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The genus *Neoromicia* (Family Vespertilionidae) in Madagascar, with the description of a new species

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Abstract

Using molecular genetics, male sexual organ morphology (baculum), and cranio-dental characters we describe a new species of the genus *Neoromicia* from Madagascar, *N. robertsi* **sp. nov**. It is presumed to be endemic to the island and is known from three specimens taken in montane areas of the eastern central region. The new species shows 1.0 % and 2.8% divergence in the 12S rRNA and 16S rRNA genes, respectively, from its nearest congener and is notably larger in cranio-dental measurements than other members of the genus occurring on Madagascar. This new species was previously identified as *N. melckorum*, which is considered a junior synonym of southern African *N. capensis*. *Neoromicia malagasyensis*, an endemic to central western Madagascar, is the sister species to *N. robertsi* and the two are best considered vicariant species. Specimens provisionally assigned to *N. malagasyensis*, but notably smaller in baculum and skull size, and with different baculum morphology, probably represent another unknown species from the island. Given the apparent rarity of *N. robertsi* compared with other Malagasy members of this genus living in the eastern portion of Madagascar, it is considered a taxon of conservation concern.

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

Résumé

Nous décrivons une nouvelle espèce de Madagascar appartenant au genre *Neoromicia*, *N. robertsi* **sp**. **nov**., sur la base de la génétique moléculaire, de la morphologie de l'organe sexuel mâle (baculum) et des caractères crânio-dentaires. Cette nouvelle espèce, supposée être endémique à l'île, est représentée par trois échantillons prélevés dans les régions montagneuses du centre-est de Madagascar. Elle montre des divergences génétiques de 1.0% (12S) et 2.8% (16S) par rapport à ses plus proches congénères, et les mesures crânio-dentaires sont plus grandes que celles des autres membres du genre rencontrés à Madagascar. Cette nouvelle espèce fut précédemment identifiée comme *N. melckorum*, ce dernier étant considéré comme un synonyme junior de *N. capensis* de l'Afrique australe. *Neoromicia malagasyensis*, une espèce endémique du centre-ouest de Madagascar, est l'espèce sœur de *N. robertsi ;* les deux espèces devraient être considérées comme des espèces vicariantes. Des spécimens provisoirement attribués à *N. malagasyensis*, mais dont le baculum et la taille des crânes sont nettement plus petits, et dont la morphologie du baculum est différente représentent probablement une autre espèce, non décrite, de l'île. Compte-tenu de la rareté apparente de *N. robertsi* en comparaison avec d'autres membres malgaches de ce genre distribués dans la partie orientale de Madagascar, le statut de conservation de cette nouvelle espèce pourrait être préoccupant.

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Introduction

Members of the family Vespertilionidae, once placed in the genus *Vespertilio* Linnaeus, 1758, have seen considerable changes in their taxonomy (cf. Koopman 1993; Simmons 2005) and now are divided into different genera including *Pipistrellus* Kaup, 1869, *Hypsugo* Kolenati, 1856, *Eptesicus* Rafinesque, 1820, and *Neoromicia* Roberts, 1926. This is in part associated with new collections being made in poorly known areas of the world, but more importantly with the advent of techniques that provide insight into the phylogenetic histories of these animals, such as male sexual organ morphology (e.g. Hill & Harrison 1987), karyological variation (e.g. Heller & Volleth 1984; McBee *et al.* 1987; Volleth *et al*. 2001; Kearney *et al*. 2002), and, most importantly, molecular genetics (e.g. Hoofer & Van Den Bussche 2003; Hoofer *et al*. 2003; Roehrs *et al*. 2010). These different techniques have provided considerable insights into the evolutionary history of vespertilionids, revealed notable levels of paraphyly and morphological convergence, and resulted in major changes in their systematics at the tribal, genus, subgenus, and species levels (Simmons 2005). Results from these different techniques have lead to the discovery and description of cryptic species from even "well-known" portions of the world, such as continental Europe (e.g. Barratt *et al*. 1997; von Helversen *et al.* 2001), and it is clear that much needs to be uncovered from more poorly studied areas. The western Indian Ocean island of Madagascar was until about a decade ago poorly known with regards to its bat fauna. Subsequently, largely based on detailed field surveys and associated molecular genetic and morphological studies, a considerable number of species new to the island and, in many cases, previously unknown to science have been documented (Goodman 2011; Goodman *et al*. 2011). Until recently, advances concerning Malagasy vespertilionids were hampered because of the few available specimens. However, based on recent collections, the measure of species diversity is notably higher than previously inferred (Goodman *et al.* 2005, 2006; Bates *et al.* 2006). While the focus of this current paper is specifically Malagasy members of the genus *Neoromicia*, in order to place this study in a clearer context, we briefly review the systematic history of the island's small vespertilionid bats (excluding members of the genus *Scotophilus* and *Myotis*).

The first review of Madagascar's bat fauna was conducted by Dorst (1947a, 1947b), and a single species of small vespertilionid was noted from the island, "*Pipistrellus nanus* ? *nanus*", collected at Morondava in the central west and a site known as "Kina-Kina". (This latter compound word is the Malagasy vernacular name for small bat and does not represent a collection locality.) In a subsequent review, Peterson *et al*. (1995) listed the following taxa from the island: *Pipistrellus* sp., *Eptesicus matroka* (Thomas & Schwann, 1905) (= *N. matroka*), and *E. somalicus* (= *N. somalicus*) (Thomas, 1901) for which they described the subspecies *malagasyensis*. Goodman & Ranivo (2004), based on new specimen material and cranio-dental characters, concluded that the form *malagasyensis* should be considered specifically distinct from *somalicus* and this proposition was subsequently supported based on bacular morphology (Bates *et al.* 2006).

The results of a bat inventory in the central west of the island by Göpfert *et al.* (1995) showed that the regional species diversity of small Malagasy vespertilionids was underestimated and they listed three taxa: *P. africanus* Rüppell, 1842 (syn. *N. nanus* [Peters, 1852]), a member of the *P. kuhlii*-group, and the third showing affinities with Oriental region pipistrelles and also allocated to the *P. kuhlii*-group. In a review of the island's bat fauna, Eger & Mitchell (2003) included the following small vespertilionids: *P. kuhlii* (Kuhl, 1817), *P. nanus* (= *N. nanus*), *Pipistrellus* sp., *E. matroka*, and *E. somalicus*.

Since the mid-1990s, new field efforts have been orchestrated to understand and document the distribution and species richness of Malagasy bats, which have given rise to new collections of bats from across the island. Bates *et al.* (2006) were able to examine over 40 specimens of Malagasy small vespertilionids and identified the following taxa: *P. raceyi*, which was described as new to science, *P. hesperidus* (Temminck, 1840) (syn. *P. kuhlii*), *Neoromicia matroka*, *N. malagasyensis*, *N. melckorum* (Roberts, 1919), and *Hypsugo anchietae* (Seabra, 1900). Bates *et al*. (2006) provided important insight into the species definitions and taxonomy of these animals, but one of the lingering questions was the identity of specimens they referred to *N. melckorum*. This taxon is generally thought to have a southern African distribution and its occurrence in eastern Madagascar is biogeographically idiosyncratic. The principal focus of this paper is to examine in detail the species relationships and identity of the Malagasy animals that Bates *et al*. (2006) assigned to *N. melckorum*.

Material and methods

Specimens. Material for the morphological and molecular genetic analyses presented herein are housed in the following museums: BMNH—The Natural History Museum (formerly The British Museum of Natural History), London; FMNH—Field Museum of Natural History, Chicago; MNHN—Muséum national d'Histoire Naturelle, Paris; ROM—Royal Ontario Museum, Toronto, and UADBA—Département de Biologie Animale, Université d'Antananarivo, Antananarivo.

Access and comparison to type specimens. We were able to directly examine the following holotypes or syntypes in association with this study: *Eptesicus somalicus malagasyensis* (ROM 42713), *Vespertilio matroka* (BMNH 97.9.32), *Vespertilio minutis somalicus* (BMNH 98.6.9.1), and *Vesperus humbloti* Milne Edwards, 1881 (MNHN 1986.1074, 1986.1075).

Morphological study. The specimens used in this portion of the study are presented in Appendix 1. 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 in grams was recorded with a Pesola spring balance with a precision of 0.5 g. Further, information on external measurements was obtained from museum specimen labels, but caution is needed in making comparisons between various field collectors associated with possible different mensuration techniques.

Seven cranial and five dental measurements were made by SMG using digital calipers 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; condyloincisive 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 tooth row (I-M^3), length from anterior alveolar border of incisors to posterior alveolar border of 3rd molar (M^3); complete canine-molar toothrow (C-M^3), length from anterior alveolar border of canine to posterior alveolar border of 3rd molar (M^3); width across upper canines (C-C), taken across the outer alveolar borders of the canines; width across 3rd upper molars (M^3-M^3), taken across the outer alveolar borders of the 3rd molars; and complete mandibular tooth row (i-m_3), length from anterior alveolar border of incisors to posterior alveolar border of 3rd molar (m_3). Tooth abbreviations include: I = incisor, C = canine, P = premolar, M = molar. Upper case abbreviations are used for upper teeth and lower case abbreviations for lower teeth.

Patterns of geographical variation. While measuring specimens of *N. matroka*, it was clear that as currently defined this species shows geographical variation in cranio-dental measurements. In order to better encompass this variation in our morphometric analyses, the different populations of *N. matroka* were divided into geographical units or Operational Taxonomic Units (OTUs): OTU 1 – the Central Highlands above 1000 m, which includes the type locality near Ambositra (Thomas & Schwann 1905; Figure 1); OTU 2 – areas in central western Madagascar less than 900 m; OTU 3 – eastern lowlands from sea-level to 200 m; and OTU 4 – eastern lowlands from 201-900 m.

Bacular study. Preparation of six bacula from males of three species (*N. matroka*, n = 3; *N. malagasyensis*, n = 1; *N.* cf. *malagasyensis*, n = 2) followed Hill & Harrison (1987), Lidicker (1968), and Kearney *et al*. (2002): penial tissue was macerated in 5% KOH and the baculum stained with alizarin red followed by dissection of the baculum and clearing with glycerine. Bacula were stored in 100% glycerine with a crystal of thymol to prevent fungal growth. Each baculum was photographed in dorsal, ventral, and lateral view, and total baculum length (TBL), measured along the axis of the shaft, was recorded using Mitutoyo digital calipers viewed under a dissecting microscope. Comparative material for *Neoromicia* described herein as new to science was redrawn from Bates *et al.* (2006, their Figure 8c).

Statistical analyses. Univariate statistical analyses were conducted for each of the measured externals and cranio-dental variables and in several cases sexual dimorphism was identified for certain taxa. Hence, in all of the morphometric comparisons presented herein the sexes of each species are separated. In order to distinguish between different members of the genus *Neoromicia*, a Principal Component Analysis (PCA) was conducted using the statistical package Statistica (version 7.0); data were log-transformed and the unrotated option was used.

Genetic study. Complete sequences for 12S rRNA and 16S rRNA genes were obtained for 21 individuals and are deposited in GenBank (Table 1). *Pipistrellus javanicus* and *P. tenuis* were designated as outgroup taxa in phylogenetic analyses, as previous study indicates that these species are outside the remainder of taxa used in this study (Hoofer & Van Den Bussche 2003). DNA sequences for these two taxa were retrieved from GenBank (AY495525, AY495529), and were originally generated by Hoofer & Van Den Bussche (2003).

