

A phylogeny for African *Pipistrellus* species with the description of a new species from West Africa

(Mammalia: Chiroptera)

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Abstract

Pipistrelloid bats are among the most poorly known bats in Africa, a status no doubt exacerbated by their small size, drab brown fur, and general similarity in external morphology. The systematic relationships of these bats have been a matter of debate for decades, and despite some recent molecular studies, much confusion remains. Adding to the confusion has been the recent discovery of numerous new species. Using two mitochondrial genes, we present a phylogeny for this group that supports the existence of three main clades in Africa: *Pipistrellus*, *Neoromicia* and the newly described *Parahypsugo*. The basal branches of the tree, however, are poorly supported. Using an integrative taxonomic approach, we describe a new species of *Pipistrellus* from West Africa, which has been labelled *Pip. cf. grandidieri* in the literature. We demonstrate that it is not closely related to *Pip. grandidieri* from East Africa, but instead is sister to *Pip. hesperidus*. Furthermore, the species *Pip. grandidieri* appears to be embedded in the newly described genus *Parahypsugo*, and therefore is better placed in it than in *Pipistrellus*. This has important taxonomic implications because a new subgenus (*Afropipistrellus*) described for *Pip. grandidieri* predates *Parahypsugo* and should therefore be used for the entire “*Parahypsugo*” clade. The Upper Guinea rainforest zone, and particularly the upland areas in the south-eastern Guinea – northern Liberia border region may represent a global hotspot for pipistrelloid bats and should receive increased conservation focus as a result.

INTRODUCTION

The mammalian fauna of Africa remains poorly known with many new species having been described over the past decade (Taylor *et al.*, 2018; Monadjem *et al.*, 2019). This is partly due to a change in the way that species are recognised and diagnosed (Taylor, Denys & Cotterill, 2019), but also as a result of increased accessibility to remote localities (Davenport *et al.*, 2006; Hart *et al.*, 2012), and the use of novel tools for capturing mammals such as harp traps and camera traps (Rovero *et al.*, 2008; Monadjem, Richards & Denys, 2016). It is now becoming clear that the species richness of African mammals has been severely under-represented; indeed, more mammals remain to be described on the African continent than on any other landmass, with an estimated 12% of species not yet discovered (Fisher *et al.*, 2018). In fact, this estimate itself is probably an underestimate considering the number of undescribed lineages of bats that have recently been reported for East Africa alone (Demos *et al.*, 2018, 2019b,a; Patterson *et al.*, 2019). This has implications for conservation, since it is difficult to conserve something that has not yet been discovered (Jones *et al.*, 2009).

The problem of undiscovered taxa is neatly exemplified by the *Pipistrellus*-like or “pipistrelloid” bats (*sensu* Volleth & Heller (1994), Monadjem *et al.* (2013)) represented in Africa by genera: *Pipistrellus* Kaup, 1829, *Neoromicia* Roberts, 1926, *Hypsugo* Kolenati, 1856, and *Parahypsugo* Hutterer *et al.*, 2019. The first two genera are widespread across the continent, whereas the third only occurs north of the Sahara Desert, and the newly described genus *Parahypsugo* is restricted to the equatorial forest zone (Hutterer *et al.*, 2019). Based on four extensive surveys conducted in the 1960s, 1970s and 1980s, six species of pipistrelloids were known to occur at Mount Nimba (Coe, 1975; Verschuren, 1976; Brosset, 1984). However, based on two 4-week surveys in 2010 and 2011, this total was increased to 10 taxa, with four of them new to science (Monadjem *et al.*, 2013), representing an under-estimate of 40% at a relatively well-surveyed site (Brosset, 1984, 2003). Of the four unnamed taxa, three have now been formally described as new species: *Neoromicia roseveari* Monadjem *et al.*, 2013, *Neoromicia isabella* Decher, Hutterer & Monadjem, 2015, and *Parahypsugo happoldorum* Hutterer *et al.*, 2019 (Monadjem *et al.*, 2013; Decher *et al.*, 2015; Hutterer *et al.*, 2019). The fourth taxon, *Pipistrellus* sp. nov., is described in this paper.

The pipistrelloid vesper bats have had a long and tortuous history of generic shuffling (Hooper & van den Bussche, 2003; Roehrs, Lack & Van Den Bussche, 2010). Generic boundaries were originally defined based on

morphological characters, particularly ear and tragus shape, features of the skull, and dentition. Specifically, the presence or absence of the upper anterior premolar (P²) was used to place this group of vesper bats into either *Pipistrellus* or *Eptesicus* Rafinesque, 1829, a practice that continued to relatively recent times (Meester *et al.*, 1986). However, an extensive review of this group based mainly on baculum morphology, recognised the generic distinction of *Eptesicus* and placed the remaining African species in the genus *Pipistrellus* (Hill & Harrison, 1987); the monophyly of *Eptesicus* has now been established by genetics (Juste *et al.*, 2013). However, Hill and Harrison (1987) noted significant variation in the bacula of African *Pipistrellus* species, and suggested that these may correspond with previously described subgenera, notably *Neoromicia*, *Pipistrellus* and *Hypsugo*; these were subsequently elevated to generic rank based on chromosomal characteristics (Volleth *et al.*, 2001). A detailed study of the baculum and chromosomes of southern African species appeared to corroborate this classification (Kearney *et al.*, 2002), at least for that region.

Recent molecular studies of African species have generally recovered two of the three clades, namely *Pipistrellus* and *Hypsugo*, but the monophyly of *Neoromicia* is not certain (Roehrs *et al.*, 2010; Goodman *et al.*, 2012; Monadjem *et al.*, 2013; Decher *et al.*, 2015; Hutterer *et al.*, 2019). Furthermore, the African “*Hypsugo*” clade has been shown to be generically and morphologically distinct from the Eurasian species of this genus, and has therefore been placed in a new genus *Parahypsugo* (Hutterer *et al.*, 2019). However, much uncertainty remains in understanding the phylogeny of African pipistrelloids, particularly since all the above-mentioned studies were focused on only part of the continent, West Africa. In addition, the relationship of the monotypic genus *Nycticeinops* Hill & Harrison, 1987 to African pipistrelloids remains unresolved. This species has a single incisor in the upper jaw, compared with two incisors in all other African pipistrelloids (Monadjem *et al.*, 2010). Yet, *Nycticeinops* shares many other morphological properties of the cranium and baculum with *Neoromicia*, *Pipistrellus*, and *Hypsugo* (Hill & Harrison, 1987). Furthermore, in a recent multi-locus phylogeny, *Nycticeinops* was located within the tribe Vespertilionini and appears to be sister to *Parahypsugo* (Amador *et al.*, 2018). The genus *Neoromicia* is also included in this tribe. In contrast, the genus *Pipistrellus* was situated in a separate tribe, the Pipistrellini (Amador *et al.*, 2018).

In addition to confusion at the generic level, the relationships between species within a genus remain poorly resolved. One such example concerns the taxon *Pipistrellus grandidieri* (Dobson, 1876) whose systematic relationships, despite having been described in 1876 from Zanzibar, are still not fully understood (Thorn, Kock

& Cuisin, 2007). Based on skull and baculum features, it was assigned to a newly described subgenus *Afropipistrellus* Thorn, Kock & Cuisin, 2007 (Thorn *et al.*, 2007), and remains the only member of that group to date. To further complicate matters, a superficially similar taxon has been infrequently collected in West Africa, over 4,500 km to the west, which has been tentatively assigned to this species, and referred to as *Pip. cf. grandidieri* (Decher *et al.*, 2015). This species is known from only two localities, both in upland rainforest, on the border zone between Liberia and Guinea (Monadjem *et al.*, 2013; Decher *et al.*, 2015).

We use an integrative approach to delimit species boundaries within African members of the *Pipistrellus* group and the related genera *Neoromicia* and *Parahypsugo*, to demonstrate that the species labelled as *Pipistrellus cf. grandidieri* from West Africa is specifically distinct from *Pipistrellus grandidieri*. Furthermore, we show that the latter species in fact is better placed in the newly described genus *Parahypsugo* (Hutterer *et al.*, 2019).

MATERIALS AND METHODS

STUDY SITES

Specimens of pipistrelloid bats of the genera *Neoromicia*, *Parahypsugo* and *Pipistrellus* were collected as part of general small mammal surveys at Mount Nimba and Wologizi National Forest (Liberia), and Zياما Man Biosphere Reserve (Guinea). The surveys at Mount Nimba have been described in detail in previous publications (Monadjem *et al.*, 2013, 2016). In brief, Mount Nimba straddles three countries: Liberia, Côte d'Ivoire and Guinea, rising to 1,768 m above sea level which represents the second highest peak west of Mount Cameroon. The lower slopes are covered by rainforest, but this gives way to savanna and then grassland above 1,500 m. On the Guinean side, the mountain was protected as a Strict Nature Reserve, which was subsequently proclaimed a Biosphere Reserve in 1980. However, in 1992, a large portion of this reserve was de-proclaimed and reserved for mining. On the Liberian side, the East Nimba Nature Reserve was proclaimed in 2003. This protected area has been supported recently by ArcelorMittal Liberia which views it as a potential off-set site for its current mining activities. For further details, see Monadjem *et al.* (2016).

In contrast, the results of the surveys at Wologizi National Forest (Wologizi hereafter) and Zياما Man Biosphere Reserve (Zياما hereafter) have only recently been submitted for publication (M. Mamba, A. Monadjem & T. Mahlaba, submitted manuscript) and are outlined here. Wologizi and Zياما form part of a

transfrontier initiative called the Zياما-Wonegizi-Wologizi Transboundary Forest Landscape (ZWW hereafter). The importance of this region has been recognized by Fauna & Flora International that has funded a short-term study which aims to strengthen protected area management, by promoting innovative cross-border collaboration between Liberia and Guinea (for further information see: <https://www.fauna-flora.org/projects/implementing-effective-management-wonegizi-landscape>). Wologizi is situated in north-western Liberia near the Guinean border, while Zياما is situated in south-eastern Guinea across the border from Wologizi. The Wonegizi mountain range runs between Wologizi and Zياما.

Wologizi and Zياما both lie at higher altitudes than the surrounding areas, ranging from about 500 m above sea level to just over 1,000 m at the highest points. The region is situated in the rainforest belt of the Upper Guinea Forest zone and receives approximately 2,000 mm of rainfall per annum falling mostly between March and October (Monadjem & Fahr, 2007).

DATA COLLECTION

Two intensive one-month long bat surveys were conducted on the Liberian side of Mount Nimba (within the ArcelorMittal concession) in December 2010 to January 2011, and in December 2011 to January 2012. A further short, two-week survey was conducted in March 2013. Daily searches were made for bat roosting sites, including mine adits, culverts (under roads or railway), tunnels, buildings, hollow trees, natural caves and cavities. Each night a new site was visited where up to five mist nets (12 m × 2.5 m, with 16 mm mesh size, Ecotone, Poland) were erected in suitable locations to maximize capture success. A 2-bank harp trap (3' Cave Catcher from Bat Conservation and Management; www.batmanagement.com) was set up alongside the mist nets. Nets and the harp trap were in place at least 30 min before sunset and were generally removed at 10 pm or once bat activity had died down, based on reduced capture rates.

Bats were surveyed in Wologizi and Zياما during an extensive 6-week survey in April and May 2019. Bats were captured with mist nets set for five consecutive nights at each site, starting at sunset and ending 3–5 hrs later with the decline of bat activity. Around 5 nets (12 m × 2.5 m, with 16 mm mesh size, Ecotone, Poland), were deployed in suitable bat flyways e.g. across small streams or gaps in the forest, near fruiting trees (to

capture fruit bats), or at suspected roosting sites (such as hollow trees, caves or crevices). Nets were typically shifted to a new location each night, except when trap success was unusually high.

