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An integrative approach to characterize Malagasy bats of the subfamily Vespertilioninae Gray, 1821, with the description of a new species of *Hypsugo*

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Although important advances have been made in recent years in the taxonomy of different families and subfamilies of Malagasy bats, those belonging to the Vespertilioninae remain partially unresolved. Herein using a mitochondrial marker (cytochrome *b*) as the point of departure for 76 specimens of Malagasy vespers and appropriate African taxa, we diagnose the six taxa of this subfamily on the island by overlaying different morphological and bioacoustic characters on the clade structure of sequenced animals. The species include: endemic *Neoromicia matroka*, which is sister to African *N. capensis*; endemics *N. malagasyensis* and *N. robertsi*, which form sister species; a member of the genus *Hypsugo*, which is sister to African *H. anchietae* and described herein as new to science; *Pipistrellus hesperidus* for which Madagascar animals are genetically close but distinct from African populations of the same species; and endemic *P. raceyi*, which shows segregation of eastern (mesic) and western (dry) populations and its sister species relationships are unresolved. While the external and craniodental measurements, as well as bioacoustic

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variables, allow only partial differentiation of these six species of Vespertilioninae, molecular characters provide definitive separation of the taxa, as do male bacular morphology.

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INTRODUCTION

The past decade has seen considerable advances in understanding aspects of the systematics and diversity of Madagascar bats based on fieldwork, resulting in new collections, and most importantly insights from molecular genetics. Peterson, Eger & Mitchell (1995) in a comprehensive monograph on the island's chiropteran fauna listed 27 forms and, in late 2013, 44 species were recognised with 77% of these being endemic to the island (Goodman, 2011; Goodman *et al*., 2011, 2012a, b). Most Malagasy bat genera or species complexes have been the subject of phylogenetic and phylogeographic studies. The last taxonomic assemblage of Malagasy bats yet to be treated in the same detailed manner is the subfamily Vespertilioninae, referred to herein as vespers or pipistrelles; exceptions include *Myotis goudoti* (A. Smith, 1834) (Weyeneth, Goodman & Ruedi, 2010) and to a lesser extent members of the genus *Scotophilus* Leach, 1821 (Trujillo *et al*., 2009). Even after the important advances presented in Bates *et al*. (2006) based on craniodental and bacular morphology of different Malagasy vespertilionids of the genera *Neoromicia* Roberts, 1926, *Pipistrellus* Kaup, 1829, and *Hypsugo* Kolenati, 1856, numerous phylogenetic and taxonomic questions remain, and until recently, these aspects could not be properly addressed due to the lack of specimen and tissue material. In sub-Saharan Africa, these three genera are known for being very difficult to identify in the hand (Monadjem *et al*., 2013a). In many cases, classical craniodental features do not allow the reliable determination to species and even in some cases to genus (Kearney & Taylor, 1997; Kearney, 2005) and only based on molecular, karyological, or bacular characters (Volleth & Heller, 1994; Kearney *et al*., 2002; Kearney, 2005) can they be definitively identified.

In what was the first review of the Malagasy bat fauna, Dorst (1947) listed a single vesper specimen from the island assigned to *Pipistrellus* sp. Nearly 50 years later, Peterson *et al*. (1995) listed the following vesper taxa from Madagascar: *Pipistrellus* sp., *Eptesicus matroka* (Thomas & Schwann, 1905) (= *N. matroka*), and *E. somalicus* (Thomas, 1901) (= *N. somalica*); for the last species they described a new subspecies, *E. s. malagasyensis*. Subsequently, a number of small vespers was collected from different areas of the island, which gave rise to two papers. The first by Bates *et al*. (2006), based on different morphological characters, highlighted the presence on Madagascar of three different Vespertilioninae genera (*Pipistrellus*, *Neoromicia*, and *Hypsugo*); three locally occurring species shared with Africa, *H. anchietae* (Seabra, 1900), *P. hesperidus* (Temminck, 1840), and *N. melckorum* Roberts, 1919; and the description of an endemic species, *P. raceyi* Bates, Ratrimomanarivo, Harrison & Goodman, 2006. The second paper investigated the relationships between African and Malagasy populations morphologically similar to *N. melckorum* and, based on molecular and different types of morphological data, it was concluded that animals from Madagascar represented a new endemic species, *N. robertsi* Goodman *et al*., 2012a. For more details on the taxonomic history of Malagasy vespertilionids see Goodman *et al*. (2012a).

While these papers helped to resolve certain taxonomic issues concerning Malagasy Vespertilioninae, a number of questions remained, particularly given recent research in the Old World that uncovered considerable cryptic diversity within vesper bats (e.g. Benda, Hulva & Gaisler, 2004; Datzmann *et al*., 2012; Kruskop *et al*., 2012; Koubínová *et al*., 2013; Monadjem *et al*., 2013a). New collections have become available since the work of Bates *et al*. (2006) and Goodman *et al*. (2012a), which provide the means to address different systematic questions. Here we use the approach of producing a molecular phylogeny and then superimposing different types of morphological and bioacoustic characters of sequenced specimens to identify the different taxa and test the utility of these different datasets in their diagnosis. Of particular interest here was discerning if the African and Malagasy populations of two species, *H. anchietae* and *P. hesperidus*, represent widespread Afro-Malagasy taxa. Using this approach, we characterize the different Vespertilioninae species occurring on Madagascar and address the question on the species limits of Afro-Malagasy populations of these two species. More details on the phylogenetic history of the Afro-Malagasy lineages of vespers, particularly

generic allocations and sister species relationships, will be presented in subsequent publications.

MATERIALS AND METHODS

Specimens

Vesper bats were collected during the course of field trips on Madagascar. This study was conducted in strict accordance with the terms of research permits issued by authorities in Madagascar (Direction du Système des Aires Protégées, Direction Générale de l'Environnement et des Forêts, and Madagascar National Parks), following national laws. Animals were captured, manipulated, and dispatched with thoracic pressure following guidelines accepted by these different national authorities and the scientific community for the handling of wild animals (Sikes *et al.*, 2011).

Specimens for the morphological and molecular genetic analyses presented herein are housed in the following museums: BMNH – The Natural History Museum (formerly The British Museum of Natural History), London; DM – Durban Natural Science Museum, Durban; FMNH – Field Museum of Natural History, Chicago; MNHN – Muséum national d'Histoire naturelle, Paris; NMZL: National Museum of Zambia, Livingstone, Zambia; ROM – Royal Ontario Museum, Toronto; TM – Ditsong Museum of Natural History (formerly Transvaal Museum), Pretoria; and UADBA – Université d'Antananarivo, Département de Biologie Animale, Antananarivo.

Systematics and species allocations

In general, we follow the arrangement of Simmons (2005) for Afro-Malagasy members of the subfamily Vespertilioninae with the following exceptions: *Eptesicus matroka* is placed in the genus *Neoromicia* based on bacular morphology (Bates *et al.*, 2006) and *N. malagasyensis* Peterson, Eger & Mitchell, 1995 is treated specifically distinct from *N. somalica* based on cranial and bacular characters (Goodman & Ranivo, 2004; Bates *et al.*, 2006). We use Simmon's arrangement of placing what was formerly referred to as *Pipistrellus nana* (Peters, 1852) in the genus *Neoromicia* and considering *N. nana* as the replacement name for *P.* [= *Neoromicia*] *africanus* (Rüppell, 1842). The placement of Seabra's *Vesperugo anchietae* in the genus *Hypsugo* is based on karyological and penis morphology characters (Volleth *et al.*, 2001; Kearney *et al.*, 2002; Kearney, 2005).

Given considerable character ambiguity in separating different taxa of Afro-Malagasy Vespertilioninae at the species and even generic level (e.g., Monadjem *et al.*, 2010), in order to characterize the taxa occurring on Madagascar, we use clade structure based on the molecular analysis presented herein and then overlay upon this the non-molecular characters. Hence, in most cases, excluding types and some African specimens, we have only analyzed specimens for which genetic data are available. This provides a solid characterization of the different taxa, allowing the formulation of subsequent systematic conclusions. The collection localities of the vespers used in the molecular genetic characterization, as well as other sites mentioned in the text, are presented in Figure 1.

Access and comparison to type specimens

We were able to directly compare sequenced Malagasy vespers with the following holotypes, paratypes or syntypes: *Eptesicus somalicus malagasyensis* [= *Neoromicia malagasyensis*] (ROM 42713); *Vespertilio matroka* [= *N. matroka*] (BMNH 97.9.32), *V. minutis somalicus* [= *N. somalica*] (BMNH 98.6.9.1), *Vesperus humbloti* Milne-Edwards, 1881 (MNHN 1986.1074, 1986.1075), *Vesperugo anchietae* de Seabra, 1900 (BMNH 1906.1.3.1, MNHN 1900-538); *N. robertsi* (UADBA 43677), and *Pipistrellus raceyi* (FMNH 185567).

DNA isolation and sequencing

Genomic DNA was extracted using a lithium chloride and chloroform method (Gemmel & Akiyama, 1996). The primers L14724/H15506 and L15171/H15915 (Irwin, Kocher & Wilson, 1991; Yoder, Vilgalys & Ruvolo, 1996) were used to amplify and sequence the cytochrome *b* gene. Template DNA was amplified by PCR in 25 μL reaction volume containing the following: 1× reaction buffer (Promega), 2.5 mM $MgCl_2$, 0.2 μM of each dNTP, 0.28 μM of each primer, 1 unit of *Taq* polymerase (Promega), and approximately 100 ng of template DNA. Cycling consisted of an initial denaturation at 94 °C for 3 min, followed by 30 cycles at 94 °C for 30 s, 50 °C for 30 s and 72 °C for 40 s, and a final extension of 72 °C for 3 min. The PCR products were directly sequenced by a commercial company (Macrogen Inc.) using the ABI Prism BigDye Cycle Sequencing kit (Applied Biosystems, Perkin-Elmer) and visualized on an ABI 3730XL (Applied Biosystems, Perkin-Elmer). The sequences were aligned using Sequencher version 4.6 (Gene Codes Corporation). All new sequences were deposited in GenBank (accession numbers KM886006-KM886099) and accession numbers of all specimens used in this study listed in Table S1.

Molecular study and tree building

The Bayesian analysis was conducted using MrBayes 3.2 (Huelsenbeck & Ronquist, 2001; Ronquist *et al.*, 2012) under the substitution model GTR + I + G. Four

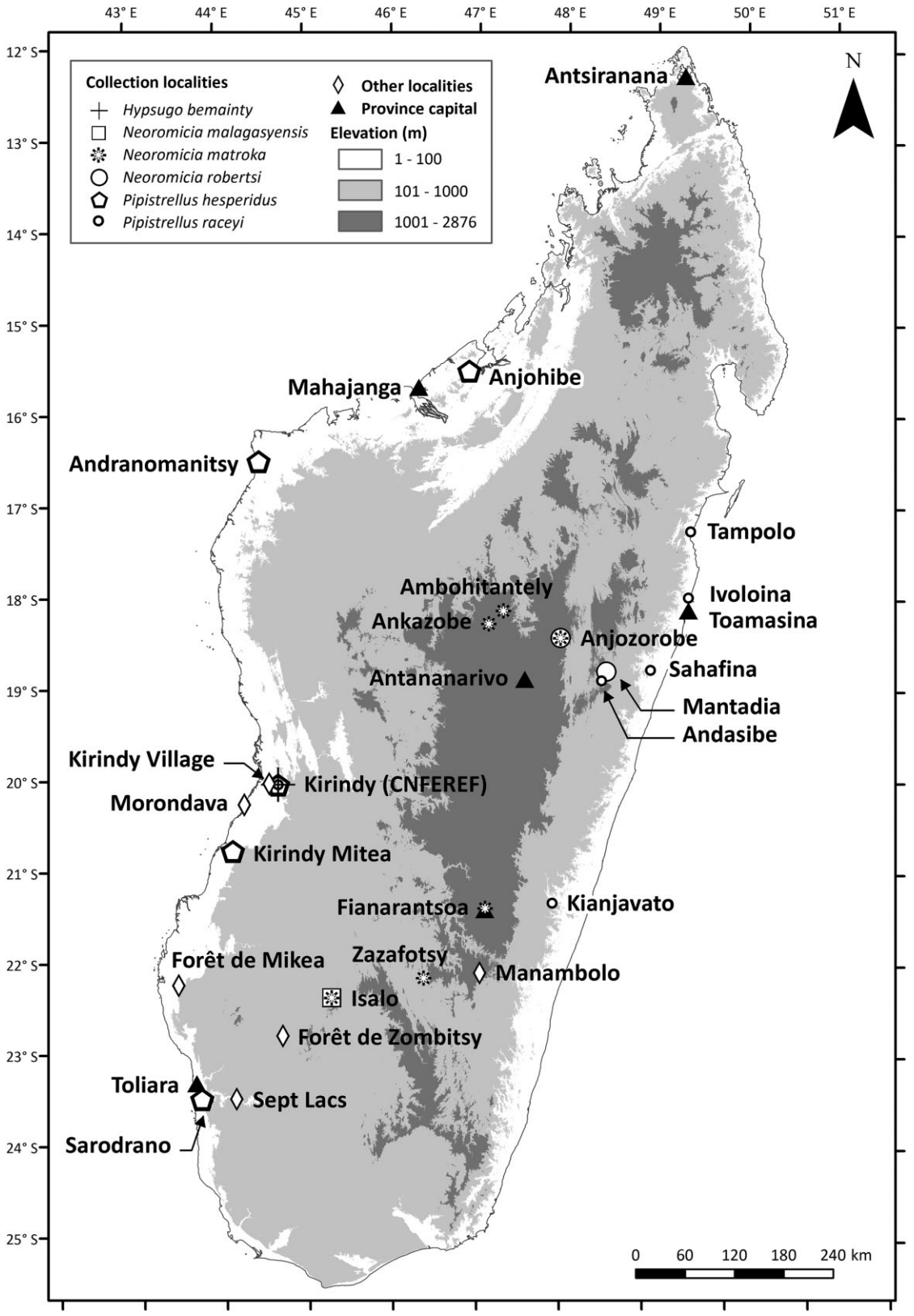

Figure 1. Map of localities mentioned in text associated with sequenced members of the genera *Neoromicia*, *Pipistrellus*, and *Hypsugo* captured on Madagascar. Other localities mentioned in the text and an elevational gradient are also shown.

chains were run under MrBayes for 2 000 000 generations with a sampling frequency of 1000. Burn-in was set at 25% of initial trees. The deviation of split frequencies was below 0.01 at the conclusion of the analysis. The maximum likelihood (ML) analysis was implemented using the RAxML program using the Black Box workbench (Stamatakis, 2006; Stamatakis, Hoover & Rougemont, 2008; Stamatakis & Ott, 2008) under the default GTR+G substitution model. Bootstrap values were estimated using 1000 pseudoreplicates. The Bayesian and ML analyses were run on a reduced data set in order to avoid the difficulties of analyzing sequences of unequal length. At least two sequences of each clade were included in the reduced dataset.

A neighbour-joining distance-based analysis (allowing for unequal sequence length) incorporating all sequences is presented in Figure 3. The overall topology was unaffected by the reduction in sequence length and sample size. A genetic distance matrix was calculated from the reduced dataset, based on Kimura 2-Parameter (K2P) distance (Kimura, 1980) and implemented in MEGA5 (Tamura *et al*., 2011).

Intraspecific diversity and divergence

Intraspecific diversity and divergence was investigated for the three most sampled lineages: *Hypsugo bemainty* sp. nov., *Pipistrellus raceyi*, and *P. hesperidus*. In addition, two species could be divided into distinct populations: (1) *P. raceyi* into an eastern population (specimens from the Provinces of Fianarantsoa and Toamasina) and a western population (specimens from Province of Toliara) and (2) *P. hesperidus* into African and Malagasy populations. Haplotype diversity (Hd) and nucleotide diversity (Pi) were calculated for all these subpopulations (except for the African *P. hesperidus* lineage due to a limited sample of two specimens), as well as for the *H. bemainty* sample as a whole. Diversity indices were calculated using DnaSP v5 (Librado & Rozas, 2009).

Morphological study

Six different external measurements were taken from animals in the field at an accuracy of 0.5 mm before being prepared as specimens: total length, tail length, hindfoot length (excluding claw), ear length, tragus length, and forearm length. Body mass was recorded with a Pesola spring balance to a precision of 0.5 g.

Throughout this paper, only dimensions taken by a single collector (SMG) are used in the analyses of external measurements. Additional information on external measurements was obtained from museum labels and associated specimen field catalogues, but caution is needed when making comparisons between various collectors associated with varying mensuration techniques. Values are reported only for adults (defined by presence of a fully erupted permanent dentition and fused basisphenoid–basioccipital suture). Tooth abbreviations include I = incisor, C = canine, P = premolar, and M = molar. Upper case abbreviations are used for upper teeth and lower case abbreviations for lower teeth and tooth number system follows Koopman (1994).

