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Patterns of morphological and genetic variation in the endemic Malagasy bat *Miniopterus gleni* (Chiroptera: Miniopteridae), with the description of a new species, *M. griffithsi*

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Abstract

Over the past decade, major advances have been made concerning the systematics and species diversity of Malagasy bats, largely based on specimens collected during inventories and associated morphological and molecular genetic studies. Herein we describe a new species of endemic bat from southern Madagascar, *Miniopterus griffithsi* sp. n., which is the sister taxa to *Miniopterus gleni*, a taxon described in 1995 (holotype from Sarodrano, just north of the Onilahy River in the southwest). Based on current information, *M. griffithsi* is found in the sub-arid bioclimatic zone, south of the Onilahy River, and *M. gleni* occurs in a variety of different bioclimatic zones, north of the Onilahy River to the northern portion of the island and on the near shore island of Ile Sainte Marie. The realization that *M. griffithsi* was a separate entity was first based on phylogeographic studies of the *M. gleni* complex. Comparisons using 397 bp of mitochondrial cytochrome *b* found a divergence of 1.2% within animals occurring across much of Madagascar north of the Onilahy River, 0.07% in those south of the Onilahy River, and 7.4% in populations separated by this river. Subsequently, morphological characters were identified that supported the specific separation of populations occurring south (*M. griffithsi*) and north of the Onilahy River (*M. gleni*), which include tragus shape, pelage coloration, and skull proportions.

Key words: Miniopteridae – *Miniopterus* – *Miniopterus gleni* – *Miniopterus griffithsi* sp. n. – Madagascar – morphological variation – phylogeography

Introduction

The bat genus *Miniopterus* Bonaparte, 1837, now placed in its own family (Hofer and Van Den Bussche 2003; Miller-Butterworth et al. 2007) is widespread and relatively speciose throughout many portions of the Old World; 19 species were recognized in the most recent synthesis of the earth's chiropteran fauna (Simmons 2005). Research over the past decade, particularly with molecular genetic tools, has found that this genus contains numerous cryptic species with remarkable cases of convergence in morphological characters between taxa that are not closely related; this phenomenon seems widespread on islands (e.g. Cardinal and Christidis 2000; Appleton et al. 2004; Goodman et al. 2007a, 2008; Juste et al. 2007).

Peterson et al. (1995) distinguished four species of *Miniopterus* on Madagascar. These include one taxon endemic to the island – *M. gleni* Peterson, Eger and Mitchell, 1995; two restricted to Madagascar and the Comoro Islands – *M. manavi* Thomas, 1906 and *M. majori* Thomas, 1906; and the fourth shared with portions of southern Africa – *M. fraterculus* Thomas and Schwann, 1906. Since the publication of that monograph, a considerable amount of fieldwork has been conducted on the bats of Madagascar, including the collection of specimens with associated tissues. Morphological studies of these new specimens, in association with comparisons to type material, and detailed phylogenetic and phylogeographic research, have considerably changed views on the species richness and levels of endemism of Malagasy bats.

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With specific regard to Malagasy *Miniopterus* spp., it is now clear that *M. manavi* is paraphyletic (Weyeneth et al. 2008; Goodman et al. in press a, b). Patterns of morphological and phylogeographic variation in *M. majori* are consistent with the single species arrangement (Maminirina et al. in press), but its occurrence in the Comoros Archipelago has been called into question (Goodman and Maminirina 2007). Recent analyses using morphological and genetic data from Malagasy specimens previously assigned to *M. fraterculus* have disclosed that these populations represent at least two endemic taxa to the island, *M. sororculus* Goodman et al. 2007a,b and *M. petersoni* Goodman et al. 2008; that are neither sister taxa nor closely related to African *M. fraterculus* (Goodman et al. 2007a, 2008). Herein we treat patterns of morphological and phylogeographic variation in the fourth and last of the taxa recognized by Peterson et al. (1995), *M. gleni*.

Animals referable to *M. gleni* have been collected from a considerable range of bioclimatic zones on Madagascar, including the far northeast in the region of the Masoala Peninsula, that receives something approaching 6 m of rainfall per year (Kremen 2003), to zones of the dry forests of Madagascar, such as Ankarana, with < 2 m of annual rainfall (Hawkins et al. 1990), and sub-arid thorn scrub in the extreme southwest where annual rainfall rarely exceeds 500 mm (Donque 1975). Given this taxon's broad distribution across dramatic bioclimatic clines and evidence of cryptic species of *Miniopterus* on Madagascar, we address the following aspects herein: (1) to examine if phylogeographic structure exists in this taxon and if so, is it associated with bioclimatic zones or other aspects (e.g. elevational variation); (2) is there morphological variation in *M. gleni* that is correlated with the ecological zones it occurs; and (3) on the basis of morpholog-

ical or molecular genetic data is there evidence of a cryptic species within animals now referred to *M. gleni*?

Taxonomic history of *Miniopterus gleni*

In 1967, the late Rudolph Peterson of the Royal Ontario Museum (ROM) and colleagues conducted fieldwork on bats in several areas of Madagascar. Amongst the specimens they obtained was a series of a large-bodied *Miniopterus* with notably dark pelage from a sea cave near Sarodrano in the southwest, to the south of Toliara (Fig. 1). These specimens, as well as some others held in different museums that had been previously assigned to the African *M. inflatus* Thomas, 1903 (Hill 1993), were described as a taxon new to science, *M. gleni* by Peterson et al. (1995). The holotype, an adult male (ROM MAM 42567), was collected 9 May 1967, at 'une grotte marine entre Sarodrano et Saint-Augustin, à 20 km au Sud de Tuléar [Toliara]' (Peterson et al. 1995).

In Peterson et al.'s (1995) description of *M. gleni*, 77 specimens were available for study, we now have had access to approximately 160 specimens, with notably broader geographic coverage of the island (Appendix S1, Fig. 1). These recently collected specimens including those from the type

locality, provide the needed material to assess patterns of morphological and phylogeographic variation in this species.

Materials and Methods

Morphological comparisons

Specimens were examined from several different 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 – Royal Ontario Museum, Toronto; and USNM – The National Museum of Natural History (formerly The United States National Museum), Washington, D.C. Animals used in the morphological comparisons are adult, defined by presence of a fully erupted permanent dentition and fused basisphenoid-basioccipital suture. Tooth abbreviations include: I, incisor; C, canine; and M, molar. Upper case abbreviations are used for upper teeth and lower case abbreviations for lower teeth.

Six standard external measurements (in millimetres) were taken from specimens using a ruler before preparation and included: total length, tail length, hind foot length (not including claw), tragus length, ear length, and forearm length. Given that field collectors often use different methods in taking external measurements, data used in the analyses and descriptive statistics are only those of a single field worker (SMG). Mass (in grams) was taken with the use of a spring balance.