Captured bats were sexed, aged (based on ossification of the epiphyses) and standard measurements taken. Voucher specimens were fixed in 10% formalin, subsequently transferred to 70% ethanol and deposited either in the Durban Natural Science Museum (South Africa) or the Eswatini Museum of Natural History (Eswatini). Before fixation in formalin, a tissue sample was taken from the pectoral muscles of each specimen and preserved in 99% ethanol.

GENOMIC DNA ISOLATION, AMPLIFICATION AND SEQUENCING

Genomic DNA was isolated from tissue samples using the QIAamp DNA Investigator Kit (Qiagen, Germany). Tissue samples were cut with a scalpel blade and were subsequently digested overnight (20–22 hours) in Proteinase K and ATL tissue lysis buffer. Following digestion, DNA was isolated according to the manufacturer's instructions. Primers were used to amplify regions of the mitochondrial genes Cytochrome Oxidase I (COI, 520 bp) and Cytochrome *b* (Cyt *b*, 570 bp). The COI gene was amplified using the universal conservative primers BatL5310 and R6036R (Hebert, Ratnasingham & DeWaard, 2003). In addition, cytb-LGL-765-F and cytb-LGL-766-R were used to amplify a region of Cyt *b* (Trujillo *et al.*, 2009). Amplification of the respective gene regions was carried out in separate PCR reactions consisting of 1 × DreamTaq Green PCR Master Mix, 0.4 μM of each primer, and approximately 20 ng template DNA in a total volume of 20 μl. The temperature profile was as follows: an initial denaturation at 95°C for 2 min, 35 cycles of 95°C for 30 s, 55–60°C for 30 s, and 72°C for 1 min, followed by a final extension at 72°C for 10 min. Successful PCR products were purified with Exonuclease I and FastAP (Thermo Fisher Scientific Inc.). Gene fragments were sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit and visualized on a 3500 Genetic Analyzer (Applied Biosystems). Sequence chromatograms were edited and assembled using Sequencing Analysis Software v.6.0 (ThermoFisher Scientific).

PHYLOGENETIC ANALYSIS

The final dataset consisted of 93 COI sequences (Table 1), which contained 35 sequences generated here and 58 reference sequences obtained from National Center for Biotechnology Information (NCBI) GenBank,

Barcode of Life Data System (BOLD) and International Barcode of Life (iBOL). The Cyt *b* dataset (Table 1) included 22 sequences generated here and 42 reference sequences. All sequences were manually trimmed and checked for ambiguous peaks and *Miniopterus fuliginosus* Hodgson, 1835 (AB085735 and HQ580335) and *M. inflatus* Thomas, 1903 (MN064735 and JF442482) were used as outgroup taxa for COI and Cyt *b*. Sequences were edited and aligned with MUSCLE (Edgar, 2004) using default parameters. We determined the best-fitting substitution model by the Bayesian Information Criterion in jModelTest 2.1 (Darrriba et al., 2012). Here, we computed a phylogenetic tree in MEGA7 (Kumar, Stecher & Tamura, 2016) by using the Maximum Likelihood (ML) method based on the Hasegawa-Kishino-Yano (HKY+G+I) model for COI and Tamura-Nei substitution model for Cyt *b*. Node support was assessed with 2000 full heuristic bootstrap replicates. A Bayesian inference of phylogeny was constructed using BEAST v1.10.4 (Drummond et al., 2012) using a Yule speciation prior for a chain length of 50 million generations, sampling every 1000 generations. Model performance was assessed by plotting likelihood scores against generations and checking the effective sample size values in Tracer 1.6 (Rambaut, Suchard & Drummond, 2013). The first 25% of the sampled trees were discarded as burnin, and the final tree was created using TreeAnnotator v1.10.4 and visualised in FigTree v1.4.4 (Rambaut, 2009). Inter- and intraspecific p-distances were calculated with MEGA7.

CRANIODENTAL MORPHOLOGY

The following standard external measurements were taken in the field: total body length, tail length, forearm length, hindfoot length (including claw), and ear length. Forearm length was taken with callipers to the closest 0.1 mm; all other measurements were at an accuracy of 1 mm. Body mass was taken with a Pesola spring balance to the closest 1 g. Nine cranial and four dental measurements were taken with callipers to the closest 0.01 mm following Monadjem et al. (2013). The cranial measurements were: greatest skull length (GSKL), from the posterior-most point of the occipital to the anterior-most point of the incisors; condylo-incisive length (CIL), from the occipital condyles to the anterior-most point of the incisors; condylo-canine length (CCL), from the occipital condyles to the anterior-most point of the canines; greatest zygomatic breadth (ZYGO), taken as the greatest width across the zygomatic arches; greatest braincase width (GBW), lateral braincase width taken posteriorly above the zygomatic arches; greatest skull height (GSH), taken from the lowest point of the bullae to the highest point of the cranium; postorbital width (POB), narrowest dorsal width posterior to the

postorbital at the constriction of the cranium; greatest mastoid breadth (MAST), greatest breadth of cranium at mastoid processes; and greatest mandible length (MAND), taken from the posterior-most point of the condyles to the anterior-most point of the incisors. The dental measurements included: width across the third molars (M3–M3), taken across the outer-most point of the alveoli of the third molars; complete upper canine–molar tooth row (C–M3), taken from the anterior-most point of the alveoli of the canine to the posterior-most point of the third molar; width across upper canines (C–C), taken across the outer-most points of the alveoli of the canines; and complete mandibular canine–molar tooth row (c–m3), taken from the anterior-most point of the alveoli of the canine to the posterior-most point of the third molar. Tooth abbreviations are as follows: C, canine; I, incisor; M, molar; P, premolar; with upper teeth presented in upper case and lower teeth in lower case.

We examined type specimens (listed in Table S1) from: The Natural History Museum (formerly The British Museum of Natural History), London (BMNH); the Muséum national d’Histoire Naturelle, Paris (MNHN); Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK); and the Durban Natural Science Museum, Durban (DM). The specimens examined were: *Vesperugo anchietae* Seabra, 1900 [BMNH 1906.1.3.1 (syntype)], *Pipistrellus crassulus* Thomas, 1904 [BMNH 1904.2.8.1 (holotype)], *Pipistrellus eisentrauti* Hill, 1968 [BMNH 1967.2129 (paratype)], *Pipistrellus musciculus* Thomas, 1913 [BMNH 1913.2.8.1 (holotype)], *Pipistrellus nanulus* Thomas, 1904 [BMNH 1904.2.8.8 (holotype)], *Scotophilus rusticus* Tomes, 1861 [BMNH 1907.1.1.419 (lectotype)], *Vesperugo brunneus* Thomas, 1880 [BMNH 1880.7.21.7 (holotype)], *Vespertilio capensis* A. Smith, 1829 [BMNH 1849.8.16.21 (lectotype)], *Vesperugo (Vesperus) flavescens* Seabra, 1900 [MNHN 1900-537 (cotype)], *Vesperugo grandidieri* Dobson, 1876 [MNHN 1996–2129 (holotype)], *Neoromicia isabella* Decher, Hutterer & Monadjem, 2015 [FMK 2008.0292 (holotype)], *Vespertilio nanus* Peters, 1852 [BMNH 1907.1.1.421 (syntype)], *Vesperugo (Vesperus) rendalli* Thomas, 1889 [BMNH 1889.3.2.3 (holotype)], *Neoromicia roseveari* Monadjem *et al.*, 2013 [DM 12617 (holotype)], *Eptesicus somalicus* Thomas, 1901 [BMNH 1898.6.9.1 (holotype)], *Eptesicus tenuipinnis* Peters, 1872 [BMNH 1889.5.1.3 (syntype)], and *Parahypsugo happoldorum* Hutterer *et al.*, 2019 [ZFMK 2009.0029 (holotype)].

We were unable to examine the type specimen of *Vespertilio hesperida* Temminck, 1840 (= *Pipistrellus hesperidus*), but this specimen has received detailed attention in the literature, including its history, type

locality, and very detailed description of characters together with craniodental measurements (Kock, 2001). The type locality is not definitely identifiable but is probably coastal Eritrea (Kock, 2001). Other names that have been synonymized with *P. hesperidus* include: *P. h. subtilis* (Sundevall, 1846) (South Africa); *P. h. fuscus* Thomas, 1901 (Kenya); and *P. h. broomi* Roberts, 1948 (South Africa). Whether any of these may represent subspecies remains uncertain, although there appears to be little difference in the size of this species across its range (Kearney, 2013).

A principal component analysis (PCA) of log-transformed values of craniodental measurements (see Table S3 for a list of which measurements) was conducted on the variance–covariance matrix in the program PAST (Hammer, Harper & Ryan, 2001) to compare the morphology of the various taxa measured in this study.

μ-COMPUTED TOMOGRAPHY (μ-CT) AND 3D RECONSTRUCTIONS

The holotype of *Pipistrellus* nov. sp. was carefully placed in a small plastic tube that does not damage the specimen but firmly holds it without allowing movements. This container was fixed with a glue gun to a specific metal stub that can be mounted in the respective μ-CT scanner (see below). The postcranial skeleton was scanned with a Skyscan 1173 scanner (Brucker) at the Museum Koenig in Bonn with the following parameters: 1 scan; 0.3° rotation steps over 360°; spatial resolution of 13.132328 μm; 31 kV; 153 μA; no filter; frame averaging of 8; random movement of 15; 1000 ms exposure time. After the scan, the container with the bat was transferred into a Skyscan 1272 (Brucker) scanner that has a smaller field of view but allows higher magnification. With this device the baculum was scanned at higher resolution with the following parameters: 1 scan; 0.2° rotation steps over 360°; spatial resolution of 2.799963 μm; 60 kV; 166 μA; no filter; frame averaging of 8; random movement of 15; 1500 ms exposure time. The skull and mandible which had been removed from the remaining body (see below) were fixed in a tube with wadding and scanned in a SkyScan 1272 Scanner with the following parameters: 2 connected scans; 0.2° rotation steps over 360°; spatial resolution of 6.000014 μm; 50 kV; 166 μA; 0.25 mm Al filter; frame averaging of 8; random movement of 15; 1400 ms exposure time. All scans were reconstructed with the software package N-recon (Bruker) into tif stacks. Subsequently these data were imported in Amira 6.5.0 (Thermo Fisher) where the relevant structures (e.g. the baculum) were segmented and isolated using the “arithmetic” function of the program. The resulting data were imported into VG Studio 3.3.4 (Volume Graphics) where volume rendering (Phong) was performed.

The created images were adjusted and assembled to plates with Photoshop CS6 and Illustrator CS6 (Adobe). All scans were transferred to the digital collection of the ZFMK (for access, please contact BW).

BACULA PREPARATION

Bacula of *Pipistrellus grandidieri*, *Pip. hesperidus*, *Pip. nanalus* and *Pip. rusticus* were prepared according to the method of Hill & Harrison (1987), with slight modification. Penile tissue was macerated in solution of 5% potassium hydroxide for 1–36 hours and bacula, in some cases, were stained with alizarin red. Bacula were photographed under a stereo microscope and measurements recorded from photographed images using the measurement tool in GNU Image Manipulation Program (GIMP version 2.10.14; Spencer Kimball, Peter Mattis and the GIMP Development Team 2019). Bacula morphological descriptions follow the convention of Hill and Harrison (1987).

RESULTS

There was broad agreement between analysis methods (ML and Bayesian & inference) for each of the two datasets. With regards to COI, several well-supported clades were observed, irrespective of the analysis method used, although basal nodes are generally poorly supported (Fig. 2A, Fig. S2). West African species of *Neoromicia* form a well-supported clade; included here are *Neo. brunnea* (Thomas, 1880), *Neo. roseveari*, *Neo. isabella* and *Neo. tenuipinnis*. Two other well-supported clades are: *Parahypsugo bellieri* and *Par. eisentrauti* (on the one hand) and the newly described *Par. happoldorum* and *Pipistrellus grandidieri* (on the other hand). There is relatively poor support for the genus *Parahypsugo*, and the position of *Nycticeinops* remains unresolved. African members of *Pipistrellus* form a poorly supported clade, with *Pip. sp. nov.* being sister to *Pip. hesperidus*. Furthermore, the genus *Laephotis* Thomas, 1901 is embedded within *Neoromicia*, as currently recognised.