Seven cranial and five dental measurements were made by SMG using digital callipers accurate to 0.1 mm (acronym for each measurement presented in parentheses). Cranial measurements include: greatest skull length (GSKL), from posterior-most part of occipital to anterior-most point of incisors; condylo-incisive length (CIL), from occipital condyle to anterior-most point of incisors; greatest zygomatic breadth (ZYGO), width taken across zygomatic arches at the widest point; postorbital width (POB), dorsal width at most constricted part of skull; breadth at mastoids (MAST), greatest breadth across skull at mastoid processes; palatal length (PAL), from posterior border of hard palate to anterior edge of premaxillary bone; and mandible length (MAND), from the posterior-most portion of the condyles to anterior-most point of upper incisors. The dental measurements comprise: complete cranial toothrow length (I-M^3), from anterior alveolar border of incisors to posterior alveolar border of third molar (M^3); canine-molar toothrow length (C-M^3), from anterior alveolar border of canine to posterior alveolar border of third molar (M^3); width across canines (C-C), taken across the outer alveolar borders of the canines; width across third molars (M^3-M^3), taken across the outer alveolar borders of the M^3; and mandibular toothrow length (i-m_3), from anterior alveolar border of incisors to posterior alveolar border of third molar (m_3). Excluding several type specimens and a few other exceptions, morphological measurements are presented only for specimens represented in our molecular genetics dataset (Table S1).

T-test analyses of morphological variables were conducted to test for sexual dimorphism in *Pipistrellus hesperidus* as well as geographical differences in eastern and western populations of *P. raceyi*. To examine possible segregation of the different species belonging to the genera *Pipistrellus*, *Neoromicia*, and *Hypsugo* groups, as well as geographic variation in *P. raceyi*, principal component analysis (PCA), based on a correlation matrix, was conducted with R software (R Development Core Team, 2012) using the Rcmdr (Fox, 2005) and FactoMineR (Lê, Josse & Husson, 2008) packages.

Bacular study

In most cases, we collected information on bacular morphology for genotyped males. Preparation of bacula for all Malagasy species (*Hypsugo bemainty* sp. nov., $N = 7$; *Neoromicia matroka*, $N = 6$; *N. malagasyensis*, $N = 2$;

N. robertsi, *N* = 1; *Pipistrellus hesperidus*, *N* = 4; *P. raceyi*, *N* = 2) and several African taxa (*H. anchietae*, *N* = 1; *N. capensis*, *N* = 2; *N.* cf. *melckorum*, *N* = 1; *N. nana*, *N* = 2; *P. hesperidus*, *N* = 2) followed the techniques of Lidicker (1968), Hill & Harrison (1987), and Kearney *et al*. (2002), with slight modifications: penile tissue was hydrated in distilled water for 12 h and subsequently macerated in 5% KOH with added alizarin red for 2–4 h. Stained bacula were dissected from the macerated tissue, cleared, and stored in 100% glycerine with a crystal of thymol to prevent fungal growth. Each baculum was photographed in dorsal, ventral, and lateral views. The total baculum length (TBL) was measured either using a 2.0 megapixel USB Digital Microscope Camera (Digimicro, USA) or using Mitutoyo digital callipers while viewed under a dissecting microscope.

Bioacoustics

Bioacoustic information presented herein is only for bats that were genotyped. The echolocation calls of 34 different vesper bats were recorded using a D-240X Pettersson bat detector (Pettersson Elektronik AB, Uppsala, Sweden) in time expansion while the individual flew within a flight cage measuring 5.4 × 1.4 × 1.5 m and in the direction of the microphone. Recorded echolocation calls were either stored directly onto a HP netbook (HPMini 210-4000, USA) or saved onto a minidisc recorder (MZ-N505 Sony, USA) for later analysis.

Time expanded echolocation calls were analyzed using BatSound 4.1.4 (Pettersson Elektronik, Uppsala, Sweden) set at 44.1 kHz (mono, 16 bit), in a Hanning window mode, Fast Fourier Transform 512. One single pulse per individual at the beginning of a set of pulses comprising one bat pass was analysed to avoid replication. The choice of the pulse was based on the ratio between signal and background noise (Schoeman & Jacobs, 2008), specifically the recorded signal needed to be at least three times greater than the background noise. The peak echolocation frequency (PF in kHz) was measured from the peak extracted from the power spectrum (Obrist, 1995). The maximum (Fmax in kHz) and minimum (Fmin in kHz) frequencies were measured at + and –18 dB, respectively, from the power spectrum (Barclay, Fullard & Jacobs, 1999). The duration (Dur in ms) and the interpulse interval (IPI in ms) were measured on the combined projection of the oscillogram and spectrogram.

Preliminary *t*-test analyses on bioacoustic parameters were conducted to test for sexual dimorphism in *Pipistrellus hesperidus* only, because in the other taxa, the sample sizes were too small for such tests. To examine segregation of the *Pipistrellus*, *Neoromicia*, and *Hypsugo* groups based on bioacoustic parameters, PCA was conducted with R software (R Development Core Team, 2012) using the Rcmdr (Fox, 2005) and FactoMineR (Lê *et al*., 2008) packages.

RESULTS

Molecular genetics

Net K2P distances between clades ranged from 4.5% to 23.2% (excluding the outgroup), while variation within clades reached a maximum of 1.6% K2P (*Neoromicia capensis*) (Table 1). Partial sequences of cytochrome *b* were obtained for 98 specimens, with the outgroup *Myotis ricketti* bringing the total to 99. However, sequencing success varied widely and for most

Table 1. Net mean Kimura 2-Parameter distance between lineages as derived from partial cytochrome *b* sequences (680 bp) and calculated using MEGA5 (Tamura *et al*., 2011) for Malagasy vespers of the genera *Hypsugo*, *Neoromicia*, and *Pipistrellus*. Italicized numbers refer to mean K2P distance within lineages. An asterisk (*) indicates that less than five samples were available from which to derive intraspecific distance. Note that only one sequence was available for *Myotis ricketti* and, thus, no intraspecific distance could be calculated. See Monadjem *et al*. (2010) for a definition of *Neoromicia* cf. *melckorum*

	N. mat	*N. c*	*N. mal*	*N. r*	*N.* cf. *me*	*H. b*	*H. a*	*N. n*	*P. r*	*P. h*
N. matroka (*N* = 11)	*0.008*									
N. capensis (*N* = 5)	0.045	*0.016*								
N. malagasyensis (*N* = 3)	0.103	0.109	**0.003*							
N. robertsi (*N* = 2)	0.107	0.110	0.040	**0.001*						
N. cf. *melckorum* (*N* = 2)	0.110	0.109	0.122	0.123	**0.000*					
H. bemainty (*N* = 4)	0.146	0.161	0.179	0.183	0.166	*0.010*				
H. anchietae (*N* = 2)	0.172	0.174	0.175	0.177	0.182	0.128	**0.000*			
N. nana (*N* = 2)	0.171	0.186	0.222	0.208	0.199	0.206	0.206	**0.006*		
P. raceyi (*N* = 7)	0.179	0.195	0.186	0.180	0.184	0.204	0.222	0.202	*0.004*	
P. hesperidus (*N* = 14)	0.204	0.205	0.221	0.196	0.223	0.223	0.225	0.232	0.202	*0.009*
M. ricketti (*N* = 1)	0.225	0.219	0.239	0.217	0.235	0.226	0.203	0.231	0.228	0.244

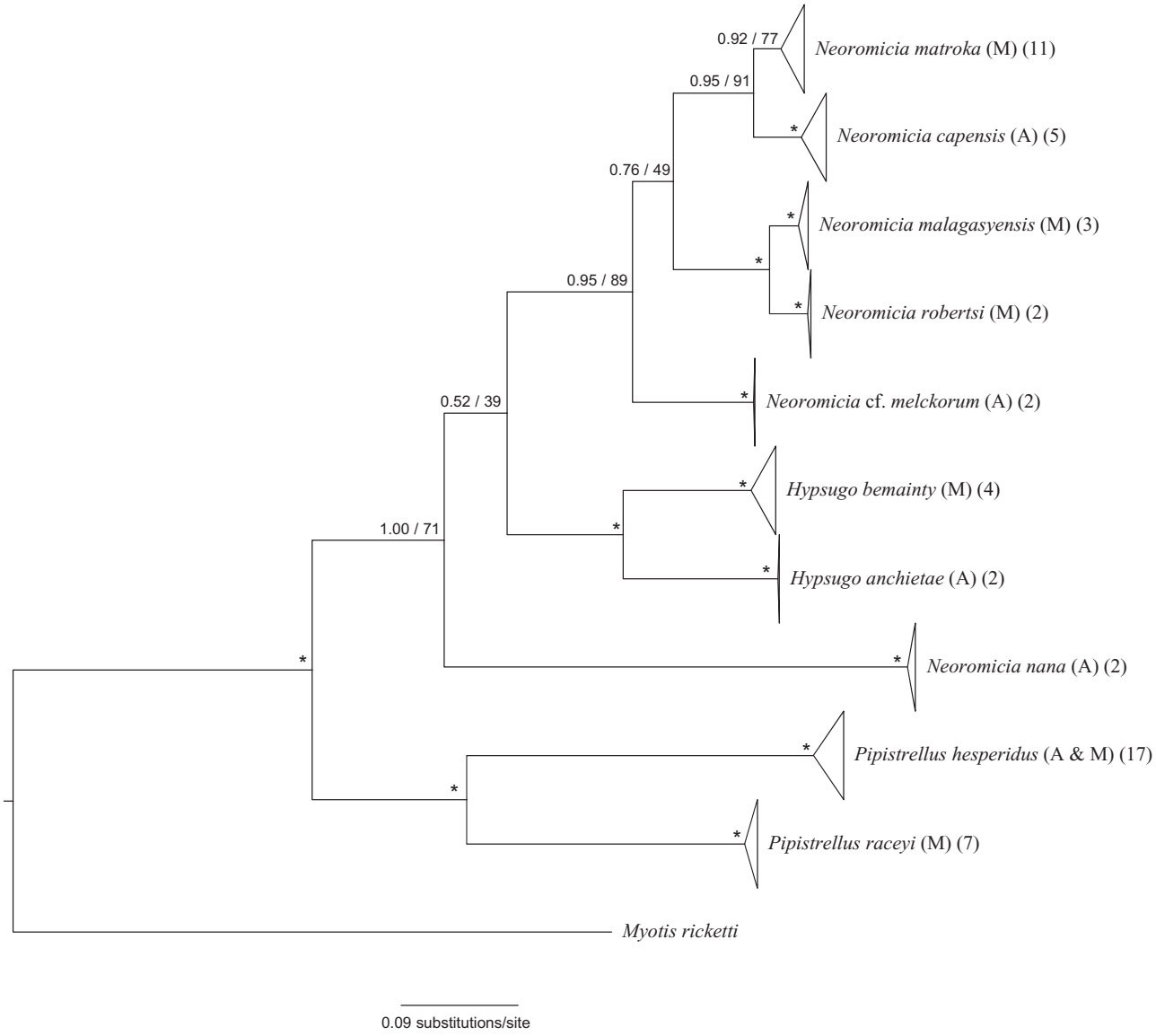

Figure 2. Consensus Bayesian tree generated from partial sequences of cytochrome *b* (680 bp), applying the model GTR + I + G and implemented in MrBayes 3.2 (Huelsenbeck & Ronquist, 2001; Ronquist *et al*., 2012). The first number at each node indicates posterior probability and the second indicates bootstrap support as generated by RAxML (Stamatakis, 2006; Stamatakis *et al*., 2008; Stamatakis & Ott, 2008), employing the model GTR+G. An asterisk (*) indicates that support for that node is greater than 0.95 posterior probabilities in the Bayesian analysis and 95% bootstrap support in the ML analysis. The clade identity of every sequenced specimen is provided in Figure 3. The geographical origins of the different samples per species are indicated as A = Africa and M = Madagascar. Numbers in parentheses indicate the number of sequences included in the analysis for that clade as per the reduced dataset. See Monadjem *et al*. (2010) for the definition of *Neoromicia* cf. *melckorum*. The clade identity of every sequenced individual is provided in Figure 3.

sequences, the targeted fragment (1201 bp) was not recovered. In order to include at least two specimens from each clade, the sequences were trimmed to 680 bp, allowing 56 sequences of equal length to be included in the final analyses.

The topology of the Bayesian and ML trees did not differ. Ten clades were recovered in the final analysis (Fig. 2), dividing into three major groups (M = Madagascar and A = Africa): (1) the genus *Pipistrellus* [including *P. hesperidus* (M and A) and *P. raceyi* (M)]; (2) the species *N. nana* (A); and (3) a larger clade consisting of *N. matroka* (M), *N. capensis* (A), *N. robertsi* (M), *N. malagasyensis* (M), *Hypsugo anchietae* (A), and *Hypsugo bemainty* sp. nov. (M), which is named below.

In the Neighbor-Joining (NJ) distance tree (Fig. 3), two species, *N. robertsi* and *N. malagasyensis*, do not show reciprocal monophyly, while their separation as distinct species is fully supported in the Bayesian and ML trees, as well as by numerous morphological characters. While the genus *Pipistrellus* has proven to be monophyletic, the position of *N. nana* does not support the reciprocal monophyly of *Neoromicia* and *Hypsugo*. However, the node including *Hypsugo* with all other *Neoromicia* spp. is poorly supported, so this relationship is uncertain.

Patterns of sexual dimorphism in morphology

Several sequenced Malagasy animals falling within the *Pipistrellus hesperidus* clade were available for morphological comparisons to assess patterns of sexual dimorphism. Three of the seven external morphological characters (total length, tail length, and body mass) showed statistically significant differences between the sexes, with females in all three cases being larger than males (Table 2). Further, for the balance of the other three variables, females on average were larger than males, with the exception of tragus length. For the seven cranial variables, females were in all cases larger on average than males, and several of these variables were statistically significant (Table 3). The same general pattern was found for the dental variables (Table 4). Hence, in populations of this taxon on Madagascar, females are in general larger than males.

Principal component analyses associated with craniodental morphology

Using measurement data from genotyped specimens, PCA were conducted for the combined cranial and dental measurements of all Malagasy members of the genera *Neoromicia*, *Pipistrellus*, and *Hypsugo*. Further, populations of *P. raceyi* from eastern and western Madagascar, which show statistically significant differences in measurements (Tables 2–4), were also segregated in these analyses. When the cranial and dental variables were separated in a series of different PCA analyses, the same general patterns described below were recovered (results not presented).

The first three components of the combined craniodental PCA explain over 88% of the variation, with PC1 accounting for nearly 75% (Table 5). Associated with PC1, all of the variables are negatively correlated with the exception of M^3-M^3. Projections of PC1 vs. PC2 (Fig. 4A) and PC1 vs. PC3 (Fig. 4B) provide a distinct separation of *N. robertsi* with respect to the other five species of Malagasy vespers. *Neoromicia matroka* shows little overlap with the other four small species (*N. malagasyensis*, *P. hesperidus*, *P. raceyi* (east and west), and *H. bemainty* sp. nov.). At certain localities in the eastern zone, *N. robertsi*, *N. matroka*, and *P. raceyi* are known to occur in sympatry and these taxa show no overlap in the PCA projections (Fig. 4). At localities in south central Madagascar, *N. malagasyensis* and *N. matroka* are known from the same sites and, based on the PCAs, they form separate groups in morphological space. In contrast, the two plots presented in Figure 4 demonstrate considerable overlap between *P. hesperidus*, western populations of *P. raceyi*, and *H. bemainty*, which occur in sympatry. In parallel to the genetic results discussed above, eastern and western populations of *P. raceyi* are notably different from one another.

Bioacoustics

Vesper bats produced low duty cycle echolocation calls with a prominent broad FM component and short Dur, where most of the energy was concentrated in the first harmonic between 49 to 58.3 kHz (*Hypsugo bemainty* sp. nov. and *Pipistrellus raceyi*, respectively; Fig. 5, Table 6). Sexual dimorphism analyses conducted on *P. hesperidus* revealed no statistically significant differences in the bioacoustic variables (Fmax: $t_{12} = 0.90$, $P = 0.38$; Fmin: $t_{12} = 2.07$, $P = 0.06$; Dur: $t_{12} = 0.83$, $P = 0.42$; IPI: $t_{12} = 1.09$, $P = 0.30$) except for PF ($t_{12} = 2.48$, $P = 0.03$). On the basis of the absence of bioacoustic differences between the sexes in *P. hesperidus* from Madagascar and other African vesper bats (Monadjem *et al.*, 2010), males and females for each of the distinct genetic clades were combined in subsequent analyses. The animals from the three genera largely overlap in all bioacoustics variables, which are reflected in the results of the PCA.

The first three PCs explained 79.7% of the total variance of the bioacoustical parameters; Fmin, Dur, and IPI loaded high on PC1, PF loaded positively high on PC2, and Fmax on PC3 (Table 7). The projection of PC1 vs. PC2 revealed broad overlap between specimens of *P. raceyi*, *Neoromicia matroka*, and *N. malagasyensis* (Fig. 6A). In contrast, with few exceptions, *H. bemainty* and *P. hesperidus* formed separate point clusters (Fig. 6A). The projections of PC1 vs. PC3, and PC2 vs. PC3 show broad overlap between specimens of *P. hesperidus*, *P. raceyi*, and *H. bemainty*, while *N. malagasyensis* and, to a lesser degree, *N. matroka* formed separate clusters (Fig. 6B, C). *Neoromicia robertsi* clustered close to *H. bemainty* in the three projections (Fig. 6A–C).