Fig. 1. Map of Madagascar showing localities mentioned in the text, and sites where specimens of *Miniopterus griffithsi* and *Miniopterus gleni* were collected. The number of specimen records and localities available for members of this species group has increased substantially since the publication of Peterson et al. (1995)

Eight cranial or mandible and seven dental measurements were taken using a digital calliper to the nearest 0.1 mm by SMG. Cranial measurements include: greatest skull length (GSKL), from posterior-most part of occipital bone 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; lachrymal width (LW), width across rostrum at lachrymal pits; palatal length (PAL), from anterior edge of upper incisors to posterior edge of palatal without posterior spike; and mandible length (MAND), from the posterior-most portion of the condyles to anterior-most alveoli of lower incisors. The dental measurements include: cranial toothrow (I^1 - M^3), length from anterior alveolar border of incisors to posterior alveolar border of 3rd molar (M^3); upper canine-molar toothrow (C - M^3), length from anterior alveolar border of canine to posterior alveolar border of third 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; lower canine-molar toothrow (c - m_3), length from anterior alveolar border of lower canine to posterior alveolar border of lower 3rd molar (m_3).

Univariate statistical analyses were conducted for each of the measured variables to examine patterns of sexual dimorphism and geographic variation. To distinguish between different populations currently assigned to *M. gleni*, a principal component analysis was performed using the statistical package STATISTICA (version 7.0, Tulsa, OK, USA); data were log-transformed, used a correlation matrix, and the unrotated option was used. The three different measurement types (external, cranial, and dental) were analysed separately, as Ranivo and Goodman (2007) found in an ecomorphological study of Malagasy dry forest bats that these different variable types do not necessarily covary.

Vegetation classification

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

Molecular comparisons

To understand patterns of phylogeographic variation in *M. gleni*, molecular genetic studies were conducted. The 5' end of cytochrome-*b* was chosen 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. 2007a). The genetic dataset includes new sequences obtained from 24 specimens of *M. gleni* taken from across its geographical range on Madagascar and the near shore island of Ile Ste Marie, as well as topotypic material from Sarodrano. In addition DNA sequences were included from the other currently recognized Malagasy species *M. majori*, *M. manavi*, *Miniopterus sororculus*, and *Miniopterus petersoni*; two South African species, *Miniopterus natalensis* and *M. fraterculus*; and the Asian '*Miniopterus australis*' as the outgroup. In agreement with other studies (Miller-Butterworth et al. 2005, 2007; Goodman et al. 2007a, 2008), the use of outgroup taxa from African or Asian Miniopterus species did not alter the tree topology significantly (Appleton et al. unpublished data).

Genomic DNA was extracted using a lithium chloride and chloroform extraction method as described by Gemmel and Akiyama (1996). A fragment of the mitochondrial cytochrome-*b* gene was amplified and sequenced using the primers L14115 and H14542 (Smith and Patton 1991). Template DNA was amplified by PCR in 25 µl reaction volume containing the following: 1x reaction buffer (Promega, Madison, WI, USA), 2.5 mM MgCl₂, 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 94°C for 3 min, followed by 30 cycles at 94°C for 30 s, 45°C for 30 s and 72°C for 40 s and a final extension of 72°C for 3 min. The single PCR product was directly sequenced by a commercial company (Macrogen Inc., Seoul, South Korea) using the ABI Prism BigDye Cycle Sequencing kit (Applied Biosystems, Perkin-Elmer, Melbourne, Australia). Sequencing products were visualized on an ABI 3730XL

(Applied Biosystems, Perkin-Elmer). The sequences were aligned using Sequencher version 4.6 (Gene Codes Corporation, Ann Arbor, MI, USA). Newly produced sequences and those collected from GenBank were overlapping but partially offset so sequences were trimmed to match, with a final 397 bp of sequence used in the analysis. All new sequences were deposited in GenBank (accession numbers FJ619509-FJ619518).

Analysis using DNA strider (Marck 1990) showed that sequences did not contain stop codons. Maximum parsimony (MP) and minimum evolution (neighbour-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 (TBR) branch-swapping algorithm. The NJ method used pairwise sequence distances estimated by HKY + G model. Nodal support of MP and NJ trees was estimated by 1000 bootstrap pseudoreplicates.

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 + 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 = 0.2705, 0.2887, 0.1615, 0.2793, -lnL = 1219.13 and shape parameter of gamma distribution = 0.1869.

All characters were equally weighted and unordered. Bayesian analysis was conducted using the program MrBayes v3.1.2 (Ronquist and 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 (0.00907) at the end of the run and the potential scale reduction factor (PSRF) of each parameter *s* within $1.000 < PSRF < 1.018$. 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 1st 2500 generations from the calculation of posterior probabilities.

A genealogical approach to representing the data from Clade 1 takes into account some characteristics of intraspecific populations, which are different to the consideration of species level phylogenies (Emerson et al. 2001). The possibility of the coexistence of ancestral and newly derived haplotypes along with the low levels of intraspecific divergence is better represented by a network (Posada and Crandall 2001). TCS version 1.21 (Clement et al. 2000) was used to create a statistical parsimony haplotype network using 95% connection limit.

Results

Molecular phylogenetics

All the phylogenetic analyses produced the same tree topology (Fig. 2). The 24 specimens referred to as *M. gleni* were recovered as a monophyletic group with respect to the other Malagasy species examined. Within the *M. gleni* group, two distinct clades were evident. The separation of the two clades received strong statistical support in all tree analyses. In terms of the genetic divergences, the average HKY + G distance between the two clades was 7.4%. Genetic divergence in closely related *Miniopterus* taxa can vary from 3% between currently recognized, well-defined subspecies (Cardinal and Christidis 2000) to 6–16% in clades thought to represent separate species (Appleton et al. 2004). The two specimens from Sarodrano (the type locality of *M. gleni*) are found in Clade 1 (Fig. 2), and as we have no evidence of multiple species of large-bodied *Miniopterus* at this site, this clade is the one associated with the name *M. gleni*. Individuals from Clade 1 were from areas north of the Onilahy River while those in Clade 2 came from south of the Onilahy River.