The Cyt *b* phylogeny (Fig. 2B, Fig. S3) was similar in topology to that based on COI (Fig. 2A), with poorly supported basal nodes. However, as with COI, there is strong support for a West African group of *Neoromicia* species (see above), but not for the entire genus *Neoromicia*. *Neoromicia capensis* is sister to *Laephotis*. Again, there is strong support for *Pipistrellus grandidieri* s.s. being sister to *Parahypsugo happoldorum*, but lower support for a clade that includes these two species and *Par. bellieri* (Fig. 2B). *Nycticeinops schlieffeni* is sister to

this entire group of *Neoromicia* and *Parahypsugo* (including *Pip. grandidieri*) species. All the *Pipistrellus* species (excluding *Pip. grandidieri*) group together, albeit with relatively low support, with *Pipistrellus (Vansonia) rueppellii* (J.B. Fischer, 1829) being sister to this group. *Pipistrellus* sp. nov. is now sister to a clade that includes *Pip. hesperidus* and *Pip. rusticus* (Tomes, 1861), with an undescribed lineage of *Pipistrellus* cf. *hesperidus* from Senegal being sister to these three *Pipistrellus* species.

Genetic K2P divergence among the main lineages; *Neoromicia*, *Pipistrellus* and *Parahypsugo* were generally high. The COI uncorrected p-distance divergence for the *Neoromicia* clade varied from 6.7 to 12.5% whereas the within variation reached a maximum of 0.4%. Genetic divergence between *Par. bellieri*, *Par. eisentrauti* and *Nyc. schlieffeni* Peters, 1860 varied from 1.9 to 18.6%. with the within divergence varying from 0.2 to 1.3%. Between *Par. happoldorum* and *Pip. grandidieri* s.s. the divergence was 9.4% and between *Pip. hesperidus* and *Pip. sp. nov.* it was 6.6%. The Cyt *b* genetic distances were comparable to those mentioned for COI (Table 2) are presented in Table S2; these distances being 8.4% between *Par. happoldorum* and *Pipistrellus grandidieri* s.s., and 7.9% between *Pip. hesperidus* and *Pip. sp. nov.*.

A PCA ordination based on craniodental morphology of African *Pipistrellus* species showed that different taxa were mostly occupying different parts of morphospace (Fig. 3). The first two principal axes accounted for 92% of the variation and are illustrated here. The first principal component represented a size gradient with high negative loadings on all craniodental measurements (Table S3). Thus, species on the right of the ordination (e.g. *Pip. nanulus* and *Pip. rusticus*) are smaller than those on the left (e.g. *Pip. grandidieri*). The second principal component had both high positive and high negative loadings, which can be interpreted as representing differences in shape; the largest negative and positive loadings were with GSH and $c\text{-m}^3$ (-0.839 and 0.220, respectively) (Table S3). Interestingly, *Pip. grandidieri* (from East Africa) did not share morphospace with *Pip. sp. nov.* (from West Africa). In fact, the latter species was craniodentally most similar to *Pip. hesperidus* (from southern Africa) (Fig. 3).

Based on the results of the molecular and morphological analyses, we describe a new species of *Pipistrellus* from Liberia and Guinea in West Africa.

TAXONOMY

FAMILY VESPERTILIONIDAE GRAY, 1821

GENUS *PIPISTRELLUS* KAUP, 1829

PIPISTRELLUS SIMANDOUENSIS SP. NOV.

MONADJEM, RICHARDS, DECHER AND HUTTERER

SIMANDOU SEROTINE

Holotype: ZMFK-MAM-2008.0302, field number: JD 614. The bat was collected by Jan Decher. It is an adult male fixed in formalin and currently preserved in 70% alcohol, with the skull extracted and cleaned.

Photographs of the head and tragus are illustrated in Figures 4 and 5, while the mandible and skull of the holotype are illustrated in Figure 6. Images of the entire skeleton of the holotype, including the skull, are presented in Figure S1. The glans penis is illustrated in Figure 8 and the dorsal, ventral and lateral views of the baculum are presented in Figure 9.

Type locality: Guinea, Macenta, Simandou Range, “Whiskey 1” as mapped in Decher *et al.* (2015) (Fig. 1). The bat was netted on 25 February 2008 across a small creek in a forested ravine on the east slope of the Simandou Range (08°34.7N, 08°53.9W) at an elevation of 1175 m above sea level.

Paratypes: Two other bats, a female (ZMFK-MAM-2008.0300; field number: JD 656) and a male (ZMFK-MAM-2008.0301; field number: JD 661) identified as belonging to this species were captured 5.9 km to the southwest of the type locality at “Foko confluence” on the west slope of the Simandou Range, on 8 and 9 March 2008, respectively, as mapped in Decher *et al.* (2015), in more humid forest habitat and at 737 m above sea level. Both these specimens have been sequenced and clearly group with the holotype (Fig. 2) and can be considered as paratypes.

Etymology: This species is named after the type region, the Simandou mountain range in eastern Guinea.

Diagnosis: A medium-sized pipistrelloid bat, assignable to the genus *Pipistrellus* on the basis of the presence of a small anterior upper premolar (Hill & Harrison, 1987) and phylogeny (Fig. 2). Some members of the genus

Parahypsugo may also have this anterior upper premolar but differ in skull morphology and shape of rhinarium (Hutterer *et al.*, 2019), the cranium being more inflated in *Pip. simandouensis* sp. nov. than in any *Parahypsugo* species. *Pip. simandouensis* is readily distinguishable from *Pip. hesperidus* (its sister taxon) by its unicoloured pelage (Fig. 4) and shape of baculum (Fig. 5); however, these two species are indistinguishable in craniodental or external measurements. It is significantly larger in external and cranial features (see below) than *Pip. nanulus* Thomas, 1904, *Pip. rusticus* (Tomes, 1861) and *Pip. deserti* Thomas, 1902. It differs from *Pip. inexpectatus* Aellen, 1959 by its unicoloured pelage (bicoloured in the latter) and lack of white on the trailing edge of the wing membrane. *Pip. (Vansonia) rueppellii* is readily distinguished from all other *Pipistrellus* species by its pure white underparts and is sometimes placed in a separate genus *Vansonia* Roberts, 1946 (Moratelli & Burgin, 2019). Finally, the poorly diagnosed *Pip. musciculus* Thomas, 1913 (which may not even be a *Pipistrellus* (Hill & Harrison, 1987)), is far smaller in external and cranial measurements.

Description: External characters: *Pip. simandouensis* sp. nov. is a medium-sized pipistrelloid bat (similar in size to that of *Pip. hesperidus*), but large for the genus *Pipistrellus* (in fact it is the largest within the genus in Africa), with a total length of 77 – 86 mm and forearm length 30–34 mm (Table 3). The pelage is bright yellowish brown and paler below than above (Fig. 4), with the individual hairs being unicoloured on both the upper and under parts. The patagium and uropatagium are both dark brown. Typically for the genus *Pipistrellus*, the ears are subtriangular in shape, rounded at the tip, and dark brown in colour (Fig. 4C). The tragus is also typical of the genus and is moderately long (roughly half of the length of the ear), relatively broad with a straight leading edge and convex outer edge (Fig. 5). It bears a markedly pointed projection, situated near the base and lying immediately below the indentation/notch of the outer margin. The rhinarium is as illustrated for the genus *Pipistrellus* in Hutterer *et al.* (2019), with the nostrils rounded in shape and obviously protruding from the snout. The external measurements of the holotype and other specimens of *Pip. simandouensis* sp. nov. are shown in Table 4.

Craniodental characters: The skull is relatively robust for a *Pipistrellus*, while the rostrum is neither particularly broad nor narrow. The brain case is moderately inflated and rises distinctly and sharply above the level of the rostrum (Fig. 6) in contrast to the relatively flatter skulls of *Parahypsugo* spp. (Hutterer *et al.*, 2019). The posterior of the skull does not end in an extended parietal/supraoccipital crest, and the sagittal and lambdoid

crests are poorly developed. The zygomatic arch is moderately robust. Cranial measurements for the holotype and other specimens of *Pip. simandouensis* sp. nov. are shown in Table 4.

The dentition of *Pip. simandouensis* sp. nov. is I 2/3, C 1/1, P 2/3, M 2/3, which is typical of the genus *Pipistrellus* (Van Cakenberghe & Happold, 2013a). In the upper tooth row, I¹ is not bifid and I² is relatively small-sized, not reaching half the length of I¹ (Fig. 7A). P¹ is relatively small in all specimens examined, and in the toothrow, creating an obvious gap between C and P² (Fig. 7B). Dental measurements for the holotype and other specimens of *Pip. simandouensis* sp. nov. are shown in Table 5.

The penis of *Pip. simandouensis* sp. nov. is long (8.4 mm in the holotype), straight, and covered by long hairs all over its length (Fig. 8). These hairs are mostly white, with only a few on the dorsal side are brown. The terminal glans is wider than the shaft (Fig. 8). Total length of the penis is about 10% of total body length (Table 3). In its long and straight shape, the penis of *Pip. simandouensis* sp. nov. is similar to other species of the genus, such as *Pip. abramus* (Temminck, 1840) and other Asian (Francis, 2019) and African *Pipistrellus* species (Benda, Hulva & Gaisler, 2004). The bacula of *Pip. simandouensis* sp. nov., *Pip. grandidieri*, *Pip. hesperidus*, *Pip. nanulus* and *Pip. rusticus* are presented in Figure 9. The baculum of *Pip. simandouensis* sp. nov. was notably shorter than the other species, with a total length of 1.32 mm. Baculum total length for the remaining species was: 2.00 mm (*Pip. grandidieri*), 1.47 mm (*Pip. hesperidus*), 2.98 mm (*Pip. nanulus*) and 1.52 mm (*Pip. rusticus*). *Pipistrellus simandouensis* sp. nov., *Pip. hesperidus* and *Pip. rusticus* were overall, similar in morphology presenting a slender-shafted baculum, with a bilobed base and an expand tip with two distinct prongs. *Pipistrellus simandouensis* sp. nov. can however, be differentiated from *Pip. hesperidus* and *Pip. rusticus*, on the basis of its robust, triangular base measuring 0.32 mm. Similarly, the projections of the tip in *Pip. simandouensis* sp. nov. are more pointed than in *Pip. hesperidus* and *Pip. rusticus*. The full suite of bacula measurements for all five species are provided in Table S4.

Biology: *Pip. simandouensis* sp. nov. is currently known from two localities in West Africa, based on the four sequenced specimens (appearing in Fig 2A) one specimen from Mount Nimba in northern Liberia (Monadjem *et al.*, 2013); and three from Simandou Range in south-eastern Guinea (Decher *et al.*, 2015). This species has also been reported from the Fouta Djallon in central Guinea (Decher *et al.*, 2015), but this has not been

confirmed genetically and we have not been able to examine these specimens. However, all these sites are from upland regions of West Africa, ranging in altitude from the foothill slopes at around 450 m above sea level, to the summit of some of the peaks at over 1200 m above sea level and higher. Therefore, we suspect that this is a species closely associated with these upland forested habitats. Since such upland habitats are relatively restricted in West Africa, and few are legally protected (Monadjem *et al.*, 2016), we furthermore predict that this is a species under threat. Hence, we recommend that the conservation status of this species be assessed as a matter of urgency.

