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The Use of Molecular Phylogenetic and Morphological Tools to Identify Cryptic and Paraphyletic Species: Examples from the Diminutive Long-fingered Bats (Chiroptera: Miniopteridae: *Miniopterus*) on Madagascar

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ABSTRACT

Based on nearly complete (1125 bp) cytochrome-*b* sequence data and morphological characters, two new endemic species of *Miniopterus* are described from Madagascar that were previously identified as *M. manavi*. Using phylogenetic analysis, the basal nodes of major lineages in the Malagasy members of this genus are weakly supported, while, in most cases, the branches leading to each of the clades are well resolved. *Miniopterus mahafaliensis*, new species, occurs in the southwestern semidesert areas and *M. brachytragos*, new species, has a broad distribution across the northern half of the island, ranging across several different biomes. Phylogenetic inference indicates that these two new taxa are not closely related to *M. manavi* sensu stricto, with average genetic distances of 9.2% and 5.7% from this taxon, respectively. On the basis of this and previous revisions, the former *M. manavi* complex is now recognized to represent at least five taxa, which do not form a monophyletic group with respect to one another, and represent extraordinary examples of convergent evolution. *Miniopterus brachytragos* is closely related to the recently named *M.*

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aelleni, while *M. mahafaliensis* is not closely associated with any of these species. Molecular phylogenetic analysis was imperative to resolve the species limits of these taxa and morphology then provided the means to corroborate the recovered clades. There are localities on the island, specifically limestone karstic zones, where four species of the former *M. manavi* sensu lato complex occur in strict sympatry. These species often use the same day-roost caves and have similar external and craniodental measurements. This raises intriguing questions as to how these animals divide their worlds with regard to dietary regimes and foraging strategies, as well as their speciation history.

INTRODUCTION

With the advent of molecular phylogenetic techniques, nearly two decades ago, new means became available to classify groups of animals that are morphologically similar to one another, but sufficiently genetically distinct to be considered separate species (Yoder et al., 2005; Bickford et al., 2006; Pfenninger and Schwenk, 2007). This research reveals cases of phylogenetic sister species possessing morphological traits that are so similar that they cannot be easily differentiated from one another, and hence are referred to as cryptic species. In such examples, the phenotypic characters used in taxonomic classification do not reflect the same level of differentiation as the genetic markers (Baker and Bradley, 2006). Further, there are numerous cases among mammals, where phenotypically similar species do not form monophyletic units and represent examples of morphological convergence and paraphyly (e.g., Pizzimenti, 1976; Ruedi and Mayer, 2001; Funk and Omland, 2003; Gaubert and Veron, 2003). Over the past few years numerous cryptic species have been described from around the world and across different biological groups (Pfenninger and Schwenk, 2007); Madagascar has produced a remarkable number of examples from the animal kingdom including, ants, beetles, amphibians, reptiles, bats, and terrestrial mammals (e.g., Olson et al., 2004; Vences and Glaw, 2005; Monaghan et al., 2005; Smith et al., 2005; Yoder et al., 2005; Goodman et al., 2007). Perhaps one of the more remarkable examples of cryptic animal species is among lemurs, arguably the best-known group of land vertebrates on the island. The mouse-lemurs of the genus *Microcebus* were thought to comprise a single broadly distributed species, until Martin (1972) recommended that a second form should be recognized. Subsequently, largely molecular studies revealed considerable fixed genetic differences and, as of September 2008, at least 19 species are proposed that are phenotypically similar to one another (Rasoloarison et al., 2000; Kappeler et al., 2005; Andriantompohavana et al., 2006; Louis et al., 2006, 2008; Olivieri et al., 2007; Radespiel et al., 2008).

Herein, we explore the species limits of another group of Malagasy vertebrates, long-fingered bats, genus *Miniopterus* Bonaparte, 1837, of the family Miniopteridae (Hoofer and Van Den Bussche, 2003; Miller-Butterworth et al., 2007). This genus contains a considerable number of cryptic species across their Old World distribution (Cardinal and Christidis, 2000; Appleton et al., 2004; Stoffberg et al., 2004; Miller-Butterworth et al., 2005; Juste et al., 2007). Peterson et al. (1995) recognized four taxa from Madagascar, two of which also occurred on the Comoros (*M. manavi* Thomas, 1906 and *M. majori* Thomas, 1906), one shared with Africa (*M. fraterculus* Thomas and Schwann, 1906), and the fourth endemic to the island (*M. gleni* Peterson et al., 1995). Recent molecular genetic and morphological studies have considerably changed this view, with *M. manavi* and *M. gleni* being paraphyletic and the Malagasy animals formerly assigned to *M. fraterculus* representing two endemic species (Goodman et al., 2007, 2008, 2009a, 2009b; Weyeneth et al., 2008). Among the original four species recognized by Peterson et al. (1995), only *M. majori* shows no notable genetic variation, based on currently available samples, and is not a composite species (Mamanirina et al., 2009). Malagasy miniopterines show numerous cases of morphological similarity, with genetic results demonstrating that certain non-sister-taxa display convergence in size and body morphology. The subject of this current paper is to further investigate other previously unrecognized clades among Malagasy *Miniopterus* formerly identified as *M. manavi*, and to describe an additional two species that are genetically and morphologically distinct.

MATERIAL AND METHODS

Morphological Comparisons

In order to resolve the taxonomic identity of different small *Miniopterus* from Madagascar, that have been classically placed within the *M. manavi* species group, specimens were examined from several different natural history museums: BMNH, the Natural History Museum, London (formerly British Museum [Natural History]); FMNH, Field Museum of Natural History, Chicago; MNHN, Muséum National d'Histoire Naturelle, Paris; ROM MAM, Department of Mammalogy, Royal Ontario Museum, Toronto; NMW = Naturhistorisches Museum, Vienna; 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. Measurements are reported for adults (defined by presence of fully erupted permanent dentition and fused basisphenoid-basioccipital suture). Tooth abbreviations include: I = incisor, C = canine, PM = premolar, and M = molar. Uppercase abbreviations are used for superior teeth and lowercase abbreviations for inferior teeth.

Six standard external measurements (in mm) were taken from specimens using a ruler before preparation and included: total length, tail length, hind foot length (not including claw), tragus, ear length, and forearm. A considerable proportion of the recent specimens from Madagascar were collected by SMG, in association with other colleagues, and the external measurements of these animals were consistently taken and, unless specified, these data are not combined with those of other field collectors. Mass was taken with the use of a spring balance and recorded in grams.

Eight cranial or mandible and seven dental measurements were taken using a digital caliper to the nearest 0.1 mm by C.P.M. and verified by S.M.G. The cranial measurements comprise: greatest skull length (GSKL), from posteriormost part of occipital bone to anteriormost 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; lachrymal width (LW), greatest width across lachrymal swellings; palatal length (PAL), from anterior edge of upper incisors to posterior edge of palatal without posterior spike; and mandible length (MAND), from the posteriormost portion of the condyles to anteriormost alveoli of lower incisors. The dental measurements include: cranial toothrow (I1–M3), length from anterior alveolar border of incisors to posterior alveolar border of third molar; upper canine-molar toothrow (C–M3), length from anterior alveolar border of canine to posterior alveolar border of third molar; width across upper canines (C1–C1), taken across the outer alveolar borders of the canines; width across third upper molars (M3–M3), taken across the outer alveolar borders of the third molars.

Univariate statistical analyses were conducted for each measured variable to examine patterns of sexual dimorphism. In order to assess multivariate overlap or separation in three-dimensional space between identified species based on molecular and morphological characters, a principal component analysis was performed using the statistical package Statistica (version 7.0); data were log-transformed, a correlation matrix was used, and the unrotated option was employed. Based on an ecomorphological study of Malagasy dry forest bats (Ranivo and Goodman, 2007), in which the same three types of variables were used as herein (external, cranial, and dental) and did not often covary, we have analyzed these different variable types separately.

Taxonomic Considerations

Recent research has demonstrated that the former *Miniopterus manavi* is a composite of several different taxa, listed below in chronological order of their description. The forearm length of each taxon is also presented, which is generally considered a good measure of body size. See figure 1 for a map of the localities mentioned on Madagascar.

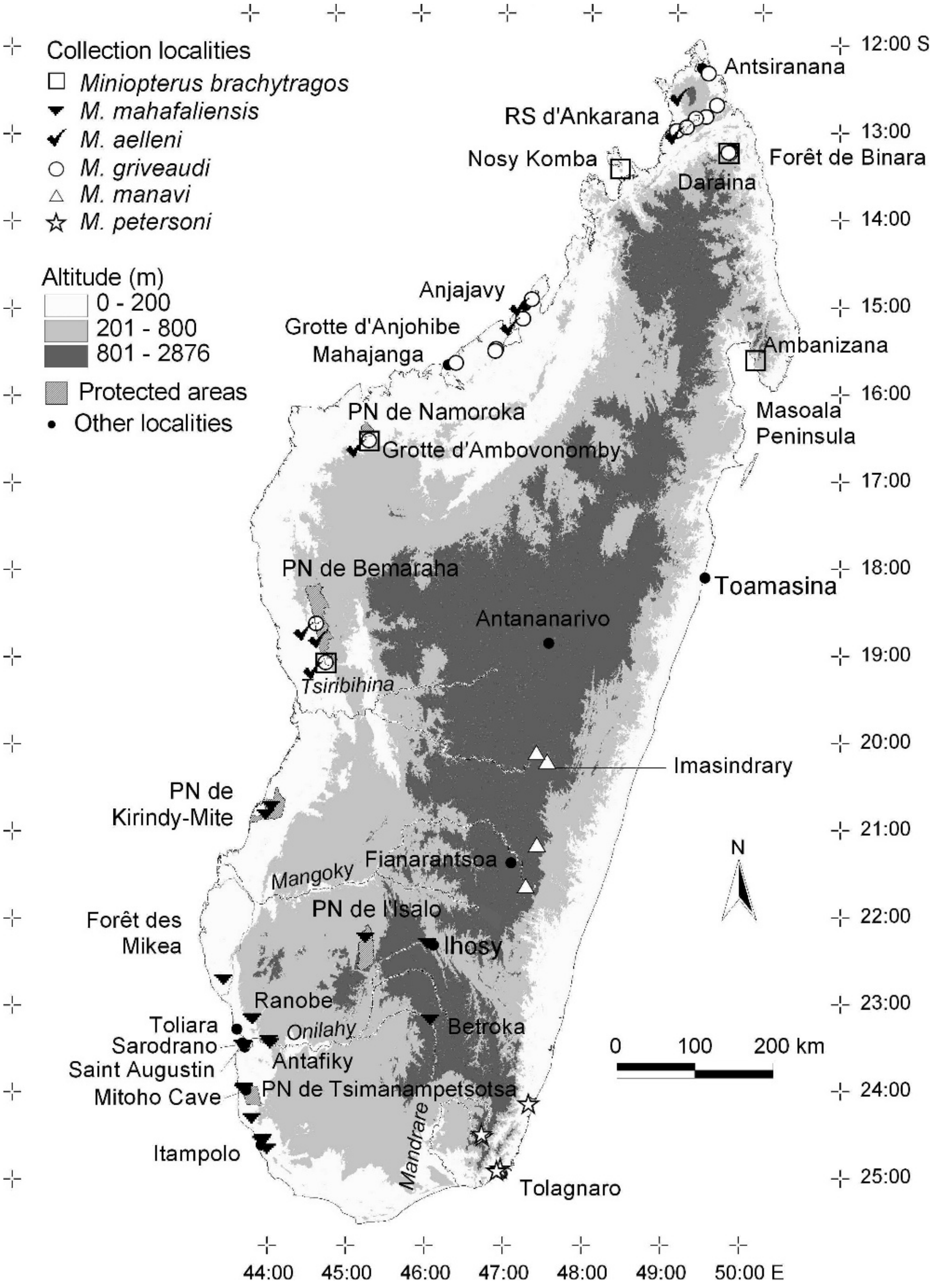

Fig. 1. Map of Madagascar showing localities mentioned in the text and sites where specimens of *Miniopterus brachytragos* sp. nov., *M. mahafaliensis* sp. nov., *M. manavi* sensu stricto, *M. petersoni*, *M. griveaudi*, and *M. aelleni* have been collected. The latter two species are also known from the Comoro Archipelago (Goodman et al., 2009b). The species symbols for the sites of Namoroka and Bemaraha have been slightly spread out to avoid obscuring information.