TABLE 1. Specimens of Vespertilionidae bats of the genera *Neoromicia*, *Hypsugo*, and *Pipistrellus* used in the molecular analysis of the study, along with associated Genbank accession numbers (JQ039200—JQ039241). FMNH—Field Museum of Natural History, TM—Ditsong National Museum of Natural History (formerly Transvaal Museum), and UADBA—Université d'Antananarivo, Département de Biologie Animale.

Species	Locality	Specimen number of voucher	12S	16S
N. capensis	Tanzania: Iringa Region, Kibebe Farms	FMNH 168093	JQ039222	JQ039223
N. capensis	Tanzania: Iringa Region, Kibebe Farms	FMNH 169094	JQ039224	JQ039225
N. capensis	Tanzania: Manyara Region, Tarangire National Park	FMNH 187221	JQ039226	JQ039227
N. capensis	South Africa: Mpumalanga Province, Marloth Park	TM 48333	JQ039228	JQ039229
N. capensis	South Africa: Mpumalanga Province, Marloth Park	FMNH 195629	JQ039230	JQ039231
N. matroka	Madagascar: Province de Fianarantsoa, Manambolo	FMNH 167660	JQ039204	JQ039205
N. matroka	Madagascar: Province de Toamasina, Parc National de Mantadia	UADBA 43674	JQ039206	JQ039207
N. malagasyensis	Madagascar: Province de Fianarantsoa, Parc National de l'Isalo	UADBA 43680	JQ039208	JQ039209
N. malagasyensis	Madagascar: Province de Fianarantsoa, Parc National de l'Isalo	UADBA 43681	JQ039210	JQ039211
N. robertsi	Madagascar: Province de Toamasina, Parc National de Mantadia	UADBA 43678	JQ039212	JQ039213
N. robertsi	Madagascar: Province de Toamasina, Parc National de Mantadia	FMNH 213931	JQ039214	JQ039215
N. zuluensis	South Africa: Mpumalanga Province, Marloth Park	TM 48334	JQ039234	JQ039235
N. zuluensis	South Africa: Mpumalanga Province, Marloth Park	FMNH 195631	JQ039236	JQ039237
N. zuluensis	South Africa: Mpumalanga Province, Marloth Park	TM 48335	JQ039238	JQ039239
N. zuluensis	South Africa: Mpumalanga Province, Marloth Park	FMNH 195633	JQ039240	JQ039241
N. zuluensis	South Africa: Mpumalanga Province, Marloth Park	FMNH 195634	JQ039232	JQ039233
N. somalicus	Tanzania: Manyara Region, Tarangire National Park	FMNH 187142	JQ039220	JQ039221
N. nanus	Tanzania: Coast Region, Mafia Island	FMNH 187390	JQ039216	JQ039217
N. nanus	Tanzania: Coast Region, Mafia Island	FMNH 187391	JQ039218	JQ039219
H. anchietae	Madagascar: Province de Toliara, Sept Lacs	FMNH 173251	JQ039200	JQ039201
H. anchietae	Madagascar: Province de Toliara, Parc National de Kirindy-Mitea	FMNH 176090	JQ039202	JQ039203
P. tenuis	Philippine Islands: Sibuyan Island	FMNH 137021	AY495529	AY495529
P. javanicus	Philippine Islands: Mindanao Island	FMNH 147069	AY495525	AY495525

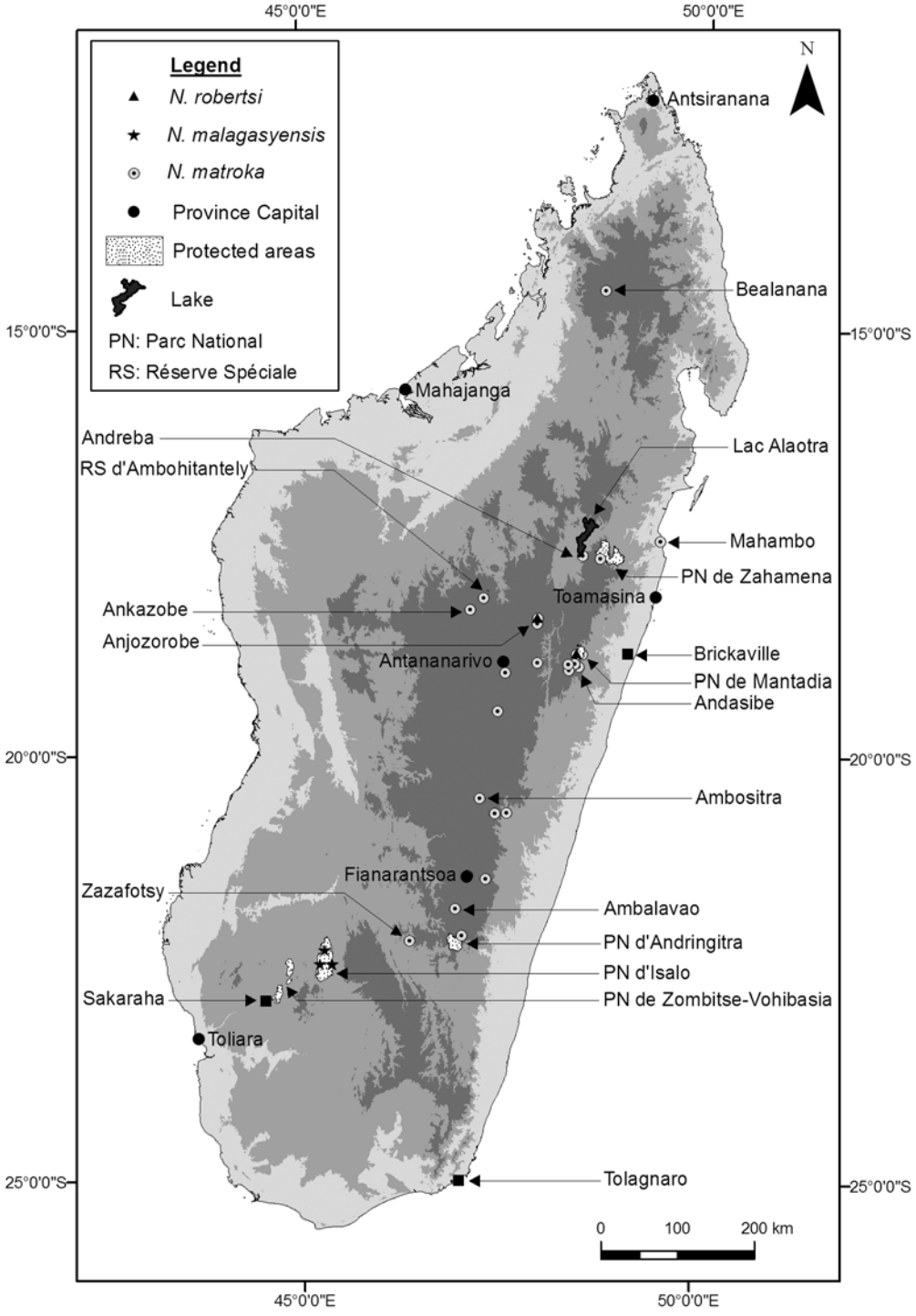

FIGURE 1. Map of localities mentioned in text associated with the known distribution of *Neoromicia robertsi* **sp. nov**., *N. matroka*, and *N. malagasyensis*. Note that *N. robertsi* occurs sympatrically with *N. matroka* at the two localities the former species is known from. Color codes associated with elevational zones depicted on map: light gray—sea-level to 200 m, medium gray—201–900 m, and dark gray—> 900 m.

Genomic DNA was isolated from skeletal muscle or organ tissue samples by standard organic methods (Longmire *et al*. 1997). Methods to amplify and sequence the 12S rRNA and 16S rRNA genes followed Van Den Bussche & Hoofer (2000). Both genes were sequenced entirely in both directions with an assortment of external and internal primers. Double-stranded PCR amplicons were purified by using a QIAquick PCR Purification Kit™ (Qiagen, Inc., Chatsworth, California), and both strands were sequenced using Big-Dye™ version 3.1 chain termi-

nators, followed by electrophoresis on a 3100-Avant Genetic Analyzer (Applied Biosystems, Inc., Foster City, California). AssemblyLIGN™ 1.0.9 software (Oxford Molecular Group PLC, Oxford, United Kingdom) was used to assemble resulting, overlapping fragments.

Multiple sequence alignment for both data sets was performed in Clustal W software (Thompson *et al*. 1997) with default parameters for costs of opening and extending gaps. Alignments were subsequently examined using MacClade software (version 4.05; Maddison & Maddison 2002), ambiguously aligned sites were delimited following methods of Hoofer & Van Den Bussche (2003), and analyses were performed with and without those sites.

Sequence analyses. Phylogenetic relationships were inferred by Maximum Parsimony and Minimum Evolution analyses implemented in PAUP* software (test version 4.0b10 – Swofford 2002) and by Bayesian analysis implemented in MrBayes 3.1.2 software (Huelsenbeck & Ronquist 2001). All nucleotide positions were treated as unordered, discrete characters (A, C, G, T), multiple states as polymorphisms, and gaps as missing. Nucleotide sequences from both 12S rRNA and 16S rRNA genes were evaluated three ways: 1) levels of phylogenetic signal were estimated using the g_1-statistic (Hillis & Huelsenbeck 1992) for 100,000 randomly drawn trees; 2) genetic distances (uncorrected "p") were obtained and compared by using pairwise comparisons of taxa; and 3) Maximum Parsimony, Minimum Evolution, and Bayesian Likelihood analyses were performed and compared among taxa.

Based on Akaike Information Criterion tests in Modeltest 3.06 software (Posada & Crandall 1998), the general time reversible (GTR) model with allowance for gamma distribution of rate variation (Γ) and for proportion of invariant sites (I) best fit the data from both genes: 12S rRNA, $r_{AC} = 42510.66$, $r_{AG} = 374442.72$, $r_{AT} = 66141.79$, $r_{CG} = 8637.57$, $r_{CT} = 1103283.50$, $\pi A = 0.35$, $\pi C = 0.21$, $\pi G = 0.19$, $\pi T = 0.25$, $\alpha = 0.61$, $P_{inv} = 0.58$; 16S rRNA, $r_{AC} = 4519072.50$, $r_{AG} = 24987952.00$, $r_{AT} = 3646013.00$, $r_{CG} = 1141400.50$, $r_{CT} = 60563272.00$, $\pi A = 0.38$, $\pi C = 0.19$, $\pi G = 0.17$, $\pi T = 0.26$, $\alpha = 0.44$, $P_{inv} = 0.40$. The GTR + Γ + I model best fit both data sets with and without ambiguous characters, although specific model parameters differed slightly; values reported were calculated without ambiguous characters.

Maximum Parsimony analyses were conducted with all characters and substitution types given equal probabilities (i.e., unweighted), full heuristic searches with 25 random additions, starting trees by simple addition, and tree-bisection-reconnection branch swapping. Minimum Evolution analyses were performed with full heuristic searches, neighbor-joining starting trees, and tree-bisection-reconnection branch swapping. Clade reliabilities were assessed in Maximum Parsimony and Minimum Evolution analyses by using bootstrapping methods with 1,000 iterations (Felsenstein 1985). Values ≥ 70 were regarded as indicative of strong support.