DISCUSSION

In this paper we present an mtDNA phylogeny for a confusing group of poorly studied and generally under-appreciated species placed in the genera *Pipistrellus*, *Neoromicia* and *Parahypsugo* (previously *Hypsugo*). Our study resolves a lingering taxonomic question as to whether *Pip. cf. grandidieri* (from West Africa) is conspecific with *Pip. grandidieri* (from East Africa). We first show that it is a valid species *Pip. simandouensis* that is sister to another species altogether *Pip. hesperidus*. Next, we demonstrate that *Pip. grandidieri* is in fact a member of the recently described genus *Parahypsugo* (Hutterer *et al.*, 2019). This raises a taxonomic problem with regards to the name “*Parahypsugo*”, because Thorn *et al.* (2007) created a new subgenus *Afropipistrellus* Thorn, Kock & Cuisin 2007 in which they placed *Pip. grandidieri*. Since *Afropipistrellus* predates *Parahypsugo*, the name of this clade should be changed to *Afropipistrellus*. We tentatively retain the use of the name *Parahypsugo* until the relationship between the taxon “*grandidieri*” and other members of *Parahypsugo* (*Par. happoldorum*, *Par. eisentrauti*, *Par. bellieri* and *Par. crassulus*) has been investigated with additional genes; preferably to also include nuclear genes as suggested by Demos *et al.* (2018) for another clade of vespertilionids, the genus *Scotophilus*.

Within *Parahypsugo*, the species *Par. eisentrauti* appears to be conspecific with *Par. bellieri* (see Fig. 2). However, this is due to a mis-identification of the two “*eisentrauti*” specimens (see Fahr (2013) for more details) which were collected at “Parc National De Mont Peko, 6 km W of Sibabli” in Côte d’Ivoire and the sequences uploaded to Genbank by J.L. Eger and colleagues (Lim & van Coeverden de Groot, 1997).

Parahypsugo eisentrauti is only known from montane areas of Cameroon (Van Cakenberghe & Happold, 2013b; Hutterer *et al.*, 2019), while *Par. bellieri* is restricted to the Upper Guinea rainforest zone (Monadjem & Fahr, 2007), which includes Parc National De Mont Peko. The taxonomy of the “*Parahypsugo*” clade, however, is further complicated by the position of *Nycticeinops* in the pipistrelloid tree (Fig. 2). In our COI phylogeny (Fig. 2A), *Nycticeinops* appears embedded within the genus *Parahypsugo* (albeit with poor support) hence rendering *Parahypsugo* paraphyletic. If future analyses based on additional genes, recover a similar relationship between these two taxa, then the “*Parahypsugo*” clade may need to be renamed *Nycticeinops* (or split into further genera). It also calls into question the usefulness of the presence or absence of the upper outer incisor (the second incisor) in systematic studies of this group as noted by others (Hofer & van den Bussche, 2003; Roehrs *et al.*, 2010). As with the presence/absence of the upper anterior premolar, the outer incisor may be useful in species identification (Monadjem *et al.*, 2020), but appears to be of little assistance in determining generic rank. We recommend that nuclear genes, as employed by Demos *et al.*, 2018, 2019b; Patterson *et al.* (2019), be incorporated in future studies of African pipistrelloid bats to resolve genus-level relationships.

Pipistrellus simandouensis is the fourth new species of pipistrelloid bat to be described from this region in the past few years, following the descriptions of *Neoromicia roseveari* (Monadjem *et al.*, 2013), *Neo. isabella* (Decher *et al.*, 2015), and *Parahypsugo happoldorum* (Hutterer *et al.*, 2019). Furthermore, these four pipistrelloid species co-occur with an additional seven species (*Neo. brunnea*, *Neo. guineensis* (Bocage, 1889), *Neo. nanus*, *Neo. somalica* (Thomas, 1901), *Neo. tenuipinnis* (Peters, 1872), *Par. bellieri*, *Pip. nanulus*) at Mount Nimba (Monadjem *et al.*, 2016), making this undoubtedly a hotspot for pipistrelloid conservation. When considering the entire upland zone in south-eastern Guinea and northern Liberia, the number of pipistrelloid species increases to 12 (with the addition of *Pip. cf. inexpectatus* which was recorded in this study). This matches the species richness at Comoé and Taï National Parks in Côte d’Ivoire, where 12 species have been reported based on comprehensive surveys (Fahr & Kalko, 2011).

The species *Pip. inexpectatus* has been poorly documented, occurring from Cameroon (the type locality) to Sierra Leone in West Africa (Van Cakenberghe & Happold, 2013c). However, west of Nigeria, single specimens have been collected from two localities, one in Sierra Leone and another in Ghana (Grubb *et al.*, 1998). A

further nine individuals have been reported captured from Comoé National Park in Côte d'Ivoire (Fahr & Kalko, 2011), but museum and/or field numbers were not communicated, and no additional information was provided to allow independent verification of their identifications. The specimen captured in this study displays all the characteristics of this enigmatic species including bicoloured fur, bicuspid upper inner incisor, the presence of a tiny anterior premolar, skull morphometrics (Fig. 3), but did not show a white stripe on the trailing edge of the wing. The absence of a white stripe on the wing is the reason why we have referred to this as *Pip. cf. inexpectatus*, because we are not completely certain that it truly represents *Pip. inexpectatus*. Rosevear (1965) questioned the usefulness of the last-named feature (white trailing edge of wing) and suggested that it may be a variable character; this needs further investigation.

In conclusion, we describe a new species of *Pipistrellus* from West Africa, present a phylogeny for the African members of this genus, and identify the upland regions of south-eastern Guinea and northern Liberia as a hotspot of species richness for pipistrelloid bats of the genera *Pipistrellus*, *Neoromicia* and *Parahypsugo* (*Afropipistrellus*).

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Table 1. Cytochrome Oxidase I (COI) and Cytochrome *b* (Cyt *b*) sequences of *Neoromicia*, *Pipistrellus* and *Parahypsugo* species, and outgroups, used in this study.

| Number | GenBank accession number | Identification number | COI | Cyt <i>b</i> | Latin name | Reference |
|--------|--------------------------|-----------------------|-----|--------------|-----------------------------|----------------------------------|
| 1 | EU797444.1 | | | √ | <i>Laephotis botswanae</i> | Trujillo, unpublished |
| 2 | EU797445.1 | | | √ | <i>Laephotis botswanae</i> | Trujillo, unpublished |
| 3 | MF038572.1 | | √ | | <i>Laephotis botswanae</i> | (Hassanin <i>et al.</i> , 2018) |
| 4 | | ZWW049 | √ | | <i>Neoromicia brunnea</i> | This study |
| 5 | | ZWW136 | √ | | <i>Neoromicia brunnea</i> | This study |
| 6 | | ZWW175 | √ | √ | <i>Neoromicia brunnea</i> | This study |
| 7 | | ZWW282 | √ | √ | <i>Neoromicia brunnea</i> | This study |
| 8 | JF444136.1 | | √ | | <i>Neoromicia brunnea</i> | Eger <i>et al.</i> , unpublished |
| 9 | JF444137.1 | | √ | | <i>Neoromicia brunnea</i> | Eger <i>et al.</i> , unpublished |
| 10 | JX508826.1 | | √ | | <i>Neoromicia capensis</i> | (Monadjem <i>et al.</i> , 2013) |
| 11 | KM886042.1 | | | √ | <i>Neoromicia capensis</i> | (Goodman <i>et al.</i> , 2015) |
| 12 | KM886073.1 | | | √ | <i>Neoromicia capensis</i> | (Goodman <i>et al.</i> , 2015) |
| 13 | JX508832.1 | | √ | | <i>Neoromicia isabella</i> | (Monadjem <i>et al.</i> , 2013) |
| 14 | KT598186.1 | | √ | | <i>Neoromicia isabella</i> | (Decher <i>et al.</i> , 2015) |
| 15 | KT598187.1 | | √ | | <i>Neoromicia isabella</i> | (Decher <i>et al.</i> , 2015) |
| 16 | MK188527.1 | | | √ | <i>Neoromicia isabella</i> | (Hutterer <i>et al.</i> , 2019) |
| 17 | | ZWW201 | √ | √ | <i>Neoromicia nana</i> | This study |
| 18 | | ZWW202 | √ | √ | <i>Neoromicia nana</i> | This study |
| 19 | | ZWW203 | √ | √ | <i>Neoromicia nana</i> | This study |
| 20 | JF442533.1 | | √ | | <i>Neoromicia nana</i> | Agwanda & Kuzmin, unpublished |
| 21 | JF442535.1 | | √ | | <i>Neoromicia nana</i> | Agwanda & Kuzmin, unpublished |
| 22 | JF444201.1 | | √ | | <i>Neoromicia nana</i> | Eger <i>et al.</i> , unpublished |
| 23 | JX508829.1 | | √ | | <i>Neoromicia nana</i> | (Monadjem <i>et al.</i> , 2013) |
| 24 | JX276206.1 | | | √ | <i>Neoromicia rendalli</i> | (Koubínová <i>et al.</i> , 2013) |
| 25 | JX276207.1 | | | √ | <i>Neoromicia rendalli</i> | (Koubínová <i>et al.</i> , 2013) |
| 26 | | ZWW017 | √ | √ | <i>Neoromicia roseveari</i> | This study |
| 27 | | ZWW045 | √ | √ | <i>Neoromicia roseveari</i> | This study |
| 28 | | ZWW048 | √ | | <i>Neoromicia roseveari</i> | This study |
| 29 | | ZWW075 | √ | | <i>Neoromicia roseveari</i> | This study |
| 30 | | ZWW247 | √ | | <i>Neoromicia roseveari</i> | This study |
| 31 | | ZWW251 | √ | | <i>Neoromicia roseveari</i> | This study |
| 32 | | ZWW263 | √ | √ | <i>Neoromicia roseveari</i> | This study |
| 33 | JX508827.1 | | √ | | <i>Neoromicia roseveari</i> | (Monadjem <i>et al.</i> , 2013) |
| 34 | JX508828.1 | | √ | | <i>Neoromicia roseveari</i> | (Monadjem <i>et al.</i> , 2013) |
| 35 | KT598188.1 | | √ | | <i>Neoromicia roseveari</i> | (Decher <i>et al.</i> , 2015) |

