Miniopterus* with typical features of the genus including an elongated second phalanx of the third digit. The tail is approximately half the total length (Table 3). The pelage is grey-brown on the upper and slightly paler on the under parts (Figure 3), with the individual hairs of the under parts having dark bases giving the pelage a bicoloured appearance. The ear is relatively short and rounded (c. 10 mm in length) and not readily distinguishable from other members of the genus in terms of length (Table 3). The tragus is long (5–6 mm) and largely straight along its length, thickening slightly towards the distal tip and constricting towards the base (Figure 6). The various external measurements of the holotype and paratype of *M. mossambicus* are given in Table 3.

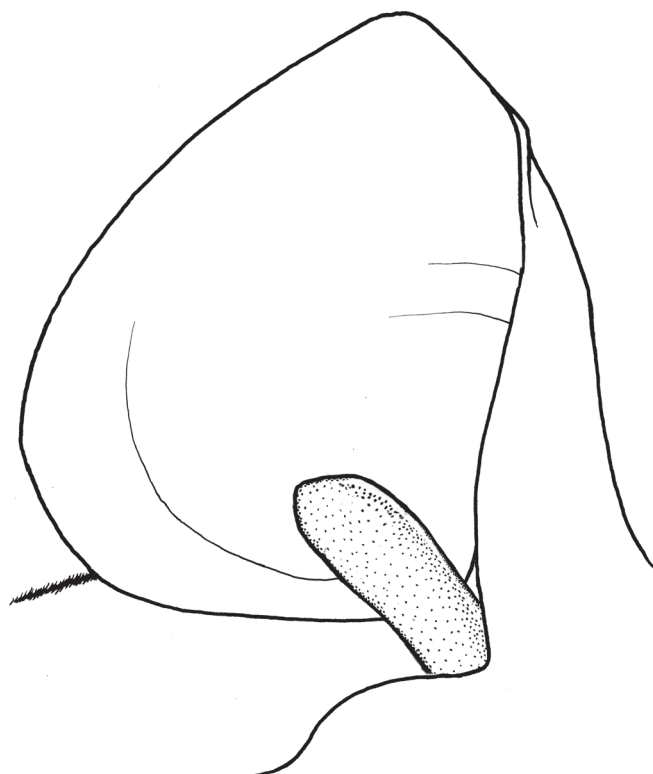


FIGURE 6. Drawing of the tragus of *M. mossambicus* sp. nov. holotype (FMNH 213651).

Cranio-dental characters. The skull of *M. mossambicus* has a broad rostrum and bulbous braincase typical of the genus (Figure 7). The cranium is intermediate in size compared with the larger *M. natalensis* and the smaller *M. fraterculus*, but showing some overlap in measurements with both (Table 4, Figure 5). Similarly, the dental measurements generally fall between those of *M. natalensis* and *M. fraterculus*, but with some overlap (Figure 4).

The dentition of *M. mossambicus* is I 2/3, C 1/1, P 2/3, M 3/3, which is typical of the genus *Miniopterus*. In the upper tooth row, the inner incisor is larger than the outer one, and the anterior premolar is relatively well developed but with a simple cusp morphology compared with the posterior premolar.