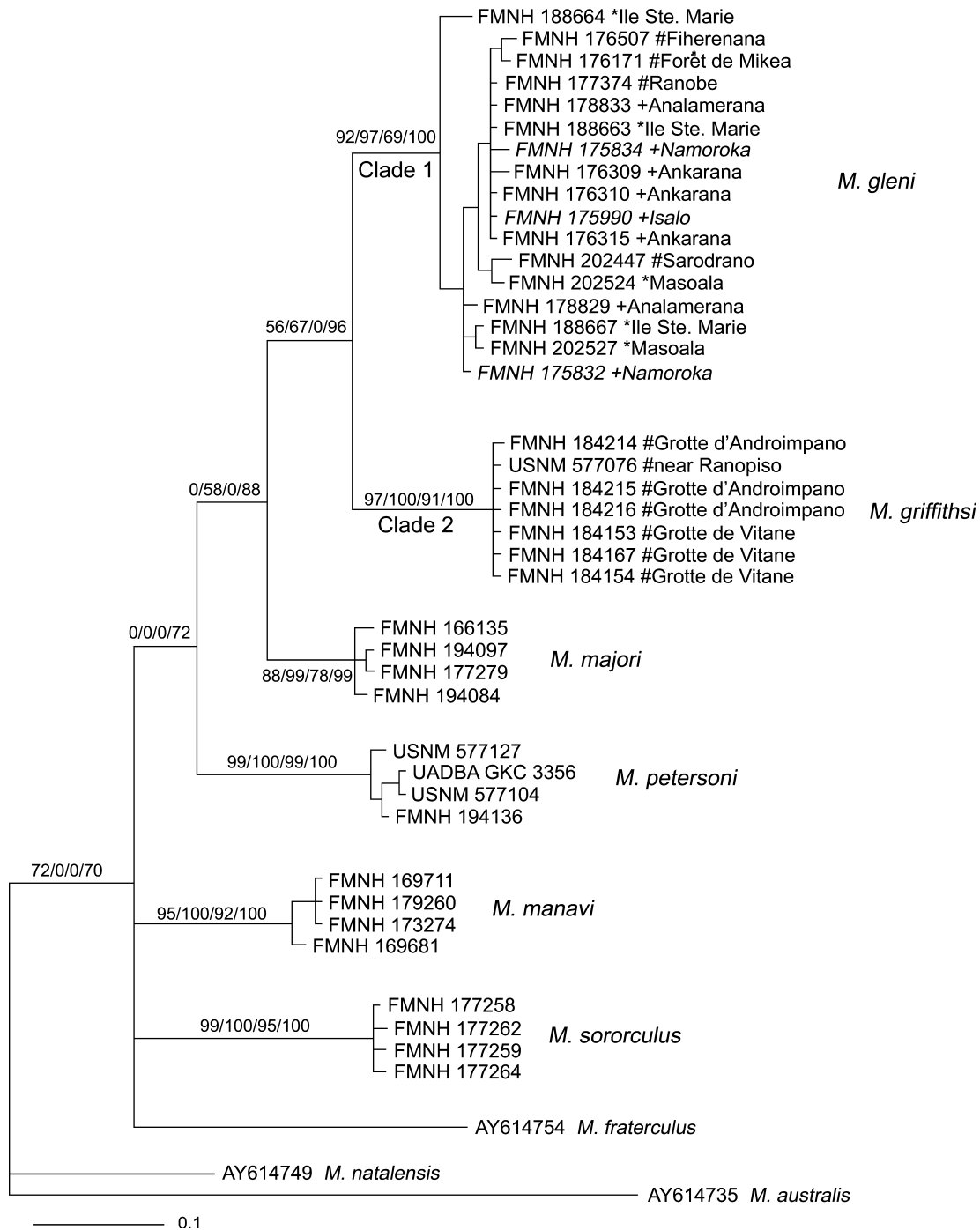


Fig. 2. Phylogenetic position of *Miniopterus gleni* (clade 1) and *Miniopterus griffithsi* (clade 2) with respect to other Malagasy Miniopteridae taxa and two African taxa (*Miniopterus fraterculus* and *Miniopterus natalensis*), based on the mitochondrial cytochrome-*b* gene. *Miniopterus australis* was selected as the outgroup. The tree presented was produced using Bayesian analysis. Posterior probabilities (Bayesian analysis) and bootstrap support (ML, NJ and MP analyses) and are indicated on the major nodes only (Bayesian/ML/NJ/MP). Labels for Clades 1 and 2 include collecting locality and catalogue number and for the other taxa only sample identification number or Genbank accession number. The species identification is included to the right of each clade. Further information on the locality for members of Clades 1 and 2 includes – biome: *humid forest, + dry forest, and #spiny bush; elevation: normal font = sea level to 120 m and italicized font = 200–900 m

Phylogeography

Within Clade 1 (Fig. 2), there were 11 haplotypes recorded amongst the 17 individuals examined. One animal from Ile Ste Marie, a near shore island, possessed a haplotype that differed by a minimum of six mutations and a maximum of 10 mutations from the other haplotypes. All the other haplotypes (including from Ile St Marie) differed by a minimum of one

mutation and a maximum of five mutations, with an average HKY + G divergence of 1.2% from the common haplotype (Fig. 3). There was no obvious phylogeographic structure amongst the haplotypes within Clade 1 with regards locality, biome or elevational zone, with the exception that all individuals were obtained north of the Onilahy River. Within some of the subclades individuals from opposite ends of the

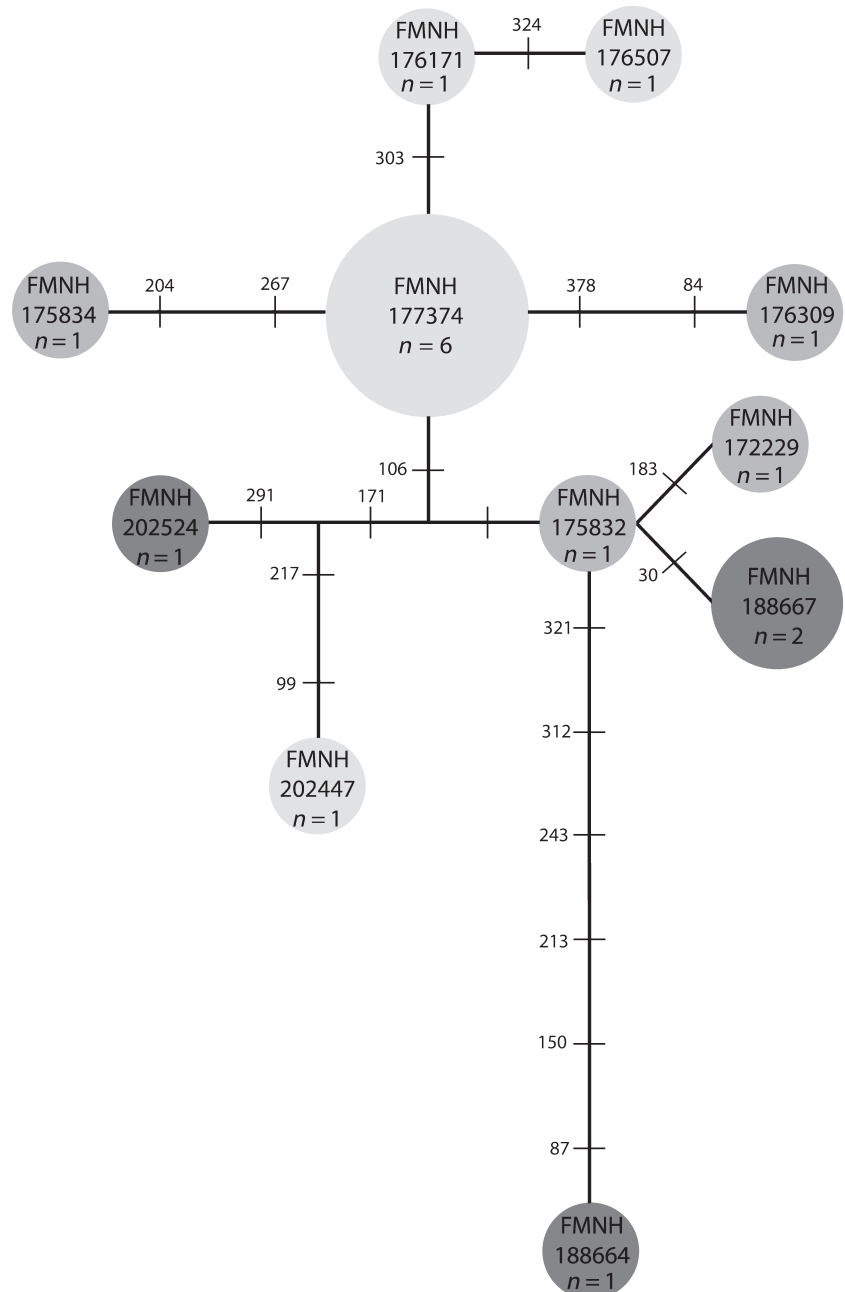


Fig. 3. Minimum spanning network of cytochrome-*b* haplotypes in Clade 1 (*Miniopterus gleni*). Circles represent haplotypes where the size is related to the number of individuals sharing the haplotype and for multiple individuals per haplotype a single specimen number is given. Numbers at lines connecting individuals represent the base positions that differ between the haplotypes. Biome code: light grey is spiny bush, grey is dry forest, and dark grey is humid forest