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|----|------------|--------|---|---|--|----------------------------------|
| 36 | KT598189.1 | | √ | | <i>Neoromicia roseveari</i> | (Decher <i>et al.</i> , 2015) |
| 37 | MK188528.1 | | | √ | <i>Neoromicia roseveari</i> | (Hutterer <i>et al.</i> , 2019) |
| 38 | JX276209.1 | | | √ | <i>Neoromicia somalica</i> | (Koubínová <i>et al.</i> , 2013) |
| 39 | JX276211.1 | | | √ | <i>Neoromicia somalica</i> | (Koubínová <i>et al.</i> , 2013) |
| 40 | JX276215.1 | | | √ | <i>Neoromicia somalica</i> | (Koubínová <i>et al.</i> , 2013) |
| 41 | JX276230.1 | | | √ | <i>Neoromicia somalica</i> | (Koubínová <i>et al.</i> , 2013) |
| 42 | JX508830.1 | | √ | | <i>Neoromicia somalica</i> | (Monadjem <i>et al.</i> , 2013) |
| 43 | JX508831.1 | | √ | | <i>Neoromicia tenuipinnis</i> | (Monadjem <i>et al.</i> , 2013) |
| 44 | KF452649.1 | | √ | | <i>Neoromicia zuluensis</i> | McCulloch, unpublished |
| 45 | KX375186.1 | | | √ | <i>Neoromicia zuluensis</i> | (Benda <i>et al.</i> , 2016) |
| 46 | KX375187.1 | | | √ | <i>Neoromicia zuluensis</i> | (Benda <i>et al.</i> , 2016) |
| 47 | JX276301.1 | | | √ | <i>Nycticeinops schlieffeni</i> | (Koubínová <i>et al.</i> , 2013) |
| 48 | JX276302.1 | | | √ | <i>Nycticeinops schlieffeni</i> | (Koubínová <i>et al.</i> , 2013) |
| 49 | JX276303.1 | | | √ | <i>Nycticeinops schlieffeni</i> | (Koubínová <i>et al.</i> , 2013) |
| 50 | JX276308.1 | | | √ | <i>Nycticeinops schlieffeni</i> | (Koubínová <i>et al.</i> , 2013) |
| 51 | KF452659.1 | | √ | | <i>Nycticeinops schlieffeni</i> | McCulloch, unpublished |
| 52 | KF452660.1 | | √ | | <i>Nycticeinops schlieffeni</i> | McCulloch, unpublished |
| 53 | KF452661.1 | | √ | | <i>Nycticeinops schlieffeni</i> | McCulloch, unpublished |
| 54 | | ZWW018 | √ | | <i>Parahypsugo bellieri</i> | This study |
| 55 | JX508834.1 | | √ | | <i>Parahypsugo bellieri</i> | (Monadjem <i>et al.</i> , 2013) |
| 56 | JX508835.1 | | √ | | <i>Parahypsugo bellieri</i> | (Monadjem <i>et al.</i> , 2013) |
| 57 | JX508836.1 | | √ | | <i>Parahypsugo bellieri</i> | (Monadjem <i>et al.</i> , 2013) |
| 58 | KT598194.1 | | √ | | <i>Parahypsugo bellieri</i> | (Decher <i>et al.</i> , 2015) |
| 59 | KT598199.1 | | √ | | <i>Parahypsugo bellieri</i> | (Decher <i>et al.</i> , 2015) |
| 60 | MK188520.1 | | | √ | <i>Parahypsugo bellieri</i> | (Hutterer <i>et al.</i> , 2019) |
| 61 | MK188521.1 | | | √ | <i>Parahypsugo bellieri</i> | (Hutterer <i>et al.</i> , 2019) |
| 62 | JF444193.1 | | √ | | <i>Parahypsugo cf. eisentrauti</i> (= <i>bellieri</i>) | Eger <i>et al.</i> , unpublished |
| 63 | JF444194.1 | | √ | | <i>Parahypsugo cf. eisentrauti</i> (= <i>bellieri</i>) | Eger <i>et al.</i> , unpublished |
| 64 | MK188531.1 | | √ | | <i>Parahypsugo eisentrauti</i> | (Hutterer <i>et al.</i> , 2019) |
| 65 | | ZWW071 | √ | √ | <i>Parahypsugo happoldorum</i> | This study |
| 66 | | ZWW086 | √ | | <i>Parahypsugo happoldorum</i> | This study |
| 67 | | ZWW258 | √ | | <i>Parahypsugo happoldorum</i> | This study |
| 68 | | ZWW268 | √ | | <i>Parahypsugo happoldorum</i> | This study |
| 69 | | ZWW269 | √ | | <i>Parahypsugo happoldorum</i> | This study |
| 70 | | ZWW275 | √ | √ | <i>Parahypsugo happoldorum</i> | This study |
| 71 | MK188523.1 | | | √ | <i>Parahypsugo happoldorum</i> | (Hutterer <i>et al.</i> , 2019) |

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|-----|-------------|--------|---|---|--------------------------------------|--|
| 72 | JX508833.1 | | √ | | <i>Parahypsugo happoldorum</i> | (Monadjem <i>et al.</i> , 2013) |
| 73 | JX276309.1 | | | √ | <i>Pipistrellus cf. hesperidus</i> | (Koubínová <i>et al.</i> , 2013) |
| 74 | JX276310.1 | | | √ | <i>Pipistrellus cf. hesperidus</i> | (Koubínová <i>et al.</i> , 2013) |
| 75 | JX276311.1 | | | √ | <i>Pipistrellus cf. hesperidus</i> | (Koubínová <i>et al.</i> , 2013) |
| 76 | JX276312.1 | | | √ | <i>Pipistrellus cf. hesperidus</i> | (Koubínová <i>et al.</i> , 2013) |
| 77 | | ZWW254 | √ | | <i>Pipistrellus cf. inexpectatus</i> | This study |
| 78 | KM252756.1 | | | √ | <i>Pipistrellus deserti</i> | (Benda, Andriollo, & Ruedi, 2015) |
| 79 | KM252757.1 | | | √ | <i>Pipistrellus deserti</i> | (Benda <i>et al.</i> , 2015) |
| 80 | KT613195.1 | | √ | | <i>Pipistrellus deserti</i> | (Andriollo, Naciri, & Ruedi, 2015) |
| 81 | | JAG430 | √ | √ | <i>Pipistrellus grandidieri</i> | This study |
| 82 | | JAG433 | √ | | <i>Pipistrellus grandidieri</i> | This study |
| 83 | | JAG439 | √ | √ | <i>Pipistrellus grandidieri</i> | This study |
| 84 | | JAG440 | √ | √ | <i>Pipistrellus grandidieri</i> | This study |
| 85 | | JAG441 | √ | √ | <i>Pipistrellus grandidieri</i> | This study |
| 86 | | JAG447 | | √ | <i>Pipistrellus hesperidus</i> | This study |
| 87 | | JAG448 | | √ | <i>Pipistrellus hesperidus</i> | This study |
| 88 | | JAG450 | | √ | <i>Pipistrellus hesperidus</i> | This study |
| 89 | AJ841968.1 | | | √ | <i>Pipistrellus hesperidus</i> | (Stadelmann <i>et al.</i> , 2004) |
| 90 | KF452663.1 | | √ | | <i>Pipistrellus hesperidus</i> | McCulloch, unpublished |
| 91 | KF452664.1 | | √ | | <i>Pipistrellus hesperidus</i> | McCulloch, unpublished |
| 92 | KF452665.1 | | √ | | <i>Pipistrellus hesperidus</i> | McCulloch, unpublished |
| 93 | KF452666.1 | | √ | | <i>Pipistrellus hesperidus</i> | McCulloch, unpublished |
| 94 | KM886007.1 | | | √ | <i>Pipistrellus hesperidus</i> | (Goodman <i>et al.</i> , 2015) |
| 95 | KM252761.1 | | | √ | <i>Pipistrellus kuhlii</i> | (Benda <i>et al.</i> , 2015) |
| 96 | KM252762.1 | | | √ | <i>Pipistrellus kuhlii</i> | (Benda <i>et al.</i> , 2015) |
| 97 | KM252763.1 | | | √ | <i>Pipistrellus kuhlii</i> | (Benda <i>et al.</i> , 2015) |
| 98 | KM252764.1 | | | √ | <i>Pipistrellus kuhlii</i> | (Benda <i>et al.</i> , 2015) |
| 99 | KM252765.1 | | | √ | <i>Pipistrellus kuhlii</i> | (Benda <i>et al.</i> , 2015) |
| 100 | CHIAA003-15 | | √ | | <i>Pipistrellus kuhlii</i> | (Andriollo <i>et al.</i> , 2015) |
| 101 | CHIAA014-15 | | √ | | <i>Pipistrellus kuhlii</i> | (Andriollo <i>et al.</i> , 2015) |
| 102 | JF443067.1 | | √ | | <i>Pipistrellus kuhlii</i> | Kruskop <i>et al.</i> , unpublished data |
| 103 | MN031798.1 | | √ | | <i>Pipistrellus kuhlii</i> | (Mifsud & Vella, 2019) |
| 104 | | ZWW044 | √ | √ | <i>Pipistrellus nanulus</i> | This study |
| 105 | | ZWW063 | √ | √ | <i>Pipistrellus nanulus</i> | This study |
| 106 | | ZWW073 | √ | | <i>Pipistrellus nanulus</i> | This study |
| 107 | | ZWW096 | √ | | <i>Pipistrellus nanulus</i> | This study |
| 108 | | ZWW097 | √ | | <i>Pipistrellus nanulus</i> | This study |
| 109 | | ZWW272 | √ | √ | <i>Pipistrellus nanulus</i> | This study |

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|----------|------------|--------|---|---|----------------------------------|-------------------------------|
| 110 | | ZWW273 | √ | √ | <i>Pipistrellus nanulus</i> | This study |
| 111 | | ZWW276 | √ | √ | <i>Pipistrellus nanulus</i> | This study |
| 112 | JF444195.1 | | √ | | <i>Pipistrellus nanulus</i> | Agwanda & Kuzmin, unpublished |
| 113 | JF444196.1 | | √ | | <i>Pipistrellus nanulus</i> | Eger et al., unpublished |
| 114 | JF444199.1 | | √ | | <i>Pipistrellus nanulus</i> | Eger et al., unpublished |
| 115 | JX508837.1 | | √ | | <i>Pipistrellus nanulus</i> | (Monadjem et al., 2013) |
| 116 | JX508838.1 | | √ | | <i>Pipistrellus nanulus</i> | (Monadjem et al., 2013) |
| 117 | MK188530.1 | | | √ | <i>Pipistrellus nanulus</i> | (Hutterer et al., 2019) |
| 118 | AJ504446.1 | | | √ | <i>Pipistrellus nathusii</i> | (Stadelmann et al., 2004) |
| 119 | DQ120850.1 | | | √ | <i>Pipistrellus nathusii</i> | (Ibáñez et al., 2006) |
| 120 | FR856769.1 | | √ | | <i>Pipistrellus nathusii</i> | (Galimberti et al., 2012) |
| 121 | GU686079.1 | | √ | | <i>Pipistrellus nathusii</i> | International Barcode of Life |
| 122 | FR856778.1 | | √ | | <i>Pipistrellus pipistrellus</i> | (Galimberti et al., 2012) |
| 123 | HM380206.1 | | √ | | <i>Pipistrellus pipistrellus</i> | International Barcode of Life |
| 124 | KM252778.1 | | | √ | <i>Pipistrellus pipistrellus</i> | (Benda et al., 2015) |
| 125 | HQ974655.1 | | √ | | <i>Pipistrellus pygmaeus</i> | International Barcode of Life |
| 126 | JX566929.1 | | | √ | <i>Pipistrellus pygmaeus</i> | Medinas, unpublished |
| 127 | KF452667.1 | | √ | | <i>Pipistrellus rusticus</i> | McCulloch, unpublished |
| 128 | KF452668.1 | | √ | | <i>Pipistrellus rusticus</i> | McCulloch, unpublished |
| 129 | KF452670.1 | | √ | | <i>Pipistrellus rusticus</i> | McCulloch, unpublished |
| 130 | KF452671.1 | | √ | | <i>Pipistrellus rusticus</i> | McCulloch, unpublished |
| 131 | KF452672.1 | | √ | | <i>Pipistrellus rusticus</i> | McCulloch, unpublished |
| 132 | KX375167.1 | | | √ | <i>Pipistrellus rusticus</i> | (Benda et al., 2016) |
| 133 | JX508839.1 | | √ | | <i>Pipistrellus</i> sp. nov. | (Monadjem et al., 2013) |
| 134 | KT598195.1 | | √ | | <i>Pipistrellus</i> sp. nov. | (Decher et al., 2015) |
| 135 | KT598196.1 | | √ | | <i>Pipistrellus</i> sp. nov. | (Decher et al., 2015) |
| 136 | KT598197.1 | | √ | | <i>Pipistrellus</i> sp. nov. | (Decher et al., 2015) |
| 137 | MK188529.1 | | | √ | <i>Pipistrellus</i> sp. nov. | (Hutterer et al., 2019) |
| 138 | JF442684.1 | | √ | | <i>Scotoecus</i> sp. | Agwanda & Kuzmin, unpublished |
| 139 | JX276315.1 | | √ | | <i>Vansonia rueppellii</i> | (Koubínová et al., 2013) |
| Outgroup | AB085735.1 | | | √ | <i>Miniopterus fuliginosus</i> | (Sakai et al., 2003) |
| Outgroup | MN064735.1 | | | √ | <i>Miniopterus inflatus</i> | (Lutz et al., 2019) |
| Outgroup | HQ580335.1 | | √ | | <i>Miniopterus fuliginosus</i> | International Barcode of Life |
| Outgroup | JF442482.1 | | √ | | <i>Miniopterus inflatus</i> | Agwanda & Kuzmin, unpublished |