Bayesian analyses were run at least 2×10^6 generations with four Markov-chains, random starting trees for each chain, and trees sampled every 100^{th} generation. For each data set, two independent analyses were run to assess whether chains converged on the same posterior probability distribution and whether likelihood values became stable (Huelsenbeck *et al*. 2002). Model parameters were treated as unknown variables (with uniform priors) to be estimated in each Bayesian analysis (Leaché & Reeder 2002). Burn-in values (initial set of unstable generations to be ignored) were based on empirical evaluation of likelihoods converging on stable values. Branch lengths and an all compatible consensus tree from the sample of stabilized trees were calculated via the "sumt" option in MrBayes 3.1.2 software (Huelsenbeck & Ronquist 2001), and viewed in PAUP* software (test version 4.0b10 – Swofford 2002). Clade reliabilities were assessed using posterior probabilities, with values ≥ 0.95 regarded as significant.

Conditional combination of the 12S rRNA and 16S rRNA data sets was assessed based on the presence of supported conflicts among phylogenetic relationships (Wiens 1998; Leaché & Reeder 2002). In combined analyses, data were partitioned with each gene sequence being analyzed with the same models and parameters (from Modeltest) determined above for each partition.

Results

Molecular phylogenetics. Complete sequences of the 12S rRNA generated in this study, along with the two retrieved from GenBank averaged 956 base pairs, ranging from 954 (*Hypsugo anchietae*, *Neoromicia robertsi*, *N. zuluensis*) to 959 (*Pipistrellus javanicus*, *P. tenuis*). Complete sequences of the 16S rRNA generated in this study, along with the two retrieved from GenBank averaged 1,562 base pairs, ranging from 1,556 (*N. zuluensis*) to 1,569 (*P. javanicus*).

Multiple sequence alignment for both genes resulted in 2,553 characters (12S rRNA, 966; 16S rRNA, 1,587), corresponding in length and similarity to other mitochondrial ribosomal gene sequences in GenBank. Alignments are available upon request from the last author. A total of 165 characters (12S rRNA, 63; 16S rRNA, 102) in 10 regions of the alignment, ranging from four base pairs to 40 base pairs, were excluded because of ambiguity in assessment of positional homology. This left 2,388 characters for analysis, of which 1,861 were constant (12S rRNA, 748; 16S rRNA, 1,113) and 445 parsimony-informative (12S rRNA, 130; 16S rRNA, 315). Levels of phylogenetic signal were significant based on the g_1 statistic ($P < 0.01$; Hillis and Huelsenbeck 1992) for the 12S rRNA (–0.69) and 16S rRNA (–0.59).

For both 12S rRNA and 16S rRNA data sets, Bayesian likelihoods reached stationarity before 500,000 generations (i.e., burn-in = 5,000), thinning the data to 15,000 sample points. Plots of generation versus the log probabilities of observing actual data revealed no trends of non-stationarity. Topology and posterior probabilities for nodes and model parameters for all runs within data sets agreed regardless of choice of particular outgroup sample. Minimum Evolution analysis resulted in six least-evolved trees (score = 0.39) and three least-evolved trees (score = 0.70) for 12S rRNA and 16S rRNA data sets, respectively. Parsimony analysis resulted in two most-parsimonious trees (length = 242, CI = 0.74, RI = 0.88) and two most-parsimonious trees (length = 645, CI = 0.72, RI = 0.86) for 12S rRNA and 16S rRNA data sets, respectively. For both data sets, differences among least evolved trees and most-parsimonious trees involved alternative arrangements of terminal branches within *N. zuluensis*. Overall, there were few or no topological differences within and between data sets and between the three optimality criteria, and none of the differences were supported. Statistically supported topologies (i.e., ≥ 70% bootstrap value, ≥ 0.95 Bayesian posterior probability) obtained from all optimality criteria agree within and between data sets (trees not shown).

Pairwise comparisons of percentage sequence distance (uncorrected "p") for both data sets are shown in Table 2. Within ingroup species, average distances ranged from 0.0% (*N. malagasyensis*) to 0.5% (*N. capensis*) in the 12S rRNA, and from 0.0% (*N. robertsi*) to 0.6% (*N. capensis*) in the 16S rRNA. Between ingroup species, average distances ranged from 1.0% (*N. robertsi* compared to *N. malagasyensis*) to 7.4% (*N. nanus* compared to *N. zuluensis*) in the 12S rRNA, and from 2.3% (*N. matroka* compared to *N. capensis*) to 9.8% (*H. anchietae* compared to *N. malagasyensis*) in the 16S rRNA.

The 12S rRNA and 16S rRNA sequences were combined because there was high degree of congruence and no supported conflicts between them (Wiens 1998). Bayesian likelihoods reached stationarity before 500,000 generations as above, and topology and posterior probabilities for nodes and model parameters for all runs agreed regardless of outgroup choice. Minimum Evolution analysis resulted in one least-evolved tree (score = 0.76) and Parsimony analysis resulted in two most-parsimonious trees (length = 1,157, CI = 0.70, RI = 0.86). Topologies and levels of nodal support obtained from all three optimality criteria were nearly identical (Fig. 2).

The species described herein, *N. robertsi*, is represented by two sequenced specimens that form a monophyletic group most closely related to *N. malagasyensis*. The level of sequence divergence separating these apparent sister species is 1.0% (12S rRNA) and 2.8% (16S rRNA). Similarly, the morphologically distinct *N. matroka* and *N. capensis* form a sister-species relationship separated by 1.8% (12S rRNA) and 2.3% (16S rRNA) sequence divergence. Hence, the levels of divergence observed between *N. matroka* and *N. capensis* are similar to those between *N. malagasyensis* and *N. robertsi*, lending further support to the recognition of the latter pair as distinct species. These two sets of apparent sister species (*N. robertsi–N. malagasyensis* and *N. matroka–N. capensis*) together shared a most recent common ancestry that is separated by approximately 3.3% (12S rRNA) and 5.1% (16S rRNA) sequence divergence. Sequence divergences between *N. robertsi* and the other more distantly related taxa are all greater than 6.0% (12S rRNA) and 8.0% (16S rRNA).

Bacular morphology. The baculum of the *Neoromicia* from Anjozorobe (FMNH 213931), previously referred to *N. melckorum* by Bates *et al*. (2006; and redrawn herein as Figure 3a), measures 2.8 mm in total length and is similar in dorsal shape to *N. matroka* (FMNH 184868), which measures 2.24 mm in total length (Figure 3b). The baculum of a second specimen of Malagasy *N. melckorum* (UADBA 43677) also measures 2.8 mm (Bates *et al.* 2006). Bacula of two *N. matroka* (FMNH 194153 and FMNH 184884) were similar in shape and size (total lengths 2.22 and 2.25 mm, respectively) to that shown in Figure 3b. In lateral view, the bacula of these two species are distinct from one another. The specimens of *N. matroka* are notably different to members of the genus *Eptesicus*, such as *E. hottentotus*, which is relatively very small and triangular (Kearney *et al*. 2002), further supporting the placement of *matroka* in *Neoromicia*, rather than *Eptesicus*. On the basis of material currently available, the bacular

morphology of *N. matroka* and *N. capensis*, as illustrated in Kearney *et al*. (2002), cannot be differentiated from one another.

TABLE 2. Pairwise comparisons of average percentage sequence distance (uncorrected "p") in the 12S ribosomal RNA (below diagonal) and 16S ribosomal RNA (above diagonal) genes of different *Neoromicia* and *Hypsugo* species. Within-taxon distances are given on the diagonal (12S / 16S).

Taxon	1	2	3	4
1 *Pipistrellus*	**0.034/0.079**	0.142	0.135	0.134
2 *H. anchietae*	0.089	**0.001/0.002**	0.083	0.098
3 *N. matroka*	0.085	0.049	**0.001/0.002**	0.052
4 *N. malagasyensis*	0.087	0.063	0.037	**0.000/0.001**
5 *N. robertsi*	0.089	0.064	0.034	0.010
6 *N. nanus*	0.082	0.063	0.055	0.063
7 *N. somalicus*	0.088	0.037	0.048	0.059
8 *N. capensis*	0.084	0.053	0.018	0.031
9 *N. zuluensis*	0.093	0.042	0.051	0.061

continued

Taxon	5	6	7	8	9
1 *Pipistrellus*	0.133	0.129	0.138	0.134	0.138
2 *H. anchietae*	0.090	0.091	0.058	0.053	0.059
3 *N. matroka*	0.045	0.093	0.077	0.023	0.083
4 *N. malagasyensis*	0.028	0.097	0.082	0.056	0.084
5 *N. robertsi*	**0.002/0.000**	0.093	0.080	0.050	0.084
6 *N. nanus*	0.060	**0.001/0.002**	0.088	0.094	0.091
7 *N. somalicus*	0.058	0.068	**na/na**	0.078	0.036
8 *N. capensis*	0.030	0.058	0.052	**0.005/0.006**	0.083
9 *N. zuluensis*	0.060	0.074	0.033	0.055	**0.001/0.002**

The specimen of *N. malagasyensis* (FMNH 157988), which measures 2.10 mm in total length (Figure 3C), is similar in overall shape to *N. matroka*, but in dorsal and lateral views has a distinct tip, reminiscent of *Hypsugo anchietae* (Kearney *et al*. 2002; Bates *et al*. 2006, their Figures 3E and 10B). However, the *N. malagasyensis* (FMNH 157988) specimen has the ventral tip deflection typical of *Neoromicia*.

Animals referred to herein as *N.* cf. *malagasyensis* (FMNH 213576 and FMNH 213577), with bacula distinctly smaller than the other taxa illustrated here (Figure 3d), measuring in total length 1.50 and 1.63 mm, respectively, have a tip similar to *N. malagasyensis* (Figure 3C, FMNH 231576), but not as notably deflected ventrally. The basal end of these specimens is emarginated in dorsal and lateral views, but also deflected ventrally. These last two features are not found in other *Neoromicia*, as this genus is currently defined based on bacular morphology (sensu Kearney *et al*. 2002), given the ventral deflection of the basal portion is more typical of *H. anchietae*. The taxonomic identity of the species referred to herein as *N.* cf. *malagasyensis* is in need of further investigation.

Conclusions based on molecular genetics and bacular morphology. Given the different points of evidence from the genetic and male sexual organ studies mentioned above, animals from Madagascar previously identified as *Neoromicia melckorum* represent an undescribed species. This conclusion is simplified with regards to African *Neoromicia*, which is restricted to sub-Saharan Africa (Simmons 2005), as bat taxonomists consider that, as currently defined, *N. melckorum* from southern Africa is a junior synonym of *N. capensis* (A. Smith, 1929) (Rautenbach *et al*. 1993; Koopman 1994; Kearney *et al*. 2002; Kearney 2005; Simmons 2005; Monadjem *et al*. 2010). Hence, in the following description it was not necessary to distinguish this new species from *N. melckorum*. In the recent literature on the bats of southern Africa mention is made to an undescribed species referred to as "*N.* cf. *melckorum*" (e.g. Monadjem *et al*. 2010). As this form currently has no official taxonomic status, it cannot be compared in the following description.