island group together (e.g. humid biome on the Masoala Peninsula in the northeast and spiny bush biome at Sarodrano in the southwest) and other individuals from these same localities are found in different subclades.

Within Clade 2, there was very little haplotype variation with only two haplotypes differing by one base pair (average HKY+G divergence of 0.07%), and consequently, little phylogeographic structure, among the seven animals. One haplotype occurred in six individuals and the other occurred in a single individual. The specimen sample included animals across spiny bush, ranging from Itampolo to Ranopiso, a distance of approximately 280 km.

Morphological comparisons

On the basis of the phylogenetic and phylogeographic results, specimens were divided into three groups: (1) those collected

on the main island north of the Onilahy River (Clade 1); (2) those collected on the main island south of the Onilahy River (Clade 2); and (3) those from Ile Ste Marie (Clade 1); the latter group was treated separately to ascertain if there are morphological differences in the population occurring on this near shore island.

Sexual dimorphism

For the three different data sets (external, cranial, and dental), the vast majority of the measured variables showed no evidence of sexual dimorphism. In the few cases where differences were found, these were either subtle, associated with large sample sizes, or best allocated to type II errors (Tables 1–3). The two exceptions were both cases of borderline significance and included total length measurement for populations north of the Onilahy River ($t = 1.67$, $p = 0.05$, $df = 62$) and mass for animals from Ile Ste Marie ($t = 2.36$,

Table 1. External measurements of adult individuals from the *Miniopterus gleni* group from three different regions of Madagascar with the sexes combined

	Total length	Tail length	Hind foot length	Tragus length	Ear length	Forearm length	Mass
Clade 1 North of Onilahy River, <i>M. gleni</i>	123.6 ± 2.39 120–131, n = 62	58.2 ± 2.43 52–63, n = 64	8.1 ± 0.55 7–9, n = 63	8.0 ± 0.47 7–9, n = 64	13.3 ± 0.72 12–15, n = 61	48.4 ± 0.76 47–50, n = 58	13.2 ± 1.49 10.5–17.5, n = 104
Clade 2 South of Onilahy River, <i>M. griffithsi</i>	125.2 ± 2.23 122–128, n = 6	57.3 ± 2.94 54–63, n = 6	8.0 ± 0.0 8–8, n = 6	8.0 ± 0.0 8–8, n = 6	13.3 ± 0.52 13–14, n = 6	48.8 ± 0.75 48–50, n = 6	13.6 ± 1.24 12.0–15.5, n = 6
Clade 1 Ile Ste Marie, <i>M. gleni</i>	121.1 ± 2.09 118–124, n = 9	53.8 ± 3.07 50–58, n = 9	7.7 ± 0.50 7–8, n = 9	7.8 ± 0.35 7–8, n = 9	13.0 ± 0.0 13–13, n = 9	47.9 ± 0.60 47–49, n = 9	13.3 ± 0.62 12.5–14.5, n = 9

Measurements are presented as mean ± SD (minimum, maximum, number of specimens).

Table 2. Cranial measurements of adults from the *Miniopterus gleni* group from three different regions of Madagascar with the sexes combined

	GKSL	ZYGO	LW	POB	GBW	MAST	PAL	MAND
Clade 1 North of Onilahy River, <i>M. gleni</i>	16.6 ± 0.21 15.9–17.2, n = 130	9.4 ± 0.15 8.9–9.7, n = 121	4.5 ± 0.16 4.1–5.2, n = 97	4.1 ± 0.12 3.8–4.4, n = 131	8.3 ± 0.16 8.0–8.8, n = 131	8.9 ± 0.15 8.6–9.4, n = 128	8.1 ± 0.16 7.7–8.5, n = 123	11.8 ± 0.21 11.3–12.4, n = 112
Clade 2 South of Onilahy River, <i>M. griffithsi</i>	16.9 ± 0.17 16.7–17.2, n = 8	9.6 ± 0.19 9.3–9.9, n = 8	4.6 ± 0.07 4.5–4.7, n = 8	3.9 ± 0.10 3.7–4.0, n = 8	8.2 ± 0.18 8.0–8.5, n = 8	9.1 ± 0.16 8.8–9.3, n = 8	8.2 ± 0.12 8.1–8.4, n = 8	12.0 ± 0.13 11.8–12.1, n = 8
Clade 1 Ile Ste Marie, <i>M. gleni</i>	16.7 ± 0.23 16.4–17.0, n = 8	9.5 ± 0.14 9.3–9.7, n = 7	4.5 ± 0.16 4.2–4.7, n = 8	4.1 ± 0.08 4.0–4.2, n = 8	8.4 ± 0.20 8.2–8.8, n = 8	9.0 ± 0.11 8.9–9.2, n = 8	8.1 ± 0.19 7.8–8.4, n = 8	11.7 ± 0.19 11.5–12.0, n = 8

Measurements are presented as mean ± SD (minimum, maximum, number of specimens).
See Materials and Methods for explanation of variable acronyms.

Table 3. Dental measurements of adults from the *Miniopterus gleni* group from three different regions of Madagascar with the sexes combined

	C ¹ -C ¹	M ³ -M ³	C-M ³	I ¹ -M ³	c-m ₃
Clade 1 North of Onilahy River, <i>M. gleni</i>	5.0 ± 0.18 4.6–5.4, n = 132	7.3 ± 0.12 6.9–7.6, n = 133	6.6 ± 0.13 6.3–7.0, n = 133	7.8 ± 0.14 7.5–8.3, n = 133	7.0 ± 0.13 6.5–7.4, n = 130
Clade 2 South of Onilahy River, <i>M. griffithsi</i>	5.0 ± 0.16 4.7–5.2, n = 8	7.3 ± 0.09 7.1–7.4, n = 8	6.6 ± 0.05 6.6–6.7, n = 8	7.9 ± 0.10 7.7–8.0, n = 8	7.1 ± 0.10 7.0–7.3, n = 8
Clade 1 Ile Ste Marie, <i>M. gleni</i>	5.0 ± 0.16 4.8–5.2, n = 8	7.4 ± 0.13 7.2–7.6, n = 8	6.7 ± 0.12 6.5–6.8, n = 8	7.8 ± 0.14 7.6–8.0, n = 8	7.1 ± 0.15 6.9–7.3, n = 8

Measurements are presented as mean ± standard deviation (minimum, maximum, number of specimens).
See Materials and Methods for explanation of variable acronyms.

p = 0.05, df = 7); this latter difference is most likely seasonal. Hence, we conclude that there was no sexual dimorphism in these mensural characters and the sexes are combined in subsequent analyses.