Table 2. Mean genetic distances based on Kimura’s two-parameter model calculated for COI in MEGA7 (Tamura *et al.*, 2013). Distance within clades is indicated in bold and between clades of *Neoromicia*, *Pipistrellus* and *Parahypsugo* species are shown below the diagonal. The presence of NA denotes cases in which it was not possible to estimate evolutionary distances.

| | <i>Laephotis botswanae</i> | <i>Neoromicia brunnea</i> | <i>Neoromicia capensis</i> | <i>Neoromicia isabella</i> | <i>Neoromicia nana</i> | <i>Neoromicia rendalli</i> | <i>Neoromicia roseveari</i> | <i>Neoromicia somalica</i> | <i>Neoromicia tenuipinnis</i> | <i>Neoromicia zuluensis</i> | <i>Nycticeinops schlieffeni</i> | <i>Parahypsugo bellieri</i> | <i>Parahypsugo eisentrauti</i> | <i>Parahypsugo happoldorum</i> | <i>Pipistrellus deserti</i> | <i>Pipistrellus grandidieri</i> | <i>Pipistrellus hesperidus</i> | <i>Pipistrellus cf. inexpectatus</i> | <i>Pipistrellus kuhlii</i> | <i>Pipistrellus nanulus</i> | <i>Pipistrellus nathusii</i> | <i>Pipistrellus pipistrellus</i> | <i>Pipistrellus pygmaeus</i> | <i>Pipistrellus rusticus</i> | <i>Pipistrellus simandouensis sp. nov.</i> | <i>Scotoecus sp.</i> | <i>Vansonia rueppellii</i> | Outgroup | |
|--|----------------------------|---------------------------|----------------------------|----------------------------|------------------------|----------------------------|-----------------------------|----------------------------|-------------------------------|-----------------------------|---------------------------------|-----------------------------|--------------------------------|--------------------------------|-----------------------------|---------------------------------|--------------------------------|--------------------------------------|----------------------------|-----------------------------|------------------------------|----------------------------------|------------------------------|------------------------------|--|----------------------|----------------------------|----------|--|
| <i>Laephotis botswanae</i> | - | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Neoromicia brunnea</i> | 0.183 | 0.004 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Neoromicia capensis</i> | 0.089 | 0.176 | - | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Neoromicia isabella</i> | 0.185 | 0.081 | 0.173 | 0.002 | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Neoromicia nana</i> | 0.155 | 0.167 | 0.138 | 0.157 | 0.016 | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Neoromicia roseveari</i> | 0.184 | 0.068 | 0.174 | 0.065 | 0.164 | 0.143 | 0.004 | | | | | | | | | | | | | | | | | | | | | | |
| <i>Neoromicia somalica</i> | 0.174 | 0.183 | 0.163 | 0.171 | 0.174 | 0.165 | 0.159 | - | | | | | | | | | | | | | | | | | | | | | |
| <i>Neoromicia tenuipinnis</i> | 0.195 | 0.113 | 0.177 | 0.122 | 0.151 | 0.149 | 0.119 | 0.190 | - | | | | | | | | | | | | | | | | | | | | |
| <i>Neoromicia zuluensis</i> | 0.180 | 0.203 | 0.158 | 0.206 | 0.190 | 0.177 | 0.184 | 0.067 | 0.194 | - | | | | | | | | | | | | | | | | | | | |
| <i>Nycticeinops schlieffeni</i> | 0.175 | 0.189 | 0.184 | 0.189 | 0.193 | 0.184 | 0.193 | 0.193 | 0.185 | 0.205 | 0.013 | | | | | | | | | | | | | | | | | | |
| <i>Parahypsugo bellieri</i> | 0.195 | 0.205 | 0.196 | 0.212 | 0.205 | 0.188 | 0.202 | 0.207 | 0.206 | 0.209 | 0.179 | 0.015 | | | | | | | | | | | | | | | | | |
| <i>Parahypsugo eisentrauti</i> | 0.181 | 0.190 | 0.172 | 0.187 | 0.172 | 0.191 | 0.183 | 0.193 | 0.167 | 0.201 | 0.193 | 0.196 | - | | | | | | | | | | | | | | | | |
| <i>Parahypsugo happoldorum</i> | 0.196 | 0.191 | 0.195 | 0.207 | 0.194 | 0.187 | 0.202 | 0.210 | 0.188 | 0.208 | 0.183 | 0.159 | 0.165 | 0.006 | | | | | | | | | | | | | | | |
| <i>Pipistrellus deserti</i> | 0.187 | 0.189 | 0.180 | 0.177 | 0.174 | 0.183 | 0.210 | 0.204 | 0.213 | 0.203 | 0.220 | 0.213 | 0.222 | 0.220 | - | | | | | | | | | | | | | | |
| <i>Pipistrellus grandidieri</i> | 0.207 | 0.176 | 0.202 | 0.190 | 0.207 | 0.191 | 0.206 | 0.211 | 0.190 | 0.225 | 0.186 | 0.206 | 0.184 | 0.091 | 0.230 | 0.002 | | | | | | | | | | | | | |
| <i>Pipistrellus hesperidus</i> | 0.180 | 0.186 | 0.169 | 0.176 | 0.171 | 0.180 | 0.187 | 0.187 | 0.189 | 0.190 | 0.189 | 0.216 | 0.208 | 0.198 | 0.149 | 0.163 | 0.009 | | | | | | | | | | | | |
| <i>Pipistrellus cf. inexpectatus</i> | 0.202 | 0.229 | 0.176 | 0.199 | 0.180 | 0.176 | 0.215 | 0.149 | 0.222 | 0.161 | 0.214 | 0.207 | 0.229 | 0.212 | 0.187 | 0.222 | 0.211 | - | | | | | | | | | | | |
| <i>Pipistrellus kuhlii</i> | 0.168 | 0.182 | 0.165 | 0.169 | 0.177 | 0.173 | 0.192 | 0.193 | 0.197 | 0.214 | 0.201 | 0.193 | 0.196 | 0.187 | 0.043 | 0.172 | 0.140 | 0.195 | 0.043 | | | | | | | | | | |
| <i>Pipistrellus nanulus</i> | 0.210 | 0.192 | 0.198 | 0.198 | 0.200 | 0.193 | 0.196 | 0.212 | 0.201 | 0.218 | 0.200 | 0.210 | 0.213 | 0.194 | 0.195 | 0.190 | 0.163 | 0.231 | 0.183 | 0.008 | | | | | | | | | |
| <i>Pipistrellus nathusii</i> | 0.190 | 0.203 | 0.147 | 0.203 | 0.181 | 0.165 | 0.197 | 0.168 | 0.184 | 0.185 | 0.186 | 0.191 | 0.191 | 0.211 | 0.192 | 0.201 | 0.156 | 0.187 | 0.181 | 0.180 | 0.002 | | | | | | | | |
| <i>Pipistrellus pipistrellus</i> | 0.176 | 0.187 | 0.154 | 0.183 | 0.174 | 0.154 | 0.185 | 0.164 | 0.196 | 0.194 | 0.183 | 0.205 | 0.179 | 0.174 | 0.155 | 0.179 | 0.152 | 0.185 | 0.152 | 0.179 | 0.162 | 0.006 | | | | | | | |
| <i>Pipistrellus pygmaeus</i> | 0.183 | 0.215 | 0.181 | 0.207 | 0.194 | 0.179 | 0.206 | 0.186 | 0.207 | 0.204 | 0.208 | 0.215 | 0.188 | 0.200 | 0.188 | 0.209 | 0.171 | 0.214 | 0.183 | 0.205 | 0.212 | 0.077 | - | | | | | | |
| <i>Pipistrellus rusticus</i> | 0.173 | 0.191 | 0.168 | 0.184 | 0.169 | 0.176 | 0.197 | 0.203 | 0.178 | 0.205 | 0.185 | 0.198 | 0.211 | 0.183 | 0.135 | 0.190 | 0.132 | 0.206 | 0.139 | 0.166 | 0.168 | 0.156 | 0.194 | 0.003 | | | | | |
| <i>Pipistrellus simandouensis sp. nov.</i> | 0.182 | 0.196 | 0.168 | 0.188 | 0.167 | 0.174 | 0.198 | 0.196 | 0.197 | 0.200 | 0.201 | 0.219 | 0.203 | 0.207 | 0.127 | 0.180 | 0.065 | 0.203 | 0.135 | 0.167 | 0.168 | 0.149 | 0.174 | 0.149 | 0.004 | | | | |
| <i>Scotoecus sp.</i> | 0.188 | 0.194 | 0.179 | 0.186 | 0.176 | 0.188 | 0.177 | 0.195 | 0.193 | 0.226 | 0.181 | 0.206 | 0.163 | 0.160 | 0.222 | 0.191 | 0.190 | 0.193 | 0.201 | 0.166 | 0.184 | 0.169 | 0.190 | 0.200 | 0.188 | - | | | |
| <i>Vansonia rueppellii</i> | 0.085 | 0.165 | 0.006 | 0.175 | 0.143 | 0.144 | 0.171 | 0.154 | 0.181 | 0.149 | 0.185 | 0.203 | 0.174 | 0.194 | 0.173 | 0.192 | 0.170 | 0.178 | 0.167 | 0.203 | 0.146 | 0.155 | 0.178 | 0.171 | 0.170 | 0.184 | - | | |
| Outgroup | 0.211 | 0.249 | 0.226 | 0.241 | 0.226 | 0.224 | 0.250 | 0.243 | 0.233 | 0.245 | 0.253 | 0.250 | 0.238 | 0.224 | 0.284 | 0.229 | 0.245 | 0.250 | 0.257 | 0.250 | 0.240 | 0.230 | 0.248 | 0.248 | 0.254 | 0.247 | 0.218 | | |

Table 3. External measurements (mm) and mass (g) of *Pipistrellus simandouensis* **sp. nov.** from Simandou Mountain Range, Guinea. Measurements presented as mean \pm standard deviation, range and sample size (n). Measurements of the holotype and other individuals of the new species *Pip. sp. nov.*, and other species of *Pipistrellus* occurring in sub-Saharan Africa and *Par. grandidieri* are shown for comparative purposes. Measurements for *Pip. (Vansonia) rueppellii*, *Pip. inexpectatus* and *Pip. musciculus* taken from Happold (2013), De Vree (1972) and Thomas (1913), respectively.

| Specimen or taxon | Total length | Tail length | Hindfoot length | Ear length | Forearm length | Body mass |
|---|--------------------------------|--------------------------------|---------------------------------|-----------------------------------|------------------------------------|---------------------------------|
| <i>Pipistrellus simandouensis</i> sp. nov. | 86 | 33 | 7 | 8 | 33.6 | 5.0 |
| Holotype ZFMK2008-0302 | | | | | | |
| <i>Pipistrellus simandouensis</i> sp. nov. | 83.0 \pm 3.00, 80–86, n = 3 | 30.3 \pm 3.80, 26–33, n = 4 | 6.6 \pm 0.53, 6.0–7.0, n = 4 | 9.8 \pm 1.30, 8.0–11.0, n = 4 | 32.9 \pm 1.00, 31.6–33.7, n = 4 | 6.0 \pm 1.73, 5.0–8.0, n = 3 |
| All specimens | | | | | | |
| <i>Pipistrellus (Parahypsugo) grandidieri</i> | 85.8 \pm 6.65, 75–95, n = 8 | 35.0 \pm 4.14, 31–42, n = 8 | 7.6 \pm 0.49, 7–8, n = 8 | 11.9 \pm 0.99, 11–13, n = 8 | 35.8 \pm 0.78, 34.6–36.8, n = 9 | 8.4 \pm 0.67, 7.7–9.6, n = 8 |
| <i>Pipistrellus hesperidus</i> | 79.3 \pm 4.80, 73–86, n = 13 | 30.5 \pm 4.22, 24–36, n = 13 | 7.2 \pm 0.64, 6.0–8.0, n = 11 | 10.2 \pm 1.29, 8.0–12.0, n = 13 | 31.4 \pm 1.80, 28.0–34.1, n = 16 | 5.3 \pm 0.93, 4.0–6.6, n = 11 |
| <i>Pipistrellus nanulus</i> | 69.6 \pm 4.15, 62–78, n = 18 | 26.2 \pm 2.48, 20–30, n = 18 | 5.9 \pm 1.13, 4.0–8.0, n = 18 | 9.4 \pm 1.29, 7.0–12.0, n = 18 | 26.6 \pm 0.79, 25.5–28.7, n = 18 | 3.9 \pm 0.36, 3.0–4.8, n = 17 |
| <i>Pipistrellus rusticus</i> | 75.8 \pm 3.53, 68–82, n = 21 | 29.5 \pm 4.17, 21–40, n = 19 | 6.0 \pm 0.89, 5–7, n = 15 | 9.8 \pm 0.82, 8.0–11.0, n = 21 | 29.0 \pm 0.98, 27.0–31.1, n = 23 | 4.3 \pm 0.65, 3.0–5.9, n = 21 |
| <i>Pipistrellus (Vansonia) rueppellii</i> | 83.1, 69–89, n = 47 | 32.4, 25–41, n = 47 | 8.9, 6–10, n = 27 | 11.9, 6–14, n = 82 | 32.7, 29–37, n = 106 | 6.3, 4–9, n = 36 |
| <i>Pipistrellus inexpectatus</i> | - | - | - | 12 | 31.5 | - |
| Holotype MNHC (954) | | | | | | |
| <i>Pipistrellus</i> cf. <i>inexpectatus</i> | 77 | 36 | 8 | 12 | 34.0 | 5 |
| This survey | | | | | | |
| <i>Pipistrellus musciculus</i> | 64 | 24 | - | 10 | 24.4 | - |
| Holotype BMNH13.2.8.1 | | | | | | |