TABLE 1
Genetic samples of *Miniopterus* spp. used in the current study and not previously published

Clade	Species	Museum catalog number	Locality	GenBank No.
Clade 2	*M. brachytragos*	FMNH 175840	Namoroka – holotype	FJ383152
Clade 2	*M. brachytragos*	FMNH 175846	Namoroka	FJ383151
Clade 2	*M. brachytragos*	FMNH 175850	Namoroka	FJ383158
Clade 2	*M. brachytragos*	FMNH 175851[1]	Namoroka	
Clade 2	*M. brachytragos*	FMNH 175852[1]	Namoroka	
Clade 2	*M. brachytragos*	FMNH 175856	Namoroka	FJ383157
Clade 2	*M. brachytragos*	FMNH 175864[1]	Namoroka	
Clade 2	*M. brachytragos*	FMNH 175865[1]	Namoroka	
Clade 2	*M. brachytragos*	FMNH 175867[1]	Namoroka	
Clade 2	*M. brachytragos*	FMNH 175870	Namoroka	FJ383156
Clade 2	*M. brachytragos*	FMNH 175871	Namoroka	FJ383155
Clade 2	*M. brachytragos*	FMNH 175872[1]	Namoroka	
Clade 2	*M. brachytragos*	FMNH 175873	Namoroka	FJ383150
Clade 2	*M. brachytragos*	FMNH 175874[1]	Namoroka	
Clade 2	*M. brachytragos*	FMNH 175875	Namoroka	FJ383153
Clade 2	*M. brachytragos*	FMNH 175877	Namoroka	FJ383154
Clade 2	*M. brachytragos*	FMNH 188651	Nosy Komba	FJ383159
Clade 2	*M. brachytragos*	FMNH 202523	Ambanizana	FJ383149
Clade 4	*M. mahafaliensis*	FMNH 172919	Sarodrano	FJ383163
Clade 4	*M. mahafaliensis*	FMNH 172930	Saint Augustin	FJ383168
Clade 4	*M. mahafaliensis*	FMNH 173197	Mitoho Cave – holotype	FJ383160
Clade 4	*M. mahafaliensis*	FMNH 175991	Isalo	FJ383166
Clade 4	*M. mahafaliensis*	FMNH 176096	Kirindy-Mite	FJ383165
Clade 4	*M. mahafaliensis*	FMNH 176100	Kirindy-Mite	FJ383161
Clade 4	*M. mahafaliensis*	FMNH 176101	Kirindy-Mite	FJ383167
Clade 4	*M. mahafaliensis*	FMNH 176103	Kirindy-Mite	FJ383164
Clade 4	*M. mahafaliensis*	FMNH 176104	Kirindy-Mite	FJ383162
Clade 4	*M. mahafaliensis*	FMNH 184470[2]	Ihosy	

[1] This animal has the same haplotype as FMNH 175877 and is not shown in fig. 2.
[2] This animal has the same haplotype as FMNH 175991 and is not shown in fig. 2.

1. *Miniopterus manavi* Thomas, 1906—described based on a holotype from "Imasindrary; N.E. Betsileo. Other specimens from Vinanitelo" [= Vinantelo]. Both of these sites are in a zone approaching the eastern edge of the Central Highlands across the elevational range of 900–1500 m (Major, 1897, 1899; Jenkins and Carleton, 2005). The holotype is BMNH 97.9.1.37 with a forearm length of 39.2 mm (Thomas, 1906).
2. *Miniopterus griveaudi* Harrison, 1959—this taxon was originally described from Grande Comore in the Comoros Archipelago as a subspecies of *M. minor*, but recent molecular work has shown that these two taxa are not phylogenetically closely related (Goodman et al., 2009b; Weyeneth et al., 2008). The holotype is BMNH 67.1231 and it has a forearm length of 37.8 mm (Harrison, 1959). A recent revision has shown that *M. griveaudi* has a broad distribution across the Malagasy region, including much of lowland northern and western Madagascar from near sea level to 480 m, as well as on Anjouan and Grande Comore from near sea level—890 m (Goodman et al., 2009b).
3. *Miniopterus petersoni* Goodman et al., 2007—this moderately small species occurs in the extreme southeastern portion of Madagascar and was originally thought to be part of *M. fraterculus*, but in fact is closely related to *M. manavi* (Goodman et al., 2009b). The holotype is FMNH 194136 from

TABLE 2

Genetic distances within and between all major clades of *Miniopterus* spp. represented in the phylogenetic tree (fig. 2)

Bold values on diagonal through table indicate the uncorrected-P distance/Kimura-2-Parameter distance. The values above the diagonal represent the pairwise uncorrected-P distances, while the values below the diagonal represent the Kimura-2-Parameter distances. Intraspecific comparisons for the three extralimital taxa (*fraterculus*, *fuliginosus*, and *schreibersii*) are not presented.

	manavi	*brachytragos*	*mahafaliensis*	*griveaudi*	*aelleni*	*petersoni*
manavi	**0.009/0.009**	0.055	0.085	0.089	0.070	0.024
brachytragos	0.057	**0.004/0.007**	0.082	0.098	0.073	0.064
mahafaliensis	0.092	0.088	**0.019/0.022**	0.096	0.105	0.090
griveaudi	0.098	0.108	0.106	**0.009/0.004**	0.099	0.096
aelleni	0.075	0.078	0.116	0.109	**0.021/0.005**	0.079
petersoni	0.025	0.068	0.098	0.105	0.085	**0.007/0.014**
minor	0.118	0.117	0.116	0.116	0.114	0.130
sororculus	0.082	0.098	0.088	0.103	0.101	0.089
gleni	0.092	0.102	0.094	0.116	0.124	0.097
fraterculus	0.111	0.129	0.135	0.125	0.134	0.108
fuliginosus	0.153	0.148	0.135	0.153	0.149	0.175
schreibersii	0.115	0.133	0.128	0.125	0.105	0.119

the Cascade de Manantantely, 5.2 km NW of Tolagnaro; this individual has a forearm length of 40.5 mm (Goodman et al., 2007).

4. *Miniopterus aelleni* Goodman et al., 2009b—the holotype (FMNH 173067) is from the Ankarana Massif in the northern portion of the island. The forearm length of this animal is 38 mm. *Miniopterus aelleni* is known to occur in northern and western Madagascar across the elevational range of near sea level—1110 m and on Anjouan from 220–690 m.

Vegetation Classification

We follow the recently proposed vegetation classification for Madagascar of Moat and Smith (2007).

Molecular Comparisons

In order to understand patterns of phylogeographic variation in specimens allocated to *Miniopterus manavi*, molecular genetic studies were conducted. Cytochrome-*b* (cyt-*b*) (1125 bp) was chosen to be sequenced as it has been shown previously to be informative at the species level in the study of miniopterine bats (Cardinal and Christidis, 2000; Miller-Butterworth et al., 2005; Goodman et al., 2007). The genetic dataset includes new sequences obtained from specimens of "*M. manavi*" taken from across its geographical range on Madagascar (table 1). *Miniopterus fraterculus* and *M. schreibersii* were included for comparison and a GenBank sequence of Asian "*M. fuliginosus*" (AY614735) was included as the outgroup. In agreement with other studies (Appleton et al., 2004; Miller-Butterworth et al., 2005, 2007; Goodman et al., 2007, 2008), use of outgroup taxa from other miniopterid genera or from Asian *Miniopterus* did not alter the tree topology significantly (data not shown). Molecular analyses were conducted as previously described (Goodman et al., 2007). Newly produced sequences were deposited in GenBank (accession numbers FJ383149–FJ383168).

Sequences did not contain insertions, deletions, or stop codons (DNA strider: Marck, 1990). 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 and the tree bisection and reconnection branch-swapping algorithm. The NJ method used pairwise sequence distances estimated by Kimura's two-parameter model, K2P (Kimura, 1980). Nodal support of MP and NJ trees was estimated by 1000 bootstrap pseudoreplicates. Uncorrected p and K2P genetic distances were calculated using Mega 3.1 (Kumar et al., 2004) (table 2).

TABLE 2
(Continued)

minor	*sororculus*	*gleni*	*fraterculus*	*fuliginosus*	*schreibersii*
0.107	0.076	0.085	0.101	0.135	0.104
0.105	0.090	0.093	0.115	0.131	0.118
0.104	0.081	0.086	0.119	0.121	0.114
0.104	0.093	0.105	0.110	0.135	0.111
0.103	0.092	0.112	0.119	0.132	0.096
0.116	0.082	0.089	0.098	0.152	0.107
0.005/0.02	0.119	0.101	0.072	0.133	0.106
0.136	**0.005/0.009**	0.090	0.128	0.124	0.092
0.113	0.098	**0.014/0.005**	0.119	0.142	0.110
0.077	0.148	0.135	—	0.158	0.099
0.150	0.139	0.162	0.185	—	0.122
0.118	0.102	0.123	0.110	0.137	—

Modeltest 3.6 (Posada and Crandall, 1998) was used to determine the most appropriate model of molecular evolution before maximum likelihood (ML) analysis was conducted in PAUP*. The model, HKY+I+G, was estimated from both the hierarchical likelihood ratio tests and Akaike information criterion and was incorporated into the heuristic searches and bootstrapping (100 pseudoreplicates) for the ML analysis. Modeltest estimated parameters settings with base frequencies = 0.2945, 0.2844, 0.1367, 0.2844, $-\ln L = 4989.2739$ shape parameter of gamma distribution = 3.1677 and proportion of invariable sites = 0.6261. All characters were equally weighted and unordered. Bayesian analysis was conducted using the program MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). The HKY+I+G model was specified, flat priors were used and starting trees were random. We ran 4 chains (3 hot, 1 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 at the end of the run and that the potential scale reduction factor (PSRF) of each parameter stayed at 1.00. Plots of generation versus the log probabilities of observing actual data did not reveal any trends for the last 75% of generations.

RESULTS

Phylogeny

The resulting trees from the Bayesian, MP, NJ, and ML analyses were all congruent with respect to the clades recovered (fig. 2). The only differences were links between the basal nodes, which were poorly supported. The phylogenetic tree of haplotypes (fig. 2) clearly shows five separated, well-supported clades, each numbered, composed of animals that were previously identified as *M. manavi* (sensu Peterson et al., 1995). A recent phylogenetic and morphological study demonstrated that Clade 1 is the real *manavi*; Clade 3 is a recently described species, *M. aelleni*; and Clade 5 is referable to *M. griveaudi* (Goodman et al., 2009b). An additional two Malagasy lineages, clades 2 and 4, are phylogenetically distinct from *M. manavi* sensu stricto.

Clade 2, presented in figure 2, includes animals obtained from three widely separate localities on the island (fig. 1; table 1)—a limestone karst zone in western central Madagascar known as the Parc National de Namoroka, with western dry deciduous forest (FMNH 175840, 175846, 175850–175852, 175856, 175864–175865, 175867, 175870–

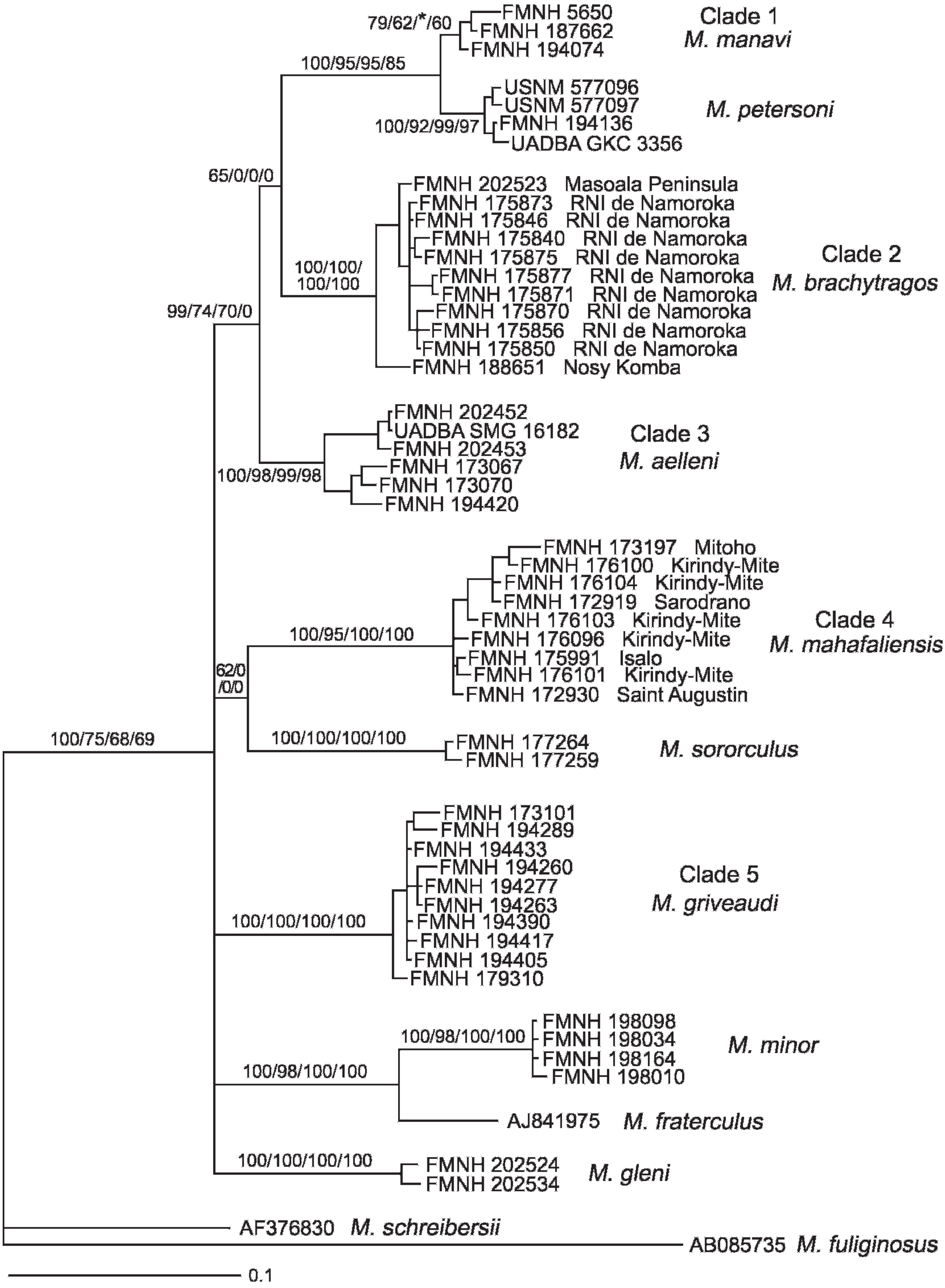

Fig. 2. Phylogenetic position of different *Miniopterus* spp. *Miniopterus fuliginosus* was selected as the outgroup. The tree presented was produced using Bayesian analysis. Posterior probabilities (Bayesian