Patterns of geographic variation in measurements and morphological characters

The univariate comparisons of the different measured variables for the samples divided into the three geographic zones yielded little in the way of statistically significant results with regards to differences between them. External measurements in animals from these three zones are largely overlapping (Table 1), although mean values for those from Ile Ste Marie tended to be smaller. The only phenotypic characters that consistently separate animals obtained on opposite sides of the Onilahy River are tragus shape and pelage coloration (see below).

There was a tendency for animals south of the Onilahy River to have slightly longer skulls with slightly narrower rostrums than those to the north of the Onilahy River, including the samples from Ile Ste Marie (Table 2), but in univariate analyses these differences are not statistically significant. In general, the shape and morphology of the skulls and mandibles of animals from north and south of the Onilahy River are similar (Fig. 4). Yet, relatively consistent differences between these different populations include for those south of the river – these characters are discussed in detail below. Animals from Ile Ste Marie show morphological similarities to those from north of the Onilahy River. The dental measurements of animals from these three different geographic regions of Madagascar are broadly overlapping (Table 3). No consistent difference was found in tooth structure and morphology between specimens from north and south of the Onilahy River or from Ile Ste Marie.

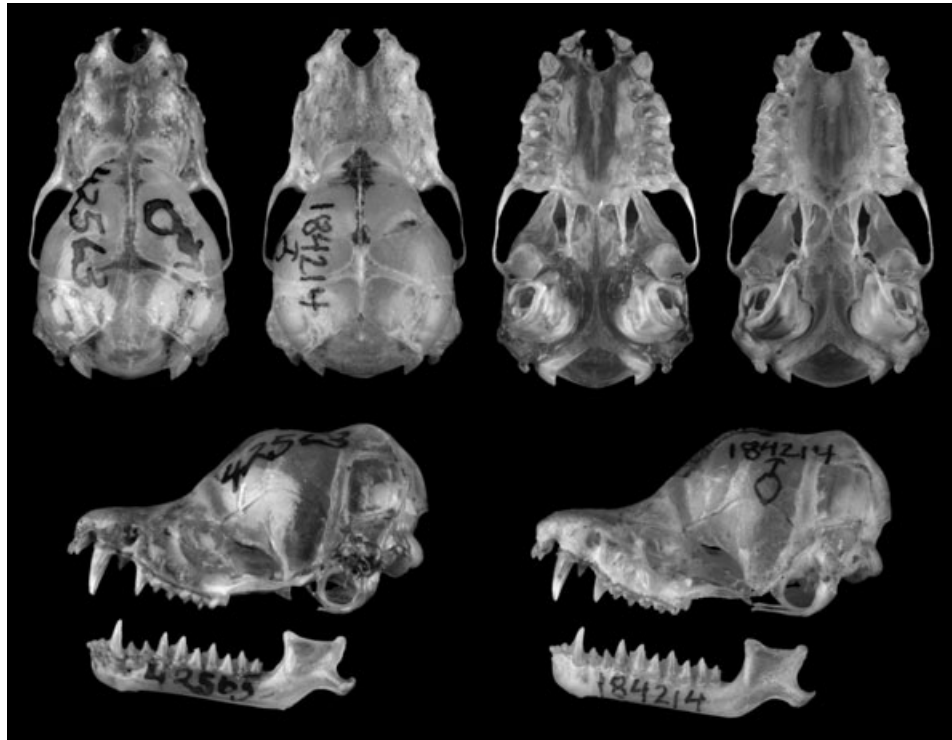


Fig. 4. Different views of skulls and mandibles of *Miniopterus* spp.: left paratype of *Miniopterus gleni* (ROM MAM 42563) from between Sarodrano and St. Augustin, north of the Onilahy River, right holotype of *Miniopterus griffithsi* (FMNH 184214) from Grotte d'Androimpano, 4.2 km NE Itampolo, south of the Onilahy River. 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 Z94481_09d.)

Principal component analyses were conducted separately on external, cranial, and dental measurements of the specimens from Clades 1 and 2, using the same classification as the univariate assessments. Of these three comparisons, the only

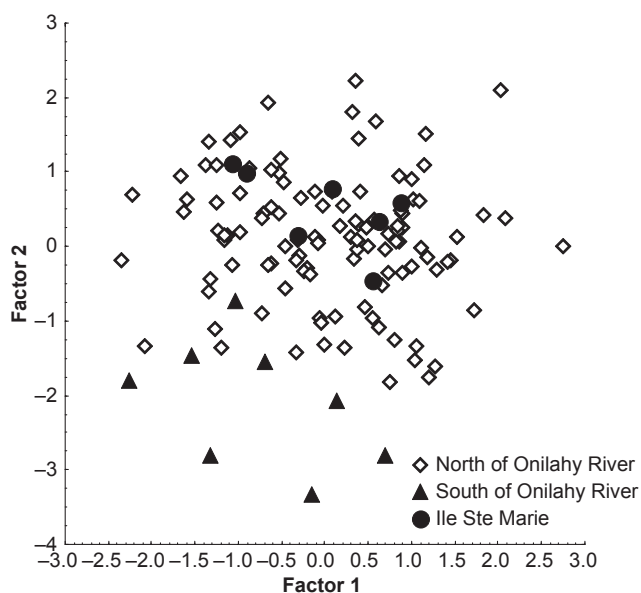


Fig. 5. Projections of factor 1 (x-axis) and factor 2 (y-axis) in principal component analysis of cranial measurements of *Miniopterus griffithsi* and *Miniopterus gleni*. Loadings of variables on each axis are shown in Table 4

one illustrated here is for the cranial measurements, which show a nearly clear separation of specimens from south of the Onilahy River, as compared with those north of this river and from Ile Ste Marie (Fig. 5). Of the six variables used in this analysis, four (greatest skull length, greatest zygomatic breadth, greatest braincase width, and mastoid breadth) show strong loadings on the first factor and one variable (postorbital breadth) strong loading on the second factor (Table 4). Factor 1 accounted for 50.8% of the explained variance and factor 2 an additional 19.9%. This would indicate that size associated with the first factor is an important component in differences between the skulls of animals from the *M. gleni* group, but also form, associated with the second factor, accounts for a considerable percentage of the variation.

Taxonomic conclusions

Given the level of genetic differentiation and consistent differences in certain aspects of morphology between individuals from Clade 1 and Clade 2 described in the above sections, we treat the two clades as different species. Given that Clade 1 includes specimens obtained at the type locality of *M. gleni*, Sarodrano, we propose to name animals from Clade 2 as new to science.