Table 4. Cranial measurements (mm) of specimens of *Pipistrellus simandouensis* **sp. nov.** from Simandou Mountain Range, Guinea. Measurements presented as mean \pm standard deviation, range and sample size (n). Measurements of the holotype and other individuals of the new species *Pip. sp. nov.*, and other species of *Pipistrellus* occurring in sub-Saharan Africa and *Par. grandidieri* are shown for comparative purposes. Measurements for *Pip. (Vansonia) rueppellii*, *Pip. inexpectatus* and *Pip. musciculus* taken from Happold (2013), De Vree (1972) and Thomas (1913), respectively.

| Specimen or taxon | GSKL | ZYGO | POB | MAST | GBW | GSH | MAND |
|---|--|---|--|--|--|--|--|
| <i>Pipistrellus simandouensis</i> sp. nov. | 13.20 | 8.90 | 3.80 | 7.50 | 7.20 | 4.65 | 10.00 |
| Holotype ZFMK2008-0302 | | | | | | | |
| <i>Pipistrellus simandouensis</i> sp. nov. | 13.06 \pm 0.41, 12.60– 13.38, n = 3 | 8.53 \pm 0.32, 8.30–8.90, n = 3 | 3.86 \pm 0.11, 3.80–3.99, n = 3 | 7.35 \pm 0.32, 7.16–7.50, n = 3 | 6.89 \pm 0.32, 6.56–7.20, n = 3 | 4.67 \pm 0.11, 4.57–4.80, n = 3 | 9.64 \pm 0.27, 9.40–10.00, n = 4 |
| All specimens | | | | | | | |
| <i>Pipistrellus (Parahypsugo)</i> <i>grandidieri</i> | 14.93 \pm 0.23, 14.50– 15.30, n = 9 | 10.14 \pm 0.27, 9.90–10.70, n = 9 | 4.17 \pm 0.17, 3.90–4.50, n = 9 | 8.40 \pm 0.25, 7.90–8.70, n = 9 | 8.08 \pm 0.16, 7.80–8.30, n = 9 | 5.34 \pm 0.21, 4.90–5.60, n = 10 | 11.53 \pm 0.14, 11.40–11.70, n = 9 |
| <i>Pipistrellus hesperidus</i> | 12.83 \pm 0.39, 12.20– 13.61, n = 17 | 8.25 \pm 0.42, 7.50–8.79, n = 10 | 3.82 \pm 0.20, 3.50–4.20, n = 12 | 7.33 \pm 0.28, 6.80–7.76, n = 14 | 6.98 \pm 0.27, 6.61–7.70, n = 12 | 4.63 \pm 0.21, 4.39–5.00, n = 8 | 9.63 \pm 0.36, 9.06–10.30, n = 11 |
| <i>Pipistrellus nanulus</i> | 11.37 \pm 0.33, 10.80– 11.93, n = 17 | 7.22 \pm 0.31, 6.70–7.70, n = 11 | 3.53 \pm 0.24, 3.20–4.06, n = 17 | 6.64 \pm 0.15, 6.47–7.00, n = 17 | 6.20 \pm 0.26, 5.87–6.60, n = 17 | 4.37 \pm 0.14, 4.14–4.55, n = 16 | 8.24 \pm 0.34, 7.60–8.70, n = 16 |
| <i>Pipistrellus rusticus</i> | 12.04 \pm 0.30, 11.50– 12.60, n = 21 | 7.79 \pm 0.27, 7.29–8.09, n = 8 | 3.48 \pm 0.15, 3.28–3.69, n = 8 | 6.95 \pm 0.23, 6.63–7.29, n = 9 | 6.54 \pm 0.22, 6.08–6.74, n = 8 | 4.23 \pm 0.18, 3.97–4.56, n = 12 | 8.73 \pm 0.20, 8.47–9.06, n = 8 |
| <i>Pipistrellus (Vansonia)</i> <i>rueppellii</i> | 13.30, 12.10– 15.00, n = 72 | 8.70, 7.80– 7.20, n = 40 | - | - | - | - | - |
| <i>Pipistrellus inexpectatus</i> | 13.10 | 8.60 | 3.50 | 7.30 | 6.40 | - | 9.20 |
| Holotype MNHC (954) | | | | | | | |
| <i>Pipistrellus cf. inexpectatus</i> | 13.00 | - | 3.60 | 7.20 | 7.00 | 5.00 | 9.50 |
| This survey | | | | | | | |

| | | | | | | | |
|--------------------------------|-------|------|---|---|------|---|---|
| <i>Pipistrellus musciculus</i> | 10.70 | 7.50 | - | - | 5.50 | - | - |
| Holotype BMNH13.2.8.1 | | | | | | | |

Table 5. Dental measurements (mm) of specimens of *Pipistrellus simandouensis* **sp. nov.** from Simandou Mountain Range, Guinea. Measurements presented as mean \pm standard deviation, range and sample size (n). Measurements of the holotype and other individuals of the new species *Pip. sp. nov.*, and other species of *Pipistrellus* occurring in sub-Saharan Africa and *Par. grandidieri* are shown for comparative purposes. Measurements for *Pip. (Vansonia) rueppellii*, *Pip. inexpectatus* and *Pip. musciculus* taken from Happold (2013), De Vree (1972) and Thomas (1913), respectively.

| Specimen or taxon | C-M ³ | C-C | M ³ -M ³ | c-m ³ |
|---|--|--|--|--|
| <i>Pipistrellus simandouensis</i> sp. nov. | 4.80 | 4.10 | 5.80 | - |
| Holotype ZFMK2008-0302 | | | | |
| <i>Pipistrellus simandouensis</i> sp. nov. | 4.72 \pm 0.09, 4.60–4.80, n = 4 | 4.05 \pm 0.17, 3.80–4.20, n = 4 | 5.63 \pm 0.13, 5.50– 5.80, n = 4 | 5.12 \pm 0.13, 4.97–5.23, n = 3 |
| All specimens | | | | |
| <i>Pipistrellus (Parahypsugo)</i> <i>grandidieri</i> | 5.27 \pm 0.12, 5.00–5.40, n = 9 | 4.98 \pm 0.10, 4.80–5.10, n = 9 | 6.78 \pm 0.19, 6.50– 7.00, n = 9 | 5.64 \pm 0.12, 5.50–5.90, n = 9 |
| <i>Pipistrellus hesperidus</i> | 4.59 \pm 0.14, 4.40–4.91, n = 17 | 4.11 \pm 0.19, 3.80–4.35, n = 14 | 5.60 \pm 0.21, 5.30– 5.93, n = 13 | 4.98 \pm 0.15, 4.78–5.23, n = 11 |
| <i>Pipistrellus nanulus</i> | 3.90 \pm 0.15, 3.70–4.21, n = 17 | 3.56 \pm 0.18, 3.20–3.84, n = 17 | 4.92 \pm 0.19, 4.50– 5.27, n = 17 | 4.27 \pm 0.41, 3.80–5.08, n = 16 |
| <i>Pipistrellus rusticus</i> | 4.19 \pm 0.12, 3.90–4.30, n = 23 | 3.77 \pm 0.12, 3.56–3.90, n = 9 | 5.25 \pm 0.20, 4.79– 5.48, n = 9 | 4.53 \pm 0.15, 4.34–4.83, n = 8 |
| <i>Pipistrellus (Vansonia)</i> <i>rueppellii</i> | 4.70, 4.10– 5.40, n = 74 | - | - | - |
| <i>Pipistrellus</i> cf. <i>inexpectatus</i> | 4.30 | 4.10 | 5.30 | - |

| | | | | |
|----------------------------------|------|------|------|------|
| Holotype MNHC (954) | | | | |
| <i>Pipistrellus inexpectatus</i> | 4.60 | 3.50 | 5.30 | 4.70 |
| This survey | | | | |
| <i>Pipistrellus musciculus</i> | 3.50 | - | - | - |
| Holotype BMNH13.2.8.1 | | | | |

Table S1. External and craniodental measurements of African *Pipistrellus* specimens used in this study.