SPECIES CHARACTERIZATION

On the basis of different characters superimposed on the molecular phylogeny of Afro-Malagasy vespers, we are able to diagnose six species occurring on

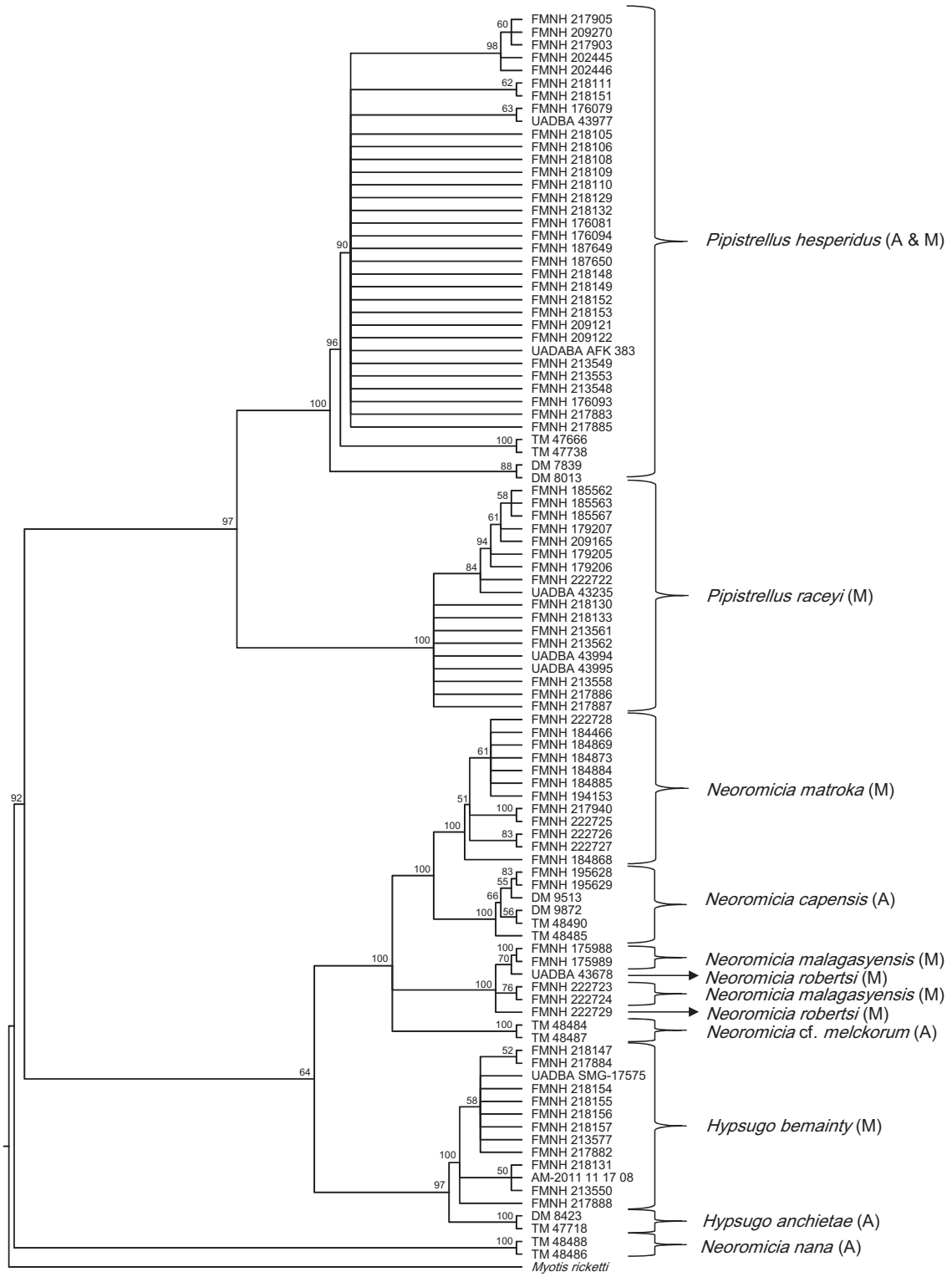

Figure 3. Majority rule 50% consensus neighbour-joining (NJ) proportional cladogram generated from partial cytochrome *b* sequences (1201 bp). The NJ analysis was conducted using Kimura 2-Parameter (K2P) distance and implemented in PAUP* 4.10b for Unix (Swofford, 2003), incorporating 1000 bootstrap pseudoreplicates. Numbers at nodes indicate bootstrap support. The geographical origins of the different samples per species are indicated as A = Africa and M = Madagascar. Specimens are designated by their voucher number or, where they have not yet been catalogued, by their field number (see Table S1 for definitions of different acronyms).

Table 2. External measurements (in millimetres) and mass (in grams) of vesper species of the genera *Hypsugo*, *Neoromicia*, and *Pipistrellus* identified from Madagascar and relevant type specimens with the holotype of ***Hypsugo bemainty* sp. nov.** having been sequenced. Measurements presented as mean ± standard deviation, minimum and maximum measurements, and number of specimens. Figures in bold are of specimens collected and measured by SMG and those in standard script by a variety of different field collectors. Statistical differences between the sexes within species with sufficient sample sizes are examined based on Student's *t*-test, n.s. = not significant

	Total length	Tail length	Hindfoot length	Tragus length	Ear length	Forearm length	Body mass
N. malagasyensis	81.3 ± 1.16	36.0 ± 1.00	4.8 ± 0.50	6.5 ± 0.58	12.0 ± 0.82	31.3 ± 0.96	4.5 ± 1.05
	80–82, *N* = 3	**35–37, *N* = 3**	**4–5, *N* = 4**	**6–7, *N* = 4**	**11–13, *N* = 4**	**30–32, *N* = 4**	**3.7–6.0, *N* = 4**
Holotype							
ROM 42713 ♀ (Peterson *et al.*, 1995)	84	27	6	–	12	31.2	9.0
N. matroka	**81.2 ± 3.13**	**32.5 ± 3.15**	**4.2 ± 0.41**	**7.1 ± 0.20**	**11.5 ± 0.84**	**31.3 ± 1.21**	5.1 ± 0.56
	77–85, *N* = 6	**27–36, *N* = 6**	**4–5, *N* = 6**	**7–7.5, *N* = 6**	**11–13, *N* = 6**	**30–33, *N* = 6**	4.0–5.8, *N* = 11
N. robertsi	91, 93, *N* = 2	35, 36, *N* = 2	5, 6, *N* = 2	6, 8, *N* = 2	13, 13, *N* = 2	38, 34.5, *N* = 2	7.8, 11.5, *N* = 2
Holotype							
UADBA 43677 ♂	85	32	6	6	13	34	6.7
P. hesperidus							
♂♂	**75.4 ± 3.32**	**32.4 ± 2.16**	**4.5 ± 0.63**	**5.7 ± 0.54**	**10.4 ± 0.50**	**29.8 ± 0.75**	3.5 ± 0.43
	68–81, *N* = 17	**29–36, *N* = 16**	**4–6, *N* = 16**	**4.6–6, *N* = 16**	**10–11, *N* = 16**	**29–31, *N* = 16**	2.8–4.5, *N* = 21
♀♀	**80.7 ± 3.40**	**34.3 ± 1.11**	**4.7 ± 0.49**	**5.4 ± 0.53**	**10.6 ± 0.53**	**30.7 ± 1.50**	4.1 ± 0.59
	77–86, *N* = 7	**33–36, *N* = 7**	**4–5, *N* = 7**	**5–6, *N* = 7**	**10–11, *N* = 7**	**29–33, *N* = 7**	3.3–5.2, *N* = 13
T-statistics	*t* = 3.58, *P* = 0.002	*t* = 2.22, *P* = 0.04	n.s.	n.s.	n.s.	n.s.	*t* = 2.02, *P* = 0.001
Combined	**77.1 ± 4.06**	**33.0 ± 2.07**	**4.6 ± 0.59**	**5.6 ± 0.54**	**10.4 ± 0.51**	**30.1 ± 1.09**	**3.8 ± 0.58**
	68–86, *N* = 23	**29–36, *N* = 23**	**4–6, *N* = 23**	**4.5–6, *N* = 23**	**10–11, *N* = 23**	**29–33, *N* = 23**	**2.8–5.2, *N* = 34**
African *P. hesperidus* (Monadjem *et al.*, 2010)							
♂♂	**80.5 ± 6.36**	**31.9 ± 3.96**	–	–	**10.2 ± 1.74**	**32.4 ± 1.29**	**5.9 ± 0.70**
	61–88, *N* = 25	**24–41, *N* = 25**			**6–13, *N* = 25**	**29.6–35.0, *N* = 27**	**4.6–7.6, *N* = 19**
♀♀	**81.7 ± 5.59**	**32.8 ± 4.18**	–	–	**10.4 ± 1.51**	**32.9 ± 1.12**	**6.2 ± 1.15**
	70–91, *N* = 21	22–37, *N* = 21			7–12, *N* = 21	30.9–34.9, *N* = 22	4.0–9.0, *N* = 22
P. raceyi	**79.1 ± 3.18**	**31.7 ± 1.58**	**4.3 ± 0.71**	**5.3 ± 0.50**	**10.3 ± 0.50**	**29.8 ± 2.11**	4.7 ± 0.68
	75–85, *N* = 9	**29–34, *N* = 9**	**4–6, *N* = 9**	**5–6, *N* = 9**	**10–11, *N* = 9**	**27–33, *N* = 9**	3.8–5.8, *N* = 16
Holotype							
FMNH 185567 ♀	80	28	4	6	11	31	5.3
West	**76.3 ± 0.96**	**31.8 ± 0.96**	**4.3 ± 0.50**	**5.0 ± 0.00**	**10.3 ± 0.50**	**27.8 ± 0.50**	**4.2 ± 0.21**
	75–77, *N* = 4	**31–33, *N* = 4**	**4–5, *N* = 4**	**5–5, *N* = 4**	**10–11, *N* = 4**	**27–28, *N* = 4**	**3.9–4.4, *N* = 4**
East	**81.4 ± 2.19**	**31.6 ± 2.07**	**4.4 ± 0.89**	**5.6 ± 0.55**	**10.4 ± 0.55**	**31.4 ± 1.14**	**5.4 ± 0.26**
	80–85, *N* = 5	**29–34, *N* = 5**	**4–6, *N* = 5**	**5–6, *N* = 5**	**10–11, *N* = 5**	**30–33, *N* = 5**	**5.1–5.8, *N* = 5**
T-tests	*t* = 4.34 *P* = 0.003	n.s.	n.s.	n.s.	n.s.	*t* = 5.90 *P* < 0.001	*t* = 7.94 *P* < 0.001
H. bemainty	79.4 ± 2.30	35.2 ± 1.92	4.6 ± 0.55	6.4 ± 0.89	11.2 ± 1.31	30.0 ± 1.41	**3.6 ± 0.39**
	77–83, *N* = 5	**33–36, *N* = 6**	**4–5, *N* = 5**	**5–7, *N* = 5**	**10–13, *N* = 5**	**29–32, *N* = 5**	2.8–4.4, *N* = 13
Holotype							
FMNH 217884 ♂	77	35	5	7	11	31	3.9
African *H. anchietae* (Monadjem *et al.*, 2010)							
♂♂	**77.3**	**34.3**	–	–	**11.3 ± 0.96**	**30.2 ± 1.36**	**4.7 ± 0.47**
	73–80, *N* = 3	**34–35, *N* = 3**			**10–12, *N* = 4**	**28.0–32.0, *N* = 13**	**4.0–5.5, *N* = 12**
♀♀	**83.0 ± 3.74**	**36.0 ± 3.16**	–	–	**11.9 ± 0.90**	**30.6 ± 1.50**	**4.9 ± 0.77**
	78–87, *N* = 4	32–39, *N* = 4			11–13, *N* = 7	28.2–32.6, *N* = 13	3.2–5.7, *N* = 11

Table 3. Cranial measurements (in millimetres) of adult vesper species of the genera *Hypsugo*, *Neoromicia*, and *Pipistrellus* identified from Madagascar and relevant type specimens with the holotype of *Hypsugo bemainty* sp. nov. having been sequenced. Measurements presented as mean ± standard deviation, minimum and maximum measurements, and number of specimens. See Methods for an explanation of variable acronyms. Statistical differences between the sexes within species with sufficient sample sizes are examined based on Student's *t*-test, n.s. = not significant

	GSKL	CIL	ZYGO	POB	MAST	PAL	MAND
N. malagasyensis	12.5 ± 0.10	11.8 ± 0.10	8.4 ± 0.23	3.4 ± 0.13	7.0 ± 0.13	4.6 ± 0.15	8.3 ± 0.13
	12.4–12.6, $N = 4$	11.7–11.9, $N = 4$	8.3–8.7, $N = 4$	3.2–3.5, $N = 4$	6.9–7.2, $N = 4$	4.4–4.7, $N = 4$	8.2–8.5, $N = 4$
Holotype							
ROM 42713 ♀	13.3	12.7	8.8	3.7	7.7	4.8	–
N. matroka	13.1 ± 0.33	12.6 ± 0.35	8.7 ± 0.27	3.5 ± 0.14	7.5 ± 0.25	4.8 ± 0.22	9.0 ± 0.21
	12.4–13.4, $N = 12$	12.0–13.0, $N = 12$	8.4–9. 2, $N = 10$	3.3–3.8, $N = 12$	7.2–7.9, $N = 12$	4.3–5.0, $N = 12$	8.7–9.3, $N = 10$
Holotype							
BMNH 97.9.1.32 ♂	13.3	12.9	8.8	3.5	7.7	4.8	9.4
N. robertsi	14.8	14.2	10.4	4.1	8.7	5.5	10.5
Holotype							
UADBA 43677 ♂	14.3	14.0	9.5	3.6	8.1	5.5	10.0
P. hesperidus (sexes combined)	12.0 ± 0.28	11.6 ± 0.25	7.7 ± 0.14	3.4 ± 0.16	6.9 ± 0.15	4.2 ± 0.21	8.4 ± 0.22
	11.5–12.9, $N = 31$	11.2–12.4, $N = 31$	7.5–8.0, $N = 12$	2.9–3.6, $N = 33$	6.6–7.2, $N = 32$	3.8–4.5, $N = 32$	7.9–8.9, $N = 32$
♂♂	12.0 ± 0.27	11.5 ± 0.24	7.7 ± 0.15	3.3 ± 0.02	6.8 ± 0.15	4.2 ± 0.19	8.3 ± 0.12
	11.4–12.5, $N = 20$	10.9–11.9, $N = 20$	7.5–8.0, $N = 10$	2.9–3.6, $N = 21$	6.6–7.0, $N = 20$	3.8–4.5, $N = 21$	7.9–8.6, $N = 21$
♀♀	12.1 ± 0.34	11.7 ± 0.28	7.8 ± 0.0	3.4 ± 0.16	6.9 ± 0.12	4.3 ± 0.22	8.5 ± 0.24
	11.8–12.9, $N = 12$	11.3–12.4, $N = 12$	7.7–7.8, $N = 3$	3.1–3.6, $N = 13$	6.8–7.2, $N = 13$	3.8–4.5, $N = 12$	8.1–8.9, $N = 12$
	n.s.	$t = 2.34$, $P = 0.02$	n.s.	n.s.	n.s.	n.s	$t = 2.64$, $P = 0.01$
'*Vesperus humbloti*'							
MNHN 1986.1074 ♀	12.1	11.5	–	3.3	6.6	4.4	8.1
MNHN 1986.1075 ♂	12.4	12.0	7.9	3.3	6.8	4.2	8.5
P. raceyi	12.0 ± 0.33	11.6 ± 0.33	8.1 ± 0.27	3.6 ± 0.24	7.1 ± 0.19	3.8 ± 0.16	8.2 ± 0.32
	11.6–12.6, $N = 16$	11.2–12.3, $N = 16$	7.7–8.6, $N = 13$	3.2–3.9, $N = 17$	6.8–7.5, $N = 17$	3.4–4.1, $N = 17$	7.6–8.6, $N = 17$
Holotype							
FMNH 185567 ♀	12.3	12.0	8.0	3.7	7.1	4.0	8.6
West	11.8 ± 0.19	11.4 ± 0.17	7.9 ± 0.16	3.4 ± 0.17	7.0 ± 0.12	3.7 ± 0.15	7.9 ± 0.18
	11.6–12.1, $N = 9$	11.2–11.7, $N = 9$	7.7–8.2, $N = 7$	3.2–3.7, $N = 9$	6.8–7.2, $N = 9$	3.4–3.9, $N = 9$	7.6–8.1, $N = 9$
East	12.3 ± 0.22	11.9 ± 0.23	8.3 ± 0.20	3.8 ± 0.12	7.3 ± 0.12	3.9 ± 0.12	8.5 ± 0.11
	12.0–12.6, $N = 7$	11.7–12.3, $N = 7$	8.0–8.6, $N = 6$	3.6–3.9, $N = 8$	7.1–7.5, $N = 8$	3.7–4.1, $N = 8$	8.3–8.6, $N = 8$
T-tests	$t = 5.03$	$t = 5.20$	$t = 4.18$	$t = 5.39$	$t = 5.10$	$t = 2.90$	$t = 7.30$
	$P < 0.001$	$P < 0.001$	$P = 0.002$	$P < 0.001$	$P < 0.001$	$P = 0.01$	$P < 0.001$
H. bemainty	12.6 ± 0.21	12.1 ± 0.26	8.2 ± 0.22	3.5 ± 0.16	7.2 ± 0.15	4.4 ± 0.17	8.5 ± 0.18
	12.3–12.9, $N = 12$	11.8–12.4, $N = 12$	7.9–8.5, $N = 5$	3.2–3.7, $N = 12$	6.9–7.4, $N = 11$	3.9–4.6, $N = 12$	8.2–8.9, $N = 12$
Holotype							
FMNH 217884 ♂	12.4	12.0	8.2	3.5	7.3	4.3	8.7
H. anchietae							
Syntype							
BMNH 6.1.3.1 ♀	12.2	12.0	–	3.4	6.7	4.1	–
Paratype							
MNHN 1900.538 ♀	–	–	–	3.5	7.2	4.2	–