Miniopterus griffithsi sp. n. (Figs. 2, 4 and 6)

Holotype

Field Museum of Natural History (FMNH) 184214 collected by S. M. Goodman (field number SMG 14593) on 23 February 2005 at Madagascar: Province de Toliara, Grotte

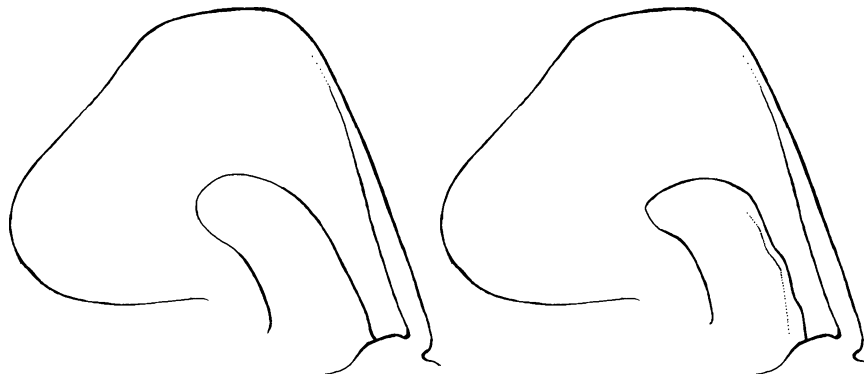


Fig. 6. Left ear and tragus of *Miniopterus* spp. presenting diagnostic characters to distinguish members of this genus: Left – adult male *Miniopterus gleni* topotypic material (FMNH 202448) from the Grotte de Sarodrano (sea cave), north of the Onilahy River; Right – adult male *Miniopterus griffithsi* holotype (FMNH 184214) from Grotte d'Androimpano, 4.2 km NE Itampolo, south of the Onilahy River. (Drawing by Rebecca Kramer.)

Table 4. Factor loadings from principal component analysis of cranial measurements of specimens of members of the *Miniopterus gleni* group (Clades 1 and 2), including *M. gleni* and *Miniopterus griffithsi*

Variable	Factor 1	Factor 2	Factor 3
GSKL	-0.762164	-0.380070	-0.152545
ZYGO	-0.750461	-0.097240	0.513398
POB	-0.279939	0.903873	-0.127769
GBW	-0.784137	0.421694	0.034918
MAST	-0.856452	-0.109024	0.189499
PAL	-0.690138	-0.184992	-0.612820
Explained variance	3.047125	1.194830	0.715850
Proportion of total explained variation (%)	50.8	70.7	82.6a

A graphical representation of the first two factors is presented in Fig. 5.

See Materials and Methods for an explanation of variable acronyms.

d'Androimpano, 4.2 km NE Itampolo (village), on old road to Ejeda, 24°39.012'S, 43°57.797'E, at 110 m above sea-level. The skull is in fine condition. Pectoral muscle samples saved in lysis buffer. The specimen has a full adult dentition and the basisphenoid-basioccipital sutures are completely fused.

Paratypes

FMNH 184215 (adult male), 184216 (adult male), 184236 (adult female), from same locality as holotype; FMNH 184153 (adult male), 184154 (adult male), 184167 (adult male), Madagascar: Province de Toliara, Grotte de Vitane (Vitany), 4.1 km SE Itampolo, 24°42'08.5"S, 43°57'49.4"E, 25 m; USNM 577076-577077, Fivondronana de Tolagnaro, 20 km WNW Ranopiso, 12 km ENE Amboasary, near Itaranta River, 25°01'S, 46°30'E, 40 m.

Measurements of the holotype

Measurements are in millimetre and body mass in grams. Total length, 124; tail length, 54; hind foot length, 8; tragus length, 8; ear length, 13; forearm length, 49; mass, 13.0; greatest skull length, 16.7; greatest zygomatic breadth, 9.5; postorbital breadth, 3.9; mastoid breadth, 9.1; greatest braincase width, 8.3; lachrymal width, 4.6; palatal length, 8.4; mandible length, 11.8; cranial toothrow, 7.8; upper canine-molar toothrow, 6.6; width across upper canines, 4.9; width across third upper molars, 7.3; lower canine-molar toothrow, 7.1 (Tables 1–3).

Diagnosis

A large-sized species of *Miniopterus* with mixed light brown anterior and medium brown middle and posterior dorsum, predominantly lighter brown ventrum, and light spotting on the uropatagium. The forearm length of the holotype is 49 mm. Tragus is relatively long, consistently 8 mm in the type series, thickset, with a distinct flange on the distal half of the external surface, slightly tapered with a downward deflection on the inner edge, and distal most portion terminating as a blunt and squarish shape (Fig. 6). The cranium has a heavier supraorbital ridge and sagittal crest, wider rostral depression, and a less open palatal emargination (Fig. 4).

Comparisons

On the basis of external measurements, members of the *M. gleni* group are notably larger than other large and medium size species in this genus on Madagascar: *M. griffithsi*, average forearm 48.8 mm (range 48–50 mm) and *M. gleni*, average forearm 48.4 mm (range 47–50 mm), as compared with *M. majori*, average forearm 45.4 mm (range 43–47 mm); *M. sororculus*, average forearm 43.5 mm (range 42–45 mm); and *M. petersoni*, average forearm length 40.5 mm (range 38–43 mm) (data presented herein; Goodman et al. 2008). Further, the phylogenetic analysis shows a clear separation of these two species from the other taxa (Fig. 2).

Individuals from south of the Onilahy River, referred here to *M. griffithsi*, have a more thickset tragus; the external edge having a distinct flange, particularly along the distal one-half; the inner edge is slightly tapered with a downward deflected; and the distal is not particularly rounded, giving a slightly squarish terminal edge (Fig. 6). In contrast, the tragus shape of animals north of the Onilahy River, *M. gleni*, lacks the prominent flange to the external edge, the distal two-thirds has an inward curve, and the distal tip is rounded and slightly downward deflected. The tragus length is on average 8.0 mm in both *M. gleni* and *M. griffithsi* (Table 1).

The holotype of *M. griffithsi* from the Grotte d'Androimpano and associated paratypes, as well as animals from the Grotte de Vitane, where preserved as fluid specimens and it is difficult to discern precise details of pelage coloration. The following description is based on individuals prepared as dried field skins. In USNM 577076, one of the paratypes of *M. griffithsi*, the dorsal and ventral pelage is slightly long

and dense. The anterior portion of the dorsum is a distinctly lighter brown than the middle and posterior dorsum, which approach medium brown in coloration. This is in contrast to specimens of *M. gleni* from the type locality of Sarodrano (e.g. ROM MAM 42548, 42560), in which the dorsum is a uniform dark chocolate brown. The ventrum in *M. griffithsi* (USNM 577076) is a mixture of predominantly lighter brown (particularly towards the membrane) with interspersed medium brown fur. In contrast, in *M. gleni* (ROM MAM 42548, 42560) the ventrum is a uniform chocolate brown, with slightly lighter coloration towards the membranes. The wing membrane and uropatagium in *M. griffithsi* (USNM 577076) are dark brown with notable light spotting on the uropatagium, while *M. gleni* (ROM MAM 42548, 42560) show no noticeable change in coloration across these membrane surfaces.

