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Morphological, bioacoustical, and genetic variation in *Miniopterus* bats from eastern Madagascar, with the description of a new species

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Abstract

Recent molecular genetic work, combined with morphological comparisons, of Malagasy members of the bat genus *Miniopterus* (Family Miniopteridae), has revealed several cryptic species. Based on new specimens and associated tissues, we examine patterns of variation in the recently described species *M. petersoni*, the holotype of which comes from extreme southeastern Madagascar, and for which specimens from more northerly portions of eastern Madagascar were noted to show some morphological divergence from typical *M. petersoni*. On the basis of morphological and genetic (cytochrome *b*) characters we described a new species, *M. egeri* **sp. nov.** This taxon also shows bioacoustical differences from *M. petersoni*. *Miniopterus egeri* is widely distributed in the eastern portion of Madagascar across an elevational range from near sea level to 550 m. The specific status of moderately small *Miniopterus* from Montagne d'Ambre in the far north remains to be determined.

Key words: taxonomy, morphology, molecular genetics, *Miniopterus,* new species, eastern Madagascar, cryptic species

Résumé

Les études moléculaires récentes combinées avec des comparaisons morphologiques des spécimens de chauves-souris malgaches du genre *Miniopterus* (Famille des Miniopteridae) ont permis de révéler l'existence d'espèces cryptiques. Basé sur des spécimens nouvellement collectés associés à leurs tissus, nous avons examiné la variation de *M. petersoni* qui est une espèce récemment décrite dont l'holotype vient de l'extrême sud-est de Madagascar. Dans la partie Est malgache, les spécimens récoltés plus au nord montrent une divergence morphologique notable par rapport au *M. petersoni* typique. En se basant sur des caractères morphologiques et génétiques (cytochrome *b*), nous avons décrit une nouvelle espèce, *M. egeri* **sp. nov.** Apparemment, des différences bioacoustiques séparent *M. egeri* **sp. nov.** de *M. petersoni*. *Miniopterus egeri* est largement distribué dans la partie Est de Madagascar à travers une gamme d'altitude allant du niveau de la mer à 550 m. Le statut taxonomique du *Miniopterus* de taille moyenne de la Montagne d'Ambre dans l'extrême nord de Madagascar demeure encore incertain.

Introduction

The bat genus *Miniopterus* Bonaparte, 1837, often referred to as "long-fingered bats", and belonging to the family Miniopteridae, is broadly distributed in the Old World. Recent research employing molecular genetics has found that the evolutionary history of the genus is far more complex than classical taxonomic studies indicate based on

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morphological characters. Numerous cryptic *Miniopterus* species have been uncovered that are not closely related and that demonstrate considerable paraphyly within the genus (Cardinal & Christidis 2000; Appleton *et al*. 2004; Miller-Butterworth *et al*. 2005; Goodman *et al*. 2007; Furman *et al*. 2010). Implicit with these studies, as well as numerous other groups of mammals, it is clear that measures of species diversity have been greatly underestimated and genetic tools provide a powerful means to recognize patterns of dispersal history, isolation, and subsequent speciation.

In the past few years, morphological and molecular genetic studies of Malagasy *Miniopterus* have disclosed an unexpected diversity of species. Peterson *et al*. (1995) in their monograph on the bats of Madagascar listed four *Miniopterus* from the island: *M. manavi* Thomas, 1906, a widely distributed species showing notable phenotypic variation; *M. majori* Thomas, 1906, largely restricted to montane zones of the central region; *M. fraterculus* Thomas & Schwann, 1906, also known mostly from montane areas and also occurring in portions of southern Africa; and *M. gleni* Peterson, Eger & Mitchell, 1995, with a relatively broad distribution on the island. Subsequently, *M. manavi* as previously configured has been found to be paraphyletic and composed of at least four species; *M. majori* has a broader range on the island than previously recognized; *M. fraterculus* is strictly African and the Malagasy populations formerly assigned to this taxon include at least two endemic species (*M. sororculus* Goodman, Ryan, Maminirina, Fahr, Christidis & Appleton, 2007; *M. petersoni* Goodman, Bradman, Maminirina, Ryan, Christidis & Appleton, 2008); and a sister species to *M. gleni* has been described (Goodman *et al*. 2007, 2008, 2009a, 2009b, 2010; Maminirina *et al*. 2009). This brings the currently recognized number of *Miniopterus* taxa on Madagascar to 10 (Table 1), of which 80% are endemic to the island and 100% are endemic to the Malagasy region (Madagascar and the Comoros).

TABLE 1. List of previously known species of *Miniopterus* from Madagascar and nearby islands.

Current name	Previously considered (Peterson *et al*. 1995)	Range
M. aelleni	*M. manavi*	Endemic to Anjouan and Madagascar
M. brachytragos	*M. manavi*	Endemic to Madagascar
M. gleni	*M. gleni*	Endemic to Madagascar
M. griffithsi	*M. gleni*	Endemic to Madagascar
M. griveaudi	*M. manavi griveaudi*	Endemic to Anjouan, Grande Comore, and Madagascar
M. mahafaliensis	*M. manavi*	Endemic to Madagascar
M. manavi	*M. manavi*	Endemic to Madagascar
M. majori	*M. majori*	Endemic to Madagascar
M. petersoni	*M. fraterculus*	Endemic to Madagascar
M. sororculus	*M. fraterculus*	Endemic to Madagascar

The *M. petersoni* holotype originated from near Tolagnaro in the extreme southeastern portion of the island (Figure 1). In its description, Goodman *et al*. (2008) noted that animals identified as this taxon from central southeastern Madagascar showed some statistically significant differences in cranial measurements as compared with individuals from the holotype locality. Further, these authors tentatively assigned a number of specimens from the central east and northeast to *M.* cf. *petersoni*. Subsequently, additional specimens, with associated tissues and bioacoustic recordings have been collected of moderately small members of this genus from a variety of eastern localities, that provide the means to examine the taxonomic identity of these animals. This is the subject of the current paper using morphology, molecular genetics, and bioacoustics.

Material and methods

Specimens. Material for the morphological and molecular genetic analyses presented herein are housed in the following museums: BMNH—The Natural History Museum (formerly The British Museum of Natural History), London; FMNH—Field Museum of Natural History, Chicago; UADBA—Département de Biologie Animale,

Université d'Antananarivo, Antananarivo; and USNM—The National Museum of Natural History (formerly The United States National Museum), Washington, D.C.

Access and comparisons to type specimens. We were able to directly compare specimens of the new species described herein to the following holotypes: *Miniopterus petersoni* (FMNH 194136) obtained at Madagascar: Province de Toliara, Cascade de Manantantely, 5.2 km NW of Tolagnaro, 24°59.343'S, 46°55.370'E, 65 m, and *M. sororculus* (FMNH 177259) collected at Madagascar: Province de Fianarantsoa, 3 km S Ambatofinandrahana, 20°34.321'S, 46°48.530'E, 1450 m. Further, we have examined and measured the holotypes of *M. manavi* (BMNH 97.0.1.37) and *M. majori* (BMNH 97.9.1.38) obtained at Imasindrary, N. E. Betsileo (Thomas 1906).

Morphological study. Six standard external measurements (in millimeters) were taken from Malagasy specimens of *Miniopterus* spp., in the field before preparation, using a ruler to an accuracy of 0.5 mm, and included: total length, tail length, hind foot length (not including claw), tragus length, ear length, and forearm length. Many of the recent specimens from Madagascar were collected by SMG and the external measurements of these animals were consistently taken and, unless specified, these data are not combined amongst different field collectors. Mass was taken with a spring balance and recorded in grams to an accuracy of 0.5 g. Measurements are reported for adults only (defined by presence of a fully erupted permanent dentition and fused basisphenoid-basioccipital suture).

Nine cranial or mandibular and five dental measurements were taken using a digital caliper to the nearest 0.1 mm. CRANIAL MEASUREMENTS include: greatest skull length (GSKL), from posterior-most part of occipital to anterior-most point of upper incisors; condyloincisive length (CIL), from occipital condyles to anterior-most point of upper incisors; greatest zygomatic breadth (ZYGO), width taken across zygomatic arches at the widest point; postorbital breadth (POB), dorsal width at most constricted part of skull; mastoid breadth (MAST), maximum width of skull across mastoid processes; greatest braincase width (GBW), breadth at widest portion of braincase; palatal length (PAL), from posterior edge of palatal emargination to anterior edge of mesopterygoid fossa; lachrymal width (LW), greatest width across rostrum at lachrymal projections; and mandible length (MAND), from the posterior-most portion of the condyles to anterior-most point of upper incisors. The DENTAL MEASUREMENTS include: complete cranial toothrow (I^1-M^3), length from anterior alveolar border of incisors to posterior alveolar border of 3rd molar (M^3); complete canine-molar toothrow (C-M^3), length from anterior alveolar border of canine to posterior alveolar border of 3rd molar (M^3); width across upper canines (C^1-C^1), taken across the outer alveolar borders of the canines; width across 3rd upper molars (M^3-M^3), taken across the outer alveolar borders of the 3rd molars; and complete mandibular toothrow (i_1-m_3), length from anterior alveolar border of incisors to posterior alveolar border of 3rd molar (m_3). Tooth abbreviations include: I = incisor, C = canine, P = premolar, and M = molar. Upper case abbreviations are used for upper teeth and lower case abbreviations for lower teeth.