Table 4. Dental measurements (in millimetres) of adult vesper species of the genera *Hypsugo*, *Neoromicia*, and *Pipistrellus* identified from Madagascar and relevant type specimens with the holotype of *Hypsugo bemainty* sp. nov. having been sequenced. Measurements presented as mean ± standard deviation, minimum and maximum measurements, and number of specimens. See Methods for an explanation of variable acronyms. Statistical differences between the sexes within species with sufficient sample sizes are examined based on Student's *t*-tests, n.s. = not significant

	I-M^3	C-M^3	C-C	M^3-M^3	c-m_3
N. malagasyensis	4.8 ± 0.13	4.2 ± 0.12	3.8 ± 0.13	5.1 ± 0.14	4.6 ± 0.12
	4.7–5.0, $N = 4$	4.1–4.3, $N = 4$	3.6–3.9, $N = 4$	5.0–5.3, $N = 4$	4.5–4.7, $N = 5$
Holotype					
ROM 42713 ♀	4.5	4.4	4.0	5.7	4.7
N. matroka	5.2 ± 0.17	4.5 ± 0.17	4.1 ± 0.17	5.6 ± 0.20	4.8 ± 0.18
	5.0–5.4, $N = 12$	4.2–4.8, $N = 12$	3.9–4.4, $N = 12$	5.3–5.9, $N = 12$	4.5–5.2, $N = 12$
Holotype					
BMNH 97.9.1.32 ♂	5.3	4.6	4.0	5.6	4.8
N. robertsi	6.2	5.4	5.0	6.5	5.7
Holotype					
UADBA 43677 ♂	6.0	4.9	4.6	6.2	5.6
P. hesperidus (sexes combined)	4.9 ± 0.14	4.3 ± 0.11	3.7 ± 0.13	5.2 ± 0.15	4.6 ± 0.13
	4.7–5.2, $N = 32$	4.1–4.5, $N = 33$	3.5–4.1, $N = 32$	4.8–5.5, $N = 33$	4.2–4.8, $N = 33$
♂♂	4.9 ± 0.15	4.3 ± 0.13	3.7 ± 0.09	5.1 ± 0.09	4.6 ± 0.12
	4.6–5.2, $N = 21$	3.9–4.4, $N = 21$	3.5–3.8, $N = 21$	4.8–5.3, $N = 21$	4.2–4.7, $N = 21$
♀♀	5.0 ± 0.14	4.3 ± 0.13	3.8 ± 0.14	5.3 ± 0.14	4.6 ± 0.12
	4.7–5.2, $N = 12$	4.1–4.5, $N = 13$	3.7–4.1, $N = 12$	5.0–5.5, $N = 13$	4.2–4.8, $N = 13$
	n.s.	n.s.	$t = 3.33$, $P = 0.004$	$t = 2.87$, $P = 0.008$	n.s.
'*Vesperus humbloti*'					
MNHN 1986.1074 ♀	4.8	4.1	3.6	5.1	4.6
MNHN 1986.1075 ♂	5.1	4.3	4.0	5.4	4.8
P. raceyi	4.9 ± 0.17	4.2 ± 0.18	3.9 ± 0.17	5.3 ± 0.25	4.5 ± 0.18
	4.7–5.2, $N = 16$	3.8–4.5, $N = 16$	3.6–4.2, $N = 17$	4.9–5.7, $N = 17$	4.2–4.7, $N = 17$
Holotype					
FMNH 185567 ♀	5.1	4.4	4.0	5.3	4.5
West	4.8 ± 0.12	4.1 ± 0.17	3.8 ± 0.10	5.1 ± 0.17	4.3 ± 0.09
	4.7–5.0, $N = 9$	3.8–4.3, $N = 9$	3.6–3.9, $N = 9$	4.9–5.5, $N = 9$	4.2–4.5, $N = 9$
East	5.1 ± 0.10	4.3 ± 0.13	4.0 ± 0.14	5.4 ± 0.25	4.6 ± 0.09
	4.9–5.2, $N = 7$	4.1–4.5, $N = 7$	3.8–4.2, $N = 8$	4.9–5.7, $N = 8$	4.5–4.7, $N = 8$
T-tests	$t = 4.49$	$t = 2.73$	$t = 3.82$	$t = 2.62$	$t = 7.11$
	$P < 0.001$	$P = 0.02$	$P = 0.002$	$P = 0.02$	$P < 0.001$
H. bemainty	5.1 ± 0.15	4.4 ± 0.19	3.8 ± 0.17	5.2 ± 0.22	4.6 ± 0.16
	4.7–5.3, $N = 12$	4.2–4.8, $N = 12$	3.5–4.1, $N = 12$	4.7–5.5, $N = 12$	4.3–4.8, $N = 12$
Holotype					
FMNH 217884 ♂	5.1	4.4	3.9	5.4	4.8
H. anchietae					
Syntype					
BMNH 6.1.3.1 ♀	5.0	4.3	3.7	5.4	4.7
Paratype					
MNHN 1900.538 ♀	5.3	4.6	3.9	5.3	4.8

Table 5. Principal components loadings, eigenvalues, and cumulative variance for 11 craniodental variables. The measurement ZYGO was removed to augment the sample size

Parameter	PC1	PC2	PC3
GSKL	−0.32	−0.17	0.31
CBL	−0.33	−0.07	0.25
POB	−0.20	0.73	−0.13
MAST	−0.31	0.24	0.43
PAL	−0.27	−0.42	0.24
MAND	−0.33	−0.10	0.06
I-M^3	−0.31	−0.01	−0.45
C-M^3	−0.31	0.18	0.53
C-C	−0.31	0.23	0.13
M^3-M^3	0.30	0.22	−0.11
c-m_3	−0.30	−0.24	−0.26
Eigenvalue	8.20	1.03	0.46
Proportion of variance	0.75	0.09	0.04
Cumulative variance (%)	74.6	83.9	88.1

Madagascar, which includes a new taxon to science provisionally placed in the genus *Hypsugo* and described below.

Neoromicia malagasyensis (Peterson *et al.*, 1995)

Molecular genetics

K2P distance within this lineage was 0.003 ($N = 3$, Table 1). Sister species to *N. robertsi* and these two taxa show 4.5% sequence divergence from one another. This clade is distinct from an apparently undescribed African species previously referred to as *N.* cf. *melckorum* (Monadjem *et al.*, 2010), from which it is separated by about 12.0% sequence divergence.

Morphometrics

Measurements presented in Table 2.

Craniodental morphology

The different species of *Neoromicia* identified from Madagascar can be differentiated from those of the genera *Pipistrellus* and *Hypsugo* based on the absence of a first upper premolar (P^3) in the former. *Neoromicia robertsi* is distinctly larger that the other two members of this genus occurring on the island with a GSKL of ≥14.3 mm, while that of *N. malagasyensis* falls between 12.4–12.6 mm and *N. matroka* between 12.4–13.4 mm (Table 3). Bates *et al.* (2006) provided a number of characters to differentiate between small species of Malagasy *Neoromicia*, including the width across the M^3–M^3 of <5.4 mm falling within the range of *N. malagasyensis* and ≥5.4 mm within that of *N. matroka*. With an increased number of samples, there is a slight overlap in the M^3–M^3 measurement between these species (Table 4). These two taxa occur in near sympatry in the Isalo region (Fig. 1, see below 'Known geographical range').

Bioacoustics

Measurements presented in Table 6.

Bacular morphology

The bacula of two males obtained from near the Parc National de l'Isalo had total lengths of 2.10 and 2.25 mm. As described by Bates *et al.* (2006), based on a single example, the distal end of the bone is flattened and deflected ventrally, similar to *N. matroka* (Fig. 7A, B). However, in *N. malagasyensis* the surface area and the lateral flanges of the ventral projection are reduced and less curved (more rectilinear) in outline (Fig. 7C). The general baculum shape in *N. malagasyensis* shows some similarities to its sister species, *N. robertsi* (Fig. 7D; Goodman *et al.*, 2012a), but its form appears intermediate between *N. matroka* and *N. robertsi*. The baculum previously assigned to *N.* cf. *malagasyensis* (FMNH 213576) in Goodman *et al.* (2012a), from specimen sequenced in the current study, is referable to *Hypsugo bemainty* sp. nov. (see below). Further, the baculum of *N.* cf. *melckorum*, which is the next group out from the *malagasyensis-robertsi* clade, is morphologically similar to this species complex (Fig. 7G).

Known geographical range

In Figure 1, localities are presented of sequenced specimens of *N. malagasyensis*. One other specimen identified as this taxon by Bates *et al.* (2006) using baculum morphology includes: Analamangabe, Parc National de l'Isalo, 22°29.13′S, 45°23.04′E (UADBA 43680, RBJ-130).

Neoromicia matroka (Thomas & Schwann, 1905)

Molecular genetics

Specimens assigned to *N. matroka* and collected across the geographical range of this species (Goodman & Ramasindrazana, 2013), form a monophyletic lineage (Fig. 2) and display little in the way of genetic variation. K2P distance within this lineage was 0.008 ($N = 11$, Table 1). This species forms the sister group to sub-Saharan *N. capensis* and these two clades are separated by 4.5% sequence divergence. *Neoromicia matroka* was formerly considered a subspecies of *N. capensis* (e.g. Koopman, 1994), but based on a variety of characters outlined herein, it warrants specific recognition and placement in the genus *Neoromicia*. In turn, two specimens from Botswana identified as *N.* cf. *melckorum* (TM 48485 and TM 48487) (see Monadjem *et al.*, 2010 for a definition of *N.* cf. *melckorum*), are sister to the *matroka-capensis* clade and separated by about 12% sequence divergence.

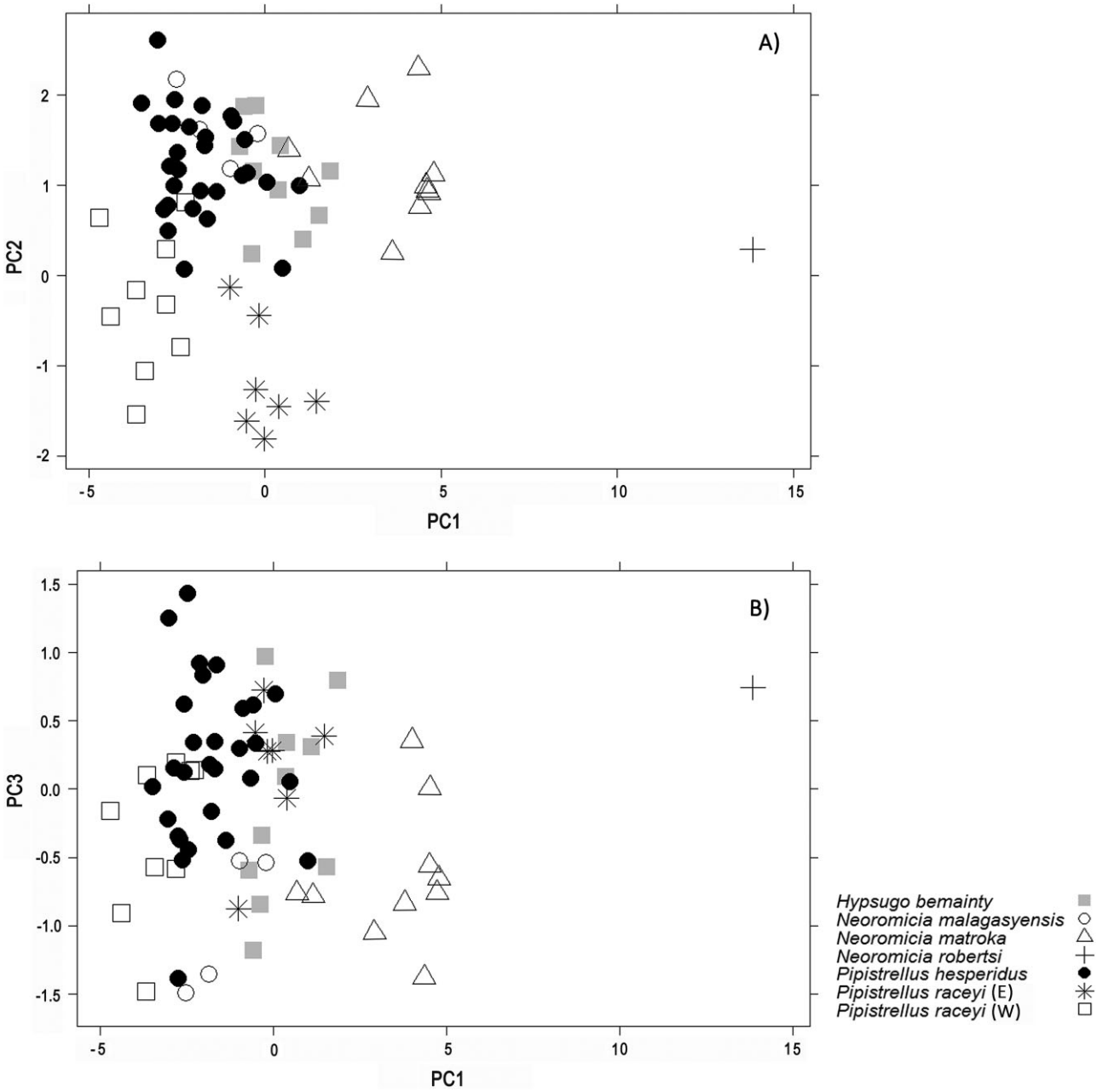

Figure 4. Principal Component Analysis plots of 11 craniodental measurements for genotyped Malagasy vesper specimens for A, PC1 vs. PC2, and B, PC1 vs. PC3. Information on component loadings is presented in Table 5.

Morphometrics
Measurements presented in Table 2.

Craniodental morphology
Neoromicia malagasyensis and *N. matroka* show little overlap in craniodental measurements and these two species are readily distinguished from *N. robertsi* (see Tables 3 and 4).

Bioacoustics
Measurements presented in Table 6.

Bacular morphology
As described by Bates *et al*. (2006), the baculum in *N. matroka* is moderate in length and averages 2.26 mm (2.12–2.49 mm, $N = 6$, see Table 8), the distal end is flattened and deflected ventrally, the dorsal surface has

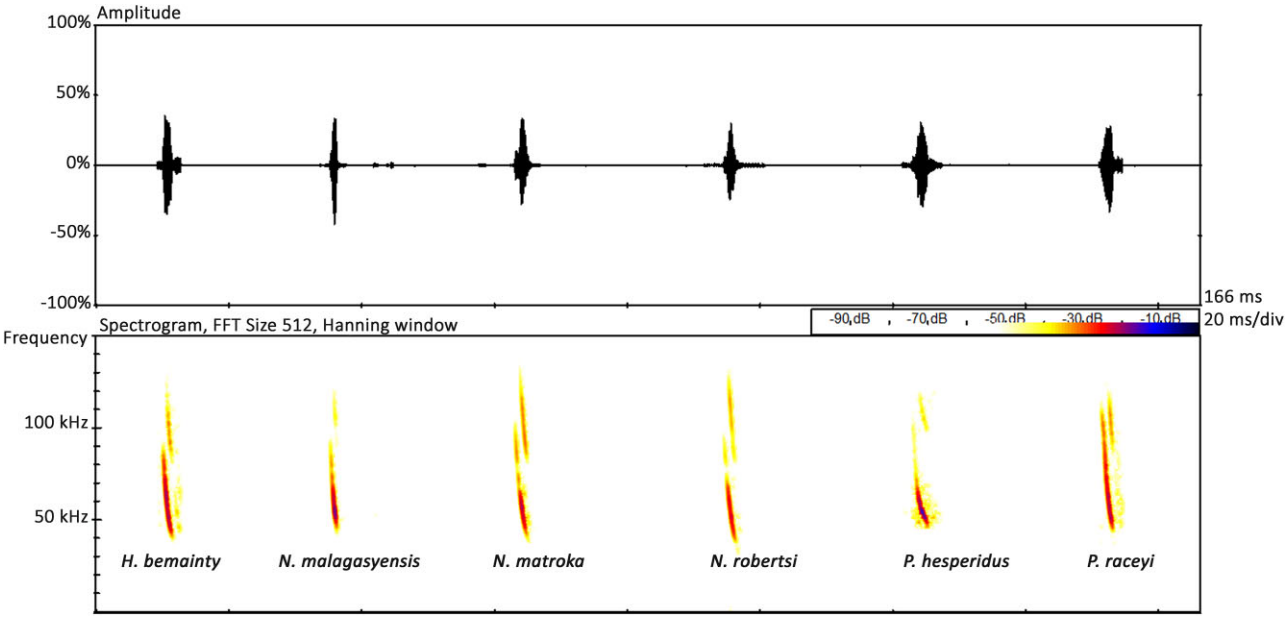

Figure 5. Echolocation calls of the holotype of *Hypsugo bemainty* sp. nov. (FMNH 217884), *Neoromicia malagasyensis* (FMNH 222724), *Neoromicia matroka* (FMNH 222727), *Neoromicia robertsi* (FMNH 222729), *Pipistrellus hesperidus* (FMNH 209270), and *Pipistrellus raceyi* (FMNH 217886).

a vertical projection, and there is some intraspecific variation in the development of lateral flanges (Fig. 7A, B). The general shape of the baculum in *N. matroka* is similar to *N. capensis* (Fig. 7E; Kearney *et al.*, 2002); the two taxa form sister species. The total length in *N. capensis* is slightly shorter, averaging 2.04 mm (1.59–2.50 mm, $N = 37$) (T. Kearney, unpublished data).