Habitat and habits

Animals from the Grotte d'Androimpano (FMNH 184214-184216, 184236) were captured along the western escarpment of the Mahafaly Plateau in a harp trap placed at a sinkhole rim. This cave opens to the surface as a large pit that drops vertically at least 130 m and is surrounded by South Western Dry Spiny Forest-thicket. The three individuals from the Grotte de Vitane (FMNH 184153, 184154, 184167) were obtained in a mist net placed close to the rim of a deep sinkhole filled with water and in South Western Coastal Bushland at the foot of the Mahafaly Plateau. The direct distance between the Grotte d'Androimpano and Grotte de Vitane is 5.7 km. The two specimens from near Ranopiso were obtained in a mist net erected in heavily disturbed gallery forest, dominated by introduced and native vegetation, and in close proximity to excavated mica mines. Hence, all sites this species has been collected have caves or rock shelters that presumably serve as day roost sites.

Reproduction and fat condition

All of the male specimens possessed abdominal testes measuring 3×2 mm or 4×2 mm and non-convoluted epididymes. The single exception was FMNH 184216, collected in late February, in which the testes had descended to a nearly scrotal position, measured 5×3 mm, and with partially convoluted epididymes. The single female (FMNH 184236) obtained in late February showed no signs of reproduction. Two of the Grotte de Vitane animals (FMNH 184153, 184167), obtained on 26 May 2005, at the start of the long dry season, had considerable subcutaneous fat and weighed 15.5 and 16.5 g (respectively). The accumulation of these fat deposits is presumably an adaptation to survive the long dry season, when food resources are reduced.

Etymology

The name *griffithsi* is a patronym in honour of Owen Griffiths, founder of Biodiversity Conservation Madagascar. He has been instrumental in preserving key important areas of habitat on Mauritius and Madagascar. He has generously supported many initiatives for documenting the rich Malagasy fauna (including genetic studies on *Miniopterus*). He is a specialist on the terrestrial mollusc fauna of the western Indian Ocean.

Conservation

The Grotte d'Androimpano site still has natural forest cover, although disturbed and associated with the removal of

hardwoods for charcoal production. The habitat in the immediate vicinity of the Grotte de Vitane is heavily perturbed by zebu cattle, goat, and sheep grazing. The natural vegetation surrounding the Ranopiso site has also been degraded by human activities. Given these habitat characteristics, it can be assumed that *M. griffithsi* is not a strict undisturbed forest-dwelling species. Hence, with regards to continued degradation of natural forests in southern Madagascar (Harper et al. 2007), there appears to be no clear direct and immediate threat.

In different portions of the Mahafaly Plateau, local hunters capture bats during periods of food scarcity as an alternative protein source. At the Grotte d'Androimpano, the holotype site of *M. griffithsi*, there is considerable seasonal hunting pressure on bats, particularly *Hipposideros commersoni* (E. Geoffroy, 1813)(Hipposideridae) and secondarily on other species occupying the cave, including *M. griffithsi* (reported as *M. gleni*) (Goodman 2006). Hence, bush meat off-take on this species may pose a threat, but this needs to be further evaluated.

Given the short distance between the two known sites for *M. griffithsi* in the western portion of its range (Grotte d'Androimpano and Grotte de Vitane), the distributional triangle formed between the three known localities for this species is distinctly narrow. The calculated area of occupancy is 740 km². On the basis of only being known from three localities and the calculated area of occupancy, the IUCN (2001) criteria would categorize *M. griffithsi* as Endangered. Yet, species almost certainly has a broader distribution in extreme southern Madagascar and its conservation status will need to be evaluated when further information is available.

Discussion

Natural history

When Peterson et al. (1995) published their monograph, in which the description of *M. gleni* appeared, few details were available on this species. On the basis of their account, it was known from several widely separated sites on the island, from Sarodrano in the southwest to the far north at Cap Diégo, as well as the Maroantsetra region in the northeast (Fig. 1). These authors also mentioned two inland south-central localities, at Maninday (22°46'S, 44°47'E), which was incorrectly reported in their gazetteer (p. 202) as '220 km au S.-E. de Tuléar' and this should read '220 km au N.-E. de Tuléar', and at Beroroha (22°37'S, 44°58'E); both of these localities are north of the Onilahy River. Their summary of its distribution was that it occurred across much of the island, particularly in coastal areas.

More recent bat survey work on Madagascar has found *M. gleni* to be more common than previously appreciated and numerous new localities have been identified that help to fill in information on its distribution. In Fig. 1 we have plotted the records from Peterson et al. (1995) and more recently collected or specimens not known to these authors. On the basis of surveys conducted during the past two decades, specimens have been collected at sites south of the Onilahy River, which are named here as *M. griffithsi*.

At virtually all of the sites where *M. gleni* and *M. griffithsi* have been found there are caves or at least rock shelters. Based on current information these two species have allopatric distributions. On Ile Ste Marie, *M. gleni* was found in a quarry excavated into bedrock, known locally as the Grotte d'Ankarana (Rakotondrasana and Goodman 2007). At Marokoloy and Tsiafabositra, near Maevatanana, this species was found

in subterranean water canals (F. Ratriomomanarivo unpublished information); these are the only examples we are aware of for this species in quasi-synanthropic day roost sites.

Nothing is known about the migratory movements of members of the *M. gleni* species group. On the label of a *M. gleni* specimen (ROM MAM 42717), obtained at Manin-day on 13 May 1967, it is written, 'Very fat-migrant. Mist netted in strip of palm swamp tract' and the animal weighed 17 g, which is one of the heaviest examples we have for this species. Whether this notably heavy animal was a migrant or had simply stored body fat before the start of the long dry season is impossible to discern based on current information.

At numerous localities on Madagascar different *Miniopterus* spp. are known to occupy the same day roost caves (Peterson et al. 1995), but not necessarily occurring in strict sympatry within a site. At the two caves in the Itampolo area where *M. griffithsi* was obtained, the only other member of this genus captured was *M. sp. n.* (Goodman et al. in press b) at the Grotte d'Androimpano (FMNH 184224, 184225). At the site near Ranopiso, several different *Miniopterus* have been recorded: *M. cf. manavi* (USNM 577079-577095), *M. sororculus* (USNM 577120, 577121), and *M. petersoni* (USNM 577072, 577073, 577122-577125). In caves within the Sarodrano area, the type locality of *M. gleni*, other *Miniopterus* that occur locally, include *M. majori* and *M. sp. n.* (Peterson et al. 1995; Goodman et al. in press b; Maminirina et al. in press).

Biogeography

On the basis of the molecular data presented herein, there appears to be little phylogeographic structure within *M. gleni* (Clade 1 in Fig. 2), with haplotypes shared across individuals occurring in dry forest (e.g. Analamerana), spiny bush (e.g. Sarodrano, Forêt des Mikea), and wet forest (Masoala) habitats, from lower (e.g. Ranobe) and moderate (e.g. Isalo) elevations, as well from a near shore island in the northeast (Ile Ste Marie). As circumscribed here, *M. gleni* only occurs north of the Onilahy River. In contrast, all of the animals identified as *M. griffithsi* (Clade 2 in Fig. 2) are from the region between the southern banks of the Onilahy River and the Mandrare River (Itampolo east to near Ranopiso; Fig. 1).