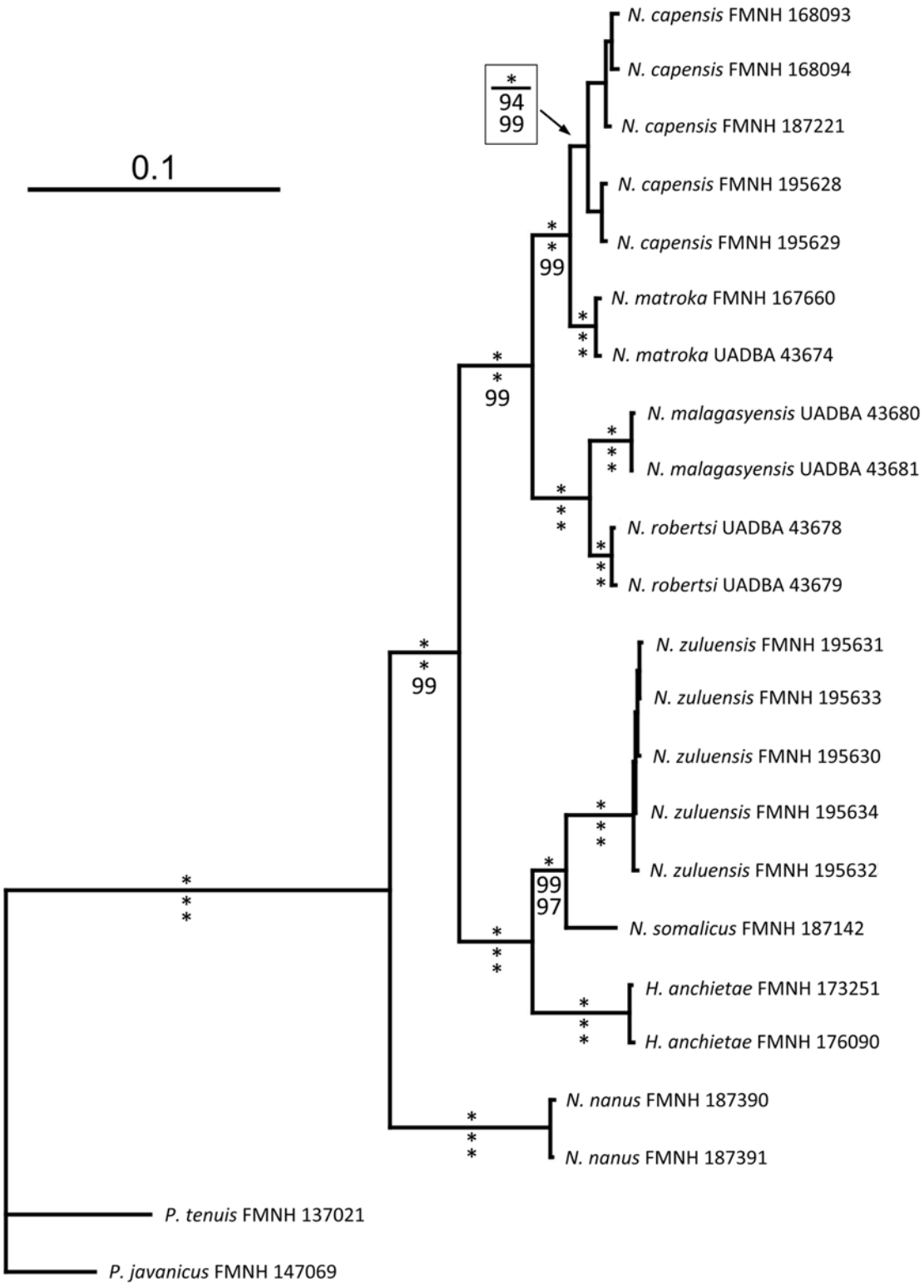

FIGURE 2. Phylogenetic position of *Neoromicia robertsi* **sp. nov.** with respect to other Malagasy and African members of this genus, based on analysis of combined sequences of 12S ribosomal RNA and 16S ribosomal RNA genes (~ 2,500 base pairs). The phylogram shown was produced by Bayesian analysis. *Pipistrellus javanicus* and *P. tenius* were designated as outgroups. Numbers above major branches are Bayesian posterior probabilities, whereas those below are bootstrap percentages from Minimum Evolution then Maximum Parsimony analyses. * = 100, *H.* = *Hypsugo*, *N.* = *Neoromicia*, *P.* = *Pipistrellus*.

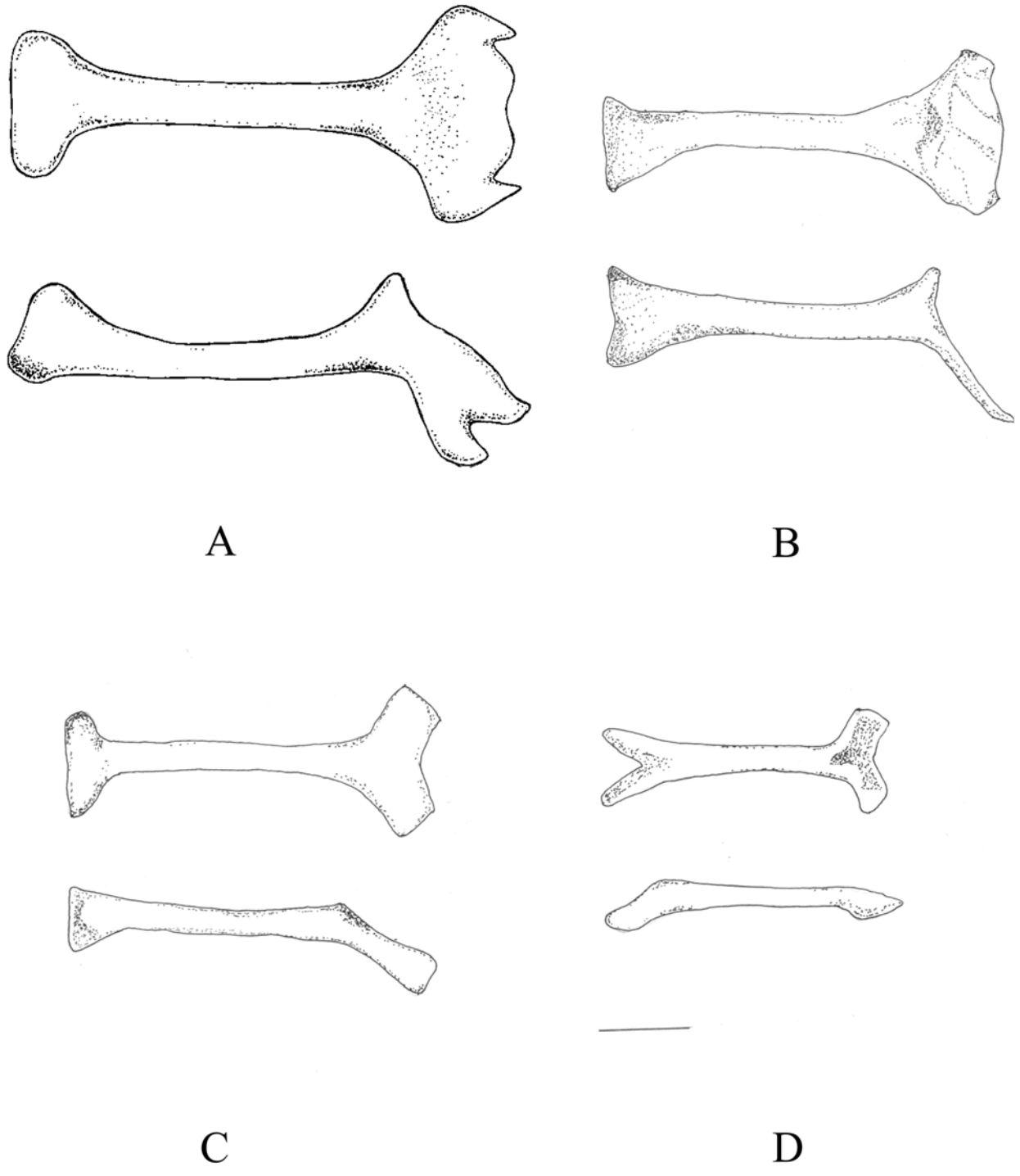

FIGURE 3. Bacula of four species of *Neoromicia*, dorsal (above) and lateral (below views): A) *N. robertsi*, **sp. nov.** (FMNH 213931, RBJ 119, total length 2.8 mm), from Province de Toamasina, Parc National de Mantadia (redrawn from Bates *et al.* 2006, Figure 8C); B) *N. matroka* (FMNH 184468, total length 2.2 mm), from Province d'Antananarivo, Réserve Spéciale d'Ambohitantely; C) *N. malagasyensis* (FMNH 175988, total length 2.1 mm), from Province de Fianarantsoa, near Parc National d'Isalo; and D) *N.* cf. *malagasyensis* (FMNH 213576, total length 1.5 mm), from Province de Toliara, Kirindy Forest (CNFEREF). Scale bar represents 0.5 mm.

Systematics

Family Vespertilionidae Gray, 1821

Genus *Neoromicia* Roberts, 1926

Neoromicia robertsi sp. nov.

Neoromicia melckorum Bates *et al*., 2006
Neoromicia capensis Goodman, 2011

Holotype. UADBA 43677, adult male, body preserved in formalin and subsequently transferred to 70% ethanol; skull removed, cleaned, and previously illustrated (Bates *et al.* 2006, their Figure 9C; baculum removed and cleared (Bates *et al*. 2006). The skull and mandible are in fine condition (Figure 4). Original field number Richard B. Jenkins (RBJ) 105. The holotype was used in the morphological and molecular comparisons.

Type locality. Madagascar: Province d'Antananarivo, Anjozorobe, Amboasary, 18°24.295'S, 47°56.699'E (Figure 1). Animal was captured on 25 September 2002 in a clearing surrounded by relatively intact montane forest.

Referred specimens. UADBA 43678 (RBJ 118) and FMNH 213931 (RBJ 119, formerly UADBA 43679), Madagascar: Province de Toamasina, Parc National de Mantadia, between PK 9 and PK 10, 18°48.941'S, 48°25.633'E (Figure 1). These two individuals were captured in an open agricultural area with some banana trees. The baculum of FMNH 213931 has been previously illustrated (Bates *et al*. 2006, their Figure 8C). The skull of UADBA 43679 appears to have been lost.

Etymology. This species is named after the late Austin Roberts, who conducted systematic research on African mammals at the Transvaal Museum in Pretoria (Brain 1998; Monadjem *et al*. 2010). A portion of his career was devoted to the study of southern Africa bats, including small vespertilionids, and he provided important insights into sorting out their generic affiliations.

Diagnosis. Medium-sized member of the genus *Neoromicia*, with a single upper premolar and a distinctly long dark chocolate brown dorsal and ventral pelage; the upper ventrum has a bi-colored appearance with certain hairs being more lightly colored. The soft parts, including the patagium, uropatgium, ear, and tragus are blackish-brown. The ears have long hair on the proximal one-half of the dorsal surface. The outer margins of the tragus run largely in parallel, and towards the distal tip curve inwards and terminate with a rounded margin. The tragus has a deep notch at the base along the posterior border. While certain external measurements overlap with populations of *N. matroka*, *N. robertsi* can be distinguished from all Malagasy members of the genus by its larger and non-overlapping cranio-dental measurements. The greatest skull length of the holotype is 14.3 mm and one of the paratypes is 14.6 mm. In *N. robertsi*, there is a distinct diastema between the 2nd upper incisor and the prominent upper canine, the upper tooth rows are positioned largely in parallel, and the 2nd upper incisor, which is approximately one-half the height of the 1st upper incisor, has a distinct single cusp on the posterior edge. Based on molecular genetic characters, *N. robertsi* forms a distinct clade from other Malagasy members of this genus and is the sister species to *N. malagasyensis*. Further, bacular characters support this relationship; whilst lateral and dorsal shape is reminiscent of *N. capensis*, total length (2.8 mm, n = 2) is larger and does not overlap at all with the smaller *N. capensis* (c. 2.1 mm) (Taylor *et al*. in prep.).