Known geographical range

In Figure 1, localities are presented of sequenced specimens of *N. matroka*. Additional specimens presented in Bates *et al.* (2006) and Goodman *et al.* (2012a) using other molecular results or bacula morphology include: Manambolo, Ambavafatra, 47°1′25″S, 22°8′58″E (FMNH 167660); Parc National de Mantadia, 18°49.94′S, 48°25.63′E (UADBA 43674); and Ambohipo, Antananarivo, 18°55.60′S, 47°33.80′E (UADBA 43675). On the basis of an unpublished study by Malalatiana Michèle Ratsimbazafy and Alexandre Hassanin of the Muséum National d'Histoire Naturelle de Paris, there is molecular evidence of this species in the Kianjavato area, 21.3818°S, 47.8928°E, 90 m asl (Goodman *et al.*, 2014) and females placed in OTU 3 of '*N. matroka*' in Goodman *et al.* (2014, Fig. 7) are actually *P. raceyi*.

Neoromicia robertsi Goodman *et al.*, 2012a

Molecular genetics

K2P distance within *N. robertsi* was 0.001 ($N = 2$, Table 1). This species forms the sister taxa of *N. malagasyensis* and these two allopatric forms show 4.0% sequence divergence. Further, this clade is distinct from *N.* cf. *melckorum* (see Monadjem *et al.*, 2010 for a definition of this taxon), from which it is separated by about 12.0% sequence divergence.

Morphometrics

Measurements presented in Table 2.

Craniodental morphology

This species is readily separated from other members of this genus occurring on Madagascar by its notably large skull and dental measurements. With the exception of POB, there is no overlap in any craniodental measurement of this species with the two smaller members of this genus on Madagascar (*N. malagasyensis* and *N. matroka*) (Tables 3 and 4). It was previously noted that the skull of UADBA 43679 was lost (Goodman *et al.*, 2012a); this is incorrect and the misplaced skull is of UADBA 43678.

Bioacoustics

Measurements presented in Table 6.

Bacular morphology

As illustrated and described by Goodman *et al.* (2012a), the baculum of *N. robertsi* is notably long for members of this genus (2.8–3.1 mm); here we add one more specimen referable to this species with a baculum length of 3.12 mm (Table 8). The distal end has a hook-like structure that is flattened and deflected ventrally, and

Table 6. Descriptive statistics of echolocation calls parameters in Malagasy vesper bats of the genera *Hypsugo*, *Neoromicia*, and *Pipistrellus* represented in the molecular genetic portion of this study (see Methods section for the definition of the variables). For species represented by ≥ 3 specimens, data are expressed as mean ± SD (standard deviation), minimum–maximum, *N*: number of individuals. For *Neoromicia robertsi*, only recordings for a single male were available

Species	Sex	PF (kHz)	Fmax (kHz)	Fmin (kHz)	Dur (ms)	IPI (ms)
N. malagasyensis	♂	57.9	78.5	46.0	1.6	30
		N = 1	*N* = 1	*N* = 1	*N* = 1	*N* = 1
	♀	57.6	70.8	43.7	1.4	18.7
		N = 1	*N* = 1	*N* = 1	*N* = 1	*N* = 1
	♂ + ♀	57.7	74.6	44.8	1.5	24.3
		57.6, 57.9	70.8, 78.5	43.7, 46.0	1.4, 1.6	18.7, 30.0
		N = 2	*N* = 2	*N* = 2	*N* = 2	*N* = 2
N. matroka	♂♂	57.5 ± 0.25	80.8 ± 8.67	43.8 ± 3.67	2.0 ± 0.15	43.5 ± 23.40
		57.3–57.8	74.3–90.6	39.6–46.5	1.9–2.2	19.9–66.7
		N = 3	*N* = 3	*N* = 3	*N* = 3	*N* = 3
	♀	54.8	115.5	43.8	2.0	25.4
		N = 1	*N* = 1	*N* = 1	*N* = 1	*N* = 1
	♂♂ + ♀	56.8 ± 1.38	89.4 ± 18.73	43.8 ± 2.99	2.0 ± 0.12	38.9 ± 21.13
		54.8–57.8	74.3–115.5	39.6–46.5	1.9–2.2	19.9–66.7
		N = 4	*N* = 4	*N* = 4	*N* = 4	*N* = 4
N. robertsi	♂	53.0	75.4	43.3	2.7	51.2
		N = 1	*N* = 1	*N* = 1	*N* = 1	*N* = 1
P. hesperidus	♂♂	54.0 ± 0.93	97.4 ± 19.79	48.8 ± 1.99	2.7 ± 0.42	49.9 ± 16.17
		52.2–54.8	67.5–115.1	44.9–55.7	2.1–3.2	17.5–65.5
		N = 7	*N* = 7	*N* = 7	*N* = 7	*N* = 7
	♀♀	52.5 ± 1.28	88.5 ± 16.90	46.2 ± 2.64	2.9 ± 0.65	61.5 ± 23.09
		50.4–54.6	71.0–112.6	40.8–49.1	2.0–4.0	22.6–94.3
		N = 7	*N* = 7	*N* = 7	*N* = 7	*N* = 7
	♂♂ + ♀♀	53.3 ± 1.32	92.9 ± 18.27	47.5 ± 2.61	2.8 ± 0.54	55.7 ± 2.26
		50.4–54.8	67.5–115.1	40.8–50.7	2.0–4.0	53.4–58.2
		N = 14	*N* = 14	*N* = 14	*N* = 14	*N* = 14
P. raceyi	♂♂	56.1 ± 2.47	122.5 ± 6.36	44.23 ± 2.27	2.7 ± 0.38	48.1 ± 29.22
		53.4–58.2	115.7–128.3	41.7–46.1	2.4–3.1	14.8, 69.5
		N = 3	*N* = 3	*N* = 3	*N* = 3	*N* = 3
	♀	58.2	91.7	51.4	2.3	125.0
		N = 1	*N* = 1	*N* = 1	*N* = 1	*N* = 1
	♂♂ + ♀	56.6 ± 2.26	114.8 ± 16.25	46.0 ± 4.04	2.6 ± 0.36	67.3 ± 45.25
		53.4–58.2	91.7–128.3	41.7–51.4	2.3–3.1	14.8–125.0
		N = 4	*N* = 4	*N* = 4	*N* = 4	*N* = 4
H. bemainty	♂♂	50.2 ± 0.75	90.3 ± 17.93	42.7 ± 2.83	2.4 ± 0.97	49.3 ± 17.97
		49.3–51.2	69.0–109.3	38.0–46.8	1.9–4.6	19.8–70.2
		N = 7	*N* = 7	*N* = 7	*N* = 7	*N* = 7
	♀♀	50.3	95.6	44	2.1	52.6
		50.2, 50.4	70.6, 120.6	43.8, 44.2	2.1, 2.1	47.1, 58.1
		N = 2	*N* = 2	*N* = 2	*N* = 2	*N* = 2
	♂♂ + ♀♀	50.2 ± 0.66	91.5 ± 20.07	43.0 ± 2.52	2.4 ± 0.85	50.1 ± 15.87
		49.3–51.2	69.0–120.6	38.0–46.8	1.9–4.6	19.8–70.2
		N = 9	*N* = 9	*N* = 9	*N* = 9	*N* = 9

the dorsal surface has a vertical projection (Fig. 7D). The general shape of the baculum shows similarities to *N. malagasyensis*, which is the sister species (Fig. 7C), but the latter is shorter in total length (2.10, 2.25, *N* = 2, see Table 8). In many ways, the structure is similar to *N.* cf. *melckorum* (Fig. 7G). The baculum illustrated by Bates *et al*. (2006, Fig. 8C) under '*N. melckorum*' is of *N. robertsi*.

Table 7. Principal components loadings, eigenvalues, and cumulative variance for five bioacoustic variables

Parameters	PC1	PC2	PC3
PF	0.06	−0.75	−0.30
Fmax	−0.09	−0.35	0.93
Fmin	0.52	−0.40	−0.10
Dur	0.58	0.40	0.14
IPI	0.62	−0.01	0.12
Eigenvalue	1.70	1.34	0.95
Proportion of variance	0.34	0.27	0.19
Cumulative variance (%)	34.0	60.7	79.7

Known geographical range
In Figure 1, localities are presented of sequenced specimens of *N. robertsi*. Other animals identified by molecular results or bacula morphology include: Amboasary, Anjozorobe, 47°56.699′S, 18°24.295′E (UADBA 43677 – holotype); Antsahabe, Anjozorobe, 18°24.26′S, 47°56.70′E (UADBA 43675, RBJ-105); and Parc National de Mantadia, 18°49.94′S, 48°25.63′E (FMNH 213931, formerly UADBA 43679); the latter two specimens were originally identified as *N. melckorum* by Bates *et al*. (2006).

PIPISTRELLUS HESPERIDUS (TEMMINCK, 1840)

Molecular genetics
K2P distance within this lineage for animals obtained in dry forest habitats of western Madagascar was 0.001 (N = 14, Table 1); hence, these populations show little genetic variation at the nucleotide level. No haplotype was shared in common between sequenced samples of *P. hesperidus* from South Africa (TM 47666) and Swaziland (TM 47738), as compared to those from Madagascar (N = 28, Table 9). These lineages exhibited a divergence of 1.89% (K2P), reflecting 22 fixed mutations in the trimmed sequence. Haplotype diversity was reasonably high in the Malagasy population (Hd 0.744), in spite of a lower nucleotide diversity (Pi 0.00181), but these indices were not calculated for the African population due to limited sample size.

Morphometrics
Measurements presented in Table 2.

Craniodental morphology
Members of this genus are easily differentiated from known Malagasy members of the genus *Neoromicia* by the presence of five post-canine teeth as compared with four in the latter genus, which lacks a first upper premolar (P^3). Bates *et al*. (2006) noted that the upper canine (C^1) was not in contact with the second upper premolar (P^4). On the basis of specimens sequenced herein, this character holds, although in some cases these two teeth are almost in direct contact.

Bioacoustics
Measurements presented in Table 6.

Bacular morphology
As described by Bates *et al*. (2006) for Malagasy specimens of *P. hesperidus*, the baculum is relatively short (1.7–1.8 mm); here we add an additional four specimens that range in total length 1.60–2.10 mm (Table 8). The distal tip and proximal base show a bifid indentation, and the shaft is distinctly recurved (Fig. 8C). This is the same configuration found in African *P. hesperidus* (Fig. 8D; Kearney *et al*., 2002), which ranges in total length from 1.7 to 2.5 mm (T. Kearney, unpublished data, N = 23).

Known geographical range
In Figure 1, localities are presented of sequenced specimens of *P. hesperidus*, which also include the sites mentioned by Bates *et al*. (2006) for verified records of this species on Madagascar.

Taxonomic comments
Different lines of evidence, including karyological distinctions (e.g. Volleth *et al*., 2001), have been published indicating that *P. hesperidus* is most likely paraphyletic and best considered a species complex. However, because of a lack of resolution in character differences of holotypes representing different proposed names currently considered synonyms of *P. hesperidus*, Simmons (2005) recognised several subspecies, which include *P. h. fuscatus* Thomas, 1901 from much of the Afrotropics and *P. h. subtilis* Sundevall, 1846 from southern Africa and Madagascar. Greater genetic sampling is needed from southern Africa to resolve the differences found in the current study between this portion of the continent and Madagascar, but given the subtle genetic differences and seemingly continuous character states associated with bacular morphology, we maintain Malagasy populations as *P. hesperidus*.

Milne-Edwards (1881) described a small species of vespertilionid from Madagascar under the name *Vespertilio humbloti*. Peterson *et al*. (1995) mentioned that the type series was apparently lost and the status of this taxon could not be assessed. In the MNHN, the syntype series associated with Milne-Edwards' description of *V. humbloti* was located (MNHN 1986.1074 to 1986.1082); most of the nine specimens were immature. Through the courtesy of Dr. Cécile Callou of the MNHN, the skulls from two adult specimens were extracted, cleaned, and allocated the numbers MNHN 1986.1074 and 1986.1075. Both of these specimens have a prominent first upper premolar (P^3), typical of the genera *Pipistrellus* and *Hypsugo*. Further, 1986.1074 has a seemingly bicuspid second upper incisor

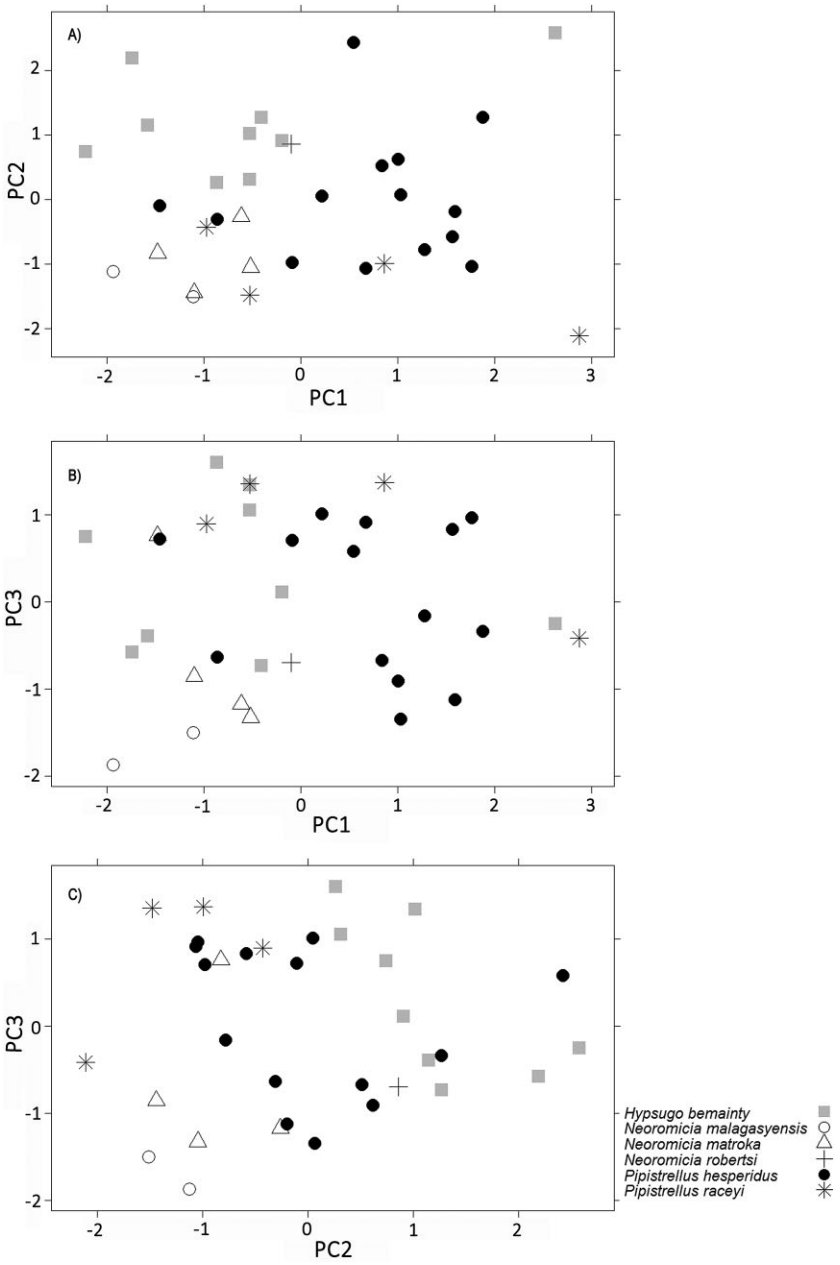

Figure 6. Plot of principal component scores associated with the five bioacoustic variables for genotyped Malagasy vesper specimens on A, PC1 vs. PC2, B, PC1 vs. PC3 and C) PC2 vs. PC3. Information on component loadings is presented in Table 7.