175875, 175877); a single individual from the western dry forests of Nosy Komba, a near-shore island (FMNH 188651); and one animal from the humid forests near Ambanizana on the Masoala Peninsula (FMNH 202523). Clade 2 is separated from Clade 3 (*M. aelleni*) by average 7.8% K2P genetic distance and from Clade 1 by average 5.5% K2P genetic distance.

The other group, Clade 4, comprises specimens obtained in several different areas of southwestern Madagascar (fig. 1; table 1). These include the inland sites of Ihosy (FMNH 184470) and Isalo (FMNH 175991) with the local anthropogenic habitat being plateau grassland–wooded grassland mosiac, and more lowland areas of Kirindy-Mite (FMNH 176096, 176100–176101, 176103–176104) with western dry deciduous forest, Forêt des Mikea (FMNH 176170) and Mitoho (FMNH 173197, 173199) with southwestern dry spiny forest-thicket, and Sarodrano (FMNH 172919) and Saint Augustin (FMNH 172930) with degraded south western dry spiny forest. Clade 4 forms a separate branch in the phylogenetic tree and is separated from Clade 1 (*M. manavi* sensu stricto) by average 9.2% K2P genetic distance and the node separating Clade 4 and Clade 2 by 8.8% K2P genetic distance (fig. 2; table 2).

Based on the five clades derived from the molecular study, the specimens previously identified as *M. manavi* sensu lato were segregated and morphological and mensural characters identified to distinguish between these animals. These analyses and the associated characters for Clade 1 (*M. manavi* sensu stricto), Clade 3 (*M. aelleni*), and Clade 5 (*M. griveaudi*) are presented elsewhere (Goodman et al., 2009b). In the case of Clade 2 and Clade 4, no previous name is available for these animals, both of which are described here as species new to science, and the associated distinguishing morphological characters are presented below.

***Miniopterus brachytragos*, new species**

Figures 3, 4A–C, 5; tables 3–5

HOLOTYPE: Field Museum of Natural History (FMNH) 175840 collected by S.M. Goodman (field number SMG 13040) on 9 October 2002. The skull is in an excellent state. Before preservation of the specimen in formaldehyde, the skull was removed, conserved in dilute ethanol, and then cleaned using Dermestidae beetles. Pectoral muscle samples were saved in lysis buffer; this animal was sequenced and was used in the phylogenetic analysis presented herein (fig. 2; table 1). The specimen has a full adult dentition and the basisphenoid-basioccipital sutures are completely fused.

Measurements are in mm and body mass in g. Total length, 86; tail length, 39; hind foot length, 6; tragus length, 4; ear length, 10; forearm length, 37; mass, 4.3; greatest skull length, 13.3; greatest zygomatic breadth, 7.0; postorbital breadth, 3.1; mastoid breadth, 7.1; greatest braincase width, 6.5; lachrymal width, 3.5; palatal length, 6.4; mandible length, 9.0; cranial toothrow, 5.9; upper canine-molar toothrow, 5.0; width across upper canines, 3.5; width across third upper molars, 4.9 (tables 3–5).

TYPE LOCALITY: Madagascar: Province de Mahajanga, Réserve Naturelle Intégrale de Namoroka [status subsequently changed to Parc National], Forêt d'Ambovonomby, 26 km NW Andranomavo, 16°28.2′S, 45°20.9′E, 200 m above sea level.

DIAGNOSIS: A notably small member of the genus *Miniopterus*, with relatively short and nondense pelage. The dorsal fur is medium to slightly dark brown in color and the ventral

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analysis) and bootstrap support (ML, NJ, and MP analyses) and are indicated on the major nodes only (Bayesian/ML/NJ/MP). Labels include collecting locality and catalog number, sample identification number or Genbank accession number. The * indicates a missing NJ bootstrap value. Sample FMNH 5650 was basal to both *M. petersoni* and Clade 1, only in the NJ analysis. This may be an artifact due to the very short length of this sequence (160 bp from old museum specimen). Within Clade 2 there are several individuals that are not illustrated and share a haplotype with FMNH 175877 and in Clade 4 there is one individual that shares a haplotype with FMNH 175991; these are identified in table 1. Within Clade 2, FMNH 175840 is the holotype of *M. brachytragos* and within Clade 4, FMNH 173197 is the holotype of *M. mahafaliensis*.

Fig. 3. Photograph of living *Miniopterus brachytragos* captured near Ambanizana, Masoala Peninsula (FMNH 202523). Note the very small tragus. (Photograph by Manuel Ruedi.)

hairs are tipped with a dark buff. Uropatagium with relatively short and dense fur on the majority of its dorsal surface, being more obvious on the proximal half. The forearm length of the holotype is 37 mm and ranges in the type series from 35–38 mm (mean = 36.6 mm). Tragus is notably short and thick, with rounded to slightly pointed tip, 4 mm in the holotype and ranges from 3–4 mm in the type series (mean = 3.9 mm), the distal portion having a few long hairs difficult to see with the naked eye. The cranium has a sagittal crest that terminates before the parietals; a short and rectangular rostrum; deep, wide, and long nasal sulcus; and broad U-shaped palatal emargination.

PARATYPES: Province de Mahajanga, Réserve Naturelle Intégrale de Namoroka [status subsequently changed to Parc National], Forêt d'Ambovonomby, 26 km NW Andranomavo, 16°28.2′S, 45°20.9′E, 200 m, 9–10 October 2002 (FMNH 175846, 175847, 175850–175852, 175856; same locality as the holotype); just outside limit of Réserve Naturelle Intégrale de Namoroka [status subsequently changed to Parc National], along Ampandra River, 22 km NW Andranomavo, 16°26.442′S, 45°24.723′E, 120 m, 11 October 2002 (FMNH 175864, 175865, 175867); Réserve Naturelle Intégrale de Namoroka [status subsequently changed to Parc National], near source of Mandevy River, 32 km NW Andranomavo, 16°22.8′S, 45°20.7′E, 100 m, 14–15 October 2002 (FMNH 175869–175875, 175877, 175879–175885); Parc National de Bemaraha, south bank Manambolo River, near Tombeau Vazimba, 3.5 km E Bekopaka, 19°08.4′S, 44°49.7′E, 100 m, 2 December 2001 (FMNH 172867). Province d'Antsiranana, Nosy Komba, 0.9 km SW

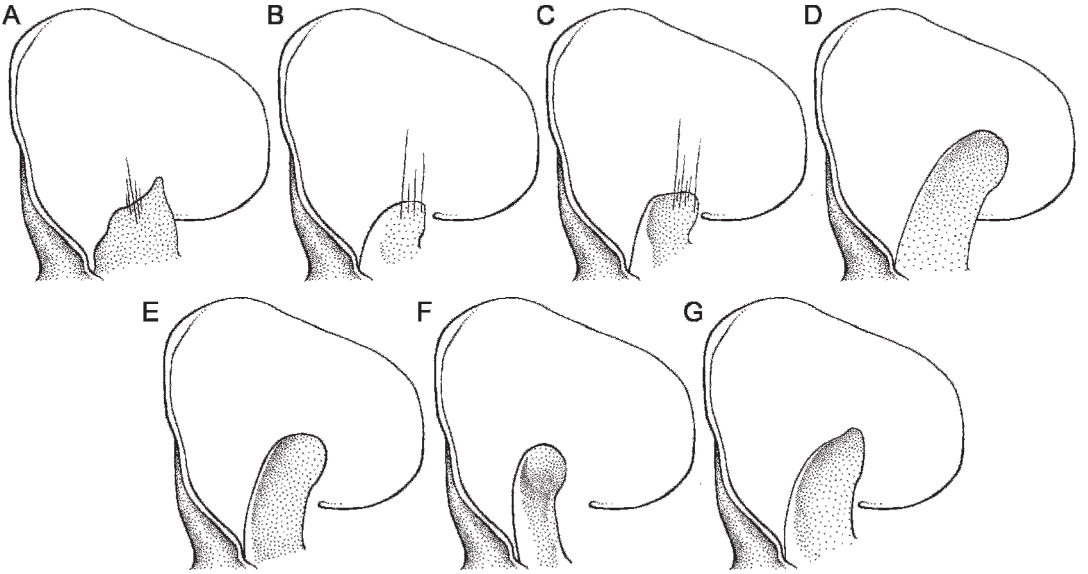

Fig. 4. Right ear and tragus of *Miniopterus* spp. from Madagascar: **A,** *M. brachytragos* (FMNH 175840, holotype), from Parc National de Namoroka, Forêt d'Ambovonomby, 26 km NW Andranomavo; **B,** *M. brachytragos* (FMNH 172685) from the Forêt de Binara, near Analamazava River, 7.5 km SW Daraina (village); **C,** *M. brachytragos* (FMNH 202523) from 1.8 km S Ambanizana, Masoala Peninsula; **D,** *M. mahafaliensis* (FMNH 173197, holotype) from Parc National de Tsimanampetsotsa, 6.5 km NE Efoetse, near Mitoho Cave; **E,** *M. mahafaliensis* (FMNH 176101) from Parc National de Kirindy-Mite, near village of Betakilotra, 11 km SE Marofihitsa; **F,** *M. griveaudi* (FMNH 184055) from Beloboka, grotte n°1, 12.5 km E Mahajanga, Madagascar; **G,** *M. aelleni* (FMNH 175842) from the Parc National de Namoroka, Forêt d'Ambovonomby, 26 km NW Andranomavo. The slightly elongated and pointed distal portion of the tragus in FMNH 175840 (A), as compared to FMNH 172685 and FMNH 202523, maybe an anomalous shape in *M. brachytragos*.

Ampangorinana, on trail to Station Forestière d'Antanampoera, 13°26.835′S, 48°20.466′E, 100 m, 14 February 2006 (FMNH 188651); Forêt de Binara, near Analamazava River, 7.5 km SW Daraina (village), 13°15.3′S, 49°37.0′E, 325–600 m, 5 November 2001 (FMNH 172685, 172686). Province de Toamasina, Masoala Peninsula, 1.8 km S Ambanizana (village), 15°38.291′S, 49°57.944′E, 5 m, 26 November 2007 (FMNH 202523).

DISTRIBUTION: Known to occur in several different biomes on Madagascar, from the Masoala Peninsula in the northeast, the Daraina region in the central northeast, and in the karstic zones of the Namoroka and Bemaraha massifs. It has also been found on the near-shore island of Nosy Komba.

DESCRIPTION: A small *Miniopterus* with tail length less than one-half the total length (table 3). The forearm length ranges from 35–38 mm, and hind foot from 5–6 mm. In the holotype and the type series, the body fur is relatively short and not particularly dense. The dorsal hairs are medium to slightly dark brown in color and the ventral hairs are tipped with a dark buff, giving a slightly mottled appearance (fig. 3). Wing membranes are medium brown, grading into a slightly lighter brown on the plagiopatagium and uropatagium; these membranes attaching to the femur at a position superior to the ankle joint. The uropatagium has a notable covering of hair across its surface, being more obvious on the dorsal surface toward the proximal half and sparser on the ventral surface.