Description. *External characters.* A moderately large *Neoromicia* with a tail less than 40% of total length (Table 3). The three specimens of *N. robertsi* comprising the type series (UADBA 43677, FMNH 213931) have notably shaggy dark chocolate drown dorsums and ventrums, longer than, slightly darker, and more saturated than typical *N. matroka*. The ventrum fur of *N. robertsi*, particularly the outer fur of the upper portion of the chest, often has a slightly lighter sheen, which gives the impression of being bi-colored and in *N. matroka* these accents are not present. The surfaces of the patagium and uropatgium are a distinct blackish-brown, similar to *N. matroka* and *N. malagasyensis*.

The ear length in the holotype of *N. robertsi* is 13 mm, which is the same measurement as in the two paratypes (Table 3), and in general falls within the range of *N. matroka* and *N. malagasyensis*. The dorsal surface of the pinna of *N. robertsi*, particularly the lower proximal half is furred, which is distinctly less developed in *N. malagasyensis* and mostly naked in *N. matroka*. *Neoromicia robertsi* has notably dark blackish-brown pinna and tragus, the later

TABLE 3. External measurements (in millimeters) and weight (in grams) of *Neoromicia robertsi* **sp. nov.**, *N. matroka*, *N. malagasyensis*, and *N. capensis*. Measurements presented as mean ± standard deviation, minimum and maximum measurements, and number of specimens. d are of specimens collected and measured by SMG and those in standard script by a variety of different field collectors. Statistical difference between the sexes within the different OTUs of *N. matroka* are examined based on student t-tests, n.s. = not significant.

	Total length	Tail length	Hindfoot length	Tragus length	Ear length		
N. robertsi[1] Holotype							
UADBA 43677 ♂	85	32	6	6	13	34	6.′
Paratypes							
FMNH 213931 ♂	84	31	6	6	13	35	7.:
UADBA 43678 ♀	93	35	6	6	13	38	11
N. matroka							
OTU 1 – Central Highlands							
♂♂	80.9 ± 1.90	30.9 ± 1.83	5.0 ± 0.50	6.7 ± 0.50	11.7 ± 0.50	31.9 ±	
	78-85, n=9	28-34, n=9	4-6, n=9	6-7, n=9	11-12, n=9		
♀♀	85.9 ± 1.66	32.5 ± 1.65	5.1 ± 0.27	6.9 ± 0.28	12.5 ± 0.52	33.9 ± (	
	82-88, n=14	30-35, n=14	5-6, n=14	6-7, n=13	12-13, n=13		
T-statistics	T=6.63, *P*<0.0001	T=2.19, *P*=0.04	n.s.	n.s.	T=3.93, *P*=0.0008	T=4.68, *P*=0.0001	T=2.78, *P*=0.01
OTU 2 – lowland central west							
♀♀	87.3 ± 2.12	34.7 ± 2.57	5.0 ± 0.14	7.0 ± 0.00	12.7 ± 0.49	32.6 ±	[2]
	85-92, n=9	30-38, n=12	4.5-5.0, n=12	7-7, n=12	12-13, n=12		
OTU 3 – eastern lowlands							
♀♀	82.8 ± 2.22	32.3 ± 1.26	4.5 ± 0.58	6.0 ± 0.00	10.8 ± 0.50	31.5 ± (	[3]
	81-86, n=4	31-34, n=4	4-5, n=4	6-6, n=4	10-11, n=4		
OTU 4 – eastern mid-elevations							
sexes combined[4]	83.2 ± 2.86	32.6 ± 1.82	5.0 ± 0.00	6.8 ± 0.45	12.4 ± 0.89	32.8 ± 0.84	6.:
	80-87, n=5	30-35, n=5	5-5, n=5	6-7, n=5	11-13, n=5		
N. malagasyensis							
FMNH 175988 ♂	82	37	4	7	11	30	3.9
FMNH 175989 ♀	82	35	5	6	12	32	6.(

[1] All external measurements made from fluid preserved specimens.
[2] Excludes pregnant females. When included 7.4 ± 1.03, 5.5-8.5, n=12.
[3] All pregnant.
[4] Composed of four females and one male.

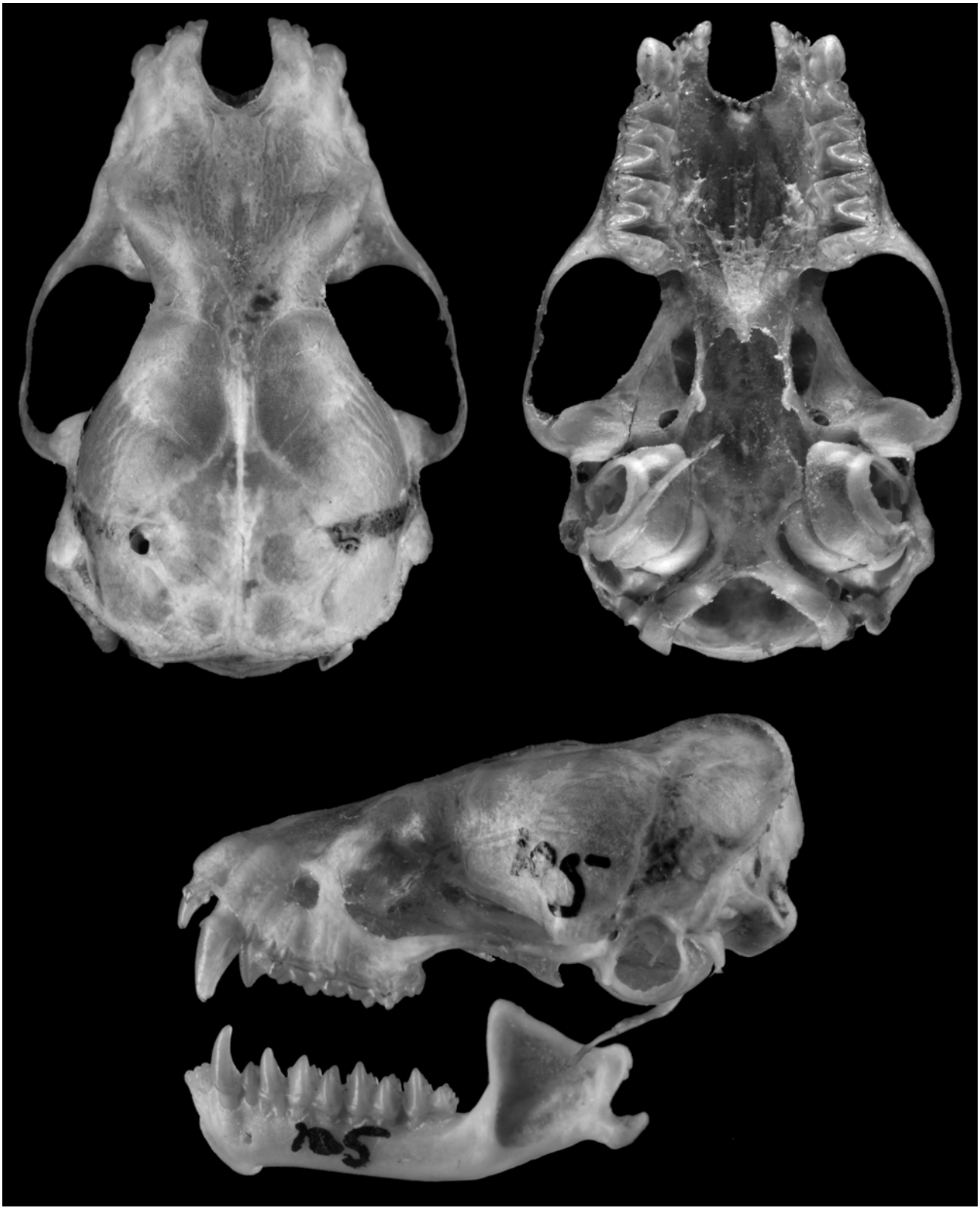

FIGURE 4. Different views of skull and mandible of *Neoromicia robertsi* **sp**. **nov.** (UADBA 43677, RBJ 105), holotype from Province d'Antananarivo, Anjozorobe, Amboasary. Pictures include dorsal view of cranium (upper row, left), ventral view of cranium (upper row, right), and lateral view of cranium and mandible (lower row). (Photograph taken by J. Weinstein, Field Museum image number Z94555_09d.)

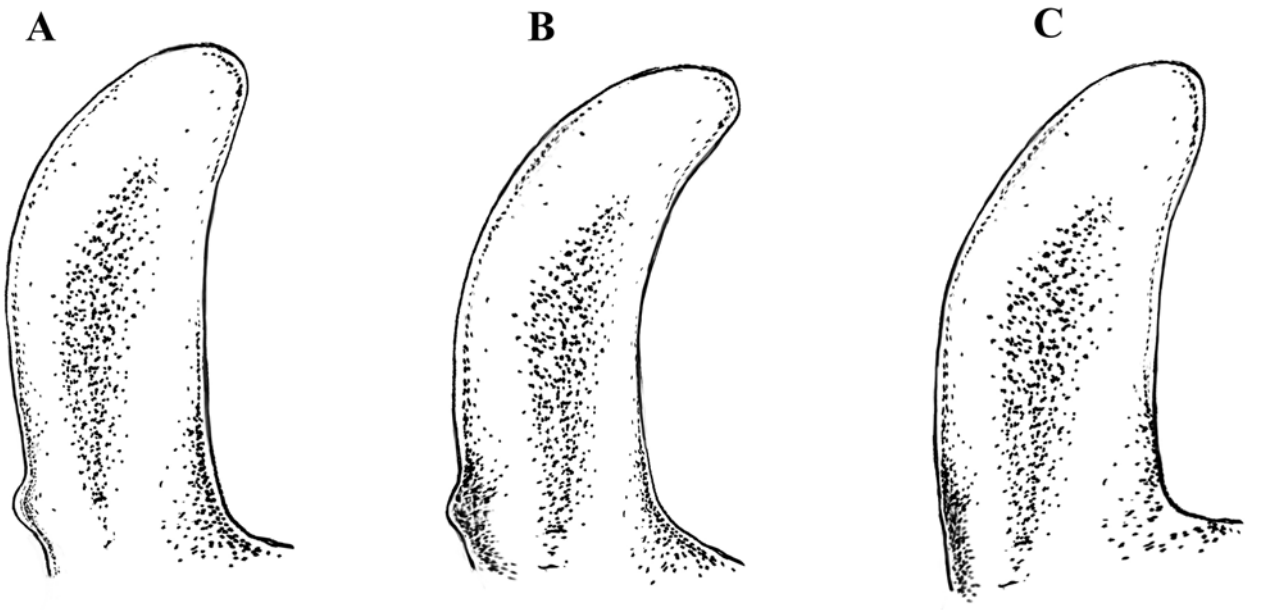

FIGURE 5. Tragus of right ear of different species of Malagasy *Neoromicia*: A) *N. robertsi* **sp. nov.** (UADBA 43677, RBJ 105), holotype, from Anjozorobe; B) *N. matroka* (FMNH 209119), from Ankazobe; and C) *N. malagasyensis* (FMNH 175989), from near Parc National d'Isalo.

representing less than 50% of the length of the former. The main body of the tragus in *N. robertsi* has the two outer margins running mostly in parallel for approximately two-thirds of the proximal length and then turns medially, curves slightly, and terminates with a rounded margin (Figure 5A). Further, there is a distinct notch at the base of the tragus along the posterior border. In comparison, *N. matroka* has a tragus length about 60% that of the ear length, the crescent-shaped tragus terminates as a rounded sickle-shape, and the notch at the posterior base of the structure is less developed than in *N. robertsi* (Figure 5B). In *N. malagasyensis* the tragus length represents between 50-65% the ear length, the tragus is slightly more crescent-shaped than in *N. robertsi*, but not as prominent as in *N. matroka*, and lacks a distinct notch at the base along the posterior border (Figure 5C).