Comparisons. The following taxa of *Miniopterus* have been described from mainland sub-Saharan Africa, several of which have been placed in synonymy (e.g. Peterson *et al.* 1995; Simmons 2005), and need to be considered in the description of *M. mossambicus* sp. nov.: *M. africanus* Sanborn, 1936, *M. fraterculus*, *M. inflatus*, *M. rufus* Sanborn, 1936, *M. minor*, *M. natalensis natalensis*, *M. natalensis arenarius* Heller, 1912, *M. breyeri breyeri* Jameson, 1909, *M. breyeri vicinior* J. A. Allen, 1917, *M. scotinus* (Sandevall, 1846), *M. dasythrix* (Temminck, 1840), *M. schreibersii smitianus* Thomas, 1927 and *M. schreibersii villiersii* Aellen, 1956. Of these, the following species have forearm lengths that are larger than those of *M. mossambicus* (41.0–44.9 mm, Table 3): *M. africanus* (45.4–51.7 mm; Peterson *et al.* 1995), *M. inflatus* (46.0–49.4 mm; Monadjem *et al.* 2010a); *M. rufus* (47.4 mm; Sanborn 1936); *M. breyeri* (46.0 mm; Jameson 1909) or smaller than *M. mossambicus*: *M. minor* (38.0–41.6 mm, Peterson *et al.* 1995). Two taxa, *M. dasythrix* and *M. scotinus* are described from the "Interior of Caffraria", corresponding to the Eastern Cape and Durban (both in South Africa), the terra typica of *M. natalensis*. This area is outside the known geographic range of *M. mossambicus* and as *M. dasythrix* and *M. scotinus* are probably junior synonyms of *M. natalensis*, they will not be considered further in these comparisons. Differences between *M. mossambicus* and the remaining species are discussed below.

Miniopterus fraterculus closely resembles *M. mossambicus*, especially in overall size and shape (see Table 3). *Miniopterus mossambicus* has a tragus measuring 5–6 mm, as compared to 4–6 mm in *M. natalensis* and 4 mm in the holotype and topotype of *M. fraterculus* (BMNH 5.5.7.18 and 5.5.7.22) (Goodman *et al.* 2007). The tragus in *M. mossambicus* is a relatively thick and linear structure, particular towards the tip and slightly constricted towards the base and is notably more prominent than in *M. fraterculus* (Figure 6). Furthermore, there is no evidence at present that these two species overlap in distribution (being currently separated by a gap of at least 400 km) (Figure 2). The skull of *M. mossambicus* is typically more gracile, and the animal is generally smaller in both external and



FIGURE 7. Skull of holotype of *Miniopterus mossambicus* sp. nov. (FMNH 213651). (Photograph taken by John Weinstein, Field Museum image number Z94640_08d.)

TABLE 4. Cranial measurements (mm) of specimens of the small *Miniopterus* species from southern Africa. Measurements presented as mean \pm standard deviation, range and sample size (n).