The dorsal surfaces of the ring-shaped ears have a fur covering across much of their surface and extending nearly to the distal margin, which is noticeable with the unaided eye, and the ventral surface is virtually naked

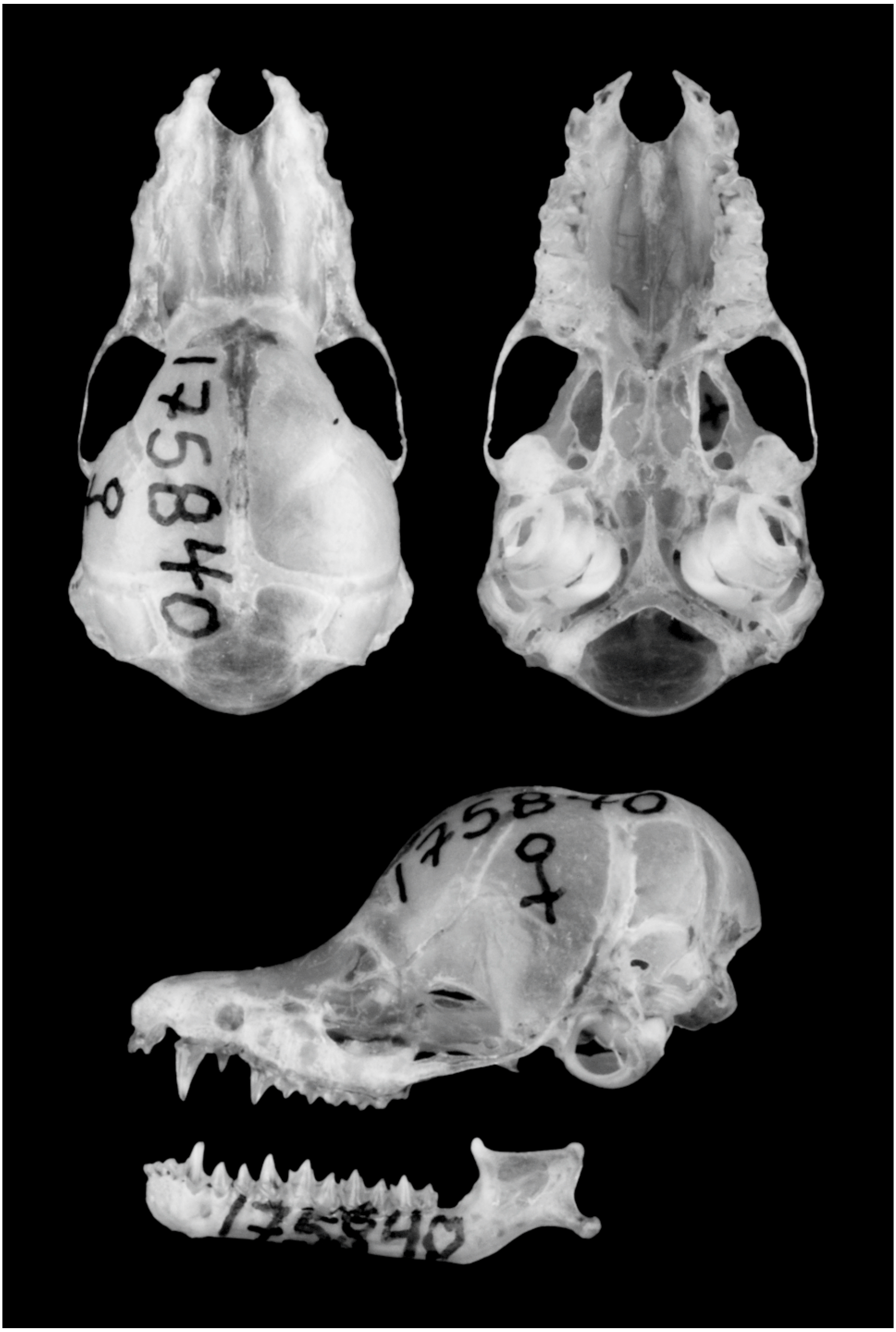

Fig. 5. Different views of skull and mandible of the holotype of *Miniopterus brachytragos* (FMNH 175840) collected in the Parc National de Namoroka, Forêt d'Ambovonomby, 26 km NW Andranomavo: (above, left) dorsal view of cranium; (above, right) ventral view of cranium; and (below) lateral view of cranium and mandible. (Photograph taken by John Weinstein, Field Museum image number Z94486_05d.)

TABLE 3

External measurements (in mm) and mass (in g) of the different diminutive species of *Miniopterus* on Madagascar that were previously considered to be *M. manavi*

All specimens are adults, the sexes are combined, and measurements are presented as mean ± standard deviation (minimum, maximum, number of specimens). Measurements were made by SMG, other than those in brackets. For measurements of *M. petersoni* see Goodman et al. (2008).

	Total length	Tail length	Hind foot length	Tragus length	Ear length	Forearm length	Mass
Type specimen of *M. brachytragos* (FMNH 175840)	86	39	6	4	10	37	4.3
Series of *M. brachytragos*	87.4 ± 2.36, 83–92, *N* = 28	40.2 ± 1.29, 38–43, *N* = 29	5.8 ± 0.44, 5–6, *N* = 29	3.9 ± 0.31, 3–4, *N* = 30	10.0 ± 0.59, 9–11, *N* = 30	36.6 ± 0.97, 35–38, *N* = 30	4.3 ± 0.83, 2.9–6.3, n = 30
Type specimen of *M. mahafaliensis* (FMNH 173197)	95	44	6	6	11	38	5.3
Series of *M. mahafaliensis*	91.1 ± 2.30, 87–96, *N* = 68	42.4 ± 2.14, 38–48, *N* = 70	6.3 ± 0.48, 6–7, *N* = 64	5.8 ± 0.40, 5–6, *N* = 70	10.4 ± 0.63, 9–11, *N* = 67	37.4 ± 0.92, 35–40, *N* = 67	4.9 ± 0.66, 3.8–7.3, *N* = 74
Series of *M. manavi* sensu stricto	90, *N* = 1	39, *N* = 1	6, *N* = 1	6, *N* = 1	10, *N* = 1	38.5 ± 0.77 37.6–39.2, *N* = 4	6.4, *N* = 1
Sequenced individuals of *M. griveaudi* from Madagascar	89.3 ± 1.75, 86–93, *N* = 18	40.1 ± 2.05 35–43, *N* = 18	5.8 ± 0.55 5–7, *N* = 18	5.9 ± 0.54 5–7, *N* = 18	10.4 ± 0.61 9–11, *N* = 18	36.9 ± 0.90 35–38, n = 18	5.4 ± 0.91 4.1–7.1, n = 18
Sequenced individuals of *M. aelleni* from Madagascar	90.7 ± 2.02 88–95, *N* = 12	42.1 ± 1.50 40–45, *N* = 12	6.1 ± 0.52 5–7, *N* = 12	6.1 ± 0.67 5–8, *N* = 12	11.1 ± 0.67 10–12, *N* = 12	38.8 ± 1.42 35–41, n = 12	4.6 ± 0.71 3.9–6.5, n = 12

TABLE 4

Cranial measurements (in mm) of the different diminutive species of *Miniopterus* on Madagascar that were previously considered to be *M. manavi*

All specimens are adults, the sexes are combined, and measurements are presented as mean ± standard deviation (minimum, maximum, number of specimens). See Methods for explanation of variable acronyms. For measurements of *M. petersoni* see Goodman et al. (2008).

	GKSL	ZYGO	LW	POB	GBW	MAST	PAL	MAND
Type specimen of *M. brachytragos* (FMNH 175840)	13.3	7.0	3.5	3.1	6.5	7.1	6.4	9.0
Series of *M. brachytragos*	13.3 ± 0.24, 12.9–14.0, *N* = 30	7.0 ± 0.17, 6.7–7.3, *N* = 30	3.6 ± 0.14, 3.2–3.8, *N* = 30	2.9 ± 0.11, 2.7–3.1, *N* = 30	6.5 ± 0.12, 6.2–6.7, *N* = 30	7.0 ± 0.17, 6.6–7.3, *N* = 29	6.3 ± 0.16, 6.1–6.7, *N* = 30	9.0 ± 0.19, 8.4–9.6, *N* = 25
Type specimen of *M. mahafaliensis* (FMNH 173197)	13.7	7.0	3.5	3.0	6.8	7.0	6.3	9.5
Series of *M. mahafaliensis*	13.8 ± 0.20, 13.4–14.2, *N* = 68	7.2 ± 0.13, 6.9–7.5, *N* = 67	3.6 ± 0.16, 3.3–4.1, *N* = 72	3.0 ± 0.10, 2.8–3.2, *N* = 74	6.7 ± 0.12, 6.4–7.0, *N* = 73	7.1 ± 0.14, 6.9–7.5, *N* = 72	6.3 ± 0.13, 6.0–6.6, *N* = 74	9.2 ± 0.17, 8.8–9.5, *N* = 66
Series of *M. manavi* sensu stricto	14.0 ± 0.12 13.8–14.1, *N* = 6	7.4 ± 0.21 7.2–7.7, *N* = 4	4.0 ± 0.17 3.8–4.2, *N* = 7	3.2 ± 0.10 3.0–3.3, *N* = 7	6.9 ± 0.20 6.6–7.2, *N* = 7	7.3 ± 0.24 7.1–7.7, *N* = 5	6.5 ± 0.09 6.3–6.4, *N* = 7	9.6 ± 0.08 9.5–9.7, *N* = 5
Sequenced individuals of *M. griveaudi* from Madagascar	13.7 ± 0.21 13.3–14.0, *N* = 18	7.4 ± 0.13 7.2–7.6, *N* = 17	4.0 ± 0.10 3.8–4.1, *N* = 18	3.2 ± 0.12 3.1–3.5, *N* = 18	6.8 ± 0.09 6.7–7.0, *N* = 18	7.3 ± 0.09 7.2–7.5, *N* = 18	6.4 ± 0.12 6.1–6.8, *N* = 18	9.2 ± 0.15 9.0–9.4, *N* = 18
Sequenced individuals of *M. aelleni* from Madagascar	14.1 ± 0.23 13.6–14.5, *N* = 12	7.3 ± 0.18 7.1–7.7, *N* = 10	3.9 ± 0.14 3.7–4.1, *N* = 12	3.1 ± 0.11 3.0–3.3, *N* = 12	6.6 ± 0.23 6.3–7.1, *N* = 12	7.2 ± 0.23 6.9–7.8, *N* = 12	6.8 ± 0.13 6.5–7.0, *N* = 12	9.7 ± 0.17 9.4–10.0, *N* = 11

TABLE 5

Dental measurements (in mm) of the different diminutive species of *Miniopterus* on Madagascar that were previously considered to be *M. manavi*

All specimens are adults, the sexes are combined, and measurements are presented as mean ± standard deviation (minimum, maximum, number of specimens). See Methods for explanation of variable acronyms. For measurements of *M. petersoni* see Goodman et al. (2008).

	I1-M3	C-M3	C1-C1	M3-M3
Type specimen of *M. brachytragos* (FMNH 175840)	5.9	5.0	3.5	4.9
Series of *M. brachytragos*	5.9 ± 0.11, 5.8–6.3, *N* = 30	4.9 ± 0.11, 4.7–5.2, *N* = 30	3.5 ± 0.14, 3.3–3.9, *N* = 30	5.0 ± 0.15, 4.7–5.4, *N* = 30
Type specimen of *M. mahafaliensis* (FMNH 173197)	6.0	5.1	3.7	5.0
Series of *M. mahafaliensis*	5.9 ± 0.10, 5.7–6.2, *N* = 74	5.0 ± 0.10, 4.7–5.1, *N* = 74	3.5 ± 0.01, 3.2–3.8, *N* = 74	5.1 ± 0.11, 4.9–5.5, *N* = 29
Series of *M. manavi* sensu stricto	6.0 ± 0.08 5.9–6.7, *N* = 7	5.1 ± 0.09 5.0–5.2, *N* = 7	3.7 ± 0.12 3.6–3.9, *N* = 7	5.3 ± 0.13 5.1–5.4, *N* = 7
Sequenced individuals of *M. griveaudi* from Madagascar	5.9 ± 0.11 5.7–6.1, *N* = 18	5.1 ± 0.09 4.9–5.1, *N* = 18	3.8 ± 0.13 3.6–4.0, *N* = 18	5.3 ± 0.10 5.1–5.4, *N* = 17
Sequenced individuals of *M. aelleni* from Madagascar	6.3 ± 0.11 6.1–6.4, *N* = 12	5.4 ± 0.11 5.2–5.5, *N* = 12	3.7 ± 0.27 3.5–4.4, *N* = 12	5.4 ± 0.16 5.1–5.7, *N* = 12

except a ring of hairs around the lateral margin. The ear terminates with a slightly elongated and rounded tip anteriomedially. The average tragus length in the holotype and other animals referred to this taxon is 3.9 mm (range 3–4 mm) (table 3). The tragus is distinct, being a proximally broad and laterally short structure, and in some specimens with a pronounced lateral flange that becomes reduced distally. Toward the tip, which varies from being rounded to slightly pointed, the tragus tapers medially and has sparse and relatively long fur, which is not easily visible to the naked eye (fig. 4A–C).