On the basis of external measurements of the type series of *N. robertsi*, this species seems to show sexual dimorphism in size, with the single female specimen being notably larger than the two males in total length, tail length, and forearm length. The two males have an average forearm length of 34.5 mm and the female measures 38 mm, as compared to 31.9 mm and 33.9 mm, respectively, in *N. matroka* from the Central Highlands (OTU 1, Table 3). On average, based on two specimens, *N. malagasyensis* is smaller than the other two Malagasy members of this genus.

Cranio-dental characters. Dental formula in *Neoromicia robertsi*, as in other members of the genus, is I 2/3 C 1/1 P 1/3 M 2/3, comprising the adult dentition of 32 teeth. *Neoromicia robertsi* is easily distinguished from other Malagasy members of this genus based on its notably larger cranial measurements (Table 4). Of the two skulls available of this taxon (both males), the greatest skull length of UADBA 43677 (holotype) is 14.3 mm and FMNH 213931 is 14.6 mm. These measurements are larger and do not overlap with the average greatest skull length of *N. matroka* from the Central Highlands (OTU 1) of 12.9 mm and in *N. malagasyensis* of 12.8 mm. Further, with the exception of postorbital width, the different cranial measurements of *N. robertsi* are larger and non-overlapping with those of *N. matroka* and *N. malagasyensis*. In *N. robertsi*, the supraorbital ridge is notably more inflated than *N. matroka* and *N. malagasyensis*.

Neoromicia robertsi is easily distinguished from other Malagasy *Neoromicia* based on its notably larger upper and lower dental measurements (Table 5). Of the two skulls available of this taxon (both males), the length of the complete cranial tooth row of UADBA 43677 (holotype) and FMNH 213931 are both 6.0 mm. These values are notably larger and do not overlap with the average length of the complete cranial tooth row of male *N. matroka* from the Central Highlands (OTU 1) of 5.1 mm and in *N. malagasyensis* of 4.7 mm. The upper tooth rows in *N. robertsi* are largely in parallel, while in *N. matroka* and *N. malagasyensis* are more of an arc shape and converge anteriorly.

TABLE 4. Cranial measurements (in millimeters) of adult *Neoromicia robertsi* **sp. nov.**, *N. matroka*, *N. malagasyensis*, and *N. capensis*. Measurements presented as mean ± standard deviation, minimum and maximum measurements, and number of specimens. See Methods f on of variable acronyms. Statistical differences between the sexes within the different OTUs of *N. matroka* are examined based on student t-tests, n.s. = not significant.

	GSKL	CIL	ZYGO	POB	MAST	P	
N. robertsi Holotype							
UADBA 43677 ♂	14.3	14.0	9.5	3.6	8.1	5.5	10.0
Paratype							
FMNH 213931 ♂	14.6	14.1	9.5	3.6	8.3	5.3	10.4
N. matroka							
Holotype							
BMNH 97.9.1.32 ♂	13.3	12.9	8.8	3.5	7.7	4.8	9.4
OTU 1 – Central Highlands[1]							
♂♂	12.9 ± 0.26 12.4-13.3, n=12	12.4 ± 0.27 12.0-12.9, n=12	8.6 ± 0.25 8.3-9.2, n=11	3.5 ± 0.12 3.3-3.7, n=13	7.5 ± 0.18 7.3-7.9, n=13	4.6 ± 0.1 4	
♀♀	13.2 ± 0.30 12.5-13.7, n=17	12.8 ± 0.31 12.2-13.3, n=17	8.8 ± 0.20 8.5-9.1, n=14	3.4 ± 0.10 3.2-3.6, n=17	7.7 ± 0.17 7.4-7.9, n=17	4.8 ± 0.1 4	
T-statistics	T=3.02, *P*=0.005	T=2.95, *P*=0.007	n.s.	n.s.	T=3.00, *P*=0.006	T=2.53, *P*=0.02	T=3.28, *P*=0.0003
OTU 2 – lowland central west							
♀♀	13.1 ± 0.35 12.5-13.6, n=12	12.7 ± 0.40 12.0-13.3, n=12	8.7 ± 0.22 8.4-9.0, n=12	3.5 ± 0.09 3.3-3.6, n=12	7.6 ± 0.22 7.2-7.9, n=12	4.7 ± 0.2 4	

continued next page

[1] Includes measurements of holotype (BMNH 97.9.1.32).

TABLE 4. (continued)

	GSKL	CIL	ZYGO	POB	MAST	P	
OTU 3 -- eastern lowlands							
♀♀	12.2 ± 0.17 12.0-12.4, n=4	11.9 ± 0.10 11.8-12.0, n=4	8.6, 8.7	3.6 ± 0.10 3.5-3.7, n=4	7.3 ± 0.06 7.2-7.3, n=4	3.8 ± 0.1 3	
OTU 4 – eastern mid-elevations							
♂♂	13.1 ± 0.31 12.8-13.4, n=3	12.6 ± 0.20 12.4-12.8, n=3	8.5 ± 0.15 8.4-8.7, n=3	3.7 ± 0.06 3.6-3.7, n=3	7.5 ± 0.15 7.3-7.6, n=3	4.7 ± 0.1 4	
♀♀	13.3 ± 0.15 13.1-13.4, n=4	12.7 ± 0.25 12.4-13.0, n=4	9.2 ± 0.45 8.7-9.6, n=3	3.6 ± 0.13 3.4-3.7, n=4	7.6 ± 0.32 7.3-8.0, n=4	4.8 ± 0.1 4	
T-statistics	n.s.	n.s.	n.s.	n.s.	n.s.	n	
N. malagasyensis Holotype ROM 42713 ♀	13.3	12.7	8.8	3.7	7.7	4.8	
Sexes combined[2]	12.8 ± 0.44 12.5-13.3, n=3	12.1 ± 0.53 11.7-12.7, n=3	8.7, 8.8	3.5 ± 0.25 3.2-3.7, n=3	7.2 ± 0.25 6.9-7.7, n=3	4.6 ± 0.21 4	8.2, 8.3
N. somalicus Holotype BMNH 98.6.9.1 unsexed	12.2	11.9	--	3.0	7.1	4	
sexes combined [3]	12.0 ± 0.21 11.6-12.2, n=8	11.6 ± 0.30 11.1-11.9, n=8	7.8 ± 0.32 7.4-8.0, n=3	3.1 ± 0.07 3.0-3.2, n=8	6.7 ± 0.21 6.5-7.1, n=7	4.3 ±0.11 4	8.2 ± 0

[2] Includes measurements of holotype (ROM 42713).
[3] Includes measurements of holotype (BMNH 98.6.9.1).

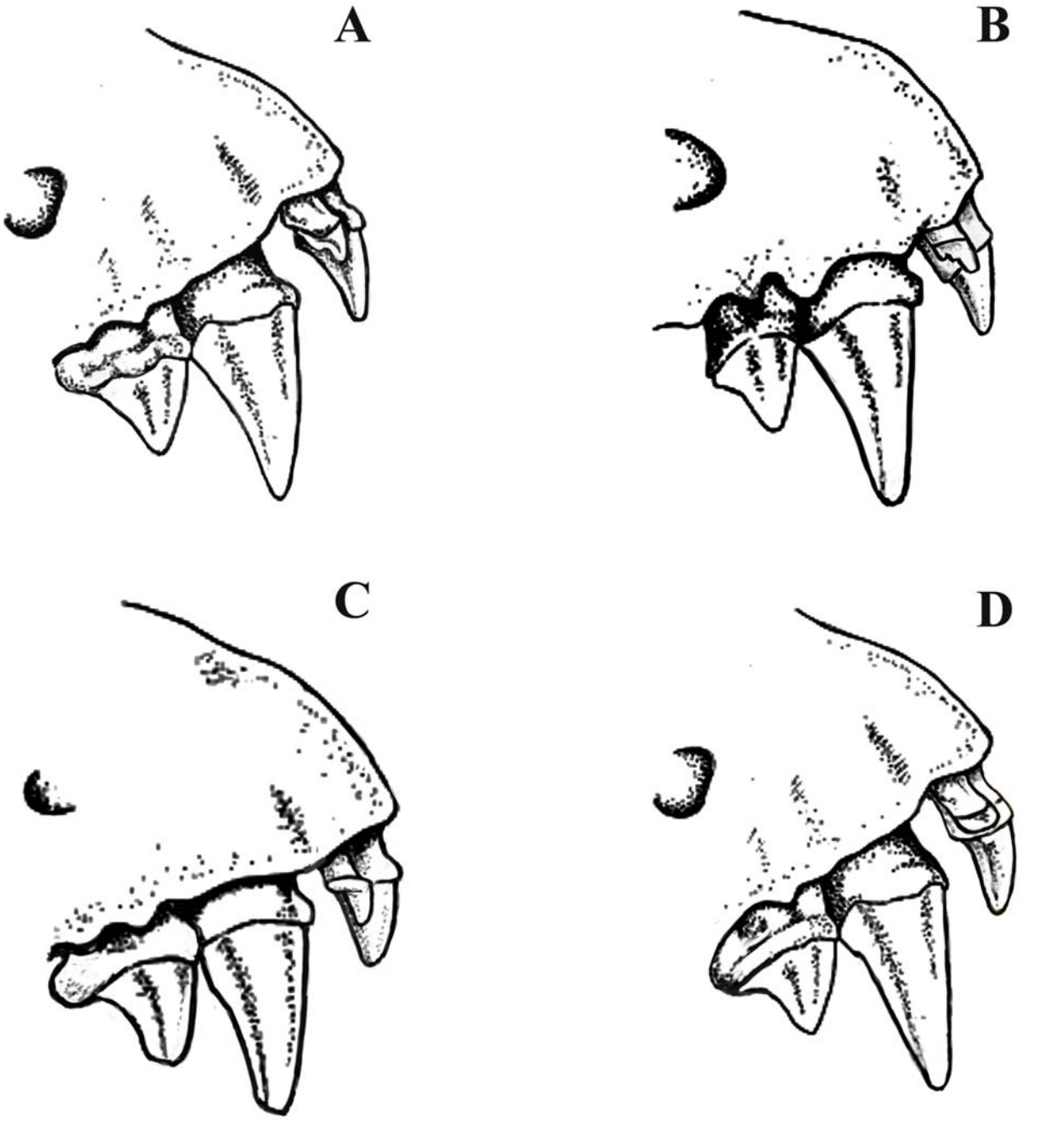

FIGURE 6. Lateral view of the right anterior portion of skull of three different species of Malagasy *Neoromicia*: A) *N. robertsi* **sp. nov.** (UADBA 43677, RBJ 105), holotype, Anjozorobe, with relatively unworn teeth; B) *N. matroka* (FMNH 194153), Ankazobe, which has relatively unworn teeth and closely matched the holotype (BMNH 97.9.1.32); C) *N. malagasyensis* (FMNH 175988), from near Parc National d'Isalo, with slightly worn teeth; and D) *N. capensis* (FMNH 195629), Marloth Park, which closely matched the lectotype (BMNH 49.8.16.21), South Africa, although with notably worn teeth. Skull images adapted and modified from Bates *et al*. (2006).