Echolocation calls recording and analysis. Echolocation calls of *Miniopterus* spp. were recorded as they flew inside one of two different style flight cages that were enclosed by fine meshed fabric. Animals were subsequently captured and retained as voucher specimens. Calls from the *Miniopterus* described herein (FMNH 209160 and 209161) were recorded directly onto an ASUS EEE 1005HA netbook (ASUSTek Computer Inc., Taiwan) with an Avisoft UltraSound Gate 116 Bat detector (Avisoft Bioacoustics, Berlin, Germany) as they flew inside a 1.8 x 1.4 x 5.4 m flight cage. Calls from *M. manavi* sensu stricto (FMNH 209178 and 209179) and *M. petersoni* (FMNH 209186) were recorded onto a Sony Net MD Walkman MZ-N505 (Sony Corporation, Japan) with a Pettersson detector (D240X, Pettersson Elektronik AB, Uppsala, Sweden) at 10x time expansion as they flew inside a 3 x 3 x 3 m flight cage. The resultant wave file was analysed in BatSound Pro (version 3.2, Pettersson Elektronik AB, Uppsala, Sweden) at a sampling rate of 500 kHz (16 bits, mono) for Avisoft recordings and 44.1 kHz (16 bits, mono) for Pettersson recordings, and at threshold of 16.

A single high quality search phase call from each individual bat was selected for analysis based on a high signal to noise ratio (Weller *et al*. 2007). From each call, peak echolocation frequency (PF), maximum frequency (Fmax), minimum frequency (Fmin), duration (Dur), and inter-pulse interval (IPI) were measured (Russ 1999). Fmax, Fmin, Dur, and IPI were measured on the spectrogram and PF on the power spectrum (Kofoky *et al*. 2009). To control for possible geographic variation in echolocation calls, bats were recorded from or near the type localities of the three species analyzed herein. Given small sample sizes of many of the compared taxa, only the PF parameter was used for statistical comparisons.

Statistical analyses. Univariate statistical analyses were conducted for each of the measured variables and no evidence of sexual dimorphism was found. Hence, in all of the comparisons presented herein the sexes of each spe-

cies are combined. In order to distinguish between morphologically similar moderately small Malagasy *Miniopterus*, Principal Component Analyses (PCA) were conducted using the statistical package Statistica (version 7.0); data were log-transformed and the unrotated option was used. Separate PCA analyses were conducted for external, cranial, and dental measurements. Associated with the bioacoustic analyses, one-way ANOVAs with post hoc Tukey tests were used to compare PF between *Miniopterus* spp.

Genetic study. Specimens were genetically analyzed to determine the level of differentiation of the *Miniopterus* described herein, and to investigate the relationships between this new taxon and previously named Malagasy species. Specimens used for genetic analysis and the location of their capture can be found in Table 2. The entire cytochrome *b* gene was chosen due to its widespread use in similar studies (e.g. Cardinal & Christidis 2000; Miller-Butterworth *et al*. 2005; Goodman *et al*. 2007, 2010). The dataset used in this article includes sequences previously described (Table 2) and new sequences of Malagasy *Miniopterus* spp. to ensure taxonomic completeness. *Miniopterus fraterculus* from South Africa was included as the outgroup.

TABLE 2. Details of *Miniopterus* specimens used in the genetic analyses. For museum acronyms see under Materials and Methods. Some specimens remain uncataloged and the field collector numbers are used, SMG = S.M. Goodman and GKC = G. K. Creighton.

Species	Specimen label	Field No.	Locality	GenBank No.	Author
M. manavi	FMNH 5650	--	Madagascar	FJ383128	Goodman *et al*. (2009a)
M. manavi	FMNH 187662	SMG 14753	Madagascar	FJ383129	Goodman *et al*. (2009a)
M. manavi	FMNH 194074	SMG 15397	Madagascar	FJ383130	Goodman *et al.* (2009a)
M. manavi	UADBA 43171	SMG 16288	Madagascar	HQ619934	This study
M. manavi	UADBA 43172	SMG 16294	Madagascar	HQ619935	This study
M. petersoni	USNM 577096	GKC 3318	Madagascar	FJ383131	Goodman *et al.* (2008)
M. petersoni	USNM 577097	GKC 3319	Madagascar	EU091258	Goodman *et al.* (2008)
M. petersoni	FMNH 194136	SMG 15833	Madagascar	EU091257	Goodman *et al.* (2008)
M. petersoni	UADBA GKC 3356	GKC 3356	Madagascar	EU091255	Goodman *et al.* (2008)
M. petersoni[1]	USNM 577098	GKC 3326	Madagascar	N/A	Goodman *et al.* (2008)
M. petersoni[2]	USNM 577102	GKC 3353	Madagascar	N/A	Goodman *et al.* (2008)
M. petersoni[3]	USNM 577104	GKC 3360	Madagascar	N/A	Goodman *et al.* (2008)
M. petersoni[1]	USNM 577106	GKC 3364	Madagascar	N/A	Goodman *et al.* (2008)
M. petersoni[1]	USNM 577127	GKC 3565	Madagascar	N/A	Goodman *et al.* (2008)
M. petersoni	FMNH 209186	SMG 16806	Madagascar	HQ619936	This study
M. aelleni	UADBA SMG 16182	SMG 16182	Madagascar	FJ383135	Goodman *et al.* (2009a)
M. aelleni	FMNH 202453	SMG 16175	Madagascar	FJ383148	Goodman *et al*. (2009a)
M. aelleni	FMNH 173070	SMG 12812	Madagascar	FJ232795	Weyeneth *et al.* (2008)
M. aelleni	FMNH 194420	SMG 15556	Anjouan	FJ232801	Weyeneth *et al.* (2008)
M. griveaudi	FMNH 173101	SMG 12902	Madagascar	FJ383136	Goodman *et al.* (2009a)
M. griveaudi	FMNH 194289	SMG 15483	Grande Comore	FJ383137	Goodman *et al.* (2009a)
M. griveaudi	FMNH 194433	SMG 15650	Anjouan	FJ383138	Goodman *et al.* (2009a)
M. griveaudi	FMNH 175839	SMG 13024	Madagascar	FJ383142	Goodman *et al.* (2009a)
M. sororculus	FMNH 177259	SMG 13573	Madagascar	DQ899771	Goodman *et al*. (2007)
M. sororculus	FMNH 177264	SMG 13560	Madagascar	DQ899773	Goodman *et al*. (2007)
M. sororculus	FMNH 177267	SMG 13576	Madagascar	HQ619937	This study
M. sororculus	FMNH 209183	SMG 16785	Madagascar	HQ619938	This study
M. majori	UADBA 43264	SMG 16800	Madagascar	HQ619953	This study
M. majori	UADBA 43261	SMG 16797	Madagascar	HQ619954	This study

continued next page

TABLE 2. (continued)

Species	Specimen label	Field No.	Locality	GenBank No.	Author
M. majori	UADBA 43198	SMG 16445	Madagascar	HQ619955	This study
M. majori	FMNH 202518	SMG 16042	Madagascar	HQ619939	This study
M. egeri	USNM 448938	GKC 2581	Madagascar	HQ619940	This study
M. egeri	USNM 449202	GKC 2728	Madagascar	HQ619941	This study
M. egeri	FMNH 202520	SMG 16057	Madagascar	HQ619942	This study
M. egeri	FMNH 202522	SMG 16067	Madagascar	HQ619943	This study
M. egeri	FMNH 202521	SMG 16058	Madagascar	HQ619944	This study
M. egeri	FMNH 202519	SMG 16056	Madagascar	HQ619945	This study
M. egeri	FMNH 187664	SMG 14765	Madagascar	HQ619946	This study
M. egeri	FMNH 187663	SMG 14764	Madagascar	HQ619947	This study
M. egeri	FMNH 209161	SMG 16603	Madagascar	HQ619948	This study
M. egeri	FMNH 202474	SMG 16128	Madagascar	HQ619949	This study
M. egeri	FMNH 209168	SMG 16634	Madagascar	HQ619950	This study
M. egeri	FMNH 209159	SMG 16601	Madagascar	HQ619951	This study
M. egeri	FMNH 209160	SMG 16602	Madagascar	HQ619952	This study
M. fraterculus	AJ841975		South Africa	AJ841975	Stadelmann *et al.* (2004)

[1] Same sequence as USNM 577096; [2] Same sequence as USNM 577097; [3] Same sequence as FMNH 194136.

Genomic DNA was extracted using a lithium chloride and chloroform extraction method as described by Gemmel and Akiyama (1996). The mitochondrial cytochrome *b* gene was amplified and sequenced using the primers L14724 and H15915 (Smith & Patton 1991). Template DNA was amplified by PCR in 25μL reaction volume containing the following: 1X reaction buffer (Promega), 2.5 mM $MgCl_2$, 0.2 μM of each dNTP, 0.28 μM of each primer, 1 unit of Taq polymerase (Promega), and approximately 100 ng of template DNA. Cycling consisted of an initial denaturation at 94ºC for 3 min, followed by 30 cycles at 94ºC for 30s, 50ºC for 30s and 72ºC for 40s, and a final extension of 72ºC for 3 min. The single PCR product was directly sequenced by a commercial company (Macrogen Inc.) using the ABI Prism BigDye Cycle Sequencing kit (Applied Biosystems, Perkin-Elmer). Sequencing products were visualized on an ABI 3730XL (Applied Biosystems, Perkin-Elmer). The sequences were aligned using Sequencher version 4.6 (Gene Codes Corporation). All new sequences were deposited in GenBank (accession numbers HQ619934-HQ619955; see also Table 2).

Sequence analyses. Analysis using DNA strider (Marck 1990) showed that sequences did not contain stop codons. Modeltest 3.6 (Posada & Crandall 1998) was used to determine the most appropriate model of molecular evolution. The model, HKY+G, was estimated from both the Hierarchical Likelihood Ratio tests and Akaike Information Criterion. Modeltest estimated parameter settings with base frequencies = 0.2873, 0.2916, 0.1396, 0.2815, and -lnL = 4078.9873, and shape parameter of gamma distribution = 0.1350.

Maximum Parsimony (MP) and Minimum Evolution (neighbor-joining, NJ) phylogenetic analyses were conducted using PAUP* 4.0 (Swofford 2003). Heuristic MP searches were conducted using the random addition option with 200 replications and the tree bisection and reconnection (TBR) branch-swapping algorithm. The NJ method used pairwise sequence distances estimated by HKY+G model. These are the distances reported in the Results section. Nodal support of MP and NJ trees was estimated by 1000 bootstrap pseudoreplicates.