| Specimen or taxon | GSKL | CIL | ZYGO | POB | MAST | GBW | LW | MAND |
|---|---|---|--|--|--|--|--|---|
| <i>Miniopterus mossambicus</i> sp. nov. | 15.12 | 14.58 | 7.92 | 3.73 | 8.18 | 7.53 | 5.64 | 11.22 |
| Holotype FMNH 213651 | | | | | | | | |
| Other possible specimens | 14.68 \pm 0.33, 14.15–15.20, n = 12 | 14.11 \pm 0.29, 13.75–14.50, n = 12 | 7.99 \pm 0.24, 7.50–8.40, n = 12 | 3.72 \pm 0.14, 3.47–4.05, n = 12 | 7.98 \pm 0.30, 7.47–8.50, n = 12 | 7.45 \pm 0.25, 7.20–8.00, n = 12 | 5.22 \pm 0.36, 4.80–5.87, n = 12 | 10.82 \pm 0.34, 10.01–11.20, n = 12 |
| <i>Miniopterus natalensis</i> | - | - | - | - | - | - | 5.30 | 10.60 |
| Holotype BMNH 1848.6.12.19 | | | | | | | | |
| Other adults | 15.44 \pm 0.19, 15.03–15.69, n = 16 | 14.95 \pm 0.21, 14.59–15.28, n = 16 | 8.56 \pm 0.14, 8.23–8.77, n = 16 | 4.09 \pm 0.12, 3.83–4.29, n = 16 | 8.57 \pm 0.15, 8.28–8.90, n = 16 | 8.00 \pm 0.15, 7.72–8.21, n = 16 | 5.85 \pm 0.16, 5.54–6.09, n = 16 | 11.41 \pm 0.25, 10.74–11.74, n = 16 |
| <i>Miniopterus fraterculus</i> | 14.60 | 13.90 | 8.20 | 3.70 | 8.15 | 7.70 | 4.70 | 10.70 |
| Holotype BMNH 1905.5.7.18 | | | | | | | | |
| Topotype BMNH 1905.5.7.22 | 14.50 | 14.00 | 8.10 | 3.80 | 8.15 | 7.60 | 4.80 | 10.50 |
| Other adults | 14.36 \pm 0.14, 14.05–14.70, n = 15 | 13.82 \pm 0.15, 13.49–14.11, n = 15 | 7.92 \pm 0.16, 7.50–8.15, n = 15 | 3.75 \pm 0.10, 3.47–3.87, n = 15 | 8.09 \pm 0.13, 7.80–8.34, n = 15 | 7.43 \pm 0.13, 7.24–7.69, n = 15 | 5.28 \pm 0.21, 4.90–5.60, n = 15 | 10.39 \pm 0.27, 10.03–10.80, n = 15 |
| <i>Miniopterus minor</i> | 14.3 \pm 0.21, 13.77–14.57, n = 10 | 13.52 \pm 0.18, 13.13–13.69, n = 10 | 7.72 \pm 0.09, 7.57–7.88, n = 10 | 3.49 \pm 0.09, 3.32–3.60, n = 10 | 7.81 \pm 0.13, 7.59–8.03, n = 10 | 7.23 \pm 0.13, 7.02–7.42, n = 10 | 3.56 \pm 0.11, 3.40–3.75, n = 10 | 9.64 \pm 0.13, 9.38–9.80, n = 10 |
| <i>Miniopterus inflatus</i> | 16.50 | 15.75 | 8.90 | 3.90 | 8.90 | 8.10 | 5.80 | 12.60 |
| Holotype BM 1903.2.4.8 | | | | | | | | |
| Other adults | 16.48 \pm 0.39, 15.60–17.10, n = 21 | 15.92 \pm 0.38, 15.20–16.70, n = 21 | 8.99 \pm 0.32, 8.30–9.66, n = 21 | 4.06 \pm 0.19, 3.60–4.34, n = 21 | 8.83 \pm 0.26, 8.20–9.10, n = 21 | 8.16 \pm 0.26, 7.40–8.45, n = 21 | 6.02 \pm 0.36, 5.20–6.73, n = 21 | 12.38 \pm 0.49, 11.40–13.27, n = 21 |

TABLE 5. Dental measurements (mm) of specimens of the small *Miniopterus* species from southern Africa. Measurements presented as mean \pm standard deviation, range and sample size (n).