The skull of *M. brachytragos* has a notably short rostrum, compared to the other diminutive Malagasy members of this genus, a slightly bulbous braincase, and not particularly constricted at the level of the interorbitals (fig. 5). In dorsal view, the rostrum is relatively linear in shape, particularly the nasals, which do not show a marked lateral expansion in their medial portions, and are about one-half longer than the width. The central sulcus of the nasal is relatively deep and wide, spanning more than the proximal two-thirds of the nasal length. Frontals somewhat rounded. The sagittal crest is not prominent and terminates distally before the junction of the parietals. In lateral view, there is a flattening or depression in the parietal region. Lambdoid crest is developed, but not prominent. The palatal emargination forms a notably open and proximally rounded V-shaped or U-shaped notch. Medial portion of palate is slightly concave and the lingual portions of toothrows are aligned in parallel. The posterior palatal spine is relatively short and blunt (broken in holotype). In most individuals, including the holotype, there are distinct and rounded palatal foramina at the level of the last upper molar.

Dental formula I 2/3 C 1/1 PM 2/3 M 3/3, comprising the adult dentition of 36 teeth, and characteristic of *Miniopterus* (Koopman, 1994). The dentition of *M. brachytragos* is typical of small members of this genus, which includes a first premolar (PM2) that is relatively small and with a more simple cusp morphology than the second premolar (PM4). In lateral view, the length of PM4 is about two-thirds that of the C (fig. 5).

On the basis of t-test comparisons, two of the 19 measured measurement variables showed evidence of sexual dimorphism, including forearm length with the mean measurement in males of 36.0 mm and in females of 36.9 mm ($P = 0.02$, $df = 27$) and mass for which the mean measurement in males was 3.8 g and females 4.5 g. In the first case, this difference is considered biologically unimportant and, in the second case, several of the females were pregnant. Hence, in *M. brachytragos* sexual dimorphism is not notable and the sexes have been combined in the analyses presented herein.

Little variation was found in the craniodental morphology and measurements within the type series of *M. brachytragos*. Two exceptions are worth mentioning, both specimens possessing the characteristic tragus of this species: (1) FMNH 172867 from the Bemaraha is notably larger in cranial measurements than the other available specimens; a tissue sample was not available for this individual for the molecular portion of this study and (2) FMNH 175871 from the Namoroka has a flatter rostrum and more rounded frontals than typical of this species, but, based on the genetic analysis, this animal is clearly referable to *M. brachytragos* (fig. 2; table 1).

MORPHOLOGICAL AND MORPHOMETRIC COMPARISONS: In the hand, *M. brachytragos* is immediately separable from other *Miniopterus* spp. known from the Malagasy region by its distinctive tragus, which is very short, stump-like structure (fig. 4A–C). In one of the illustrated tragi (fig. 4A) the distal tip is pointed, rather than rounded in the other individuals, and this maybe an anomalous condition in this species. Other characters to differentiate *M. brachytragos* from the different regional diminutive members of this genus are presented in the Comparisons section under the next species.

PHYLOGEOGRAPHY: *Miniopterus brachytragos* is characterized by an average intraclade divergence of average 0.7% K2P. Most of the analyzed tissue samples come from Namoroka, for which one haplotype was shared among eight individuals, and animals from this locality showed a mean sequence divergence of 1.0%. The most distinctive haplotype (mean 2.1% divergence) was FMNH 188651, which came from Nosy Komba, a near-shore island separated from the main island by 5 km of water. In the same regard, a specimen from the Masoala Peninsula (FMNH 202523) differed by a mean divergence of 1.1% from the others. These apparent subclades might imply some phylogeographic structure in this taxon, but current sampling is insufficient to draw any clear conclusion.

ECOLOGICAL NOTES: The holotype and the associated paratypes from the same locality were captured in the limestone Namoroka Massif soon after dusk in a harp trap placed within slightly disturbed western dry forest and in close proximity to one of the entrances of the Grotte d'Ambovonomby. This portion of the massif has heavily eroded limestone karst, with deeply sculpted rock pinnacles, known as tsingy. *Miniopterus brachytragos* was also obtained at two other nearby sites in the Namoroka Massif, both gallery forests surrounded by anthropogenic savannah and with flowing water—the Ampandra River site was just outside the limit of the park and the Mandevy River site at a locality where an artesian water source percolates from the ground. The elevation range of *M. brachytragos* in the Namoroka region is from 100 to 200 m. The second limestone karst location where this species has been documented is in the Bemaraha Massif at 100 m elevation. Here a single individual was netted within a canyon in undisturbed tsingy forest just south of the Tombeau Vazimba and over a temporary water pool.

Miniopterus brachytragos has been recorded at three other sites with notably different habitats. In the Forêt de Binara, near the village of Daraina, in the central northeast, it was netted over the Analamazava River in a zone dominated by lowland humid forest with elements of western dry forest. Some exposed sedimentary rock occurs along the river with eroded crevices. This locality is at about 320 m elevation. The second site was on the offshore island of Nosy Komba, less than 5 km over water distance from the main island, in a mixed habitat composed of introduced mango (*Mangifera indica*) and western dry forest. The final locality, in the northeast on the Masoala Peninsula and just south of Ambanizana, was in disturbed gallery lowland Humid Forest within close distance to the sea.

ETYMOLOGY: The name is derived from the Greek *brachys* ("short") and *tragos*, meaning "goat," a word ultimately borrowed into New Latin to mean "tragus," and has been chosen as this taxon is easily distinguished from its congeners by its diminutive tragus.

Miniopterus mahafaliensis, new species

Figures 4D–E, 6, 7; tables 3–5

HOLOTYPE: Field Museum of Natural History (FMNH) 173197 collected by S.M. Goodman (field number SMG 12649) on 2 March 2002. The skull is in fine condition. Before preservation of the specimen in formaldehyde, the skull was removed, placed in dilute ethanol, and then cleaned using dermestid beetles. Pectoral muscle samples saved in lysis buffer; this animal was sequenced and was used in the phylogenetic analysis presented herein (fig. 2; table 1). The specimen has a full adult dentition and the basisphenoid-basioccipital sutures are completely fused.

Measurements are in mm and body mass in g. Total length, 95; tail length, 44; hind foot length, 6; tragus length, 6; ear length, 11; forearm length, 38; mass, 5.3; greatest skull length, 13.7; greatest zygomatic breadth, 7.0; postorbital breadth, 3.0; mastoid breadth, 7.0; greatest braincase width, 6.8; lachrymal width, 3.5; palatal length, 6.3; mandible length, 9.5; cranial toothrow, 6.0; upper canine-molar toothrow, 5.1; width across upper canines, 3.7; width across third upper molars, 5.0 (tables 3–5).

TYPE LOCALITY: Madagascar: Province de Toliara, Parc National de Tsimanampetsotsa, 6.5 km NE Efoetse, near Mitoho Cave, 24°03.0′S, 43°45.0′E, at 50 m above sea level.

DIAGNOSIS: A diminutive species of *Miniopterus* with relatively dense pelage and medium-brown dorsum and light gray-tipped ventrum. Uropatagium with relatively dense fur on the dorsal surface. The holotype forearm length is 38 mm and in the type series ranges from 35–40 mm (mean = 37.4 mm). Tragus is relatively long, 6 mm in the holotype and in the type specimens ranges from 5–6 mm (mean = 5.8 mm). The tragus is moderately wide, has parallel margins along most of its length, and the distal portion curves medially into a slightly expanded and rounded tip. The cranium has a prominent sagittal crest in contact with the parietals, relatively short and wide nasal sulcus, and deep cut V-shaped palatal emargination.

PARATYPES: Province de Fianarantsoa, Parc National de l'Isalo, along Sahanafa River, at foot of Bevato, 28 km SE Berenty-Betsileo, 22°19.0′S, 45°17.6′E, 550 m, 9 December 2002 (FMNH 175991); Ihosy, commune rurale d'Akily, Grotte d'Andranomilitry, 22°23.111′S, 46°03.355′E, 950 m, 1 November 2004 (FMNH 184470–184473). Province de Toliara, Parc National de Kirindy-Mite, 13 km W Marofihitsa, 20°47.4′S, 44°08.8′E, 30 m, 11 November 2002 (FMNH 176096–176099); Parc National de Kirindy-Mite, near village of Betakilotra, 11 km SE Marofihitsa, 20°53.2′S, 44°04.8′E, 35 m, 16–17 November 2002 (FMNH 176100–176110); Forêt des Mikea, 9.5 km W Ankililoaka, 22°46.7′S, 43°31.4′E, 80 m, 14, 15, and 18 February 2003 (FMNH 176167–176170); Ranobe, 23°14.033′S, 43°52.493′E, approximately 50 m, 16 August 2003 (FMNH 177376); Fiherenana, 23°14′17″S, 43°52′23″E, 27 November 2002 (FMNH 176509, 176510); Betroka, 23°15′50″S, 46°05′30″E, 800 m, 18 June 1953 (FMNH 75774); Antafiky, 23°29′16.0″S, 044°04′39.1″E, 50 m, 24 July 2001, 4 February 2002 (FMNH 173252–173253); Grotte d'Ambanilia, 3.7 km SSE Sarodrano, 23°32.397′S, 43°44.763′E, sea level, 7 May 2002 (FMNH 172918–172928); Grotte de Bisihiko, 0.75 km E St. Augustin, 23°32.933′S, 43°46.044′E, 10 m, 8 May 2002 (FMNH 172929–172933, 202484–202485); Mahaleotse, 23°31′38.8″S, 44°05′16.1″E, 70 m, 4 June 2002 (FMNH 176508); Parc National de Tsimanampetsotsa, Malaza Manga Aven, 24°1.827′S, 43°45.283′E, 80 m, 4 March 2002 (FMNH 173204); Parc National de Tsimanampetsotsa, 6.5 km NE Efoetse, near Mitoho Cave, 24°03.0′S, 43°45.0′E,

Fig. 6. Photograph of living *Miniopterus mahafaliensis* captured 6.5 km NE Efoetse, near Mitoho Cave, Parc National de Tsimanampetsotsa (FMNH 173191), taken at the same locality as the holotype (FMNH 173197). Note the gray grizzled hair to the ventrum. (Photograph by Harald Schütz.)

Fig. 7. Different views of skull and mandible of the holotype of *Miniopterus mahafaliensis* (FMNH 173197) collected in the Parc National de Tsimanampetsotsa, 6.5 km NE Efoetse, near Mitoho Cave: (above, left) dorsal view of cranium; (above, right) ventral view of cranium; and (below) lateral view of cranium and mandible. (Photograph taken by John Weinstein, Field Museum image number Z94487_05d.)

50 m, 28 February 2002, 2 March 2002 (FMNH 173154, 173189–173196, 173198–173203; same locality as the holotype); Grotte d'Antagneotsy, 5.0 km NE Vohombe, 24°23.001′S, 43°50.742′E, 100 m, 26 February 2005 (FMNH 184226); Grotte d'Amborombe, 4.0 km Itampolo, 24°37′42.7″S, 43°58′56.9″E, 70 m, 29 May 2005 (FMNH 184160); Grotte d'Andraimpano, 4.2 km NE Itampolo, 24°39.012′S, 43°57.797′E, 110 m, 22–23 February 2005, 28 May 2005 (FMNH 184155–184159, 184168, 184222–184225); 10.5 km SE Itampolo (village), 24°44.2′S, 44°1.79′E, 120 m, 18 February 2005 (FMNH 184217–184221).

DISTRIBUTION: *Miniopterus mahafaliensis* is known from numerous sites on or in close proximity to the limestone Mahafaly Plateau in the southwestern portion of the island, including the zone from Itampolo north across the Onilahy River to Sarodrano spanning the elevational range from sea level to 120 m (fig. 1). Its distribution then continues further north to the Forêt des Mikea (70–80 m) and based on current data ends in the Kirindy-Mite region (30–35 m). Further, it is known from the more inland and upland sites of Ihosy and near Betroka from 800–950 m and within the Isalo formation at 550 m.

DESCRIPTION: A small species of *Miniopterus* with the tail length less than one-half the total length (table 3). Forearm length ranging in the type series from 35–40 mm and the hind foot length from 6–7 mm. In the holotype and associated type series, the body fur is relatively long and dense, and the dorsum coloration is a medium brown and the ventrum hairs are tipped with a light gray, giving a notably lighter appearance to the underside (fig. 6). Wing membranes are medium brown, grading into a slightly lighter brown in the uropatagium. This latter membrane has a relatively dense covering of hair across its surface, being more obvious on the dorsal side toward the proximal half of the membrane and sparser on the ventral side. Uropatagium and plagiopatagium attaching to the femur at a position superior to the ankle joint.