In *N. robertsi*, the 2nd upper incisor, which is approximately one-half the height of the 1st upper incisor, has a distinct single cusp on the posterior margin, as compared to *N. matroka*, which has a more complicated bicuspid 2nd incisor (Figures 6A and 6B). The incisors in *N. malagasyensis* are distinctly different with the 2nd upper incisor without a distinct secondary cusp and the 1st upper incisor being about two-thirds the length of 2nd (Figure 6C). Further, in *N. capensis*, including the holotype (BMNH 97.9.1.32), the 2nd upper incisor is a peg-like tooth with no clear secondary structure (Figure 6D). The diastema between the 2nd upper incisor and the upper canine is most prominent in *N. robertsi* as compared to the other Malagasy members of this genus.

TABLE 5. Dental measurements (in millimeters) of adult *Neoromicia robertsi* **sp. nov.**, *N. matroka*, *N. malagasyensis*, and *N. capensis*. Measurements presented as mean ± standard deviation, minimum and maximum measurements, and number of specimens. See Methods for an expl able acronyms. Statistical differences between the sexes within the different OTUs of *N. matroka* are examined based on student t-tests, n.s. = not significant.

	I-M^3	C-M^3	C-C	M^3-M^3	c-m_3
N. robertsi Holotype					
UADBA 43677 ♂	6.0	4.9	4.6	6.2	5.6
Paratype					
FMNH 213931 ♂	6.0	5.2	4.6	6.4	5.5
N. matroka					
Holotype					
BMNH 97.9.1.32 ♂	5.3	4.6	4.0	5.6	4.8
OTU 1 – Central Highlands[1]					
♂♂	5.1 ± 0.11	4.4 ± 0.14	4.0 ± 0.16	5.5 ± 0.17	4.7 ± 0.13
	5.0-5.3, n=13	4.2-4.7, n=13	3.8-4.3, n=13	5.3-5.9, n=13	4.5·
♀♀	5.1 ± 0.17	4.5 ± 0.17	4.1 ± 0.14	5.6 ± 0.13	4.9 ± 0.14
	4.9-5.4, n=17	4.2-4.8, n=16	3.7-4.3, n=16	5.4-6.0, n=17	4.7·
T-statistics	n.s.	n.s.	n.s.	n.s.	T=; *P*=0.01
OTU 2 – lowland central west					
♀♀	5.2 ± 0.13	4.5 ± 0.14	4.1 ± 0.11	5.6 ± 0.12	4.9 ± 0.07
	5.0-5.5, n=12	4.2-4.7, n=12	3.9-4.3, n=12	5.4-5.8, n=12	4.7·

continued next page

[1] Includes measurements of holotype (BMNH 97.9.1.32).

TABLE 5. (continued)

	I-M^3	C-M^3	C-C	M^3-M^3	c-m$_3$
OTU 3 -- eastern Lowlands					
♀♀	4.9 ± 0.08 4.8-5.0, n=4	4.1 ± 0.05 4.1-4.2, n=4	4.0 ± 0.10 3.9-4.1, n=4	5.3 ± 0.10 5.2-5.4, n=4	4.5 ± 0.02 4.4·
OTU 4 – eastern mid-elevations					
♂♂	5.1 ± 0.10 5.0-5.2, n=3	4.5 ± 0.10 4.4-4.6, n=3	4.1 ± 0.06 4.1-4.2, n=3	5.7 ± 0.00 5.7-5.7, n=3	4.7 ± 0.12 4.6·
♀♀	5.3 ± 0.09 5.0-5.4, n=4	4.5 ± 0.04 4.4-4.6, n=4	4.2 ± 0.09 4.0-4.4, n=4	6.0 ± 0.17 5.7-6.1, n=4	4.9 ± 0.17 4.8·
T-statistics	n.s.	n.s.	n.s.	n.s.	n.s.
N. malagasyensis Holotype ROM 42713 ♀	4.5	4.4	4.0	5.7	4.7
Sexes combined[2]	4.7 ± 0.15 4.5-4.8, n=3	4.3 ± 0.15 4.1-4.4, n=3	3.8 ± 0.21 3.6-4.0, n=3	5.3 ± 0.38 5.0-5.7, n=3	4.6 ± 0.12 4.5·
N. somalicus Holotype BMNH 98.6.9.1 unsexed	5.0	4.3	3.8	5.2	4.7
Sexes combined[3]	4.8 ± 0.21 4.5-5.0, n=8	4.1 ± 0.18 3.9-4.3, n=8	3.6 ± 0.15 3.4-3.8, n=7	4.9 ± 0.27 4.6-5.3, n=8	4.5 ± 0.16 4.3·

[2] Includes measurements of holotype (ROM 42713).
[3] Includes measurements of holotype (BMNH 98.6.9.1).

Male baculum characters. The baculum of *N. robertsi* is typical of the genus *Neoromicia*, with strong ventral deflection of the tip, but diagnostically larger than any described species.

Natural history, distribution, and conservation status. *Neoromicia robertsi* is only known from two localities in central eastern Madagascar, where it occurs in sympatry with *N. matroka* (Figure 1). The specimens of *N. robertsi* from the Parc National de Mantadia were obtained at an elevation between 900 and 1000 m, and the holotype from Anjozorobe, between 1200 and 1300 m. These two sites are both associated with partially degraded habitats, one of which is in close proximity to a forested zone. Hence, *N. robertsi* does not appear to be a forest restricted species, but has not been found roosting in a synanthropic context, which is known for *N. matroka* (Goodman 2011).

No precise details are known about the reproductive ecology of *N. robertsi*. The pair obtained on 8 November 2002 at Mantadia (UADBA 43678, FMNH 213931) included a male with relatively large testes and, based on the form and shape of the mammae, a female that recently gave birth and lactated. The holotype (UADBA 43677) was an adult male.

Bioacoustic information of animals that can be definitively identified as *N. robertsi* is not available. Kofoky *et al.* (2009) made recordings of hand-released individuals of *Neoromicia* at Mantadia and Anjozorobe, sites *N. matroka* and *N. robertsi* are known to occur, and based on the forearm length of released individuals it is not possible to determine which species was involved. *Neoromicia* at these sites had an average frequency of maximum energy of 41.5 kHz (range 39.2-44.3 kHz); average maximum frequency of 69.2 kHz (range 60.3-88.2 kHz); average minimum frequency of 37.7 kHz (range 35.5-39.3 kHz); average duration of 5.7 ms (range 3.8-8.0 ms); and average interpulse interval of 109.9 ms (range 65.2-196.7 ms), as compared to *N. malagasyensis* near Isalo where these values were 45.7 kHz (range 41.4-51.0 kHz), 79.8 kHz (range 60.3-100.0 kHz), 40.5 kHz (range 32.4-45.5 kHz), 4.9 ms (range 3.6-6.3 ms), and 34.2 ms (range 34.2-94.4 ms), respectively. Thus, while it is not apparent if differences exist between *N. matroka* and *N. robertsi* in bioacoustic parameters, *N. malagasyensis* shows some divergent aspects in their echolocation calls.

An older mummified specimen (MNHN 1882.1964) was obtained by Jean Auguste Lantz in the "interieur de Madagascar C. est, mai 1881" at a locality that cannot be properly read from the specimen label but appears to be "Ambohiramiane". The skull still remains in the specimen, but based on the coloration of the notably long pelage, the partially furred proximal portions of the upper surface of the ears, and a forearm length of 34 mm, it may be referable to *N. robertsi*.

In total, we have examined over 60 specimens of *N. matroka*, as compared to three specimens of *N. robertsi*. The disproportional number of specimens of the former species may be in part associated with its broader distribution and use of buildings for day roost sites, providing easier access to field collectors. However, based on current information, *N. robertsi* has a limited distribution and appears notably less common than *N. matroka*. Further, information is needed to assess its distribution and population size, in order to properly evaluate its conservation status.

Discussion

In the context of this current study, the decision that *Neoromicia robertsi,* previously identified as a Malagasy population of *N. melckorum* by Bates *et al*. (2006), was an undescribed species was based on an integrated approach of diagnostic characters associated with molecular, cranio-dental morphology, and bacula size and shape. One of the important points of departure is that the name *melckorum*, as currently configured, is a junior synonym of *N. capensis* based on cranio-dental, bacular, and karyological characters (Rautenbach *et al*. 1993; Kearney *et al*. 2002; Kearney 2005; Monadjem *et al*. 2010). Secondly, our molecular phylogeny indicates that southern African animals referred to *N. capensis* are the sister species of *N. matroka*. Finally, the animals from Madagascar previously referred to *N. melckorum* by Bates *et al*. (2006) are sister to *N. malagasyensis*; these two species can be easily differentiated based on molecular genetics, bacular characters, and cranio-dental differences.

Kearney *et al*. (2002) showed that, whilst extremely useful for species delimitation, bacular morphology failed to yield unequivocal synapomorphies for *Neoromicia* as defined chromosomally. While a well developed baculum tip (relative to base) and ventral deflection of the tip are typical of most members of *Neoromicia* (but not for example, *N. nana*), an identical shape has been found in *Laephotis* cf. *wintoni* Thomas, 1901 and to lesser extent, *Hyp-*

sugo anchietae (Kearney *et al*. 2002), suggesting convergent evolution or possible paraphyly. Nevertheless, in the absence of chromosomal characters but as supported by molecular data, the prevalence of the above-cited *Neoromicia*-type characters in the Malagasy species *matroka*, *malagasyensis*, and *robertsi* certainly provides support for their inclusion in *Neoromicia*. The conservation of the *capensis*-type baculum in its sister-species, *N. matroka*, in our study demonstrates that baculum morphology can be conserved across sister species. Nevertheless, in the species-pair *robertsi* – *malagasyensis*, although the baculum morphology shares some characteristics (enlarged and ventrally-deflected tip), there are several subtle differences such as the *anchietae*-like shape and absence of dorsal projection of the tip in *malagasyensis*. Baculum morphology can reveal cryptic species, as indicated by the extremely novel and *H. anchietae*-like morphology displayed by small *N*. cf. *malagasyensis* individuals from Kirindy Forest (CNFEREF).