(I^3) and 1986.1075 an unicuspid I^3 that, in both cases, the longest portion of the I^3 reaches the length of the first upper incisor (I^2) cingulum; a diastema between C and second upper premolar (P^4), a prominent first upper premolar (P^3) in lateral view (similar to the illustration in Bates *et al*., 2006, Fig. 5B); and craniodental measurements falling within the range of *P. hesperidus* (Tables 3 and 4). On the basis of these different features, we identify the Milne-Edwards series of *V. humbloti* as *P. hesperidus*; this awaits confirmation based on bacular structure or molecular genetics. Hence, *V. humbloti* is considered a junior synonym of *P. hesperidus*.

Two unsexed specimens held in the BMNH (28.1.24.1, 28.1.24.2) were collected by Capt. K. Parcon in June 1922 on Europa Island (22.3683°S, 40.3633°E) positioned halfway across the Mozambique Channel between Mozambique and Madagascar. Using the craniodental

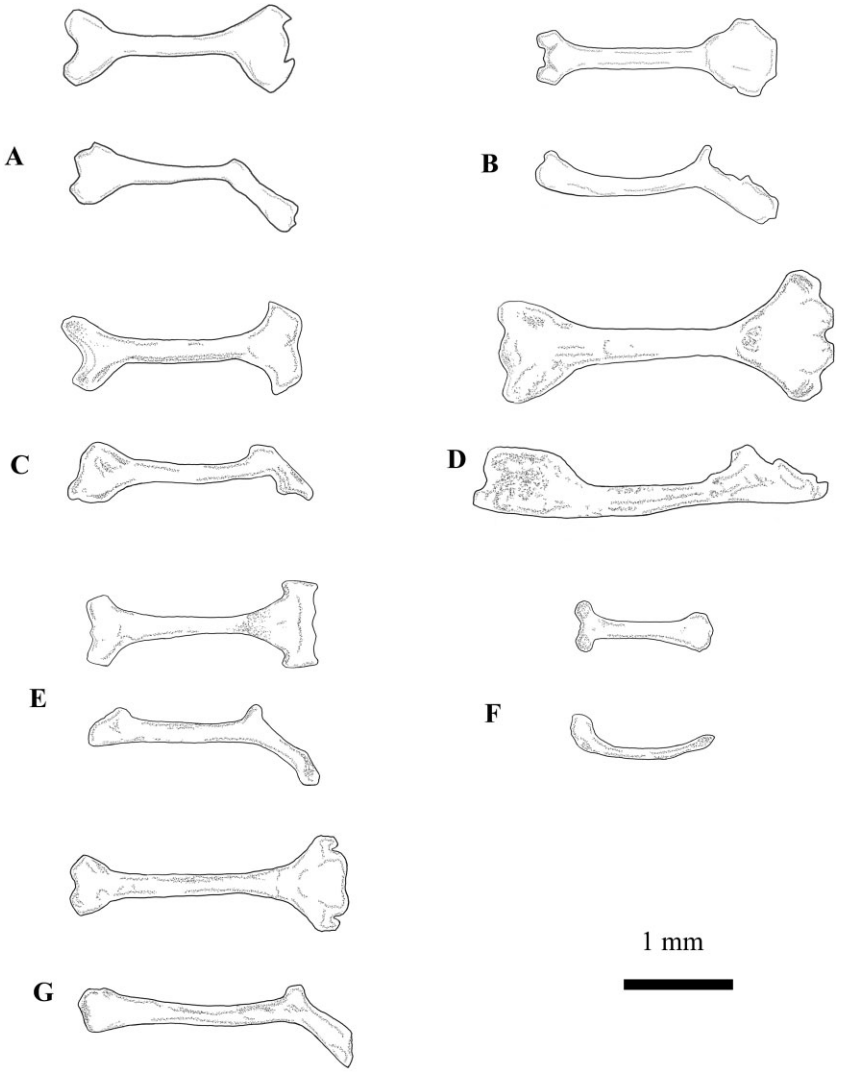

Figure 7. Dorsal (above) and lateral (below) views of bacula from six species of *Neoromicia*. A, *Neoromicia matroka* (FMNH 222725, total length 2.12 mm) from Mantadia; B, *Neoromicia matroka* (FMNH 222728, total length 2.26 mm) from Anjozorobe; C, *Neoromicia malagasyensis* (FMNH 222724, total length 2.25 mm) from Isalo; D, *Neoromicia robertsi* (FMNH 222729, total length 3.12 mm), from Anjozorobe; E, *Neoromicia capensis*, dorsal view (TM 48490, ECJS-112/2009, total length 2.04 mm) and lateral view (TM 48485, ECJS-03/2009, lateral view, total length 2.01 mm) from Botswana; F: *Neoromicia nana* (TM 48573, UP-923, total length 1.32 mm), from South Africa; G, *Neoromicia* cf. *melckorum* (NMZL ECJS-114/2010, total length 2.55 mm) from Zambia.

characters outlined in Bates *et al*. (2006) and Monadjem *et al*. (2010), the two animals are referable to *Pipistrellus*, and one of the specimens in relatively good shape (BMNH 28.1.24.2), based on the form of the first upper premolar (P^3) and the second upper incisor (I^3), as well as cranial shape, is identified as *P. hesperidus* and not *Neoromicia nana*, as written on the specimen label. This identification needs to be verified based on molecular data or potentially bacular morphology. In the same accession of the *Pipistrellus* material is a specimen of *Coleura seychellensis* Peters, 1868 (BMNH 28.1.24.3) obtained by the same captain on 4 October 1924 near Amirante Island, Seychelles (6.000°S, 53.1667°E), indicating that Capt. Parcon navigated boats in the western Indian Ocean. The context that the *Pipistrellus* specimens were obtained is ambiguous and they could have been stowaways on the vessel after docking in a coastal area and then transported to Europa Island; the direction of travel from Africa towards Madagascar or vice versa is unknown. However, if they represent a natural occurrence on Europa Island, this would provide evidence of movements for this species spanning at least half the distance between southern Africa and Madagascar.

PIPISTRELLUS RACEYI BATES *ET AL*., 2006

Molecular genetics

This endemic species is known from two distinct portions of Madagascar. The samples used in the intraspecific genetic analysis include ten animals from dry deciduous western forests and nine specimens from humid eastern area. The K2P distance within *P. raceyi* when these lineages were pooled was 0.004 ($N = 7$, Table 1). No haplotypes were shared between the eastern and western populations ($N = 17$, Table 9). These populations exhibited a divergence of 0.74% (K2P), reflecting five fixed mutations in the trimmed cytochrome *b* sequences (680 bp). When the full sequence set of *P. raceyi* was examined ($N = 17$, Table 9), diversity was reasonably high in the western population (Hd 0.722, Pi 0.00287), but was noticeably low in the eastern population (Hd 0.429, Pi 0.00057), which was only represented by two different haplotypes and distinguished by a single base change.

This species is notably divergent from the other Vespertilioninae samples included in the analysis, specifically members of the genus *Pipistrellus*, and without more extensive taxonomic and geographic sampling, little can be advanced concerning possible sister taxa or the geographical region the ancestral form that colonized Madagascar was from. Bates *et al*. (2006) suggested, based on baculum morphology, that *P. raceyi* might be closely related to Asiatic *P. endoi* Imaizumi, 1959. This interesting hypothesis warrants further molecular investigation.

Table 8. Descriptive statistics of baculum total length (TL) measurements in Malagasy vesper bats of the genera *Hypsugo*, *Neoromicia*, and *Pipistrellus*, with most individuals represented in the molecular genetic portion of this study. For species represented by ≥ 3 individuals, data are expressed as mean ± SD (standard deviation), minimum–maximum, N = number of individuals.

Species	TL
N. malagasyensis	2.10, 2.25, $N = 2$
N. matroka	2.26 ± 0.128
	2.12–2.49, $N = 6$
N. robertsi	3.12, $N = 1$
P. hesperidus	1.73 ± 0.153
	1.60–2.10, $N = 4$
P. raceyi (east)	6.10, 10.00, $N = 2$
P. raceyi (west)	8.90, $N = 1$
H. bemainty	1.70 ± 0.097
	1.50–1.79, $N = 7$

Morphometrics

Measurements presented in Table 2. Animals from eastern populations are in general larger than those from western populations.

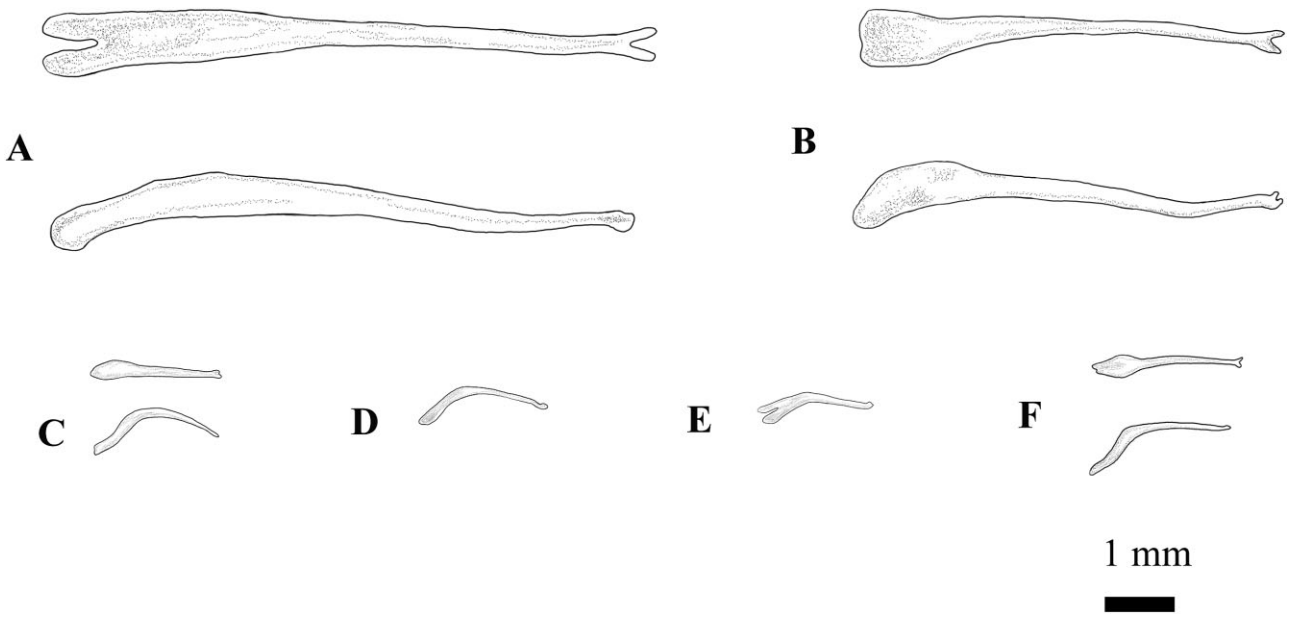

Figure 8. Dorsal (above) and lateral (below) views of bacula from two species of *Pipistrellus*. A, *Pipistrellus raceyi* (FMNH 217886, total length 8.90 mm) from Kirindy Forest (CNFEREF); B, *Pipistrellus raceyi* (FMNH 222722, total length 6.10 mm) from near Andasibe; C, *Pipistrellus hesperidus* (FMNH 176094, average total length of four specimens 1.9 mm) from Parc National de Kirindy Mitea (redrawn from Bates *et al*., 2006, Fig. 10A); D, *Pipistrellus hesperidus* (FMNH 209270, total length 1.90 mm) from Grotte de Sarodrano; E, *Pipistrellus hesperidus* (FMNH 217905, total length 1.70 mm) from Grotte de Sarodrano; F: *Pipistrellus hesperidus* (TM 48624, UP-840, total length 2.18 mm) from South Africa.

Table 9. Intraspecific diversity and divergence for the three most intensively sampled taxa in the genera *Hypsugo* and *Pipistrellus*. Numbers in parentheses indicate standard deviation (SD) for estimates of haplotype diversity (Hd) and nucleotide diversity (Pi). There were no shared haplotypes between the east and west populations of *Pipistrellus raceyi* (five fixed mutations between lineages) or between the African and Malagasy populations of *Pipistrellus hesperidus* (22 fixed mutations between lineages)

Species	Population	Hd (SD)	Pi (SD)	% divergence (K2P)
H. bemainty	n/a* (N = 13)	0.962 (0.050)	0.00519 (0.00072)	n/a
P. raceyi	East (N = 8)	0.429 (0.028)	0.00057 (0.00022)	0.74%
	West (N = 9)	0.722 (0.159)	0.00287 (0.00439)	
P. hesperidus	Madagascar (N = 28)	0.744 (0.078)	0.00181 (0.00035)	1.89%
	Africa†	n/a	n/a	

**Hypsugo bemainty* was only sampled as a single population.
†Only two samples were available from the African population of *Pipistrellus hesperidus* and intraspecific diversity could not be calculated.

Craniodental morphology

As mentioned under this header for *P. hesperidus*, members of this genus occurring on Madagascar are easily differentiated from *Neoromicia* based on upper toothrow counts. Further, Bates *et al*. (2006) have provided a few characters to differentiate *P. hesperidus* from *P. raceyi*, the other member of the genus occurring on Madagascar: (1) the second upper incisor (I^3) ≤½ the length of the first upper incisor (I^2) (in *hesperidus*) vs. I^3 nearly the length of I^2 (in *raceyi*); (2) I^2 is unicuspid (in *hesperidus*) vs. bicuspid (in *raceyi*); and (3) that the first upper premolar (P^3) is notably small (in *hesperidus*) as compared to large and prominent (in *raceyi*). A reevaluation of these characters based on the specimens of *P. hesperidus* sequenced in this study (N = 13) found: characters 1 – consistent in all cases, character 2 – almost uniformly consistent, with the exception of MNHN 1986.1074 with a seemingly bicuspid I^2, character 3 – consistent in all cases, with some variation in size of the P^3, which was always smaller than in *P. raceyi*. Bates *et al*. (2006) also noted that the relative length of I^3 to I^2 between *P. hesperidus* and *Hypsugo anchietae* [= *H. bemainty* sp. nov., see below] were similar and the best means to differentiate these two taxa based on craniodental characters was the former has a distinctly thin zygomata and without a jugal eminence (fig. 11 in Bates *et al*., 2006).

Bioacoustics

Bioacoustics (average values, sexes combined, N = 4, which include from the west 2 ♂♂ and 1 ♀ and from the east 1 ♂): PF: 56.6 (range 53.4–58.2) kHz, Fmax: 114.8 (range 91.7–128.3) kHz, Fmin: 46.0 (range 41.7–51.4) kHz, Dur: 2.6 (range 2.3–3.1) ms, IPI: 67.3 (range 14.8–125.0) ms (Table 6). The single sequenced female with associated bioacoustic recordings had several parameters that fell outside the range for three males (Fig. 6). Further work is needed to verify this pattern.

Bacular morphology

In their description of *P. raceyi*, Bates *et al*. (2006) provided details on its bacular morphology. The length of this structure in *P. raceyi* is distinctly longer than any other Malagasy vesper and measures 8.90 and 10.00 mm (Table 8). It has a long and narrow shaft, with a slight curvature, and variable deflection at the distal end that seems to indicate some intraspecific variation (Fig. 8A, B). A specimen obtained outside of Andasibe (FMNH 222722) had a distinctly shorter baculum (6.10 mm), which may be related to age and lack of ossification of the structure (Table 8, Fig. 8B).

Known geographical range

Localities from which sequenced specimens of this species are known are presented in Figure 1. One specimen from another site referred to this species includes Forêt de Mikea, 22°16.00′S, 43°28.70′E (FMNH 176165) (Bates *et al*., 2006).

Taxonomic comments

Notable genetic differences were found between eastern and western populations of *P. raceyi*, with the type specimen being from the eastern locality of Kianjavato (Fig. 1). Further, statistically significant differences were identified between specimens from these two different portions of the island in several external measurements (Table 2) and in all cranial and dental measurements (Tables 3 and 4). Further work is needed to assess the level of genetic divergence and possible reproductive isolation between eastern and western populations; herein we maintain them as a single taxon, with divergent populations.