On the dorsal surface of the ring-shaped ears, the proximal one-half has a covering of hair across much of its surface and the distal half is sparsely furred. In contrast, the ventral surface is sparsely covered with fur, being concentrated around the distal ear margin. The ear terminates with a slightly elongated tip, rounded anteriomedially. The average tragus length in the holotype and other animals in the type series is 5.8 mm (range 5–6 mm) (table 3). The proximal three-quarters of the moderately wide tragus have a shaft with largely parallel sides, distally curving slightly medially, and expanding into a rounded tip (fig. 4D–4E). In some individuals, there is a lateral flange along the proximal three-quarters of the tragus.

The skull of *M. mahafaliensis* has a slightly elongated rostrum, slightly bulbous braincase, and not notably constricted at the level of the interorbitals (fig. 7). In dorsal view, the rostrum is relatively linear in shape, particularly the nasals, which do not show a marked lateral expansion in their medial portions. The central sulcus of the nasal region is relatively narrow and spanning, in most cases, slightly more than the proximal one-half of the nasal length. Frontals somewhat rounded with prominent sagittal crest passing distally to the parietals; in lateral view, there is a slight flattening of the cranium, without a notable depression, in the parietal region. Lambdoid crest is prominent. When viewed dorsally, the palatal emargination forms a deeply cut V-shaped notch. Medial portion of palate is slightly concave with lateral lingual sides of the toothrows forming two elongated and parallel surfaces, and posterior palatal spine relatively long and thin. In most individuals, the palatal foramina, located at the level of the last upper molar, are indistinct or not present.

Dental formula I 2/3 C 1/1 PM 2/3 M 3/3, comprising the adult dentition of 36 teeth, and characteristic of *Miniopterus* (Koopman, 1994). The dentition of *M. mahafaliensis* is typical of small members of this genus, with, for example, the first premolar (PM2) being relatively small and with simpler cusp morphology than the second premolar (PM4). In lateral view, the length of PM4 is approximately two-thirds that of the C.

On the basis of t-test comparisons, four of the 19 measured variables for *M. mahafaliensis* demonstrated evidence of sexual dimorphism.

Among the external measurements listed in table 3, only mass showed a difference between the sexes—on average 5.0 g in females, several of which were pregnant, and 4.7 g in males. None of the cranial measurements demonstrated sexual dimorphism. In contrast, three of the four dental measurements were sexually dimorphic: cranial toothrow, in males 6.0 mm and females 5.9 mm (t = 2.00, P = 0.05, df = 71); upper canine-molar toothrow, in males 5.0 mm and females 4.9 mm (t = 2.07, P = 0.04, df = 71); and width across upper canines, in males 3.6 mm and females 3.5 mm (t = 4.21, P = 0.0001, df = 71). While statistically significant, in part based on the large sample sizes, the differences between the sexes are rather subtle and we consider them unimportant with regard to the analyses presented herein and the sexes have been combined.

MORPHOLOGICAL AND MORPHOMETRIC COMPARISONS: There are at least six species of diminutive *Miniopterus* within the Malagasy region (Madagascar and the Comoros Archipelago) (Goodman et al., 2008, 2009b; Weyeneth et al., 2008): *M. petersoni* (average forearm length of 39.8 mm, range 38–43 mm); *M. manavi* sensu stricto (average forearm length of 38.6 mm, range 38–39 mm); *M. griveaudi* (average forearm length of 36.9 mm, range 35–38 mm); *M. aelleni* (average forearm length of 38.8 mm, range 35–41 mm); *M. brachytragos* (average forearm length of 36.6 mm, range 35–38 mm); and *M. mahafaliensis* (average forearm length of 37.4 mm, range 35–38 mm) (all of these measurements were made by the same collector, with the exception of *M. manavi*). Of these six species, four are endemic to Madagascar and the other two (*M. aelleni* and *M. griveaudi*) restricted to Madagascar, Anjouan, and Grande Comore. These species can be differentiated at the first level based on molecular genetic data (fig. 2) and differences in tragus shape (fig. 4; fig. 3 in Goodman et al., 2009b), and at a second level using a combination of other external and craniodental characters and morphology.

The only diminutive East African member of this genus, *M. minor*, has been shown to be genetically separate from the Malagasy species (fig. 2; Goodman et al., 2009b; Weyeneth et al., 2008) and does not need to be considered further. Several Indomalayan, Australian, and Oceanian taxa fall into the same size range as the Malagasy animals, but based on geographic arguments can be removed from evaluation. Hence, species necessary to demonstrate the distinctiveness of *M. brachytragos* and *M. mahafaliensis* are all restricted to the Malagasy region. Given its slightly larger size, *M. petersoni* can also be eliminated from these comparisons (Goodman et al., 2008).

Miniopterus brachytragos can be distinguished from the other regional members of this genus based on tragus shape, which is notably short and blunt, as well as being the only one species with sparse hairs occurring on this structure (fig. 4A–C). Further, *M. mahafaliensis* has a tragus morphology that separates it from the other four diminutive Malagasy *Miniopterus* spp., and can be possibly confused only with *M. griveaudi* and *M. aelleni*. The tragus in *M. mahafaliensis* is a moderately thick structure with two largely parallel sides that terminate distally with a slightly expanded rounded tip that curves medially (fig. 4D–E); *M. griveaudi* has a notably thinner tragus that has a relatively straight shaft and the structure terminates with a slightly retracted and rounded head (fig. 4F); *M. aelleni* has a thickset tragus, distinctly wider at the base, that tapers toward the distal slightly pointed tip (fig. 4G); and *M. manavi* has a relatively thin tragus and the medial margin along the distal two-thirds of its length has a flange, the distal lateral portion is slightly enlarged and rounded, and the distal medial tip terminates with an angular, straight edge (fig. 3a in Goodman et al., 2009b). In some photographic images we have examined of agitated handheld animals, the tragus is bent anteriorly compared to fluid-preserved specimens; this aspect needs to be taken into consideration when making comparisons to fig. 4.

Of these five taxa, *M. manavi* has the darkest dorsal pelage coloration, tending toward black or a slightly lighter dark brownish black. The fur of *M. petersoni*, *M. griveaudi*, and *M. aelleni*, particularly the dorsum, have different tints of dark brown and in a few adults approach reddish brown, while in *M. mahafaliensis* and *M. brachytragos* the dorsum tends to be a lighter medium brown. In all five species, the venter is paler in coloration than the dorsum, specifically on the distal portions of the hairs, giving a slightly mottled appearance; the exception is *M. mahafaliensis*, which is distinctly grizzled. The wing and tail membrane coloration in *M. manavi* is largely a uniform dark brownish-black, while these surfaces in the remaining four taxa are generally dark to medium brown on the wings, grading into a lighter brown on the interfemoral membrane. The amount of fur covering the uropatagium also helps to distinguish these species: in *M. manavi*, *M. mahafaliensis*, and *M. brachytragos* there is relatively dense fur over more than half the proximal dorsal surface and more sparse on the proximal ventral surface; in *M. aelleni* there is thin fur across the surface of this membrane, being more obvious on the dorsal than ventral portions; and in *M. griveaudi* the membrane is largely naked, with the exception of thin hairs often difficult to see with the naked eye.

There are a number of cranial characters that help to separate these five taxa and here we restrict comparisons to the most obvious features. In general, from dorsal view, the rostrum of *M. aelleni* is linear in shape and distinctly longer than *M. brachytragos*, *M. griveaudi*, *M. mahafaliensis*, and *M. manavi*, which are characterized by reduced lateral expansion on the medial portion of nasals (fig. 8). *Miniopterus griveaudi* tends to have an even more rounded rostrum than *M. manavi*. In *M. aelleni*, *M. griveaudi*, and *M. mahafaliensis* the sagittal crest passes distally to the parietal region, while in *M. brachytragos* and *M. manavi* it extends two-thirds of the distance to the parietals. The lambdoid crest in *M. aelleni*, *M. griveaudi*, and *M. mahafaliensis* is more prominent than in the remaining two taxa. The palatal emargination in *M. aelleni* forms a deeply cut V-shaped notch, in *M. griveaudi*, *M. mahafaliensis*, and *M. manavi* this is a more open V-shape, and in *M. brachytragos* a notably open and proximally rounded U shape.

The medial portion of the palate in *M. aelleni* and *M. manavi* is notably flat, while in *M. brachytragos*, *M. griveaudi*, and *M. mahafaliensis* the palate forms a slightly curved basin (fig. 9). In all five taxa, the basisphenoid pits are shallow and narrow in shape, with some subtle variation between them and a tendency to be more elongated in *M. manavi*. If palatal foramina are present, they tend to be more regular in *M. brachytragos* and are located at the level of the last upper molar. In general, the dentitions of these five species are similar (figs. 9, 10).

Principal component analyses were conducted separately on cranial and dental measurements of the five species that had been classically placed under the name *manavi*: *M. aelleni*, *M. brachytragos*, *M. griveaudi*, *M. mahafaliensis*, and *M. manavi* sensu stricto, as well as the slightly larger *M. petersoni*. When factors 1 and 2 are plotted for the cranial variables (fig. 11A), individuals of *M. petersoni* show a clear separation from the balance of the taxa, including *M. manavi* sensu stricto, individuals of *M. aelleni* form largely a separate group, and *M. griveaudi* and *M. brachytragos* show no overlap. Certain taxa (*M. brachytragos*, *M. griveaudi*, *M. mahafaliensis*, and *M. manavi* sensu stricto) show broad overlap in their spatial positions, which emphasizes the similar cranial size and morphology. All of the cranial variables showed heavy loadings on the first factor, which accounted for 71.5% of the explained variation (table 6). On the second factor, no variable displayed an important loading score and an additional 13.2% of the variation was explained. In general, *M. petersoni* and *M. aelleni* have relatively long and narrow skulls and, at the opposite extreme, *M. griveaudi* short and wide skulls.

The results of the principal component analysis for the dental variables (fig. 11B) show the same basic distribution for the plot of factors 1 and 2 as with the cranial variables. The exceptions include one individual of *M. petersoni* that falls within the multispecies cluster. Based on this analysis, *M. brachytragos*, *M. griveaudi*, *M. mahafaliensis*, and *M. manavi* sensu stricto, show considerable similarity in dental size and shape. Of the four variables used in this analysis, all showed heavy loadings on factor 1, which explains 81.7% of the variance (table 6). With the addition of factor 2, and additional 11.7% of the variance is explained and not one of the variables demonstrated heavy loading.