Bayesian analysis was conducted using the program MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). The HKY+G model was specified, flat priors were used and starting trees were random. We ran four chains (three hot, one cold) for 1,000,000 generations, sampling trees every 100 generations. We made sure that our Bayesian runs achieved sufficient convergence by ascertaining that the average standard deviation of split frequencies between chains had reached below 0.01 (0.007369) at the end of the run and the potential scale reduction factor (PSRF) of each parameter s within $1.000 < PSRF < 1.006$. Plots of generation versus the log probabilities of observing actual data did not reveal any trends for the last 75% of generations. We excluded the first 2,500 generations from the calculation of posterior probabilities.

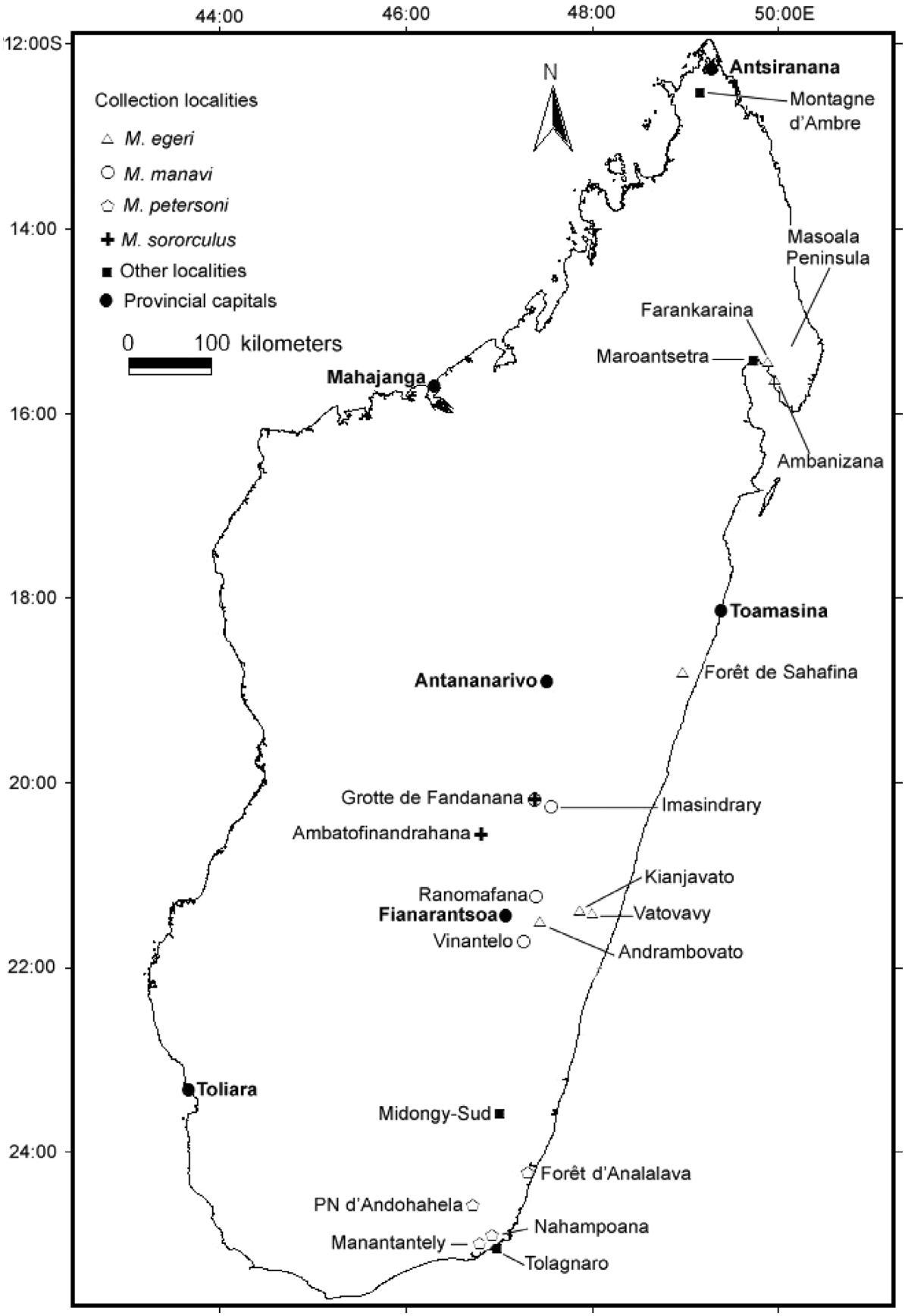

FIGURE 1. Map of localities mentioned in text associated with the known distribution of *Miniopterus egeri* **sp. nov.**, *M. manavi*, *M. petersoni*, and *M. sororculus*.

Results

To properly assess the level of differentiation of the moderately small individuals of *Miniopterus* obtained from a variety of localities in eastern Madagascar, different analyses using molecular and bioacoustic data sets are presented. Subsequently, this taxon is described as a new species, *M. egeri*, and comparative aspects of its morphology are discussed.

Molecular phylogenetics and phylogeography

The same tree topology was produced using three different phylogenetic analysis methods (Figure 2). The species described herein, *Miniopterus egeri*, consists of a monophyletic group that is most closely related to both the recently described *M. petersoni* and re-defined *M. manavi* (Goodman *et al.* 2008, 2009a, 2009b). The number of specimens of *M. manavi* sensu stricto is small due to difficulties in obtaining samples of this species. A minimum of 4.3% sequence divergence separates *M. egeri* from all other *Miniopterus* species used in the analysis. This level of sequence divergence (4.3%) is found only in the comparisons between *M. egeri*, *M. petersoni*, and *M. manavi* sensu stricto (Table 3). Sequence divergences in any other pairwise combination of Malagasy *Miniopterus* spp. are all greater than 8%.

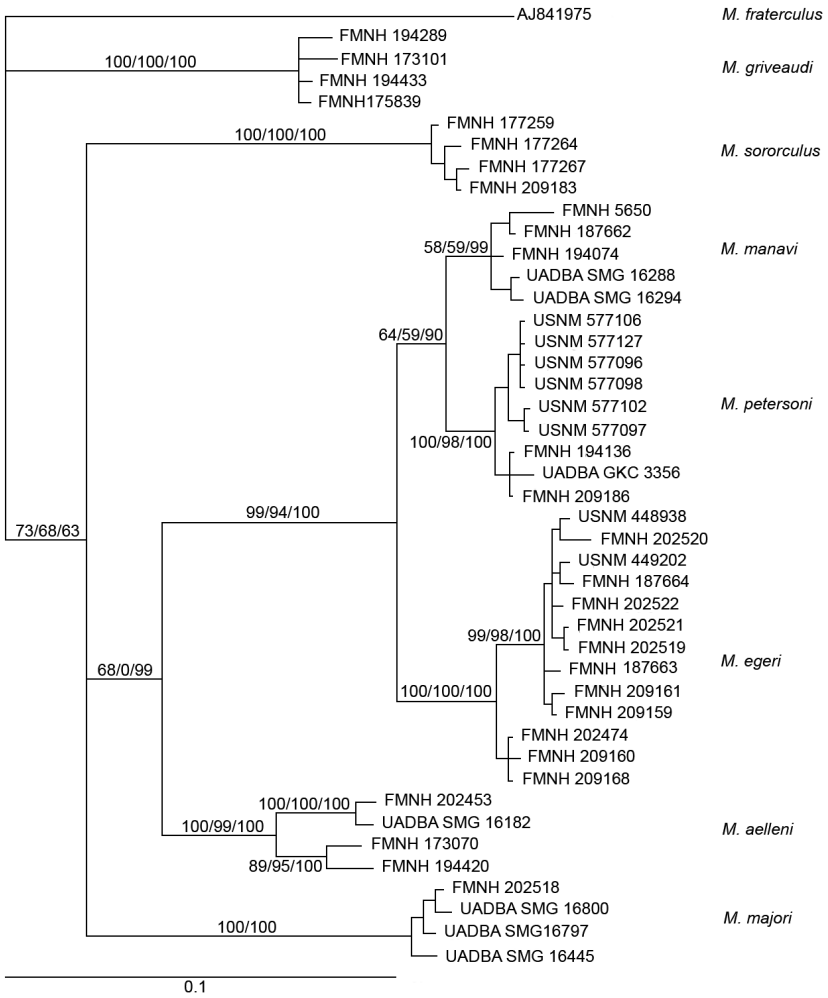

FIGURE 2. Phylogenetic position of *Miniopterus egeri* **sp. nov.** with respect to other Malagasy Miniopteridae taxa and the African outgroup *M. fraterculus*, based on the mitochondrial cytochrome *b* gene. The tree presented was produced using Bayesian analysis. Neighbor-joining, Maximum Parsimony bootstrap, and Bayesian posterior probabilities are indicated on the major nodes (NJ/PARS/BAYES). Labels include museum catalogue number and species identification is included to the right of each clade.