HYPSUGO BEMAINTY, SP. NOV. (FIGS 9–12)

syn. *Hypsugo anchietae* Bates *et al*., 2006
syn. *Hypsugo anchietae* Goodman, 2011

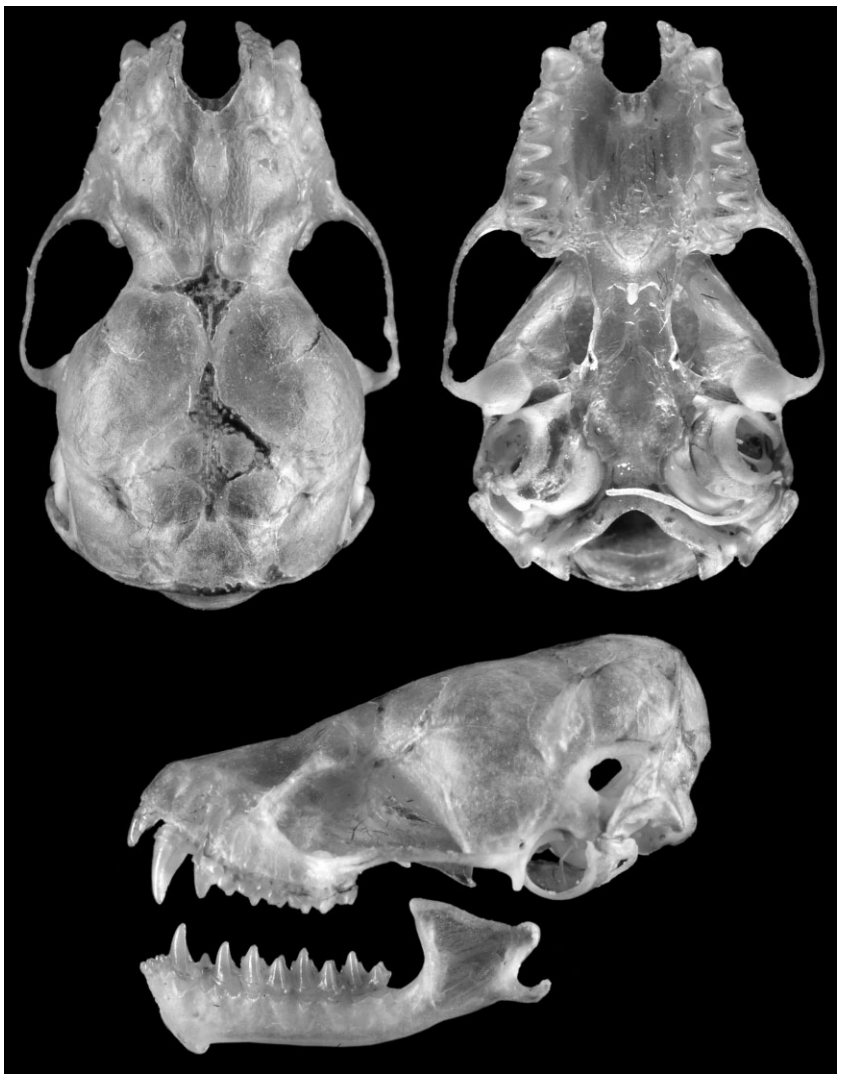

Figure 9. Different views of skull and mandible of *Hypsugo bemainty* sp. nov. (FMNH 217884, SMG 17029), holotype from Province de Toliara, Kirindy (CNFEREF). Pictures include dorsal view of cranium (upper row, left), ventral view of cranium (upper row, right), and lateral view of cranium and mandible (lower row). (Photograph taken by J. Weinstein, Field Museum image number Z95227_d.)

Holotype

Adult male, FMNH 217884 (field number SMG 17029), collected 2 November 2011 by S. M. Goodman & Ara Monadjem. The specimen was conserved in 12% formaldehyde and subsequently transferred to 70% ethanol. Before preservation, the skull was removed via small incisions on both sides of the mouth, conserved in 60% ethanol, and then cleaned by dermestid beetles. Samples of pectoral muscle were collected and saved in lysis buffer. Other information obtained from this individual includes bioacoustic recordings, cleared and stained baculum, and photographs of the living animal. The specimen, with a full adult dentition and the basisphenoid–basioccipital suture completely fused, had scrotal testes measuring 2 × 3 mm and slightly convoluted epididymides. External measurements (in mm) include total length 77, tail length 35, hindfoot length (without claws) 7, ear length 11, tragus length 7, forearm length 31, and body mass 3.9 g (Table 2).

Type locality

Madagascar: Province de Toliara, Kirindy Forest (CNFEREF), 60 km N Morondava, along the Kirindy River, 20°4′29.1″S, 44°40′14.4″E, 45 m asl.

Paratypes

Other information obtained from the associated type series included bioacoustic recordings (br), cleared and stained baculum (bac), and photographs (ph). Madagascar: Province de Toliara, 0.8 km N Kirindy village,

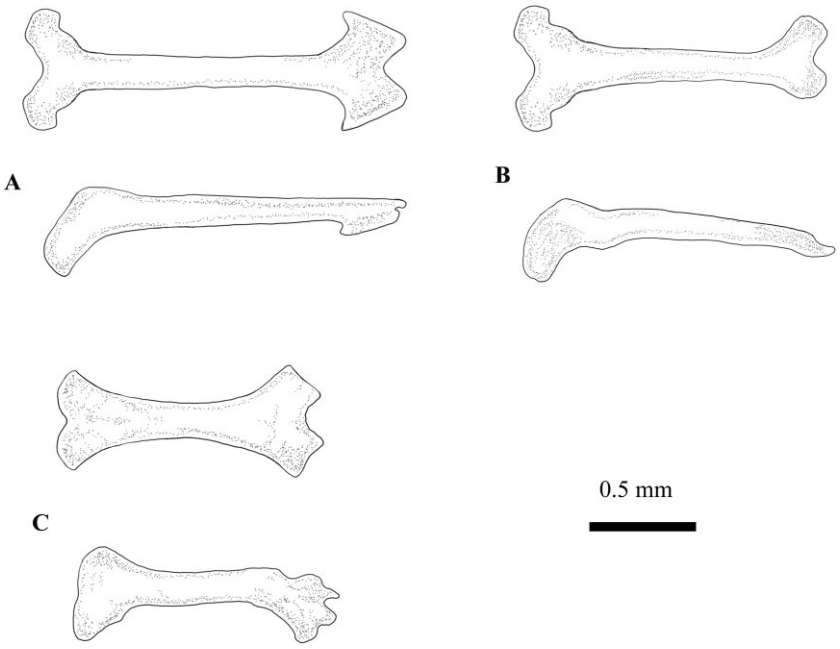

Figure 10. Dorsal (above) and lateral (below) views of bacula from two species of *Hypsugo*. A, *Hypsugo bemainty* sp. nov. (FMNH 217884, holotype, 1.79 mm) from Kirindy (CNFEREF); B, *Hypsugo bemainty* (FMNH 213577, total length 1.50 mm) from Kirindy (CNFEREF); C, *Hypsugo anchietae* (TM 48489, ECJS-120/2009, total length 1.25 mm) from Botswana.

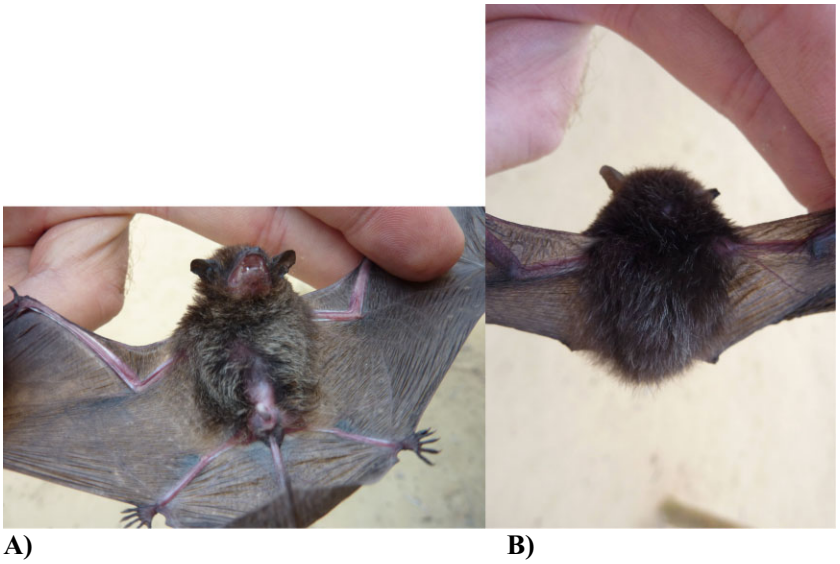

Figure 11. Photographs of *Hypsugo bemainty* sp. nov. holotype (FMNH 217884) from Kirindy (CNFEREF) A, ventral view and B, dorsal view (photographs by Steven M. Goodman). Note multicoloured shaggy fur and dark brown wing and tail membranes.

20.06222°S, 44.60126°E, 50 m asl, FMNH 218131 (br), UADBA SMG-17575, B. Ramasindrazana and S. M. Goodman (fluid preserved with skull removed); Madagascar: Province de Toliara, Kirindy Forest (CNFEREF), along Kirindy River, site 4, 20.07475°S, 44.67069°E, 70 m asl, FMNH 213577 (bac, ph), C. F. Rakotondramanana (fluid preserved with skull removed); Madagascar: Province de Toliara, Kirindy Forest (CNFEREF), along Kirindy River, site 3, 20.07861°S, 44.67469°E, 75 m asl, FMNH 213550 (ph), C. F. Rakotondramanana

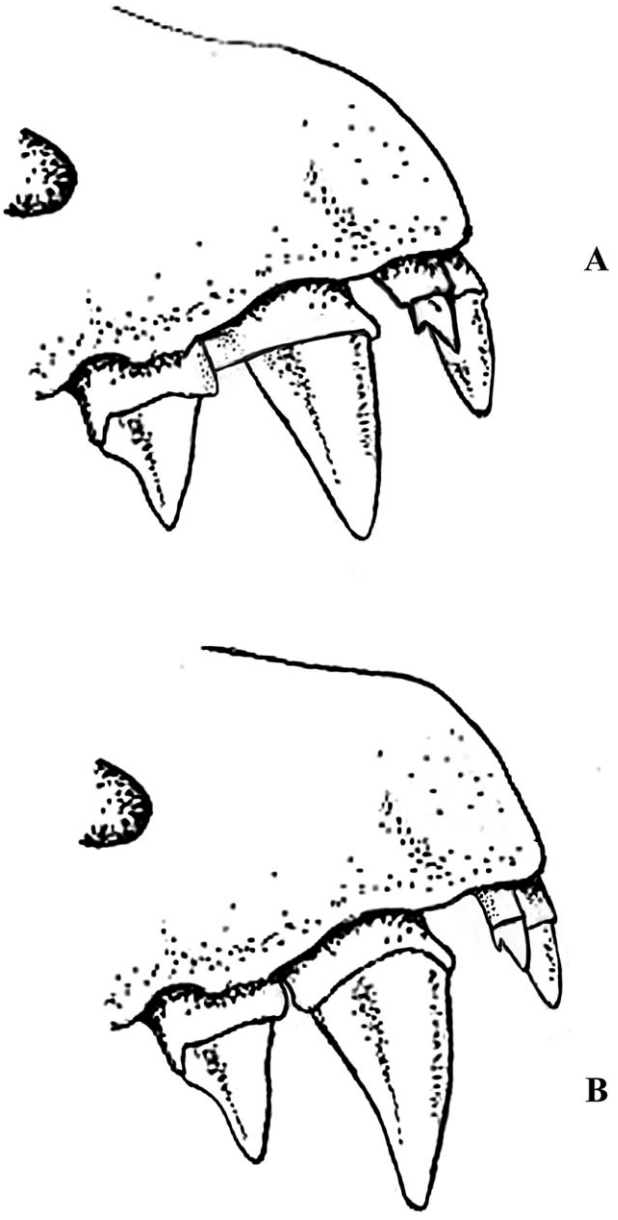

Figure 12. Lateral view of the right anterior portion of skull of two different species of *Hypsugo*: A, *Hypsugo bemainty* sp. nov. (FMNH 217884), holotype, Kirindy (CNFEREF), with relatively unworn teeth; B, *Hypsugo anchietae* (BMNH 6.1.3.1), syntype, Angola. Skull images adapted and modified from Bates *et al*. (2006).

(fluid preserved with skull removed); Madagascar: Province de Toliara, Kirindy Forest (CNFEREF), along Kirindy River, site 1, 20.079°S, 44.7169°E, 50 m asl, FMNH 213576 (bac, ph), C. F. Rakotondramanana (fluid preserved with skull removed); Madagascar: Province de Toliara, Kirindy Forest (CNFEREF), along Kirindy River, 20°4′35.1″S, 44°44′49.4″E, 45–55 m asl, FMNH 217882 (ph), 217888 (ph), S. M. Goodman, FMNH 218147 (br, ph), FMNH 218154 (br, bac, ph), FMNH 218155 (br, bac, ph), FMNH 218156 (br, bac, ph), FMNH 218157 (br, bac, ph), UADBA AM 2011.11.17.08 (br, bac, ph), Ara Monadjem and Melanie Dammhahn (fluid preserved with skulls removed).

Etymology

The name *bemainty* is derived from the Malagasy, with '*be*' meaning notably or considerably and '*mainty*' referring to dark coloration, hence, meaning 'notably dark'. The name *bemainty* is also used by local guides in the Kirindy Forest for the spirit of a medicine man (*ombiasy*) that lives in the forest and with notably dark skin colour.

Diagnosis

Hypsugo bemainty is a small species of Vespertilioninae (forearm 29–32 mm), similar in external and cranial measurements, and dental morphology and measurements to *P. hesperidus*, which is known from Madagascar and southern Africa, and *P. raceyi*, a Madagascar endemic (Tables 3 and 4); all three species occur in sympatry at the type locality, the Kirindy Forest (CNFEREF). *Hypsugo bemainty* is separated from members of the genus *Neoromicia* occurring on Madagascar based on dental formula, specifically by the presence of a first upper premolar (P^3). *Hypsugo bemainty* has a unique cytochrome *b* sequence combination differing from its African sister species, *H. anchietae*, by 12.8%.

The pelage coloration is typical for a small vesper bat, although with notably shaggy fur (Fig. 11). Dorsum fur coloured dark chocolate brown to dark tan brown with some gray streaking and ventrum fur coloured medium tan brown with dark underfur. The soft parts, including the patagium, uropatagium, ear, and tragus are dark brown, although the ventral surface of the patagium sometimes has white venation. The ears have moderately long hair on the proximal half of the dorsal surface. The margins of the proximal lower half of the tragus run in parallel and then taper medially towards a rounded tip.

Skull is notably fragile with thin zygomatic arches, broad rostrum, and rounded and partially flattened braincase (Fig. 9). The GSKL in the holotype is 12.4 mm. The palate is relatively long and proportionately not broad. From dorsal view, the nasal emargination has an angular tapered indentation, forming a slightly open 'V-shaped'. In unworn adult dentitions, the first upper incisor (I^2) is bifid and the second upper incisor (I^3) is trifid. In lateral view, I^2 passes beyond the cingulum of I^2 and is slightly longer than half the total exposed tooth length of I^2. The first upper premolar (P^3) is notably reduced in size and not always visible from lateral view. A small to moderate gap between the C

and the second upper premolar (P^4) is generally present. The baculum of this species is a short structure ranging from 1.5–1.8 mm (Table 8) with a distinct bifurcation at the broad proximal and narrow distal extremities (Fig. 10A).

Description and comparisons

The important difference in adults of the three genera of vespers treated herein and occurring on Madagascar is the number of upper teeth, which is 16 (addition of a first upper premolar [P^3] in *Pipistrellus* and *Hypsugo*) and 14 (lacking a P^3 in *Neoromicia*). African *N. nana*, which is unknown on Madagascar, has 16 upper teeth and a different baculum structure (Fig. 7F). The only Malagasy vesper of the six species known to occur on the island that can be readily separated based on external, cranial, and dental measurements is *N. robertsi*; it is distinctly larger than the other taxa (Tables 2–4). As demonstrated by the PCA discussed above, the remaining Malagasy vesper taxa (*N. malagasyensis*, *N. matroka*, *P. hesperidus*, *P. raceyi*, and *H. bemainty*) show broad overlap in craniodental measurements. The single exception is *N. matroka* (Fig. 4), which is larger than the other four taxa in certain cranial measurements, although there is some overlap with *H. bemainty*.

External characters

A small vesper bat and in the holotype the tail length is 45% of total length, similar to the type series (Table 2). All sequenced specimens referable to this taxon have slightly to notably shaggy fur, particularly on the upper dorsum, which ranges in coloration from dark chocolate brown to dark tan brown (Fig. 11). In certain specimens there are notable light gray streaks interspersed in the dorsal fur. In notable contrast, the underside of *H. bemainty*, running from the lower chin to lower ventrum, is a medium tan brown, with dark underfur. The patagium and uropatagium are dark brown in coloration, with the exception that the ventral surface of the uropatagium has considerable whitish venation, which gives the impression that this surface is lighter in coloration. The ears and tragus are also dark brown in coloration. Most of these external characteristics do not readily separate *H. bemainty* from other species of Malagasy vespers, with the exception of *N. robertsi* and *N. matroka*, which have dark chocolate brown coloured venters.

The tragus and ear length of the *H. bemainty* holotype are 7 and 11 mm, respectively, and the latter measurement falls within the range of African *H. anchietae* (Table 2). In general, the tragus and ear measurements of *H. bemainty* are within the general range of *P. hesperidus* and *P. raceyi*, although the average measures in *H. bemainty* are greater. Further, in Malagasy members of the genera *Pipistrellus* and *Neoromicia*, the tragus tends to be shorter and broader than in *H. bemainty*. In the later taxon, the margins of the proximal lower half of the tragus run in parallel to one another, then taper medially, and terminate with a rounded margin. The ears have moderately long hair on the proximal half of the dorsal surface.

In the field, this species can be unambiguously separated based on external measurements from *N. matroka.* On the basis of measurements of sequenced animals in the type series of *H. bemainty*, and non-sequenced animals measured by the same collector (SMG), this species seems to show some sexual dimorphism in size, with the single female being larger than the four males in total length (mean of ♂♂ = 78.5 and ♀ = 83 mm, no overlap between sexes), forearm length (mean of ♂♂ = 29.5 and single ♀ = 32 mm, no overlap between sexes), and body mass (mean of ♂♂ = 3.7 and ♀ = 4.4 g, no overlap between sexes).