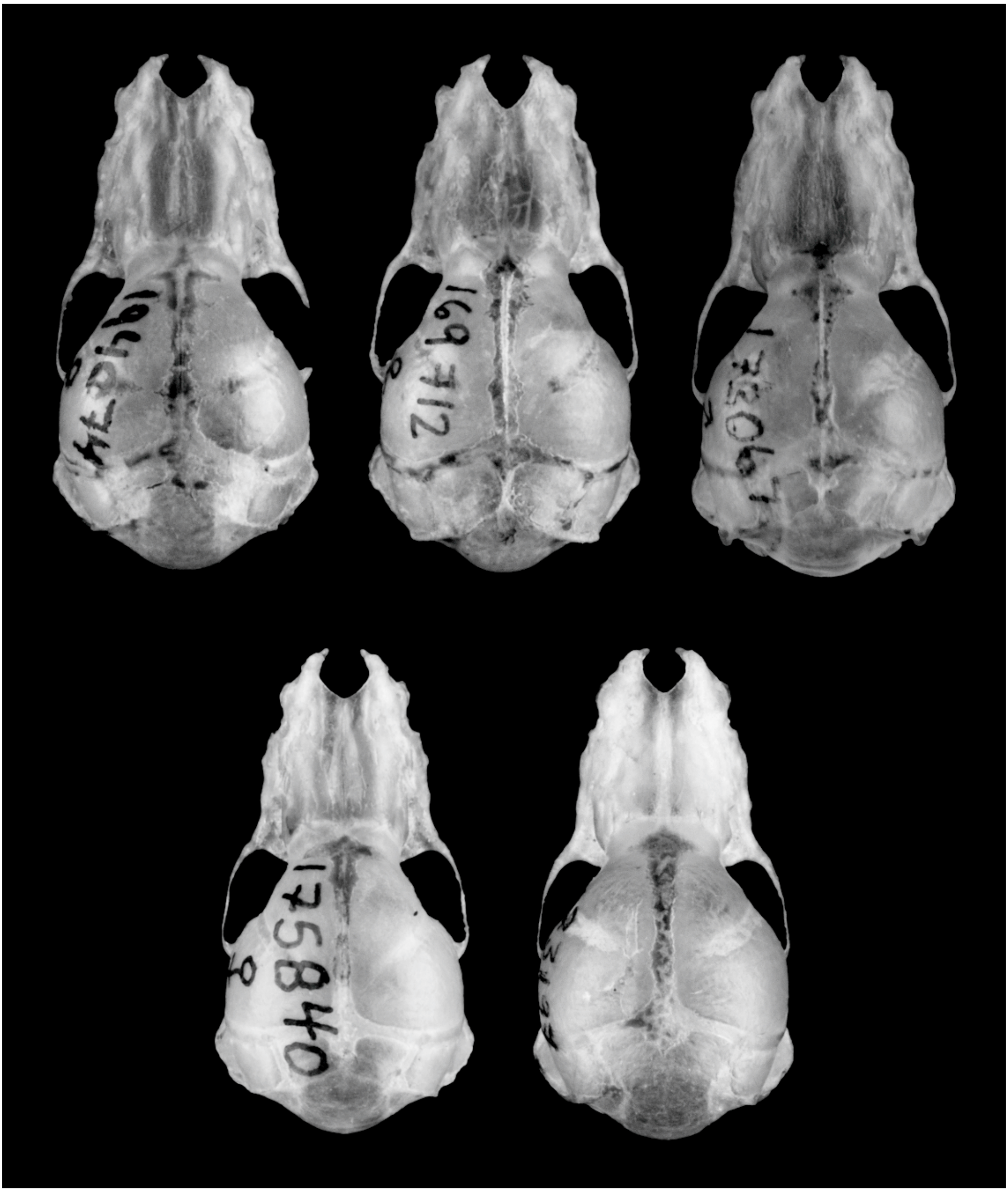

Fig. 8. Dorsal views of skulls of *Miniopterus* spp. from Madagascar: (above, from left to right) *M. manavi* (FMNH 194074) from the Grotte de Fandanana, near Fandriana; *M. griveaudi* (FMNH 169712) from near the Andrafiabe Cave, Ankarana; holotype of *M. aelleni* (FMNH 173067), from the Canyon d'Antsiroandoa, Ankarana; below, left) holotype of *M. brachytragos* (FMNH 175840), from the Forêt d'Ambovonomby, Parc National de Namoroka; and (below, right) holotype of *M. mahafaliensis* (FMNH 173197), from near Mitoho Cave, Parc National de Tsimanampetsotsa. (Photograph taken by J. Weinstein, Field Museum image number Z94488_01d.)

Fig. 9. Ventral views of skulls of *Miniopterus* spp. from Madagascar: (above, from left to right) *M. manavi* (FMNH 194074) from the Grotte de Fandanana, near Fandriana; *M. griveaudi* (FMNH 169712) from near the Andrafiabe Cave, Ankarana; holotype of *M. aelleni* (FMNH 173067), from the Canyon d'Antsiroandoa, Ankarana; (below, left) holotype of *M. brachytragos* (FMNH 175840), from the Forêt d'Ambovonomby, Parc National de Namoroka, Forêt d'Ambovonomby; and (below, right) holotype of *M. mahafaliensis* (FMNH 173197), from near Mitoho Cave, Parc National de Tsimanampetsotsa. (Photograph taken by J. Weinstein, Field Museum image number Z94488_02d.)

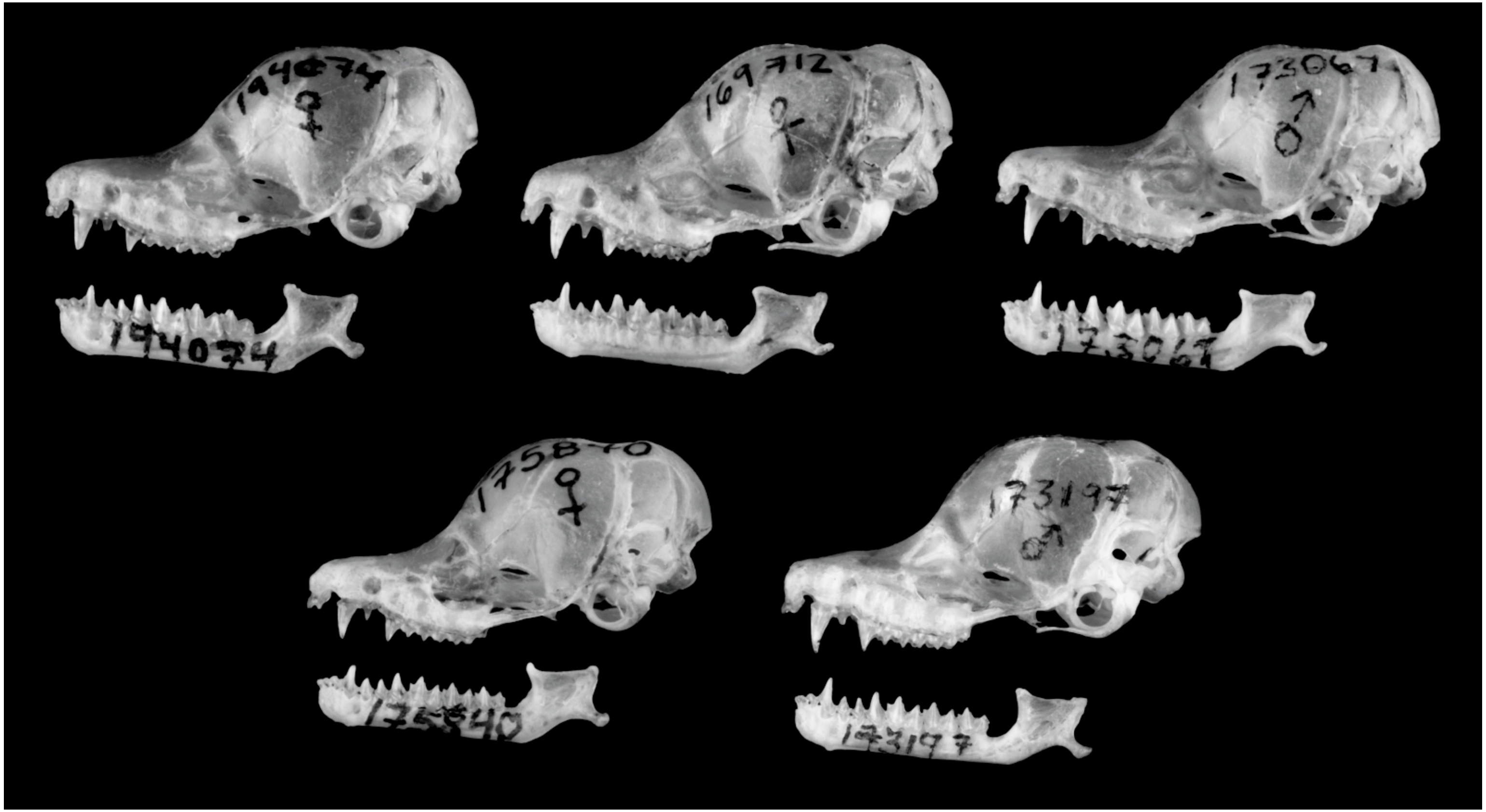

Fig. 10. Lateral views of skulls and mandibles of *Miniopterus* spp. from Madagascar: (above, from left to right) *M. manavi* (FMNH 194074) from the Grotte de Fandanana, near Fandriana; *M. griveaudi* (FMNH 169712) from near the Andrafiabe Cave, Ankarana; holotype of *M. aelleni* (FMNH 173067), from the Canyon d'Antsiroandoa, Ankarana; (below, left) holotype of *M. brachytragos* (FMNH 175840), from the Forêt d'Ambovonomby, Parc National de Namoroka, Forêt d'Ambovonomby; and (below, right) holotype of *M. mahafaliensis* (FMNH 173197), from near Mitoho Cave, Parc National de Tsimanampetsotsa. (Photograph taken by J. Weinstein, Field Museum image number Z94488_03d.)

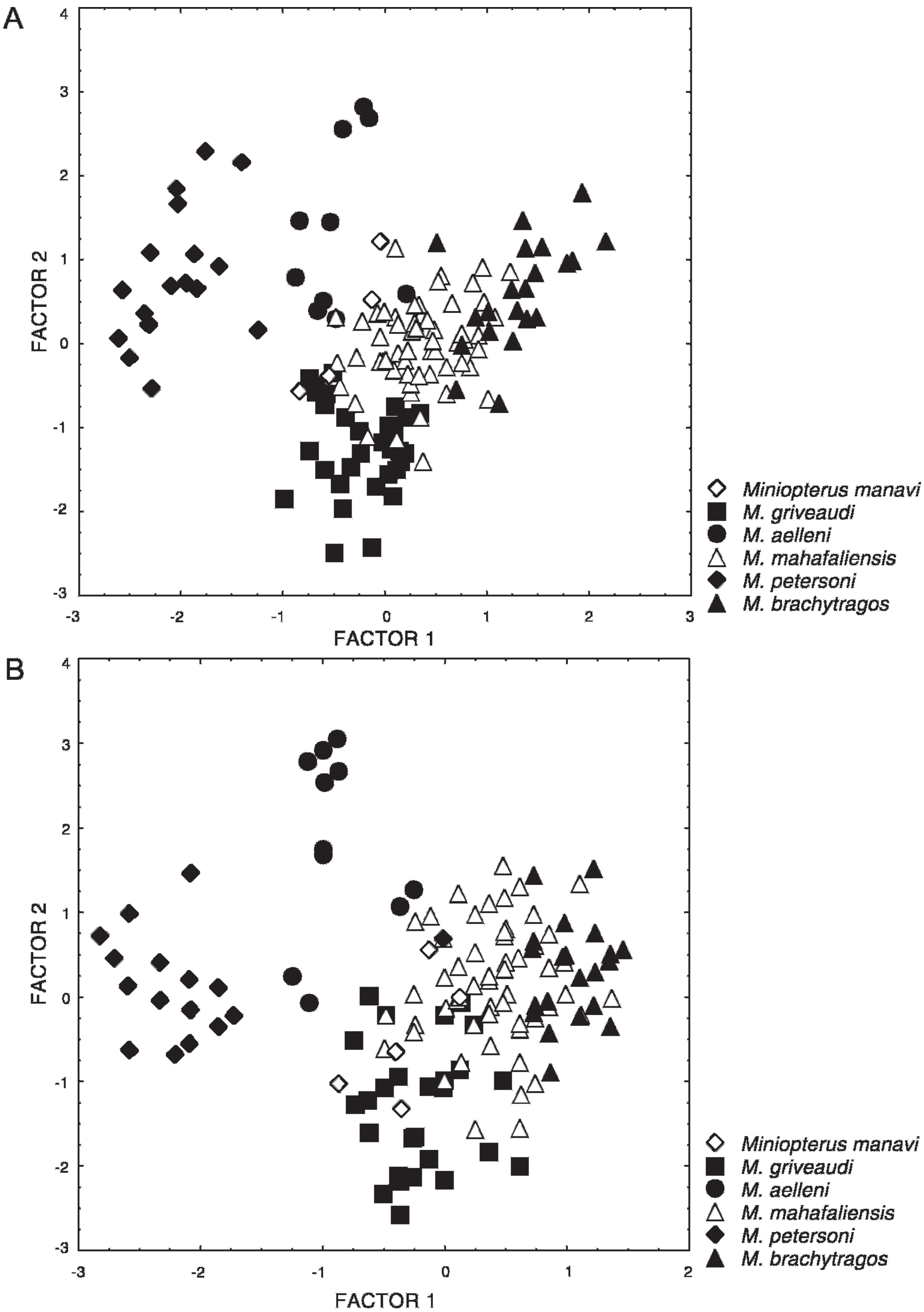

Fig. 11. Projections of factor 1 (x-axis) and factor 2 (y-axis) in principal component analysis of **A,** cranial measurements and **B,** dental measurements of different diminutive species of *Miniopterus* from Madagascar, all of which were previously considered to be *M. manavi*, as well as *M. petersoni*. Loadings of variables on each axis are shown in table 6.

TABLE 6

Factor loadings from principal component analysis of cranial and dental measurements of specimens of *Miniopterus manavi*, *M. griveaudi*, *M. aelleni*, *M. mafaliensis*, *M. petersoni*, and *M. brachytragos*

The measurement greatest zygomatic breadth (ZYGO) has been removed from the analysis to augment sample sizes. A graphical representation of the first two factors is presented separately for cranial and dental variables in fig. 11. See Methods for an explanation of variable acronyms.

	Factor 1	Factor 2	Factor 3
Cranial			
GSKL	−0.919	0.249	−0.136
LW	−0.860	−0.151	−0.346
POB	−0.727	−0.517	0.361
GBW	−0.762	−0.401	−0.467
MAST	−0.901	−0.186	−0.192
PAL	−0.856	0.439	0.176
MAND	−0.877	0.428	−0.064
Explained Variance	5.01	0.92	0.56
Proportion of Total Explained Variation (%)	71.5	84.7	92.7
Dental			
I1-M3	−0.907	0.355	0.146
C-M3	−0.942	0.253	0.012
C1-C1	−0.837	−0.506	0.207
M3-M3	−0.926	−0.147	−0.343
Explained Variance	3.27	0.47	0.18
Proportion of Total Explained Variation (%)	81.7	93.4	98.0

PHYLOGEOGRAPHY: *Miniopterus mahafaliensis* has an intraclade divergence of 2.2% K2P. Among the sequenced animals, there appears to be no clear phylogeographic structure, as, for example, among animals from Kirindy-Mite, which are distributed among the different subclades (fig. 2).