TABLE 3. Genetic distances within and between all major clades of *Miniopterus* spp. represented in the phylogenetic tree (Figure 2). Bold values on diagonal through table indicate the within species HKY85 distances, while the values above the diagonal represent the mean HKY85 distances between species. Intraspecific comparison for the single extralimital taxon (*fraterculus*) is not presented.

	manavi	*petersoni*	*egeri*	*aelleni*	*sororculus*	*majori*	*griveaudi*	*fraterculus*
manavi	**0.0067**	0.0253	0.0446	0.0821	0.1082	0.088	0.1074	0.1166
petersoni		**0.0052**	0.0433	0.0886	0.1118	0.0985	0.1072	0.1223
egeri			**0.0081**	0.0833	0.1182	0.1018	0.1162	0.1315
aelleni				**0.0263**	0.0934	0.0982	0.0932	0.1151
sororculus					**0.0046**	0.1078	0.1105	0.1217
majori						**0.0065**	0.1081	0.1164
griveaudi							**0.0039**	0.1072
fraterculus								--

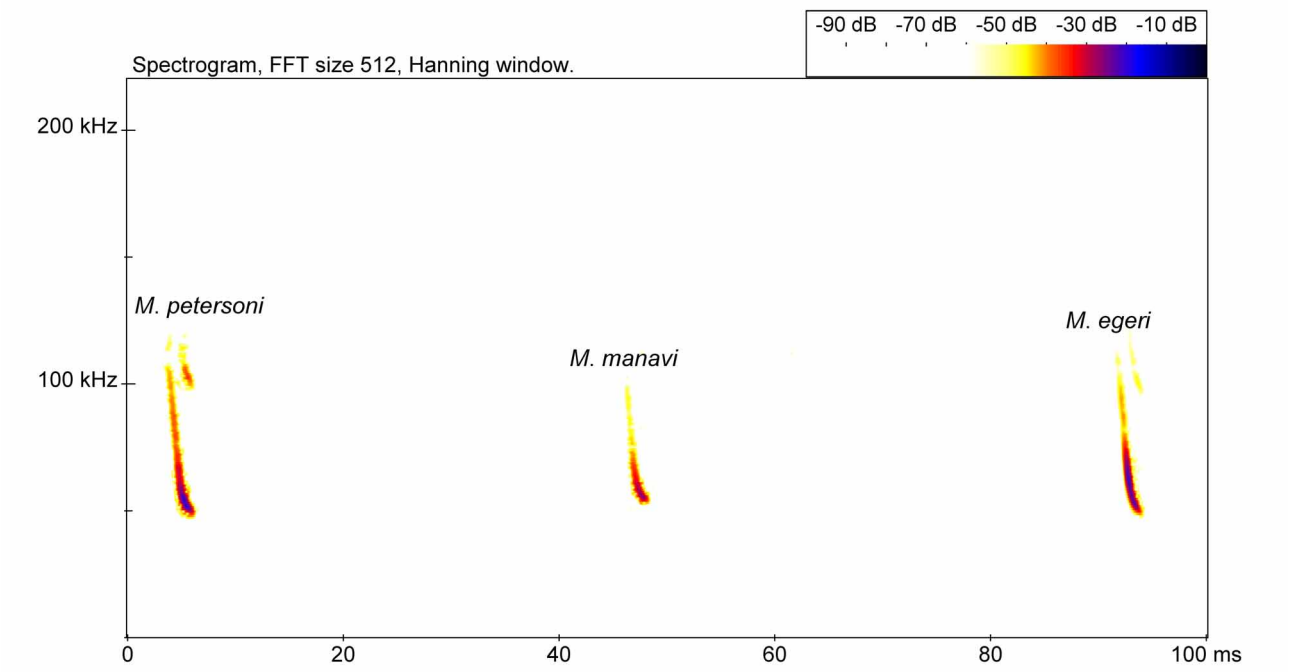

FIGURE 3. Low-duty-cycle, FM/QCF echolocation calls of *Miniopterus petersoni* (FMNH 209186), *M. manavi* sensu stricto (FMNH 209179), and *M. egeri* **sp. nov.** (FMNH 209160) recorded inside flight cages. PF of *M. manavi* (57.2 kHz) is significantly higher than the PFs of *M. egeri* (54.7 kHz) and *M. petersoni* (53.2 kHz). The power (or amplitude) of the echolocation calls are coded as different colors.

Within *M. egeri*, two distinct sub-clades were evident. The separation of the two sub-clades received strong statistical support in all phylogenetic analyses, but the average genetic distance was small (1.2%). Given that animals from the same locality (Sahafina) are morphologically indistinguishable from one another and are represented in both *M. egeri* sub-clades (FMNH 209159 and 209161 in one sub-clade and FMNH 202474, 209160, and 209168 in the other), this level of divergence is best interpreted as intraspecific variation.

Bioacoustics

Low-duty-cycle, frequency modulated/quasi-constant frequency (FM/QCF) echolocation calls of *Miniopterus egeri* (FMNH 209160, 209161), *M. petersoni*, and *M. manavi* typically swept from a maximum frequency ~100 kHz to a minimum frequency of ~50 kHz (Figure 3; Table 4). Species could be identified from calls based on PF:

mean PF was highest for *M. manavi* calls and lowest for *M. petersoni* calls (ANOVA: F=39.45, P<0.001; post hoc Tukey: *M. egeri* and *M. petersoni*, P=0.005; *M. manavi* and *M. petersoni*, P<0.001; and *M. manavi* and *M. egeri*, P<0.001; Figure 3, Table 4). Certain other measured bioacoustic variables showed differences between *M. egeri* and *M. petersoni* (Fmax) and between *M. manavi* and *M. egeri/petersoni* (Fmax, Dur, Fmin) (Table 4). However, given the small sample sizes we prefer not to use these differences in the diagnosis of the species described herein. The present study provides the first data on the echolocation calls of *M. manavi* sensu stricto obtained near its type locality (Fandriana in the Central Highlands), as well as *M. egeri* and *M. petersoni* also obtained at their respective type localities. Echolocation call characteristics of the three species are consistent with recordings from other *Miniopterus* species from southern and central Africa (Schoeman & Jacobs 2008; Monadjem *et al*. 2010). Furthermore, a previous study of bat bioacoustics showed little variation in the PF of calls from *Miniopterus* "*manavi*" bats recorded in flight cages, on zip lines, and during hand release (Kofoky *et al*. 2009) from different areas of Madagascar and almost certainly these animals represent other members of this genus than those used in our study. Nonetheless, echolocation characteristics of bats flying inside flight cages are probably similar to those of bats foraging or commuting in clutter habitats. Because call characteristics of clutter-edge bats such as miniopterids may change in different habitats (Obrist 1995; Barclay 1999; Schnitzler & Kalko 2001), it is possible that echolocation calls used by the three *Miniopterus* species may be more different or more similar to each other in clutter-edge or open habitats. Future studies should record Malagasy miniopterids in various habitats using different recording techniques to elucidate intra-specific or inter-specific variability of calls.

TABLE 4. Echolocation parameters (mean ± SD; minimum–maximum) of *Miniopterus egeri* **sp. nov.**, *M. manavi* sensu stricto, and *M. petersoni* recorded in flight cages. PF: peak echolocation frequency, Fmax: maximum frequency, Fmin: minimum frequency, Dur: duration and IPI: interpulse interval. Sample sizes are shown as number of bats and pulses analyzed.

Species	Number of bats/ number pulses	PF (kHz)	Fmax (kHz)	Fmin (kHz)	Dur (ms)	IPI (ms)
M. egeri	2/16	54.7 ± 1.02 53.2–56.3	113.8 ± 3.62 107.0–123.0	49.0 ± 0.52 48.0–50.0	2.9 ± 0.26 2.5–3.4	62.6 ± 12.57 43.2–81.1
M. manavi	2/9	57.2 ± 0.77 55.5–58.2	98.4 ± 7.60 89.0–110.0	53.0 ± 0.00 53.0–53.0	2.5 ± 0.32 2.1–3.0	66.0 ± 8.70 54.1–84.7
M. petersoni	1/6	53.2 ± 0.75 52.0–53.9	106.5 ± 6.66 95.0–115.0	49.0 ± 0.63 48.0–50.0	2.9 ± 0.32 2.5–3.3	71.0 ± 4.63 63.8–76.7

Systematics

Family Miniopteridae, Dobson, 1875

Genus *Miniopterus* Bonaparte, 1837

***Miniopterus egeri* sp. nov.**
Eger's long-fingered bat
Minioptère d'Eger
(Figures 4–7)

Synonyms
Miniopterus manavi Peterson, Eger & Mitchell, 1995, in part
Miniopterus petersoni Goodman, Bradman, Maminirina, Ryan, Christidis & Appleton, 2008, in part
Miniopterus cf. *petersoni* Goodman, Bradman, Maminirina, Ryan, Christidis & Appleton, 2008, in part

Holotype. FMNH 209160, adult female in reproductive condition, lactating and with one placental scar, body preserved in 12% formalin and subsequently transferred to 70% ethanol, skull removed and cleaned by Dermestidae beetles. The skull is in fine condition. Original field number Steven M. Goodman (SMG) 16602. This specimen was used in the morphological and molecular analyses, and bioacoustic information is also available.

Type locality. Madagascar: Province de Toamasina, Forêt de Sahafina, 9.5 km W Brickaville, 18º48’37”S, 48º58’48”E, 50 m. Captured in a harp trap along a trail in degraded lowland forest on 5 December 2009, collected by S.M. Goodman and B. Ramasindrazana.

FIGURE 4. Photograph of *Miniopterus egeri* **sp. nov.** (FMNH 209168) obtained at the type locality of Forêt de Sahafina, 9.5 km W Brickaville. (Image taken by Corrie Schoeman).