| Specimen or taxon | I-M ³ | C-M ³ | C-C | M ² -M ³ | UPMOLS | i-m ³ | LWMOLS |
|---|--|--|--|--|--|--|--|
| <i>Miniopterus mossambicus</i> sp. nov. | 7.03 | 5.78 | 4.29 | 5.96 | 4.79 | 7.34 | 5.77 |
| Holotype FMNH 213651 | | | | | | | |
| Other possible specimens | 6.68 \pm 0.18, 6.40–7.00, n = 12 | 5.49 \pm 0.15, 5.20–5.73, n = 12 | 4.10 \pm 0.15, 3.90–4.40, n = 12 | 5.95 \pm 0.14, 5.67–6.12, n = 12 | 5.02 \pm 0.34, 4.49–5.50, n = 12 | 7.11 \pm 0.18, 6.90–7.40, n = 12 | 5.60 \pm 0.20, 5.30–5.83, n = 12 |
| <i>Miniopterus natalensis</i> | 6.80 | 5.60 | 3.90 | 5.95 | 5.20 | 6.90 | 6.00 |
| Holotype BMNH 1848.6.12.19 | | | | | | | |
| Other adults | 6.85 \pm 0.20, 6.40–7.13, n = 16 | 5.74 \pm 0.10, 5.58–5.93, n = 16 | 4.42 \pm 0.14, 4.08–4.61, n = 16 | 6.29 \pm 0.15, 5.87–6.45, n = 16 | 4.88 \pm 0.13, 4.67–5.12, n = 16 | 7.54 \pm 0.17, 7.26–7.93, n = 16 | 5.97 \pm 18, 5.68–6.23, n = 16 |
| <i>Miniopterus fraterculus</i> | 6.35 | 5.10 | 3.80 | 5.50 | 4.75 | 6.70 | 5.10 |
| Holotype BMNH 1905.5.7.18 | | | | | | | |
| Topotype BMNH 1905.5.7.22 | | | | | | | |
| Other adults | 6.35 | 5.15 | 3.90 | 5.60 | 4.80 | 6.90 | 5.20 |
| | 6.44 \pm 0.05, 6.36–6.51, n = 15 | 5.32 \pm 0.06, 5.20–5.43, n = 15 | 3.92 \pm 0.09, 3.78–4.10, n = 15 | 5.70 \pm 0.13, 5.51–5.90, n = 15 | 4.57 \pm 0.25, 4.27–5.20, n = 15 | 6.98 \pm 0.15, 6.77–7.24, n = 15 | 5.47 \pm 0.12, 5.30–5.66, n = 15 |
| <i>Miniopterus minor</i> | 6.29 \pm 0.10, 6.08–6.41, n = 10 | 5.29 \pm 0.08, 5.14–5.40, n = 10 | 4.01 \pm 0.06, 3.92–4.09, n = 10 | 5.62 \pm 0.11, 5.36–5.76, n = 10 | 4.45 \pm 0.09, 4.26–4.56, n = 10 | 6.59 \pm 0.09, 6.36–6.68, n = 10 | 5.01 \pm 0.08, 4.86–5.14, n = 10 |
| Other adults | 7.60 | 6.10 | 4.80 | 6.70 | 6.00 | 8.00 | 6.60 |
| <i>Miniopterus inflatus</i> | | | | | | | |
| Holotype BM 1903.2.4.8 | | | | | | | |
| Other adults | 7.56 \pm 0.24, 7.10–8.02, n = 21 | 6.28 \pm 0.22, 5.75–6.65, n = 21 | 4.77 \pm 0.21, 4.40–5.25, n = 21 | 6.77 \pm 0.23, 6.30–7.22, n = 21 | 5.54 \pm 0.30, 5.16–6.20, n = 21 | 8.05 \pm 0.34, 7.30–8.67, n = 21 | 6.41 \pm 0.26, 5.90–6.83, n = 21 |

cranio-dental measurements (Tables 3 and 4) than *M. natalensis*. While there is considerable overlap of cranio-dental measurements in univariate space (Figure 4), these two species separate out in multidimensional space (Figure 5). Further, these two taxa show 17.7% genetic divergence (Table 2). These two species almost certainly overlap in distribution, although the northern limits of *M. natalensis* are not yet clearly defined (Monadjem *et al.* 2010a).