ECOLOGICAL NOTES: The specimens of *M. mahafaliensis* captured at the holotype site, near Mitoho Cave in the PN de Tsimanampetsotsa, were in largely undisturbed southwestern dry spiny forest-thicket resting on exposed limestone at the edge of the Mahafaly Plateau. The series was obtained in mist nets erected near the Grotte d'Andranoilovy, which is less than 100 m from the entrance of the Mitoho Cave; these two caves open horizontally and are presumed to be part of the same complex. At the nearby Malaza Manga Aven, in similar habitat, a single individual was captured in a harp trap as it exited a relatively deep sinkhole after dusk. A number of other individuals of this taxon were obtained in similar limestone cave settings on the Mahafaly Plateau to the south of Mitoho Cave, including the Grotte d'Antagneotsy, Grotte d'Amborombe, and Grotte d'Andraimpano, and to the north at the sea cave of Ambanilia and in the coastal littoral at the Grotte de Bisihiko. The habitat surrounding these different cave sites ranged from relatively undisturbed to heavily disturbed southwestern dry spiny forest-thicket and southwestern coastal bushland habitats.

Further to the north and away from any large blocks of exposed sedimentary rock, but still in lowland habitat, *M. mahafaliensis* is known from a site in the Forêt des Mikea in disturbed transitional southwestern dry spiny forest-thicket/western dry forest resting on red sand substrate. Several individuals were captured further to the north in the Parc National de Kirindy-Mite in slightly disturbed western dry forest. At a site near the village of Betakilotra and within the same park, several animals were netted as they descended into a narrow dug well shaft, presumably to drink water. This latter locality was in heavily degraded mixed open habitat and secondary dry deciduous forest.

Miniopterus mahafaliensis is known from two inland and upland central southwest sites with eroded sandstone outcrops. It was captured in the Parc National de l'Isalo in a mist net traversing the Sahanafa River, a zone with degraded gallery forest dominated by introduced mangos and within a few hundred meters from exposed sandstone cliffs. The second sedimentary rock site is the Grotte d'Andranomilitry, near Ihosy, is a shallow cave with a small river passing through the interior and surrounded by open savannah. An older specimen was obtained near Betroka and we have no specific details on the collection site.

ETYMOLOGY: The name *mahafaliensis* is derived from the Malagasy word *mahafaly*, meaning "to make taboos," but here specifically referring to one of the local cultural groups in southwestern Madagascar, the Mahafaly, and the Mahafaly Plateau, a limestone karst area, from whence many of the specimens of this taxon were collected. In the region of the Forêt des Mikea, the Malagasy name for this bat was given by a local hunter as *kitrotroke*.

DISCUSSION

Using a combination of molecular phylogenetic and morphological tools, we demonstrate that the previously recognized species, *Miniopterus manavi*, is composed of several species, two of which are named herein—*M. brachytragos* and *M. mahafaliensis*. Including *M. manavi* sensu stricto, this brings the number of described cryptic species within this former paraphyletic complex to five, four of which have been recently named or resurrected from synonymy (Goodman et al., 2009b; herein). What is remarkable about these discoveries is that although these four species are morphologically very similar to one another, they are not closely related to *M. manavi* sensu stricto, and represent cases of convergent evolution. The resolution of this complicated systematic puzzle was greatly aided by molecular clade-defining phylogenetic tools. Subsequently, the specimens were separated into clade groupings and corroborative morphological characters were identified. Another extraordinary finding is that there are localities on Madagascar where up to four of these morphologically similar species occur in strict sympatry.

When Rudolph Peterson was preparing the manuscript for the monograph on Malagasy bats (Peterson et al., 1995), which was completed after his death by Judith Eger and Loralie Mitchell, he had divided different populations, originally identified as *M. manavi*, into new species (notes written on specimen labels in ROM MAMM; J. Eger, personal commun.). Given that the proposed new taxa had allopatric distributions, Peterson's collaborators decided to take a more conservative approach in interpreting these patterns and did not publish the new species descriptions in the monograph. They specifically mentioned that this would need to be reassessed (p. 137, our translation), "when their [different forms attributed to *M. manavi*] sympatric occurrence can be eventually demonstrated or other means to study the relationships between the specimens can be applied." After more than 15 years of fieldwork obtaining samples with associated tissue samples of *M. manavi* from different portions of Madagascar and the Comoros Archipelago, we have now been able to address the point, using both molecular and morphological techniques, to resolve patterns of variation in animals previously assigned to this taxon.

Cases of sympatry species of *Miniopterus* in the dry forest regions of Madagascar and bioclimatic correlates of species diversity

The distributional patterns on Madagascar of the five species of small *Miniopterus* and other endemic congeners are complicated. On the basis of specimens examined during the course of this project, some preliminary insights can be presented in this regard and here we restrict our comparisons to reasonably well-surveyed sites in the dry forest regions of the western half of Madagascar. In table 7 we present a summary of the known distribution of *Miniopterus* spp. starting in the southwest and passing toward the north, following a cline of increasing annual rainfall. In the extreme southern portion of the island, the natural vegetation is dominated by southwestern dry spiny forest-thicket in inland areas, particularly limestone zones such as the Mahafaly Plateau,

TABLE 7

Distributional patterns of *Miniopterus* spp. in the lowland dry forest regions of Madagascar and associated ecological and bioclimatic correlates

Sites presented from south to north following an increasing rainfall cline. Rainfall data is from Donque (1975) and Chaperon et al. (1993) and dry season data from Cornet (1974). See fig. 1 for location of different sites. Species are ordered for each site from largest to smallest based on average forearm length. Habitat codes: SWDSFT = southwestern dry spiny forest-thicket, SWCB = south western coastal bushland, WDF = western dry forest.

Region	Average rainfall	Dry season (months)		Species and associated documentation	Exposed sedimentary rock present
Mahafaly Plateau (south of Onilahy River)	less than 390 mm	12	SWDSFT & SWCB	*M. griffithsi* sp.[1] *M. mahafaliensis*[2]	Yes
Sarodrano	~390 mm	12	SWDSFT & SWCB	*M. gleni*[1] *M. majori*[3] *M. mahafaliensis*[2]	Yes
Forêt des Mikea	496 mm	12	Transitional SWDSFT & WDF	*M. gleni*[1] *M. majori*[4] *M. mahafaliensis*[2]	Isolated
Kirindy-Mite	554 mm	9–10	WDF	*M. mahafaliensis*[2]	No
Bemaraha	1629 mm	8	WDF	*M. gleni*[1] *M. aelleni*[5] *M. griveaudi*[5] *M. brachytragos*[2]	Yes
Namoroka	1420 mm	8	WDF	*M. gleni*[1] *M. manavi* group[6] *M. aelleni*[5] *M. griveaudi*[5] *M. brachytragos*[2]	Yes
Mahajanga karst (Anjohibe and Anjajavy complexes)	1503 mm	7–8	WDF	*M. gleni*[1] *M. majori*[4] *M. aelleni*[5] *M. griveaudi*[5]	Yes
Ankarana	1890 mm	7	WDF	*M. gleni*[1] *M. aelleni*[5] *M. griveaudi*[5]	Yes

[1] Goodman et al. (2009a)

[2] Herein.

[3] Contrary to previous assertions (Goodman et al., 2005), there is specimen evidence for the occurrence of this species at Sarodrano (ROM MAMM 42610; Maminirina et al., 2009). This site has been revisited on several occasions and to our knowledge no further evidence of *M. majori* has been found; it may be a vagrant to the zone.

[4] Maminirina et al. (2009).

[5] Goodman et al. (2009b).

[6] Includes specimens in the FMNH (175838, 175841, 175843, 175845, 175849, 175853, 175855, 175858, 175859), which have been sequenced for cyt-*b*.

and the coastal area has a largely degraded southwestern coastal bushland. Slightly further to the north, at the level of the Forêt des Mikea, there is a transitional zone between these vegetational types and western dry forest (Moat and Smith, 2007). The latter habitat continues to the north end of the island in the vicinity of Ankarana. A previous analysis of bat species richness in the dry forests of Madagascar showed that diversity is highest in areas of exposed sedimentary rock, in which caves and crevices presumably provide day roost sites (Goodman et al., 2005).

In the Mahafaly Plateau area, with notably arid conditions, *Miniopterus* species richness includes two taxa south of the Onilahy River and three taxa just to the north of the river, near Sarodrano (table 7). This is despite the fact that this zone is limestone karst with a considerable number of potential day roost sites for cave dwelling bats and extensively sampled. Further to the north, in the Forêt des Mikea, the amount of annual precipitation increases slightly, but species diversity remains as near Sarodrano. Caves are present in the Forêt des Mikea, but are more limited than areas further south, several of which are unexplored for bats. In the region of Kirindy-Mite, where there is no exposed sedimentary rock near our inventory zones and slightly more mesic conditions, only *M. mahafaliensis* is known to occur and it is presumed to roost during the day in large trees, such as baobabs (*Adansonia*).

The next two sites to the north, the Bemahara and Namoroka limestone massifs, possess a considerable number of caves, often with complex systems and some with subterranean water, and deep cut canyons. Further, within these two zones there are dramatic increases in annual precipitation and the length of the rainy season as compared to the sites to the south. At Bemaraha four species and at Namoroka five species of *Miniopterus* are known, including three and four species, respectively, that were formerly placed in the *M. manavi* complex.

In the karstic area to the north of Mahajanga, specifically the sites of Anjajavy and Anjohibe, four species of *Minopterus* have been recorded, including two species previously considered *M. manavi*. This zone has slightly moister conditions than Bemaraha and Namoroka, but lower species diversity among *Miniopterus* taxa. This would imply that in western Madagascar increasing annual precipitation and a shorter dry season are not clearly correlated with increasing species richness in this genus. Further support for this hypothesis comes from the Ankarana Massif, another heavily eroded limestone plateau with a labyrinth of caves and canyons, the most intensively surveyed zone in the western half of the island, and with the most mesic conditions of the sites listed in table 7; here only three species of *Miniopterus* have been recorded, including two diminutive species. Hence, while clinal differences in yearly rainfall and the length of the dry season are positively associated with *Miniopterus* spp. diversity in the southern half of western Madagascar, this relation does not stand for the northern half. Some other factor(s) than these two variables might influence patterns of species richness in this genus in western Madagascar. With the use of ecological modeling tools, based on a finer data set of the distribution of these taxa, it may be possible to discern these patterns.

CONCLUSIONS AND FUTURE RESEARCH

We have been able to demonstrate that the previously recognized taxon, *Miniopterus manavi*, is paraphyletic and is composed of at least five taxa, several of which occur in sympatry. These different species include one recently described, *M. aelleni*, and another recently resurrected from synonymy, *M. griveaudi*, which occur in the lowland areas of Madagascar and islands in the Comoros Archipelago (Goodman et al., 2009b). Herein we name an additional two species, *M. brachytragos*, known from several biomes in the northern half of the island, and *M. mahafaliensis*, collected in the southwest. The phylogeny and species limits of *M. manavi* sensu stricto, named from the central highlands, is in need of further investigations with respect to populations other than in the vicinity of the terra typica and this work is currently underway.

Given the taxonomic changes to animals previously assigned to *M. manavi* sensu lato, it will be necessary to reevaluate different citations in the literature for records of this "taxon" from around the island associated with behavioral, ecological, and faunal inventory studies (e.g. Bennett and Russ, 2001; Goodman et al., 2005; Razakarivony et al., 2005; Kofoky et al., 2007), as virtually all of the original identifications are no longer valid. This aspect further underlines in a country such as Madagascar, for which the chiropteran fauna is not well known, the need for voucher specimens or at least wing punches of released bats for molecular studies in order to make proper species identifications. Without such materials, it will be impossible to update published information associated with recent and ongoing taxonomic revisions. We have identified some external characters, most notably tragus shape, that can be used in the identification of captured small *Miniopterus* on Madagascar and the Comoros.

One of the fascinating aspects that comes out of this current revision and warrants more detailed investigations is how the different sympatrically occurring diminutive *Miniopterus* species, which have remarkably similar body size and craniodental morphology, divide and share portions of their habitats. Detailed studies with associated voucher specimens on roosting and foraging ecology, vocalizations, and dietary regimes, in zones of sympatry and allopatry, should provide intriguing answers to these questions and identify the constraints that have allowed the evolution of such extraordinary levels of convergence among not necessarily phylogenetically closely related species.

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