Referred specimens (specimens used in molecular analysis are marked in **bold**). Province de Fianarantsoa: USNM **448938**-448939, 0.5 km N Kianjavato, 21º23'S, 47º52'E, 300 m, 12 August 1987, G.K. Creighton and L.H. Emmons; FMNH **187663-187664**, Kianjavato, FOFIFA Station, 21º22’674”S, 47º52’152”E, 75 m, 25 September 2005, S.M. Goodman and F. Ratrimomanarivo; USNM 449204-449205, 2 km NE Andrambovato, 21º30'S, 47º54'E, 550 m, 19 August 1988, G.K. Creighton and J.M. Ryan; USNM **449202**-449203, Vatovavy, 9 km ESE Kianjavato, 21º24'S, 47º57'E, 150 m, 30 July 1988, G.K. Creighton and J.M. Ryan. Province de Toamasina: FMNH **202474**, Forêt de Sahafina, 9.5 km W Brickaville, 18º48.442’S, 48º58.924’E, 105 m, 9 December 2007, S.M. Goodman, M. Ruedi, and N. Weyeneth; FMNH **209159, 209160, 209161**, **209168**, Forêt de Sahafina, 9.5 km W Brickaville, 18º48’37”S, 48º58’48”E, 50 m, 5 & 10 December 2009, S.M. Goodman and B. Ramasindrazana; FMNH **202519**, **202520**, **202521**, Poste Forestier de Farankaraina, 7.5 km SW Mahalevona, 15º25.694’S,

49º50.717'E, 50 m, 24 November 2007, M. Ruedi and S.M. Goodman; FMNH **202522**, 1.1 km S Ambanizana, along Androka River, 15º38.053'S, 49º58.114'E, 5 m, 25 November 2007, S.M. Goodman, M. Ruedi, and N. Weyeneth. Some of these specimens were previously used in the diagnosis of *M. petersoni* (Goodman *et al*. 2008).

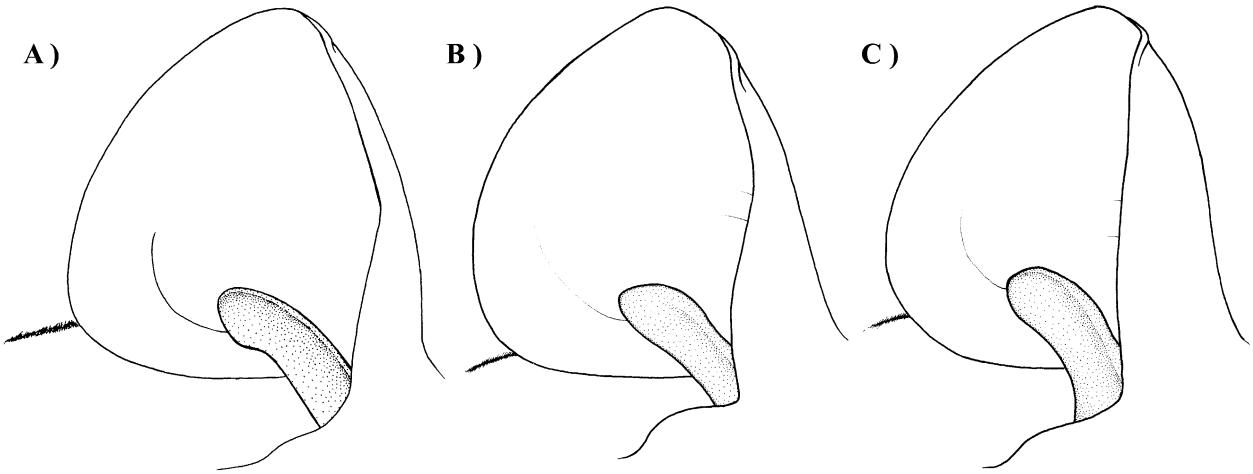

FIGURE 5. Lateral view of external ear and tragus of different Malagasy *Miniopterus* spp.: **A**—*M. egeri* **sp. nov.** (FMNH 209168) from Province de Toamasina, Forêt de Sahafina, 9.5 km W Brickaville; **B**—*M. petersoni* (FMNH 209186) from Province de Toliara, Cascade de Manantantely, 5.2 km NW of Tolagnaro; **C**—*M. sororculus* (FMNH 209181) from Province de Fianarantsoa, Grotte de Fandanana, 4.1 km NE de Fandriana.

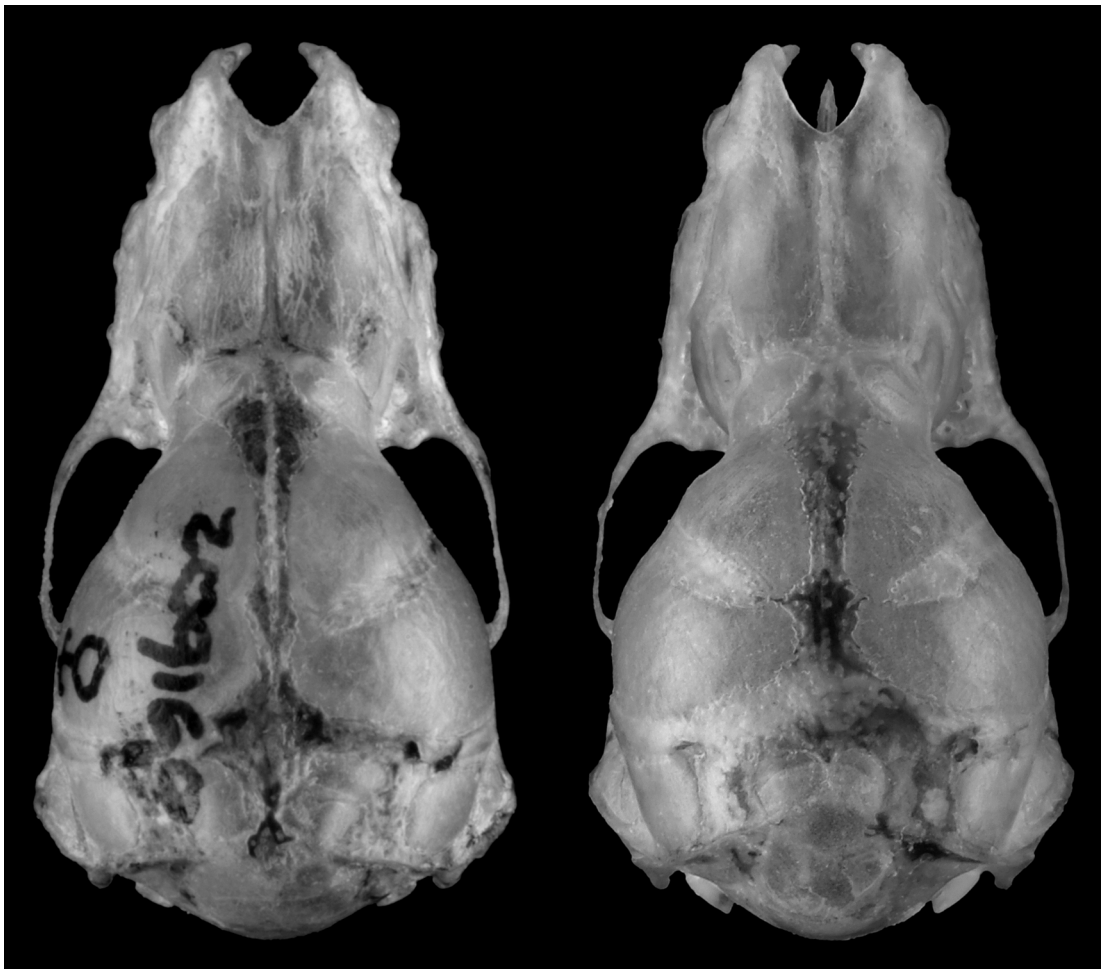

FIGURE 6. Dorsal view of skulls of *Miniopterus* spp. from Madagascar: left—*M. egeri* **sp. nov.** (FMNH 209160), holotype from Province de Toamasina, Forêt de Sahafina, 9.5 km W Brickaville and right—*M. petersoni* (FMNH 194316) holotype from Province de Toliara, Cascade de Manantantely, 5.2 km NW of Tolagnaro. (Photograph taken by J. Weinstein, Field Museum image number Z94502_06d.)