Craniodental characters

The skull of *H. bemainty* is notably delicate and short with the average GSKL of 12.6 mm (range 12.3–12.9 mm), overlapping with *P. hesperidus* with its average length of 12.0 mm (range 11.5–12.9 mm) and *P. raceyi* with its average length of 12.0 mm (range 11.6–12.6 mm). The zygomatic arch is a thin structure, typical of small vesper bats. The emargination of the nasal is proportionately longer than broader and terminating proximally in a 'V-shaped' structure with angular sides (Fig. 9).

Cranial measurements of *H. bemainty* show broad overlap with other Malagasy vespers with the same dental formula, namely *P. hesperidus* and *P. raceyi*: these latter two species can be separated from *H. bemainty* in most cases based on the shape and relative lengths of the first (I^2) and second upper incisor (I^3), the position of the first upper premolar (P^3), and the diastema between the C and second upper premolar (P^4) (based on characters outlined in Bates *et al*., 2006 and elaborated on herein).

Dental formula in adult *H. bemainty*, as in other members of the genus: I 2/3 C 1/1 P 2/3 M 2/3 for a compliment of 34 teeth. Upper post-incisor toothrows are largely in parallel, although with a slight bow, laterally orientated, running from the I to M^3 (Fig. 9). Amongst the specimens falling within the *H. bemainty* clade and with unworn upper incisors, the I^2 is bifid and the I^3 is trifid, as in the holotype. Further, in lateral view the I^2 passes beyond the cingulum of the I^2 and is slightly more than half the total exposed tooth length of I^2. Amongst the 12 sequenced specimens of *H. bemainty*, in 10 cases the P^3 is present and not visible in lateral view (including the holotype) and in two cases present and slightly visible in lateral view. In one specimen (FMNH 213576), the P^3 is distinctly small and without careful examination under a scope is not readily

visible. Of these 12 specimens, in nine cases, the diastema between the C and P^4 is relatively wide (including the holotype) or with a slight space and in three cases the two teeth are in direct contact. In lateral view of the paratype (MNHN 1900.538) and syntype (BMNH 6.1.3.1) of *H. anchietae*, the I^2 extends notably beyond the cingulum of I^3 and is nearly 3/4 the total exposed tooth length of I^3 (Fig. 12). The P^3 in these two specimens is small, more nested between the C and the P^4 then in the holotype of *H. bemainty* and these teeth are nearly touching. In lateral view, the P^3 is not visible.

Bacular morphology

As described by Bates *et al*. (2006) for Malagasy populations of *H. anchietae*, which is named herein as *H. bemainty*, this species has a short baculum (1.7–1.8 mm). Subsequently, sample sizes have increased and the length ranges from 1.50–1.79 mm (Table 8). In this species, the broad proximal base and narrow distal tip are bifid (Fig. 10A, B). The proximal base is slightly deflected ventrally but the distal tip is horizontal without any ventral deflection, distinguishing it from all other members of Malagasy Vespertilioninae. In dorsal view, the baculum of the holotype (FMNH 217884), which measures 1.79 mm in total length, shows some finer structure and the proximal bifid base has a pair of distal projections (Fig. 10A). One specimen (FMNH 213577) had a baculum length of 1.50 mm and the structure may not have been completely ossified (Fig. 10B). The general shape of the *H. bemainty* baculum is similar to that of *H. anchietae* (Kearney *et al*., 2002; Kearney, 2005; Fig. 10C), but the tips are angled more acutely inward and meet closer together at the distal end. The baculum total length for the two taxa is similar, with the measurement in *H. anchietae* being 1.4–1.6 mm (T. Kearney, unpublished data, $N = 5$).

The distinctly short (1.50–1.79 mm) and largely straight baculum in *H. bemainty* separates it from the slightly longer on average (1.60–2.10 mm) and recurved form of *P. hesperidus* (Fig. 8C–E) and the distinctly longer (8.8–10.0 mm) and differently shaped form in *P. raceyi* (Fig. 8A, B).

Common name

We propose 'Dark Madagascar pipistrelle' for the English vernacular name for this species and 'Pipistrelle sombre de Madagascar' in French.

Molecular genetics

On the basis of genetic samples available for this study, *H. bemainty* is notably divergent to animals assigned to African populations of *H. anchietae*, with mean K2P distance between these lineages measuring 12.8%. Intraspecific genetic diversity (cytochrome *b*) was notably higher in the *H. bemainty* sample, as compared to the other Malagasy species of vespers, and within this lineage exhibited a K2P distance of 0.010 ($N = 4$, Table 1), haplotype diversity of 0.962, and a nucleotide diversity of 0.00519 – nearly double that of the nearest value ($N = 13$, Table 9). One haplotype occurred in three specimens, while the remainder was composed of singletons.

Morphometrics

Measurements presented in Table 2. Measurements of holotype are presented above.

Bioacoustics

Measurements presented in Table 6. The echolocation call of the holotype is presented in Figure 5.

Known geographical range

Based on identifications made from molecular and bacular characters, *H. bemainty* has been documented in the immediate vicinity of the Kirindy Forest (CNFEREF) and nearby Kirindy village (see type series), all to the north of Morondava. Further, using features of cleared and stained bacula, it has also been documented in the Province of Toliara, Sept Lacs (UADBA 43682, RBJ-186), 23°31′15″S, 44°08′35″E, about 110 m asl (Bates *et al*., 2006). Several specimens have been referred to this taxon based on craniodental characters from Province de Toliara, Parc National de Kirindy Mitea, 0.75 km SW Manahy, 22°52.07′S, 43°54.45′E, 5 m asl (FMNH 176090–176095); Province de Toliara, Forêt de Zombitsy (= Parc National de Zombitse-Vohibasia), 22°50′S, 44°42′E, 870 m asl (FMNH 151940) (Bates *et al*., 2006).

Comparisons of Hypsugo bemainty *and* H. anchietae, *specifically associated with type specimens:* General external measurements of the animals sequenced herein and assigned to *H. bemainty* show considerable overlap to specimens allocated to *H. anchietae* from southern Africa (Table 2). Some caution needs to be given to not over interpreting these comparisons, as the Malagasy and African animals were not necessarily measured in the same fashion.

The skull of the *H. bemainty* holotype (FMNH 217884) is longer and broader than that of the *H. anchietae* syntype (BMNH 6.1.3.1) (Table 3). These differences are particularly noticeable for two different measurements: (1) the GSKL, which on average in the *H. bemainty* series measures 12.6 mm (range 12.3–12.9 mm), including 12.4 mm in the holotype, as compared to 12.2 mm in the *H. anchietae* syntype; and (2) the mastoid breadth, which on average in the *H. bemainty* series measures 7.2 mm (6.9–7.4 mm), including 7.3 mm in the holotype, as compared to 6.9 mm in the *H. anchietae* syntype. The posterior

portion of the cranium in the paratype (MNHN 1900.538) is damaged limiting certain craniodental measurements.

In the *H. anchietae* syntype (BMNH 6.1.3.1), the ventral portion of the skull is damaged, particularly the posterior portion of the palate, zygomatic region, and outer auditory bullae, which does not allow certain structural comparisons between this specimen and the *H. bemainty* holotype. The nasal emargination and palatal emargination in *H. bemainty* is distinctly 'V-shaped', while in the syntype and paratype of *H. anchietae*, this structure is a more rounded and open 'U-shape'. The exposed palate of *H. bemainty* is proportionately long and not particularly broad, and is similar to the syntype of *H. anchietae*. Although not physically compared with each another, based on notes, the syntype (BMNH 6.1.3.1) and paratype (MNHN 1900.538) of *H. anchietae* show some differences in cranial proportions and structural aspects of the palate.

The holotype of *H. bemainty* does not have worn incisors and the first upper incisor (I^2) is bifid and the second upper incisor (I^3) is trifid, and, in lateral view, the I^2 passes beyond the cingulum of I^3 and is slightly more than half the total exposed tooth length of I^3 (Fig. 12). In the *H. anchietae* syntype, the upper incisors are distinctly worn, but I^2 is bifid and I^3 trifid. In lateral view, I^3 passes to the level of the I^2 cingulum and is about half the total exposed tooth length of I^2. In lateral view, the first upper premolar (P^3) in the *H. bemainty* holotype is not evident in lateral view, while in the *H. anchietae* syntype, this tooth is present and slightly visible (Fig. 12). Further, the diastema between the C and the second upper premolar (P^4) is relatively wide in *H. bemainty* and a narrow diastema is present in the syntype of *H. anchietae*. The upper post-incisor toothrows in the holotype of *H. bemainty* are largely in parallel, with a slight mid-toothrow lateral bow. In contrast, the upper post-incisor toothrows in the syntype and paratype of *H. anchietae* show a slight posterior convergence.

DISCUSSION

On the basis of molecular genetic data, we have identified six species of Vespertilioninae on Madagascar: (1) the endemic *Neoromicia matroka*, which forms a sister species with African *N. capensis*; (2) *N. malagasyensis* and (3) *N. robertsi*, both endemic to the island and sister to each another; (4) the endemic *Hypsugo bemainty* sp. nov., described herein, which forms a sister species to African *H. anchietae*; (5) *Pipistrellus hesperidus*, for which African and Malagasy populations show slight genetic differences and herein considered conspecific; and (6) the endemic *P. raceyi*, which falls within *Pipistrellus* and notably distant from *P. hesperidus*. Even though nearly 100 Malagasy vespers were sequenced in the context of this study, we did not find evidence on Madagascar of the widespread African *N. nana*, which has been previously reported from the island [e.g. Koopman, 1994 and named *P.* (= *Neoromicia*) *africanus*]; we propose that this species does not occur on Madagascar.

Volleth *et al*. (2001) reported from the central western Kirindy Forest (Fig. 1), also amongst our principal study sites and where we documented three species of vesper, a form that they referred to as *P. kuhlii* (= *P. hesperidus*, sensu Kock, 2001) with a diploid chromosome number of 42, as compared to the true European *P. kuhlii* (sensu Simmons, 2005) with a diploid number of 44. While European *P. kuhlii* was not included in our molecular phylogeny, given our considerable sampling of vespers from the Kirindy Forest and nearby Kirindy village ($N = 38$), we suspect that the *P. kuhlii*-like species is not a fourth locally occurring taxon and almost certainly represents *P. hesperidus*. Specimens from South Africa that were previously called *P.* cf. *kuhlii*, and latter assigned to *P. hesperidus*, also had a diploid number of 42 (Kearney *et al*., 2002). Chromosome counts in *P. raceyi* have yet to be described and African *H. anchietae* have a diploid chromosome number of 26 (Kearney *et al*., 2002).

Herein, in order to characterize the six different vesper species identified from Madagascar, we used a molecular phylogeny of 99 animals including appropriate African taxa, to provide a structure to overlay different types of morphological and bioacoustic datasets to determine if these different characters sorted based on the relationships derived from the genetic data. In general, external and craniodental morphology and bioacoustics do not differentiate most of the Malagasy vesper species distinguished in our molecular dataset. In contrast, bacular morphology allowed separation of species in complete concordance with the genetic data. Hence, most of these taxa when in the hand, particularly *H. bemainty*, *P. hesperidus*, and *P. raceyi* in the dry west, cannot be readily distinguished from one another. This is in contrast with areas of eastern Madagascar with distinctly more mesic conditions, where *N. matroka* and *N. robertsi* can be easily differentiated from each another based on morphological and bioacoustic characters.

Patterns of sympatry on Madagascar

At our three principal field sites with intensive to moderately intensive bat surveys, local species diversity was highest in the lowland western dry deciduous Kirindy Forest (CNFEREF), with three sympatric species (*Hypsugo bemainty*, *Pipistrellus hesperidus*, and *P. raceyi*), followed by two species in the south central dry-humid transitional habitats of the Isalo National Park (*Neoromicia malagasyensis* and *N. matroka*) and

the highland eastern zones near Anjozorobe Forest (*N. robertsi* and *N. matroka*) (Fig. 1). Given the relatively restricted ranges of two species (*N. robertsi* and *N. malagasyensis*) and considerable portions of the island that have not been inventoried for vesper bats, it is possible that other species remain to be discovered and described.

Generic allocation of species

Some molecular phylogenies of the family Vespertilionidae or subfamily Vespertilioninae encompassing broad geographical zones have been published that demonstrate cases of paraphyly at the generic level (e.g. Hoofer & Van Den Bussche, 2003; Roehrs, Lack & Van Den Bussche, 2010). In light of the complexity of vesper evolution, the extraordinary level of cryptic speciation, and the complications of resolving the higher relationships of these animals, including African taxa (e.g. Lack & Van Den Bussche, 2010; Datzmann *et al*., 2012; Koubínová *et al*., 2013; Monadjem *et al*., 2013a), the number of cases of paraphyly at the generic level in these different datasets is remarkably few.

In the genetic analyses presented herein for Malagasy species and to a lesser extent African species, no clear problem of paraphyly was uncovered. This is particularly the case if *nana* is considered a member of the genus *Neoromicia* (e.g. Roehrs *et al*., 2010; Koubínová *et al*., 2013), rather than a *Hypsugo* (e.g. Hoofer & Van Den Bussche, 2003; Lack & Van Den Bussche, 2010). Hence, based on a wide variety of data types used to define genera of African Vespertilioninae, which include molecular genetics, karyotypes (banding), bacular morphology, etc. (e.g. Volleth *et al*., 2001; Kearney *et al*., 2002; Roehrs *et al*., 2010; Koubínová *et al*., 2013; Reeder *et al*., 2013), relationships at the generic level seem largely in order.

The only problem along these lines that should be mentioned is the placement of *bemainty* and *anchietae* in the genus *Hypsugo*. The type species of this genus is *H. savii* Bonaparte, 1837, found in the Palaearctic and western Asia. Recently generated molecular phylogenies of African vespertilionids found this genus to be polyphyletic, with *H. savii* and Asiatic *H. cadorne* (Thomas, 1916) showing different sister group relationships than African *H. eisentrautii* (Hill, 1968) (Roehrs *et al*., 2010; Koubínová *et al*., 2013). Further, Lack & Van Den Bussche (2010) found notable levels of paraphyly in animals identified as *Hypsugo*; these different datasets cast some doubts about the monophyly of this genus. Further, notable differences in chromosome counts occur between *H. savii* (2n = 44 and FN = 50) and African species currently placed in this genus [*H. anchietae* (2n = 26 and FN = 36), *H. eisentrautii* (2n = 42 and FN = 58), and *H. crassulus* (Thomas, 1904) (2n = 30 and FN = 56)] (Volleth *et al*., 2001; Kearney *et al*., 2002). If *Hypsugo* is not monophyletic, *H. savii* and related species will be retained in this genus. With this point in mind, it is plausible that African '*Hypsugo*', at least *H. anchietae* and *H. bemainty*, named herein, belong in the genus *Pipistrellus* or *Neoromicia*; further detailed phylogenetic work with broad geographic and taxonomic sampling of correctly identified animals should resolve this question.

Resolving the taxonomy of cryptic vesper species

In recent phylogenetic studies of African vespers, there are numerous cases of relatively deep branches between specimens assigned to the same species based on phenotypic characters (e.g. Koubínová *et al*., 2013; Monadjem *et al*., 2013a, b). This is in part because external or craniodental morphological characters classically used to define vesper species are not necessarily reflective of the subtleties in the true evolution of these animals. Virtually every molecular study conducted on African vespers at a broad geographical scale, or even at a relatively local level, opens 'Pandora's box' and highlights the frequency of unrecognized cryptic species (e.g. Hulva *et al*., 2004; Ibáñez *et al*., 2006; Kruskop *et al*., 2012; Santos *et al*., 2014). On the basis of these inferences, we suspect that numerous species of vespers warrant formal description or resurrection of certain names in synonymy.

In many of these cases, resolution of species level taxonomy of paraphyletic lineages is rather problematic, as it is often unclear how to pigeonhole which clade correctly represents a named taxon. The principal means to resolve these issues is to obtain genetic data from type specimens, allowing the unambiguous identification of the clade representing the taxon in question. This technique has been successfully applied to a number of African-Malagasy bat groups (e.g. Goodman *et al*., 2009, 2010; Monadjem *et al*., 2013b), allowing for animals incorrectly identified based on morphological characters to be accurately diagnosed. As a further complication, caution needs to be given to the sequencing of paratypes, as such specimens given numerous documented cases of morphological difficult to distinguish cryptic species occurring in sympatry (Monadjem *et al*., 2013a, b) are not necessarily the same species as the holotype.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Bat specimens of the genera *Hypsugo*, *Neoromicia*, and *Pipistrellus* (Vespertilionidae) used in the molecular analysis of this study. Certain species are not yet catalogued at the depository institution and are presented with field numbers. AFK = Amyot F. Kofoky, AM = Ara Monadjem, CFR = Claude Fabienne Rakotondramanana, ECJS = Ernest C. J. Seamark, RB = Beza Ramasindrazana, RBJ = Richard B. Jenkins, RHF = Fanja H. Ratrimomanarivo, SMG = Steven M. Goodman.