The type specimen of *M. natalensis arenarius* (USNM 181811) taken at Guaso Nyuki, Kenya, has cranial measurements (GSKL = 14.8 mm, CIL = 14.3 mm) within the range of *M. mossambicus*, but a larger forearm length (45 mm). The paratype has an even larger forearm length (47 mm). Hence, based on this information, *M. n. arenarius* has different body proportions to *M. mossambicus*. Recently, a distinct taxon has been identified from south-western Arabia and Ethiopia which was labelled *Miniopterus cf. arenarius* (Sramek *et al.* 2013). The authors were unable to confirm whether this species referred to *M. arenarius* since they did not have comparative material from the type location. We note that *M. mossambicus* has similar cranio-dental measurements to *M. cf. arenarius*, but no external measurements were reported for the latter taxon. Since *M. mossambicus* is clearly distinguishable from the type specimen of *M. arenarius* based on forearm length, we suspect that it will also be shown to be similarly separable from *M. cf. arenarius*. If *M. cf. arenarius* is shown to be distinct from *M. arenarius*, then it is also probably distinct from *M. mossambicus* since the distributions of the two species are separated by that of *M. arenarius*. Therefore, biogeographically, it would be highly unlikely for *M. cf. arenarius* and *M. mossambicus* to be conspecific if either was distinct from *M. arenarius*. The type specimen of *M. n. vicinior* (AMNH 49019) from Aba, north-eastern DRC, has a forearm length (43.2 mm) within the range of that of *M. mossambicus*; however the former taxon is distinctly smaller in dental measurements (C-M³ = 5.1 mm, C-C = 3.8 mm), which fall within the range of *M. minor*. The taxon *M. s. smitianus* is only known from the arid parts of Namibia. The specimens in the BMNH (1935.1.6.44, 1935.9.1.80, 1935.9.1.82) identified as this form had CIL = 14.8–14.9 mm, well outside the range of *M. mossambicus* (see Table 4). *Miniopterus s. villiersii* is known from rainforests of Central and West Africa (Simmons 2005) and, based on the type series, has an overlapping forearm length (44.2–46.0 mm) with *M. mossambicus*, but larger and non-overlapping GSKL (15.3–15.9 mm) (Aellen 1956). On the basis of these comparisons, it was possible to eliminate other named taxa of *Miniopterus* from mainland sub-Saharan Africa as possible candidates for the animals we name here as *M. mossambicus*.

TABLE 6. Eigenvector loadings for the first three components of the principal component analysis of log-transformed cranial and dental measurements of *Miniopterus mossambicus* sp. nov., *M. natalensis*, *M. fraterculus* and *M. minor*.

| | PC1 | PC2 | PC3 |
|--------------------------------------|--------|---------|---------|
| GSKL | 0.3750 | 0.4395 | 0.0617 |
| CIL | 0.4268 | 0.2239 | 0.0965 |
| ZYGO | 0.2386 | 0.1243 | -0.3488 |
| POB | 0.1583 | -0.0015 | -0.3315 |
| MAST | 0.2158 | 0.0357 | -0.4226 |
| GBW | 0.2246 | 0.0567 | -0.3932 |
| MAND | 0.4844 | -0.5423 | -0.1282 |
| I ¹ -M ³ | 0.1720 | 0.0405 | 0.2101 |
| C-M ³ | 0.1470 | 0.1001 | 0.3039 |
| C-C | 0.1491 | 0.3526 | 0.2083 |
| M ³ -M ³ | 0.2151 | 0.2426 | 0.1588 |
| UPMOLS | 0.1323 | 0.0092 | 0.1932 |
| i ¹ -m ³ | 0.2512 | -0.2974 | 0.3314 |
| LWMOLS | 0.2517 | -0.3991 | 0.2309 |
| Cumulative total variation explained | 89.2% | 92.4% | 94.6% |

The PCA analysis showed that morphologically similar or geographically overlapping *Miniopterus* spp. (*M. minor*, *M. natalensis* and *M. fraterculus*), as compared to *M. mossambicus*, have measurements that occupied

different regions of morphospace (Figure 4). The first two axes of the PCA accounted for 90.2% of the total variance (Table 6). All variable loadings on PC1 were positive indicating a general size vector, whereas PC2 represented a shape vector contrasting high positive (LW) and high negative (UPMOLS, MAND) character loadings (Table 6). *Miniopterus mossambicus* occupies a region of morphospace intermediate between *M. natalensis* and *M. fraterculus* (Figure 4).

Biology. *Miniopterus mossambicus* is definitively known from two specimens collected on the outskirts of Nampula, northern Mozambique. However, we have examined 12 other *Miniopterus* specimens from Mozambique and neighbouring countries that share cranial and dental features with *M. mossambicus*. We therefore tentatively identify these specimens as *M. mossambicus*; the majority of which were collected from highland areas of Mozambique, Malawi and Zimbabwe, although the two type specimens were obtained at lower altitudes.