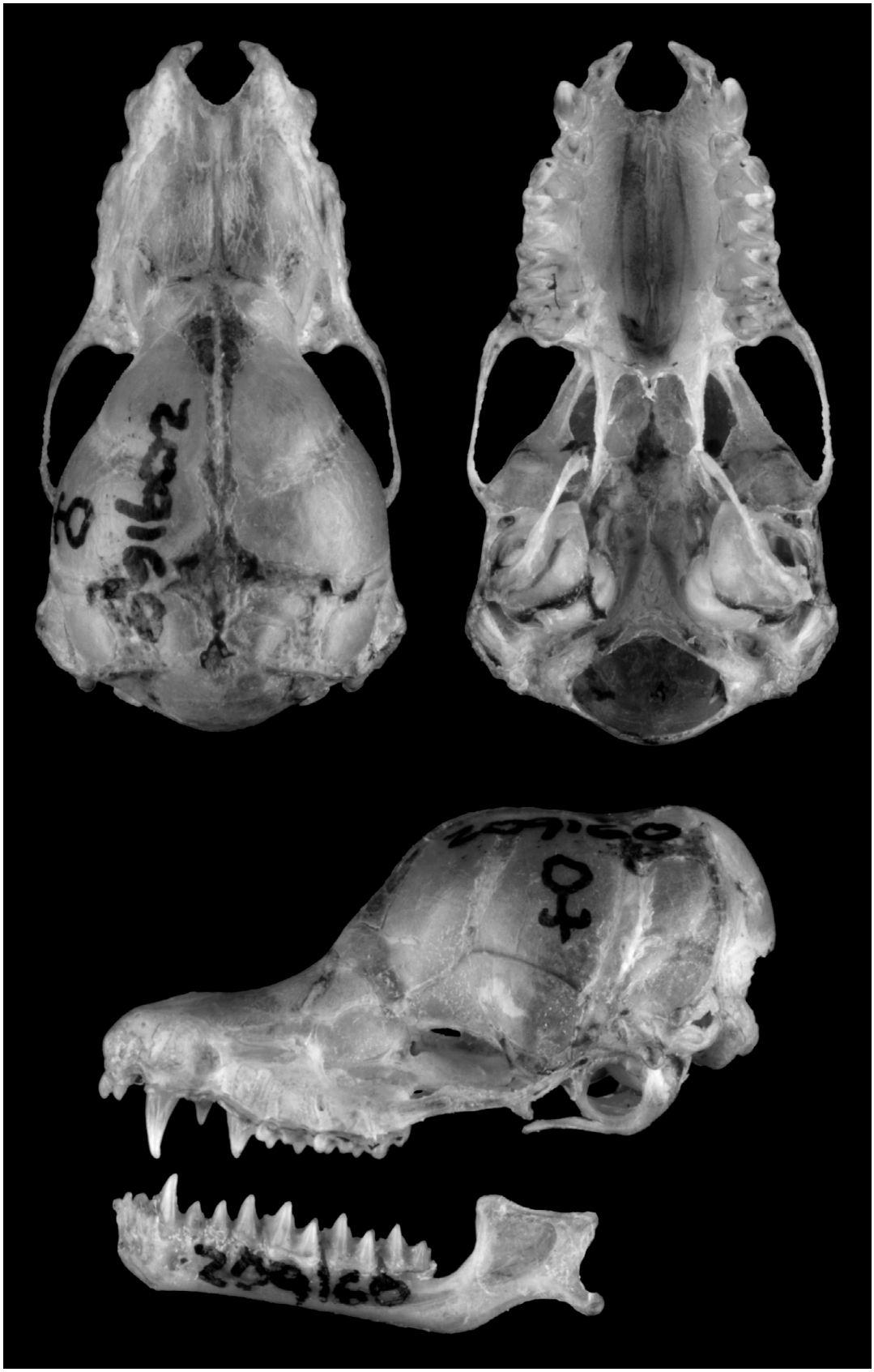

FIGURE 7. Different views of skull and mandible of *Miniopterus egeri* **sp**. **nov.** (FMNH 209160), holotype from Province de Toamasina, Forêt de Sahafina, 9.5 km W Brickaville. Pictures include dorsal view of cranium (upper row, left), ventral view of cranium (upper row, right), and lateral view of cranium and mandible (lower row). (Photograph taken by J. Weinstein, Field Museum image number Z94502_05d.)

Etymology. This species in named in honor of Dr. Judith Eger, Senior Curator, Department of Mammalogy, Royal Ontario Museum, Toronto, for her contribution to taxonomic studies of Old World bats, including Madagascar (see Monadjem *et al*. 2010 for further details).

Diagnosis. A moderately small species of *Miniopterus* with mixed medium and dark brown colored pelage (Figure 4). The holotype has a forearm length of 39 mm and in the species this measurement ranges from 37–40 mm (mean=38.5 mm). Tragus relatively short, 5–6 mm in length, slightly constricted on lower distal side, and distal portion notably thickened (Figure 5). From dorsal view, cranial palatal emargination with angularly tapered base, forming a slightly open "V-shaped" (Figure 6). Rostrum and interorbital region narrow. Rostral depression narrow, relatively shallow and long. Notable medial inflation of supraorbital ridge. Based on molecular characters, *M. egeri* forms a distinct clade from other Malagasy moderately small members of the genus.

Description. We have directly compared the holotypes of *M. egeri* (FMNH 209160) and *M. petersoni* (FMNH 194136) for external and cranio-dental characters, as well as series referable to these two species and other moderately small Malagasy members of the genus, including *M. sororculus* and *M. majori*.

TABLE 5. External measurements (in millimeters) and mass (in grams) of *Miniopterus egeri* **sp. nov.**, *M. petersoni*, *M. sororculus* (Central Highlands), and *M. majori*. Measurements presented as mean ± standard deviation, minimum and maximum measurements, and number of specimens. Figures in bold are of specimens collected and measured by SMG and those in standard script by a variety of different field collectors. Statistical differences between *M. egeri* and *M. petersoni* are examined based on student t-tests, n.s. = not significant.

	Total length	Tail length	Hindfoot length	Tragus length	Ear length	Forearm length	Body mass
M. egeri Holotype FMNH 209160 ♀	**91**	**43**	**7**	**6**	**10**	**39**	**6.1**
M. egeri Central east	**92.3 ± 1.51 91–95, n=6**	**41.7 ± 1.51 40–44, n=6**	**6.3 ± 0.82 5–7, n=6**	**5.8 ± 0.41 5–6, n=6**	**10.3 ± 0.52 10–11, n=6**	**38.3 ± 0.76 37–39, n=6**	**6.3 ± 0.69 5.6–7.6, n=6**
M. egeri Northeast	**91.0 ± 2.16 89–94, n=4**	**41.8 ± 0.96 41–43, n=4**	**6.8 ± 0.50 6–7, n=4**	**6.0 ± 0.0 6–6, n=4**	**11.0 ± 0.0 11–11, n=4**	**39.0 ± 0.82 38–40, n=4**	**5.4 ± 0.25 5.2–5.7, n=4**
M. egeri Across range	**91.8 ± 1.81 89–95, n=10**	**41.7 ± 1.25 40–44, n=10**	**6.5 ± 0.71 5–7, n=10**	**5.9 ± 0.32 5–6, n=10**	**10.6 ± 0.52 10–11, n=10**	38.5 ± 0.73 37–40, n=15	5.7 ± 0.75 4.2–7.6, n=15
M. petersoni Holotype FMNH 194136 ♂	**98**	**45**	**6.5**	**6**	**10**	**40.5**	**6.5**
M. petersoni Southeastern Madagascar	93.9 ± 3.06 89–99, n=15	43.6 ± 2.44 39–50, n=15	6.0–6.5, n=2	6.7 ± 0.46 6–7, n=15	11.7 ± 0.82 10–13, n=15	40.0 ± 0.70 39–41, n=14	6.3 ± 0.80 5.5–8.2, n=15
T-statistics for *M. egeri* compared to *M. petersoni*	n.s.	T=2.06 *P* =0.05	n.s.	T=5.19 *P*<0.00001	T=3.98 *P*=0.0006	T=5.63 *P*< 0.00001	n.s.
M. sororculus	**110.8 ± 2.71 105–115, n=23**	**54.7 ± 1.84 51–58, n=23**	**6.7 ± 0.65 6–8, n=23**	**6.7 ± 0.65 6–8, n=23**	**10.7 ± 0.62 10–12, n=23**	**43.5 ± 0.85 42–45, n=23**	**7.9 ± 0.62 7.0–9.1, n=22**
M. majori	**115.6 ± 2.42 112–120, n=39**	**55.5 ± 1.90 51–60, n=39**	**7.7 ± 0.54 7–9, n=37**	**7.3 ± 0.46 7–8, n=39**	**11.9 ± 0.47 11–13, n=39**	**45.5 ± 0.82 44–47, n=39**	**9.7 ± 0.93 8.4–12.5, n=37**

External characters. A moderately small *Miniopterus* with a tail less than 50% of total length (Table 5). In the holotype, which was preserved in fluid in late 2009, the dorsal and ventral pelage is slightly long, dense and a mix of medium brown interspersed with a distinctly lighter brown colored fur (Figure 4). This individual was compared

to five specimens prepared as dry skins (USNM 448938-448939, 449203-449205). USNM 448939, in which the back and upper ventrum coloration approaches a dark brown, and USNM 449204, in which the back is a slightly lighter brown, show slightly different fur coloration than the holotype. Across the type series, the wing membrane and uropatagium are dark brownish-black, largely naked, and show no noticeable change in coloration across their surface area.

The ear average length of the holotype of *M. egeri* is 10 mm, and the range for the ten specimens referred to this species is 10–11 mm (average 10.6 mm), which is smaller than the average values of 11.7 mm for animals referred to *M. petersoni*; these differences are statistically significant (Table 5; all measured by same field collector). The tragus length in the holotype of *M. egeri* is 6 mm and this species shows little variation with all animals falling with the range 5–6 mm (average 5.9 mm); it does not show extensive overlap with *M. petersoni* with minimum–maximum values of 6–7 mm (average 6.7 mm; this difference is statistically significant (Table 5; all measured by same field collector).

TABLE 6. Cranial measurements (in millimeters) of adult *Miniopterus egeri* **sp. nov.**, *M. petersoni*, *M. sororculus* (Central Highlands), and *M. majori*. Measurements presented as mean ± standard deviation, minimum and maximum measurements, and number of specimens. See Methods and materials for an explanation of variable acronyms. Statistical differences between *M. egeri* and *M. petersoni* are examined based on student t-tests, n.s. = not significant.