To examine further patterns of morphological variability in Malagasy species of *Neoromicia*, we conducted a PCA analysis on cranio-dental measurements of *N. robertsi*, *N. matroka*, *N. malagasyensis*, as well as *N. somalicus* for which the form *malagasyensis* was previously considered a subspecies of. In these analyses, holotypes of these taxa were included to provide a point of comparison to assess intra-specific variation. The first two unrotated principal components (PCs) accounted for 86.1% of the total variance of the cranio-dental morphology (Figure 7; Table 6). All of the variables, excluding POB, loaded heavily on PC1. The only variable that showed a high factor loading on another PC was POB and this was on PC2. In the analysis, *N. robertsi* is notably separate from the balance of these taxa, based on its relatively large cranial and dental measurements. Further, the specimens identified as *N. somalicus*, including the holotype, have proportionately small skulls and form a distinct, although slightly diffused cluster. In comparison, the animals referred to *N. malagasyensis* show considerable size variation in cranio-dental measurements, which might be related to sexual dimorphism or the specimens used in this analysis might represent more than one species. Animals referred to *N. matroka* and divided into different geographical units (OTUs) demonstrate considerable variability in cranial and dental measurements, forming a large and slightly dispersed cluster. Most divergent amongst these different OTUs is the lowland eastern population (OTU 3) and this variation warrants some further investigation. In any case, on the basis of the PCA analysis, there is no overlap in two Malagasy species, *N. matroka* and *N. robertsi* (Figure 1), which are known to occur in at least partial sympatry.

TABLE 6. Factor loadings from principal component analysis of log-transformed cranial and dental measurements of specimens of *Neoromicia robertsi* **sp. nov.**, *N. matroka*, *N. malagasyensis*, and *N. somalicus*. A graphical representation of the first two factors for this analysis is presented in Figure 7. See Methods and materials for the acronym definitions. The variables ZYGO and MAND have not been used in the analysis to maximize the number of specimens that could be used, particularly holotypes. The values in bold indicate the variables that are notably correlated with a given factor (<0.75).

Cranial	PC1	PC2	PC3
GSKL	**-0.961**	0.123	0.128
CIL	**-0.978**	0.062	0.055
POB	-0.550	**-0.760**	0.251
MAST	**-0.892**	-0.172	0.015
PAL	**-0.767**	0.405	0.463
I-M^3	**-0.880**	0.068	-0.325
C-M^3	**-0.924**	0.175	-0.039
C-C	**-0.892**	-0.253	-0.131
M^3-M^3	**-0.918**	-0.135	-0.122
i-m_3	**-0.904**	0.233	-0.145
Eigenvalue	7.652	0.962	0.457
Proportional sum of total explained variation	76.5%	86.1%	90.7%

Milne Edwards (1881) described a small species of vespertilionid from Madagascar under the name *Vespertilio humbloti*, but the status of this taxon could not be previously assessed, as the type series remained unlocated (Peterson *et al.* 1995). In the MNHN, a jar was found in the fluid preserved collection holding the syntype series of spec-

imens used by Milne Edwards in the description of *V. humbloti* and with the catalog numbers MNHN 1986.1074 to 1986.1082. The majority of the animals were immature. Through the courtesy of Dr. Cécile Callou of the MNHN, the skulls from two of the adult specimens were extracted, cleaned, and allocated the numbers MNHN 1986.1074 and 1986.1075. These specimens have upper premolars notably different from *N. robertsi*. Hence, in this current revision, *V. humbloti* does not need to be considered as a possible suitable name for the species described herein.

On the basis of the bacular and molecular genetic analyses, there is good evidence that *N. malagasyensis* and *N. robertsi* represent sister species. The former taxon has a distribution limited to the transitional dry-humid forests surrounding the Isalo Massif in south-central Madagascar and the latter to upland of the central east characterized by montane humid forest formations. Associated with climatic shifts that took place in recent geological history (Wilmé *et al*. 2006; Vences *et al.* 2009) former humid forest corridors that linked these two areas were severed and gave rise to numerous examples of vicariant species resembling the relationships uncovered here between *N. malagasyensis* and *N. robertsi* (e.g., Boumans *et al.* 2007; Carleton & Goodman 2007; Mercurio *et al.* 2008).

Even though only known from three specimens taken in montane areas in the eastern central portion of Madagascar, as compared to the notably common and widespread *N. matroka*, *N. robertsi* is a well-defined taxon based on different characters. Now that it is described, fieldwork needs to be conducted to obtain data on its distribution, ecology, and bioacoustics. This information is critical to help define this species' conservation status.

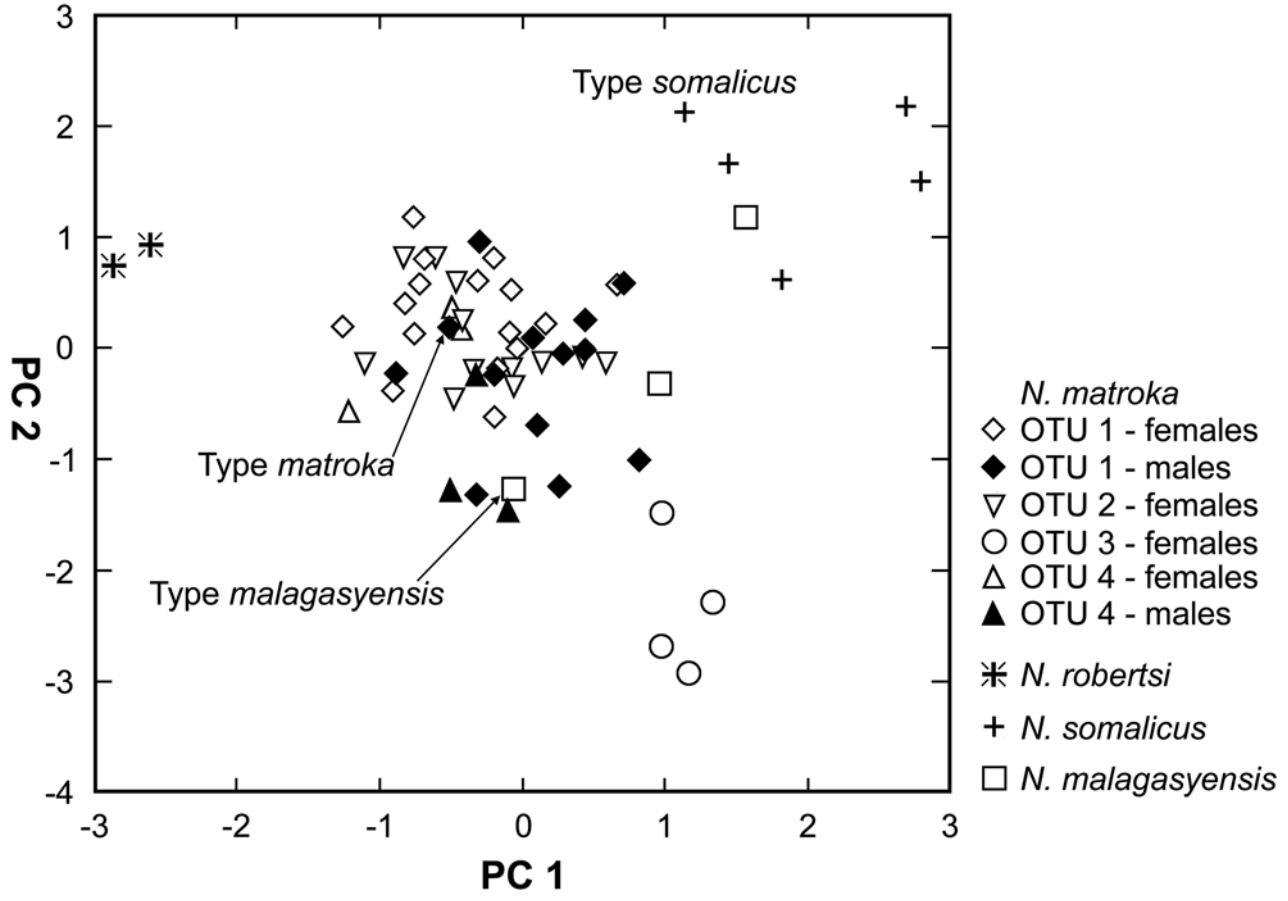

FIGURE 7. Projection of first two unrotated principal components for morphologically similar or phylogenetically closely related species of *Neoromicia* spp. from Madagascar and continental Africa. Of the two specimens of *N. robertsi* used in the analysis, the one to the lower left is a male and the other a female. PC1 and PC2 clearly separate the sympatrically occurring Malagasy species *N. robertsi* **sp. nov.** and *N. matroka*. This latter species shows considerable morphological variation, which needs to be investigated in further detail. See Table 6 for the variables used in the analysis and the associated loadings.

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APPENDIX 1. Details of specimens used in the morphological analyses. The definitions of museum acronyms are: **BMNH**: British Museum (Natural History), currently The Natural History Museum; **FMNH**: Field Museum of Natural History; **MCZ**: Museum of Comparative Zoology, Harvard University; **MNHN**: Muséum national d'Histoire naturelle (Paris); **ROM**: Royal Ontario Museum; **USNM**: The United States National Museum, currently National Museum of Natural History.

Neoromicia malagasyensis
220 km NE Tulear (ROM 42713 – HOLOTYPE); Parc National de Isalo (FMNH 175988, 175989).

Neoromicia cf. *malagasyensis*
Forêt de Kirindy (CNFEREF) (FMNH 213576, 213577).

Neoromicia matroka
OTU 1 --- Ambohimitombo (BMNH 66.6052, FMNH 5646); Ambositra (BMNH 97.9.1.34, 97.9.32 – HOLOTYPE, 1989.16, MCZ 45115); Ampitambe (BMNH 97.9.1.33); Ankazobe (FMNH 184467-184485, 209119); Antananarivo (MNHN 1957.145, ROM 41943, 41944, 41986); Fianarantsoa (FMNH 184465, 184466); Forêt de Maromizaha (FMNH 184884), Ivohimanitra (BMNH 97.9.1.36); Manambolo, Ambavafatra (FMNH 167660); Réserve Spéciale d'Ambohitantely (FMNH 194153).
OTU 2 – Ambalavao (FMNH 184464); Zazafotsy (FMNH 184453-184463).
OTU 3 – Andasibe (FMNH 184601-184603); 0.5 km N. Kianjavato (USNM 448936, 448937); Mahabo (FMNH 188055-188058).
OTU 4 – Anjiro (FMNH 188221, 188222); Amparafara (FMNH 108983, 108984).
Not allocated to OTU – Andreba (MNHN 1985.105); Bealanana (BMNH25.12.9.15, 25.12.9.17, 25.12.9.20).

Neoromicia roberti
Anjozorobe, Amboasary (UADBA 43677 – HOLOTYPE); Parc National de Mantadia (FMNH 213931, UADBA 43678).

Neoromicia somalicus
Ethiopia, 10 km W. Mabil, Blue Nile Gorge (BMNH 70.486); Ethiopia, mouth of the River Fineha (BMNH 70.484, 70.485); Ethiopia, [Waderc Ogaden] (BMNH 54.1015); Somalia, Harigisa (BMNH 98.6.9.1 – HOLOTYPE); Somalia, Berbera (BMNH 98.6.8.5); Tanzania, Tanangire (FMNH 187142); Tanzania, Tandamanga (BMNH 64.1789).

Vespertilio humbloti
MNHN 1986.1074, 1986.1075.