Miniopterus natalensis in South Africa is known to migrate between high-altitude hibernacula and low-altitude maternity roosts (van der Merwe 1975). Five of the 14 specimens of *M. mossambicus* were collected from old mines, indicating that it uses caves as day roosts, typical for the genus (Monadjem *et al.* 2010a). The possibility that *M. mossambicus* employs a similar migratory strategy needs to be investigated. Nothing can be inferred about the reproductive biology of the species.

Release calls were recorded (using an Anabat II bat detector) from a single putative specimen of *M. mossambicus* captured at Gurue, northern Mozambique (forearm length = 44.0 mm). The frequency of the knee of its echolocation call was 55 kHz, which is similar to that of *M. natalensis* (55.6 kHz) but significantly lower than that of *M. fraterculus* (58.4 kHz) from Swaziland (Monadjem *et al.* 2010a).

Discussion

In this paper, we describe a new species of *Miniopterus*, *M. mossambicus*, based on material obtained in the Nampula Province of Mozambique, which has been characterized using molecular genetics and different morphological characters. Further, specimens from other areas of Mozambique, as well as Malawi and Zimbabwe, are tentatively assigned to this taxon based on morphological characters. While research has been conducted on South African *Miniopterus* (Stoffberg *et al.* 2004; Miller-Butterworth *et al.* 2005) to re-evaluate historical species definitions, the current paper is one of the first attempts to resolve such questions for members of this genus in other areas of sub-Saharan Africa. Reference to an undescribed species was also made by Miller-Butterworth *et al.* (2005) based on two specimens from Zambia, which appear to refer to *M. mossambicus* (see Figure 1). This suggests that *M. mossambicus* may have a wide distribution in south-central Africa.

Morphologically, *M. mossambicus* closely resembles *M. natalensis*, but is typically larger than *M. fraterculus* and *M. minor*. As has been shown for *Miniopterus* on islands in the Western Indian Ocean, molecular tools have been paramount to separate aspects of intraspecific variation from morphological convergence, which is prevalent amongst members of this genus (Weyeneth *et al.* 2008; Goodman *et al.* 2010, 2011). On the basis of clade assignment, associated specimens can be sorted and concordant morphological characters, such as tragus shape or differences in body size, defined. Across its Old World range, two to three body forms of *Miniopterus* can be found in sympatry: medium- and large-sized, often with a small-sized form occurring together. On the basis of the 11 species of *Miniopterus* known from Madagascar, body size is not a plesiomorphic character, with small species, for example, falling out in different portions of the phylogeny and mixed together with medium- and large-sized taxa (Goodman *et al.* 2011). This underlines an important level of plasticity in phylogenetic constraints related with size, presumably associated with reducing competition with sympatrically occurring taxa, but, on the other hand, considerable morphological canalization in the evolution of body form, with only three generalized evolutionary solutions.

With the description of *M. mossambicus* herein, we have cracked open Pandora's box associated with the systematics of sub-Saharan *Miniopterus*. Numerous problems remain to be resolved and significant portions of the currently recognized taxa are probably paraphyletic. For example, *M. minor* was described from coastal Tanzania and populations assigned to this taxon from the Congo Basin maybe specifically distinct (Juste & Ibáñez 1992; Monadjem *et al.* 2010a). *Miniopterus natalensis*, described from the Knysna region of South Africa, is currently considered to range into Sudan and the Arabian Peninsula (Simmons 2005). Given the considerable habitat variation across the range of this species and broad geographical distribution, molecular data are needed to test the question of monophyly in this taxon.

With the description of a new species from south-eastern Africa herein, some advances are being made in the regional systematics of this genus. However, portions of tropical central and western Africa remained to be studied based on specimens with associated tissues. As has been demonstrated for Malagasy *Miniopterus* (Goodman *et al.* 2007), a critical step in resolving species limits and attaching binomial names to different clades is the sequencing of type or topotypic specimens. This will be a key step in resolving the systematics of sub-Saharan *Miniopterus*.

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