	GSKL	CIL	ZYGO	POB	MAST	GBW	PAL	LW	MAND
M. egeri Holotype FMNH 209160 ♀	14.8	14.3	7.7	3.3	7.9	7.2	5.8	4.7	10.4
M. egeri Central east	14.4 ± 0.38 13.9–14.8, n=9	13.7 ± 0.38 13.3–14.3, n=8	7.5 ± 0.15 7.3–7.7, n=7	3.1 ± 0.13 2.9–3.3, n=9	7.6 ± 0.23 7.3–7.9, n=9	6.9 ± 0.20 6.7–7.2, n=9	5.4 ± 0.31 5.0–5.9, n=9	4.5 ± 0.16 4.2–4.7, n=10	10.2 ± 0.38 9.7–11.0, n=10
M. egeri Northeast	14.2 ± 0.18 14.0–14.4, n=4	13.5 ± 0.12 13.4–13.6, n=4	7.7 ± 0.06 7.6–7.7, n=4	3.3 ± 0.06 3.2–3.3, n=4	7.6 ± 0.08 7.5–7.7, n=4	7.0 ± 0.05 6.9–7.0, n=4	5.1 ± 0.06 5.0–5.2, n=3	4.6 ± 0.10 4.5–4.7, n=4	9.9 ± 0.10 9.8–10.0, n=4
M. egeri Across range	14.3 ± 0.30 13.9–14.8, n=13	13.6 ± 0.32 13.3–14.3, n=12	7.6 ± 0.13 7.3–7.7, n=11	3.2 ± 0.15 2.9–3.3, n=13	7.6 ± 0.20 7.3–7.9, n=13	6.9 ± 0.17 6.7–7.2, n=13	5.3 ± 0.31 5.0–5.9, n=12	4.5 ± 0.15 4.2–4.7, n=14	10.1 ± 0.34 9.7–11.0, n=14
M. petersoni Holotype FMNH 194136 ♂	14.7	14.1	7.8	3.2	7.8	7.0	5.6	4.6	10.3
M. petersoni Southeastern Madagascar	14.5 ± 0.19 14.1–14.9, n=34	13.9 ± 0.22 13.2–14.1, n=34	7.7 ± 0.16 7.4–8.1, n=28	3.1 ± 0.12 2.9–3.4, n=34	7.6 ± 0.15 7.3–7.9, n=31	6.8 ± 0.19 6.5–7.3, n=31	5.4 ± 0.12 5.2–5.6, n=31	4.5 ± 0.17 4.2–4.8, n=33	10.2 ± 0.20 9.8–10.6, n=31
T-statistics for *M. egeri* compared to *M. petersoni*	T=2.33 P=0.03	T=2.76 P=0.008	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
M. sororculus	14.5 ± 0.24 13.8–14.9, n=52	14.0 ± 0.25 13.5–14.4, n=52	8.1 ± 0.15 7.7–8.3, n=44	3.4 ± 0.11 3.1–3.6, n=54	8.0 ± 0.19 7.7–8.2, n=52	7.4 ± 0.14 7.1–7.7, n=51	5.4 ± 0.20 5.2–6.0, n=41	4.7 ± 0.16 4.5–4.9, n=55	10.0 ± 0.15 9.7–10.3, n=28
M. majori	15.4 ± 0.26 14.9–15.8, n=40	15.1 ± 0.19 14.7–15.4, n=40	8.5 ± 0.14 8.2–8.8, n=40	3.6 ± 0.11 3.3–3.8, n=40	8.3 ± 0.16 7.9–8.7, n=40	7.7 ± 0.14 7.4–8.0, n=40	5.9 ± 0.12 5.7–6.3, n=39	5.3 ± 0.16 4.9–5.5, n=40	10.9 ± 0.20 10.5–11.3, n=36

The tragus of *M. egeri* is notably thick along the shaft, slightly constricted on lower distal side, the length of the proximal edge has a distinct flange that folds slightly anterior-medially, and the downward deflected distal tip is notably thickened and slightly fleshy (Figure 5a). In comparison, *M. petersoni* has a distinctly thinner tragus shaft, the constriction of the lower proximal portion notably more pronounced, and the distal tip has a slightly raised and rounded structure (Figure 5b). The tragus in *M. sororculus* is similar in length to *M. petersoni* (Table 5), but distinctly more spatulate-shaped and without a notable tapered distal head (Figure 5c).

On the basis of most external measurements, *M. egeri* is more diminutive than other moderately small Malagasy *Miniopterus* (Table 5). For example, the average forearm length in *M. egeri* is 38.5 mm (range 37–40 mm), *M. petersoni* 40.0 mm (range 39–41 mm), *M. sororculus* 43.5 mm (range 42–45 mm), and *M. majori* 45.5 mm (range 44–47 mm). The difference between the first two taxa is statistically significant. In contrast, the hindfoot length in *M. egeri* and *M. petersoni* broadly overlap, but this measurement is slightly longer in *M. sororculus* and notably longer in *M. majori* (Table 5).

Cranio-dental characters. The skull of *M. egeri* is notably short with a greatest skull length of 14.3 mm on average (range 13.9–14.8 mm), overlapping with *M. petersoni* with its average length of 14.5 mm (range 14.1–14.9 mm); this slight difference is statistically significant (Table 6). *Miniopterus sororculus* has approximately the same skull length as *M. egeri* and *M. petersoni*, but *M. majori* is notably longer. *Miniopterus egeri* has a notably short and narrow rostrum and not a particularly bulbous braincase, similar to *M. petersoni* (Figure 6), although there are a few statistical differences between these two taxa (Table 6). In *M. egeri*, the interorbital region is narrow and the rostral depression tends to be proportionately narrow and relatively shallow across most of its length as compared to *M. petersoni*. In the former species, the supraorbital ridge shows a notable medial inflation, similar to *M. petersoni* (Figure 6). When viewed from above, the palatal emargination in *M. egeri* is relatively narrow and forms a slightly open "V-shape"; in contrast, the emargination in *M. petersoni* is a more closed "V-shape" (Figure 6).

The upper toothrows in *M. egeri* are relatively short for an animal of its size, largely in parallel, although slightly posteriorly diverging (Figure 7); the upper toothrow is similar in length to *M. petersoni* and *M. sororculus* (Table 7). The lower tooth row in *M. egeri* is 6.6 mm on average (range 6.4–7.0 mm), which is shorter than the average of *M. petersoni* at 6.8 mm (range 6.4–7.0), and this difference is statistically significant (Table 7). Dental formula I 2/3 C 1/1 P 2/3 M 3/3, comprising the adult dentition of 36 teeth. First upper premolar (P^2) is relatively small and with more simple cusp morphology than second upper premolar (P^3), typical of members of this genus (Koopman 1994). The dentition of *M. egeri* is similar to *M. petersoni* and other moderately small *Miniopterus*.

Distribution and conservation status. Over the past decade, largely based on faunal surveys and associated specimens and tissues obtained, important advances have been made in understanding the species limits and distribution of Malagasy region *Miniopterus*. On the basis of current information, which by no means should be considered complete, it is possible to delineate the distributions of members of this genus occurring in the more mesic eastern portions of the island (Goodman 2011): *M. brachytragos* Goodman, Maminirina, Bradman, Christidis, Appleton, 2009—largely a western species but has been captured near Maroantsetra from near sea level; *M. gleni*—Masoala Peninsula to Tolagnaro and scattered records from the Central Highlands from sea level to 1200 m; *M. egeri*—Masoala Peninsula to Vatovavy from 50 to 550 m; *M. majori*—largely confined to the Central Highlands, but has also been found near Maroantsetra south to Tolagnaro from sea level to over 1550 m; *M. manavi*—Central Highlands, near Fandriana, south to Ranomafana (Ifanadiana) and Vinantelo from 900 to 1500 m; *M. petersoni*—lowland portions of the southeast in the vicinity of Tolagnaro from 10 to 550 m; and *M. sororculus*—Central Highlands from 950 to 2200 m. Hence, amongst these small to medium sized species, with the exception of *M. sororculus* and *M. majori*, none are known to occur in sympatry in the east. *Miniopterus egeri* is allopatric with *M. petersoni*, which in turn do not have overlapping elevational distributions with *M. manavi*. Approximately 300 km separate the known northern limit of *M. petersoni* and the southern limit of *M. egeri* (Figure 1). Medium to small *Miniopterus* are known form this intermediate zone (e.g., Midongy-Sud), but based on molecular genetics data represent other clades than those species mentioned herein (Appleton *et al*. unpublished data).

Most of the sites where *M. egeri* occurs are within intact or partially degraded forest ecosystems and few details are available about this species' natural history. On the basis of current information, it is known from scattered sites occurring across two-thirds of Madagascar's lowland area from near sea level to 550 m, often associated with disturbed forests with mixed native and introduced trees or at the ecotone between degraded anthropogenic habitats and native forest. A day roost site of this species was found in a natural rock shelter in the Poste Forestier de Farankaraina surrounded by slightly disturbed natural lowland humid forest. At least in the short-term, this species does not appear to face a risk of dramatic population decline. However, further information is needed on its distribution and size of existing colonies to properly assess its conservation status.

TABLE 7. Dental measurements (in millimeters) of *Miniopterus egeri* **sp. nov.**, *M. petersoni*, *M. sororculus* (Central Highlands), and *M. majori*. Measurements presented as mean ± standard deviation, minimum and maximum measurements, and number of specimens. See Methods and materials for an explanation of variable acronyms. Statistical differences between *M. egeri* and *M. petersoni* are examined based on student t-tests, n.s. = not significant.

	I^1-M^3	C-M^3	C^1-C^1	M^3-M^3	i_1-m_3
M. egeri Holotype FMNH 209160 ♀	6.8	5.6	4.0	5.8	7.0
M. egeri Central east	6.4 ± 0.23 6.2–6.8, n=10	5.4 ± 0.14 5.2–5.6, n=10	3.9 ± 0.11 3.8–4.1, n=10	5.5 ± 0.22 5.2–5.8, n=10	6.7 ± 0.21 6.4–7.0, n=10
M. egeri Northeast	6.4 ± 0.10 6.3–6.5, n=4	5.4 ±0.13 5.2–5.5, n=4	4.0 ± 0.08 3.9–4.1, n=4	5.5 ± 0.06 5.4–5.5, n=4	6.5 ± 0.03 6.5–6.6, n=4
M. egeri Across range	6.4 ± 0.20 6.2–6.8, n=14	5.4 ± 0.13 5.2–5.6, n=14	4.0 ± 0.10 3.8–4.1, n=14	5.5 ± 0.18 5.2–5.8, n=14	6.6 ± 0.19 6.4 –7.0, n=14
M. petersoni Holotype FMNH 194136 ♂	6.7	5.6	3.9	5.8	6.8
M. petersoni Southeastern Madagascar	6.5 ± 0.14 6.1–6.7, n=34	5.4 ± 0.13 5.1–5.6, n=34	3.9 ± 0.11 3.5–4.1, n=34	5.5 ± 0.13 5.1–5.8, n=34	6.8 ± 0.12 6.4–7.0, n=34
T–statistics for *M. egeri* compared to *M. petersoni*	n.s.	n.s.	n.s.	n.s.	T=2.75 P=0.009
M. sororculus	6.4 ± 0.13 6.0–6.6, n=54	5.4 ± 0.11 5.1–5.6, n=54	4.0 ± 0.15 3.6–4.0, n=51	5.7 ± 0.15 5.2–6.0, n=54	6.9 ± 0.14 6.5–7.1, n=52
M. majori	7.2 ± 0.14 6.9–7.5, n=40	6.0 ± 0.10 5.8–6.2, n=40	4.4 ± 0.10 4.2–4.6, n=38	6.5 ± 0.12 6.3–6.7, n=40	7.5 ± 0.15 7.2–7.9, n=42

Discussion

Across its range from the Maroantsetra region south to near Vatovavy (Figure 1), a zone of approximately 700 km in length, there is no noticeable variation in the morphometrics of *M. egeri*. In Tables 5, 6, and 7, different external, cranial, and dental measurements (respectively) of specimens are segregated into two different geographic zones, the southeast (Vatovavy to Sahafina) and northeast (Masoala Peninsula). In general, although sample sizes are not large, measurements are consistent between the two zones. Further sampling in other areas, specifically the zone north of Toamasina and south of Maroantsetra, will need to be conducted to verify if *M. egeri* has a continuous distribution across much of the eastern portion of the island.