Wilmé et al. (2006) formulated a hypothesis to clarify patterns of micro-endemism on Madagascar and explain much localized cases of speciation using an analysis of watersheds in the context of Quaternary climatic changes. This mechanistic model postulates that organisms occupying river catchments with their water sources at relatively low elevations resulted in increased rates of micro-endemism as compared with other river catchments with their sources at higher elevations (at least 2000 m). In the latter case, these riverine habitats would have provided retreat-dispersal corridors to montane areas during periods of glaciation, which were cooler and dryer, while watersheds with their sources at lower elevations would have been notably isolated. A number of zones on the island were proposed as centres of endemism by Wilmé et al. (2006). One of these zones, referred to as Centre of Endemism 6 (CE 6), south of the Mangoky River, occupies the area from the southern bank of the Mangoky and then west to the western foothills of the Anosy Mountains close to Ranopiso. This extensive area has a complicated hydrology, with the Mandrare and Onilahy Rivers, which fall within the CE 6, having their headwaters slightly below 2000 m. As a result, CE 6 was

divided into several different portions: CE 6 (south), essentially the area south of the Onilahy River and CE d6 being formed by the Mandrare watershed. The distribution of *M. griffithsi* coincides with the zones CE 6 (south) and d6.

The lack of phylogeographic structure in *M. gleni* suggests that they have the capacity to disperse considerable distances, including over-water crossings. Now the quandary is why individuals of *M. griffithsi* have not dispersed further north, particularly across the Onilahy watershed, which is a relatively wide river near its mouth, but not approaching the 7 km distance separating Ile Ste Marie and Madagascar, which has been traversed by *M. gleni*.

Cornet (1974) classified Madagascar into bioclimatic zones, which are based on calculations of several different parameters, derived from a series of meteorological stations: hydric deficit (precipitation minus potential evapotranspiration), mean minimum temperature in the coldest month, and the length of the dry season. Four different broad zones are delineated within this system: humid (largely the eastern region and Montagne d'Ambre in the far north), sub-humid (montane eastern region, including the Central Highlands), dry (western region), and sub-arid (southern and southwestern region). The sub-arid zone, which largely coincides with Wilmé et al.'s (2006) zones CE 6 (south) and CE d6, was further subdivided by Cornet into sub-zones. One of these sub-zones has at least 700 mm of annual precipitation and a 12 month dry season and is delineated by the region from Morombe south, passing across the Onilahy basin, to the southern tip of the island and then picking up again along the coast to the west of the Anosy Mountains to the west of Tolagnaro (Fig. 1). All of the known records of *M. griffithsi* fall within the southern portion of this sub-zone, that is to say the section south of the Onilahy River.

Given the correlation between the current distribution of *M. griffithsi* and the southern portion of one of Cornet's bioclimatic sub-zones, this can be interpreted as a causal explanation for the modern distribution of this species. Different problems arise, at various temporal scales, in this interpretation. The first is if indeed the distribution of this bat is correlated with bioclimatic factors, why does it not occur north of the Onilahy River in the extension of this zone towards Morombe? On the basis of the dispersal ability of members of this species complex, it would appear that they have the capacity to traverse this river valley. Hence, it should have a distribution at least to the Morombe region based on the Cornet hypothesis, but it is *M. gleni* that picks up at Sarodrano, just to the north of the Onilahy River, and continues further to the north. Given that current samples from the region immediately to the north of the Onilahy River are not extensive, it is possible with further survey efforts in this region individuals of *M. griffithsi* will be located.

The second confounding aspect is that based on subfossils dating from the Holocene, there is good evidence that the coastal section of extreme southern and southwestern Madagascar were distinctly more mesic a few millennia ago. Animal bone remains recovered from sites close to Itampolo (e.g. Mahafaly Plateau) and Ranopiso (Andrahomana Cave), provide evidence of substantial faunal shifts, including local extinction events, in these regions during the past 6000 years (MacPhee 1986; Burney et al. 2004, 2008; Goodman et al. 2006, 2007b). Hence, the explanation that some aspect inherent in the post-Pleistocene climatic regime of the sub-arid zone of the southern portion of Madagascar is associated with the isolation and differentiation of *M. griffithsi* is not

supported in its entirety by Cornet's bioclimatic system, as recent climatic vicissitudes indicate a notably fluidity in certain abiotic aspects of the region.

The third confounding aspect is the period of cladogenesis between *M. gleni* and *M. griffithsi*. Using the estimate of 2% divergence per 1 million years (Brown et al. 1979), this event would have taken place approximately 3.7 million years ago. This is a period that no information is available from the southern portion of Madagascar concerning fossil vertebrate or pollen deposits, and hence no direct inference that can be made on climatic regimes that might be correlated with their speciation. In the final assessment, we do not have a clear explanation of why the distributions of these two species are allopatric and the biotic and abiotic factors that lead to their separation. Perhaps once more detailed information is available on the distribution of *M. griffithsi*, analyses using different aspects of niche-modelling will yield more coherent correlates to explain their distribution and isolation from its sister taxa, *M. gleni*.

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Résumé

Modèles de variations morphologique et génétique de la chauve-souris endémique malgache Miniopterus gleni (Chiroptera: Miniopteridae), avec la description d'une nouvelle espèce, M. griffithsi

Au cours de la dernière décennie, d'importants progrès ont été accomplis en ce qui concerne la compréhension de la systématique et de la diversité des espèces de chauves-souris malgaches, en grande partie basée sur des spécimens collectés au cours des inventaires ainsi que sur les études morphologiques et génétiques moléculaires associées.

Nous décrivons ici une nouvelle espèce de chauve-souris du sud de Madagascar, *Miniopterus griffithsi* sp. n., qui est la sœur de *M. gleni*, un taxon décrit en 1995 (holotype de Sarodrano, juste au nord de la rivière Onilahy). Sur la base des informations actuelles, *M. griffithsi* se trouve dans la zone bioclimatique sub-aride de l'île, au sud de la rivière Onilahy, et *M. gleni* se produit dans une variété de zones bioclimatiques, du nord de la rivière Onilahy jusqu'au nord de l'île et sur l'île Sainte Marie. Les deux espèces semblent utiliser des grottes et des abris sous roche comme gîtes diurnes. Le fait que *M. griffithsi* soit une entité distincte est fondé sur des études phylogéographiques du complexe *M. gleni*. Les comparaisons avec les 397 bp du mitochondrial cytochrome *b* montre une divergence de 1,2% dans les animaux qui se

produisent dans la majeure partie de Madagascar au nord de la rivière Onilahy, 0,07% dans celles au sud de la rivière Onilahy et 7,4% dans les populations séparées par cette rivière. Par la suite des caractères morphologiques ont été identifiés, comprenant la forme du tragus, la coloration du pelage et les proportions du crâne, soutenant ainsi la séparation des populations qui se produisent au sud (*M. griffithsi*) et au nord de la rivière Onilahy (*M. gleni*). La zone d'occupation connue pour *M. griffithsi* est d'environ 740 km², mais ce n'est certainement pas représentatif de la distribution de cette espèce.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1. Specimens of *Miniopterus gleni* and *Miniopterus griffithsi* used in this study.

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