In their description of *M. petersoni*, Goodman *et al*. (2008) presented several characters that clearly separated that species from the other Afro-Malagasy *Miniopterus*. Subsequently, a number of species have been described or elevated from subspecies level known from Madagascar and surrounding islands, which are distinctly larger (*M. griffithsi*; Goodman *et al*. 2010) or smaller (*M. aelleni* Goodman, Maminirina, Weyeneth, Bradman, Christidis, Ruedi & Appleton, 2009, *M. brachytragos*, and *M. mahafaliensis* Goodman, Maminirina, Bradman, Christidis, Appleton, 2009) than *M. petersoni* and its morphologically similar species *M. egeri*. All of these recently recognized *Miniopterus* species can be differentiated from *M. egeri* based on tragus shape and other morphological characters and, hence, these taxa do not need to be considered further. The remaining needed comparison is the morphological distinction between *M. petersoni* and *M. egeri*.

Goodman *et al*. (2008) noted that specimens allocated to *M. petersoni* from the Tolagnaro region, which includes the holotype locality, showed some measurement and morphological differences to those obtained from the central southeast, as well as the northeast portions of the island. As molecular genetics have been of primary importance for the recognition of cryptic *Miniopterus* species from the Madagascar region (see for example, Goodman *et al*. 2009a; Weyeneth *et al*. 2008), and given that tissue samples were not associated with the Goodman *et al.* (2008) study for specimens from the central southeast and northeast allocated to *M. petersoni*, the resolution of their specific status was uncertain. On the basis of the comparisons presented above, it is now clear that populations previously assigned to *M. petersoni* from the zone between the central southeast (Vatovavy to Sahafina) and northeast (Masoala Peninsula) show genetic sequence divergence as well as morphological characters, such as tragus shape, that separate them from extreme southeastern populations of *M. petersoni*. There also appear to be bioacoustic differences that separate *M. egeri* and *M. petersoni*. However, echolocation data recorded in different natural habitats are needed to confirm this.

To further explore morphological differences between genetically or morphologically similar Malagasy *Miniopterus* spp. we conducted PCAs on external, cranial, and dental measurements of *M. egeri*, *M. petersoni*, *M. sororculus*, *M. majori*, and the diminutive *M. manavi* sensu stricto. The comparisons of external, cranial, and dental measurements generally showed clear separation of specimens assigned to *M. sororculus*, *M. majori*, *M. manavi*, and members of the *M. egeri/M. petersoni* complex; only the cranial analysis is presented here (Figure 8). The first two unrotated principal components (PCs) accounted for 94% of the total variance of the cranial morphology, and separated the specimens into four groups: *M. sororculus*, *M. majori*, *M. manavi*, and the *M. egeri/M. petersoni* complex (Figure 8; Table 8). All of the eight cranial variables showed heavy loadings on PC1, but only three cranial variables, POB, GBW, and MAND loaded high on PC2. These patterns are interpreted as follows. PC1 was a measure of cranial size and bats that loaded low on PC1 (*M. majori*) had relatively larger skulls than bats that loaded high on PC1 (*M. egeri* and *M. manavi*). PC2 was a measure of cranial shape, and bats that loaded low on PC2 (*M. sororculus*) had relatively broader brain cases and crania with shorter mandibular lengths compared to bats that loaded high on PC2 (*M. petersoni*). In the case of the sister taxa *M. egeri* and *M. petersoni*, which have allopatric distributions, there is notable overlap in the PCA plot of cranial variables for PC1 and PC2. In contrast, the sympatrically occurring and phylogenetically distinct *M. majori* and *M. sororculus* (Figure 2) show complete separation in the PCA analysis for both PC1 and PC2. These differences in size and shape might be associated with interspecific competition between *Miniopterus* spp. of similar ecology and overlapping distributions.

TABLE 8. Factor loadings from principal component analysis of log-transformed cranial measurements of specimens of *Miniopterus egeri* **sp. nov.**, *M. manavi* sensu stricto, *M. petersoni*, *M. sororculus*, and *M. majori*. A graphical representation of the first two factors is presented in Figure 8. See Methods and materials for the acronym definitions.

Variable	PC 1	PC 2	PC 3
GSKL	-0.934	0.269	-0.159
CIL	-0.962	0.204	-0.106
ZYGO	-0.971	-0.132	-0.051
POB	-0.884	-0.376	0.106
MAST	-0.967	-0.160	-0.073
GBW	-0.918	-0.342	-0.087
LW	-0.905	0.138	0.387
MAND	-0.893	0.401	0.007
Eigenvalue	6.917	0.595	0.213
Proportion of total explained variation	86.4%	93.8%	96.5%

In a more practical sense, in the hand *M. egeri* can be separated from other moderately small to medium-sized Malagasy members of this genus, particularly *M. petersoni* and *M. sororculus*, by tragus shape (Figure 5). Based on current information there is no evidence that *M. egeri* and *M. petersoni* occur in sympatry. Hence, the geographical zone a given animal was captured is also informative as to its specific designation.

One of the remaining questions is the specific status of the moderately small *Miniopterus* from the upper slopes of Montagne d'Ambre in the far north that were allocated to *M.* cf. *petersoni* by Goodman *et al.* (2008), but

showed notable measural differences with typical *M. petersoni* from the southeast. The animals from Montagne d'Ambre have relatively broader rostrums, approaching that of *M. sororculus*. In order to resolve this question, further specimens and associated tissue are needed from the Montagne d'Ambre population.

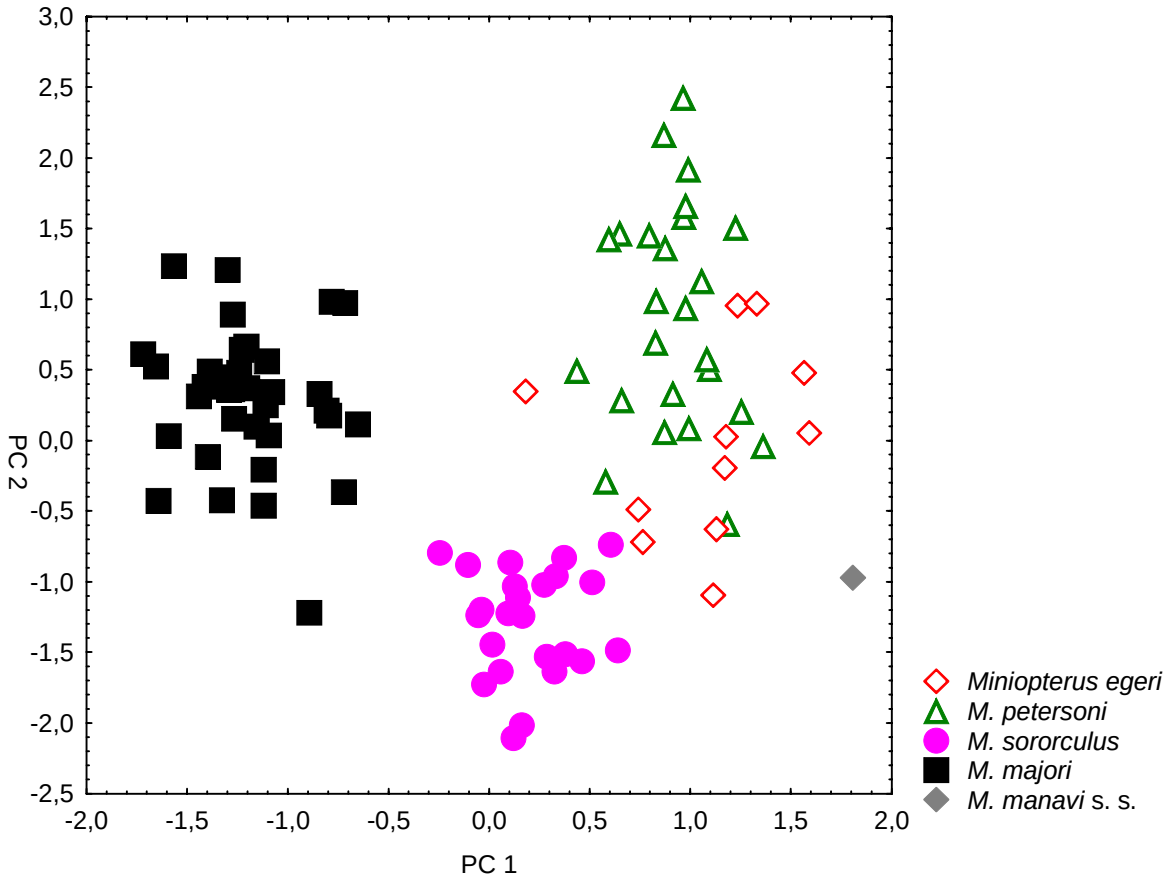

FIGURE 8. Projection of first two unrotated principal components for morphologically similar or phylogenetically close species of *Miniopterus* spp. from Madagascar. PC1 and PC2 clearly separate the sympatrically occurring species *M. majori* and *M. sororculus*, while neither axis separates the allopatric species *M. egeri* and *M. petersoni*. See Table 8 for the variables used in the analysis and the associated loadings.

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