| Taxon | Museum/Field No | Holotype | Country | Sex | Age | Total | Tail | HF/cu | Ear | FA | Mass | GSKL | ZYGO | MAST | POB | GBW | GSH | C-M3 | C-C | M3-M3 | Mand | c-m3 |
|----------------------|--------------------|----------|--------------|-----|----------|-------|------|-------|------|------|-------|-------|-------|------|------|------|------|------|------|-------|-------|------|
| <i>simandouensis</i> | DM13220 | | Liberia | M | Adult | | | 6.2 | 10 | 32.7 | | 13.38 | 8.39 | 7.16 | 3.99 | 6.56 | 4.79 | 4.78 | 4.09 | 5.61 | 9.46 | 5.23 |
| <i>simandouensis</i> | ZMFK-MAM-2008.0300 | | Guinea | F | Adult | 83.0 | 32.0 | 6.0 | 11.0 | 33.7 | 8.0 | 12.60 | 8.30 | 7.40 | 3.80 | 6.90 | 4.58 | 4.70 | 4.20 | 5.50 | 9.70 | 4.97 |
| <i>simandouensis</i> | ZMFK-MAM-2008.0301 | | Guinea | M | Adult | 80.0 | 26.0 | 7.0 | 10.0 | 31.6 | 5.0 | | | | | | | 4.60 | 3.80 | 5.60 | 9.40 | |
| <i>simandouensis</i> | ZMFK-MAM-2008.0302 | Holotype | Guinea | M | Adult | 86.0 | 33.0 | 7.0 | 8.0 | 33.6 | 5.0 | 13.20 | 8.90 | 7.50 | 3.80 | 7.20 | 4.65 | 4.80 | 4.10 | 5.80 | 10.00 | 5.15 |
| <i>grandidieri</i> | CH-C12-040 | | Mozambique | F | Adult | 95 | 41 | | 13 | 35.6 | 9.1 | 15.00 | 10.20 | 8.10 | 4.00 | 8.10 | 5.20 | 5.20 | 4.80 | 6.50 | 11.70 | 5.50 |
| <i>grandidieri</i> | CH-C12-041 | | Mozambique | F | Adult | 95 | 42 | | 13 | 36.5 | 9.6 | 14.80 | 10.30 | 8.50 | 4.30 | 8.10 | 5.50 | 5.30 | 4.90 | 6.60 | 11.60 | 5.60 |
| <i>grandidieri</i> | JAG_349 | | Mozambique | F | Adult | 84 | 33 | 8 | 11 | 34.6 | 8.0 | 14.50 | 9.90 | 8.40 | 4.20 | 7.90 | 5.60 | 5.20 | 5.00 | 6.80 | 11.40 | 5.50 |
| <i>grandidieri</i> | JAG_406 | | Mozambique | F | Adult | 75 | | | 11 | 35.8 | 8.0 | 14.80 | 9.90 | 8.50 | 4.20 | 8.00 | 5.50 | 5.30 | 5.00 | 6.70 | 11.40 | 5.90 |
| <i>grandidieri</i> | JAG_430 | | Mozambique | F | Subadult | 83 | 34 | 7 | | 36.3 | 7.7 | 15.10 | 10.70 | 8.70 | 4.10 | 8.30 | 5.30 | 5.40 | 5.10 | 7.00 | 11.70 | 5.70 |
| <i>grandidieri</i> | JAG_433 | | Mozambique | F | Adult | | 31 | 7 | 12 | 36.8 | 8.8 | 15.10 | 10.10 | 8.60 | 4.20 | 8.20 | 5.50 | 5.30 | 5.00 | 6.90 | 11.70 | 5.70 |
| <i>grandidieri</i> | JAG_439 | | Mozambique | F | Adult | 84 | 33 | 8 | 11 | 34.6 | 8.0 | 14.80 | 9.90 | 7.90 | 3.90 | 7.80 | 4.90 | 5.00 | 4.90 | 6.60 | 11.50 | 5.60 |
| <i>grandidieri</i> | JAG_440 | | Mozambique | M | Adult | 87 | 34 | 8 | 13 | 36.3 | 8.8 | 15.30 | 10.30 | 8.50 | 4.50 | 8.20 | 5.30 | 5.30 | 5.00 | 7.00 | 11.40 | 5.60 |
| <i>grandidieri</i> | JAG_441 | | Mozambique | M | Adult | 83 | 32 | 8 | 11 | 35.9 | 7.8 | 15.00 | 10.00 | 8.40 | 4.10 | 8.10 | 5.30 | 5.40 | 5.10 | 6.90 | 11.40 | 5.70 |
| <i>grandidieri</i> | MNHN2129 | Holotype | Tanzania | F | Adult | | | 7 | | 33.0 | | 12.30 | 9.10 | 7.10 | 3.50 | 7.30 | 5.90 | 4.80 | 4.10 | 5.90 | 10.20 | 5.10 |
| <i>hesperidus</i> | Bulembu_13 | | Eswatini | F | Adult | 86 | 34 | 7 | 10 | 31.3 | 6 | 13.05 | 8.25 | 7.25 | 3.70 | 6.75 | 4.75 | 4.55 | 3.95 | 5.50 | 9.35 | 4.75 |
| <i>hesperidus</i> | JAG_447 | | Mozambique | M | Adult | 76 | 24 | 7 | 11 | 29.0 | 4.2 | 12.60 | 7.70 | 7.10 | 3.50 | 6.90 | 4.40 | 4.60 | 4.10 | 5.50 | 9.50 | 4.80 |
| <i>hesperidus</i> | JAG_448 | | Mozambique | F | Adult | 76 | 30 | 8 | 9 | 29.8 | 4.5 | 12.50 | 7.50 | 7.20 | 4.00 | 6.90 | 4.40 | 4.40 | 4.00 | 5.50 | | 4.90 |
| <i>hesperidus</i> | JAG_450 | | Mozambique | M | Adult | 80 | 27 | 8 | 9 | 31.1 | 4.8 | 12.70 | 8.30 | 7.70 | 4.20 | | 4.40 | 4.60 | 4.20 | 5.90 | 10.30 | 4.90 |
| <i>hesperidus</i> | Jilobi_11 | | Eswatini | F | Adult | 86 | 36 | 7 | 11 | 33.2 | 6 | 13.00 | 8.50 | 7.50 | 3.50 | 7.00 | 5.00 | 4.35 | 4.00 | 5.60 | 9.40 | 4.60 |
| <i>hesperidus</i> | Jilobi_12 | | Eswatini | M | Adult | 79 | 35 | 8 | 11 | 32.5 | 5 | 12.70 | 8.20 | 7.60 | 3.75 | 6.90 | 5.00 | 4.25 | 3.80 | 5.30 | 9.15 | 4.55 |
| <i>hesperidus</i> | Dom_M_18 | | Eswatini | M | Adult | 76 | 27 | 6 | | 28.0 | 5 | 12.40 | | 7.30 | | | | 4.40 | 3.80 | 5.40 | | |
| <i>hesperidus</i> | Dom_M_22 | | Eswatini | M | Adult | 75 | 29 | 7 | | 28.5 | 4 | 12.20 | | 6.80 | | | | 4.50 | 3.80 | 5.30 | | |
| <i>hesperidus</i> | DM10063 | | UAE | | | | | | | | 12.86 | | 8.26 | 7.11 | 3.55 | 6.72 | 4.62 | 4.60 | 4.07 | | 9.89 | 5.10 |
| <i>hesperidus</i> | DM10832 | | Mozambique | M | | 73 | 25 | 7.4 | 8 | 31.8 | | 12.50 | | 7.31 | 3.60 | 6.90 | 4.41 | 4.57 | 3.90 | 5.39 | 9.36 | 4.85 |
| <i>hesperidus</i> | DM10837 | | Mozambique | M | | | | 6.5 | 8.8 | 32.2 | | 12.60 | | 7.41 | 3.75 | 6.90 | 4.72 | 4.46 | 4.01 | 5.53 | 9.06 | 4.78 |
| <i>hesperidus</i> | DM4692 | | Zimbabwe | M | | 80 | 35 | | 11 | 32.0 | | 13.08 | 8.25 | 7.56 | 4.00 | 7.08 | 4.84 | 4.57 | 4.30 | 5.83 | 9.46 | 5.04 |
| <i>hesperidus</i> | DM5382 | | South Africa | F | | 84 | 34 | | 11 | 31.0 | 6.11 | 12.74 | 8.55 | 7.40 | 3.91 | 6.90 | 4.55 | 4.67 | 4.20 | 5.68 | 9.63 | 5.06 |
| <i>hesperidus</i> | DM5403 | | South Africa | M | | 86 | 33 | | 12 | 34.1 | 6.59 | 13.61 | 8.56 | 7.61 | 3.85 | 7.13 | 4.77 | 4.91 | 4.27 | 5.93 | 9.95 | 5.15 |
| <i>hesperidus</i> | DM5868 | | South Africa | M | | | | | 12 | 32.0 | 6.47 | 13.54 | 8.79 | 7.76 | 3.82 | 7.09 | 4.70 | 4.87 | 4.26 | 5.80 | 9.87 | 5.23 |
| <i>hesperidus</i> | DM8477 | | Mozambique | M | Adult | | | 7.2 | 9.4 | 31.2 | | 12.56 | 7.97 | 6.96 | 3.85 | 6.61 | 4.39 | 4.55 | 4.35 | 5.50 | 9.25 | |
| <i>hesperidus</i> | DM8860 | | South Africa | M | | 74 | 27 | | | 34.0 | | 13.26 | 8.61 | 7.44 | 3.85 | 6.91 | 4.56 | 4.67 | 4.34 | 5.50 | 9.65 | 4.95 |
| <i>nanulus</i> | ZMFK-MAM-2003.1048 | | Ghana | M | Adult | 68.0 | 20.0 | 6.0 | 7.0 | 26.0 | 3.6 | 11.70 | 7.60 | 6.80 | 3.50 | 6.40 | | 4.00 | 3.70 | 5.10 | 8.10 | |
| <i>nanulus</i> | ZMFK-MAM-2008.0298 | | Guinea | M | Adult | 71.0 | 23.0 | 5.0 | 7.0 | 26.8 | 3.5 | 11.20 | 7.70 | 6.70 | 3.40 | 6.50 | | 3.90 | 3.70 | 4.90 | 8.00 | |
| <i>nanulus</i> | ZMFK-MAM-2009.0031 | | Guinea | M | Adult | 73.0 | 24.0 | 7.0 | 9.0 | 26.3 | 4.0 | | | | | | | | | | | |
| <i>nanulus</i> | ZWW_272 | | Guinea | M | Adult | 67 | 25 | 6 | 8 | 25.7 | 3.0 | 11.30 | | 6.50 | 3.30 | 6.10 | 4.40 | 3.80 | 3.50 | 4.80 | 8.30 | 4.10 |
| <i>nanulus</i> | ZWW_273 | | Guinea | F | Adult | 62 | 26 | 7 | 10 | 26.6 | 4.0 | 10.90 | | 6.50 | 3.30 | 5.90 | 4.30 | 3.70 | 3.20 | 4.50 | 7.60 | 3.90 |
| <i>nanulus</i> | ZWW_276 | | Guinea | F | Adult | 67 | 30 | 7 | 9 | 27.0 | 4.0 | 11.40 | | 6.80 | 3.20 | 6.00 | 4.20 | 3.80 | 3.40 | 5.00 | 8.70 | 3.80 |
| <i>nanulus</i> | DM13230 | | Liberia | M | Adult | 67 | 27 | 5 | 9 | 25.9 | 3.7 | 10.94 | 6.76 | 6.59 | 3.75 | 5.91 | | 3.87 | 3.51 | 4.73 | 8.52 | 4.42 |
| <i>nanulus</i> | DM13231 | | Liberia | F | Adult | 66 | 29 | 4 | 10 | 27.0 | 3.7 | 11.40 | 7.19 | 6.86 | 3.76 | 6.03 | | 4.03 | 3.68 | 5.23 | 8.70 | 4.26 |
| <i>nanulus</i> | DM13234 | | Liberia | M | Adult | 71 | 25 | 5 | 10 | 26.7 | 11.41 | 7.40 | 6.49 | 3.95 | 5.87 | | 3.93 | 3.60 | 5.03 | 8.53 | 4.22 | |
| <i>nanulus</i> | DM14196 | | Liberia | F | Adult | 78 | 25 | 4 | 10 | 28.1 | 4.8 | 11.52 | 7.02 | 6.63 | 3.60 | 5.95 | 4.30 | 4.17 | 3.62 | 5.27 | 8.21 | 4.59 |
| <i>nanulus</i> | DM14197 | | Liberia | F | Adult | 71 | 26 | 5 | 9 | 25.5 | 3.9 | 11.53 | 7.19 | 6.58 | 3.53 | 6.31 | 4.24 | 4.00 | 3.41 | 5.01 | 8.29 | 4.80 |
| <i>nanulus</i> | DM14198 | | Liberia | M | Adult | 77 | 26 | 5 | 9 | 26.8 | 4.0 | 11.51 | 7.32 | 6.47 | 3.62 | 6.13 | 4.14 | 3.95 | 3.71 | 4.90 | 8.24 | 4.76 |
| <i>nanulus</i> | ZWW_44 | | Liberia | F | Adult | 72 | 28 | 7 | 11 | 26.1 | 4.0 | 11.40 | | 6.60 | 3.50 | 6.10 | 4.40 | 3.70 | 3.20 | 4.80 | 7.90 | 3.80 |

| | | | | | | | | | | | | | | | | | | | | | | |
|---------------------|----------------|----------|--------------|---|----------|----|----|-----|------|------|------|-------|------|------|------|------|------|------|------|------|------|------|
| <i>nanulus</i> | ZWW_63 | | Liberia | F | Adult | 71 | 28 | 6 | 11 | 26.3 | 4.0 | 11.50 | 7.30 | 7.00 | 3.40 | 6.60 | 4.50 | 3.90 | 3.60 | 4.90 | 8.20 | 3.90 |
| <i>nanulus</i> | ZWW_73 | | Liberia | M | Adult | 69 | 30 | 7 | 10 | 26.3 | 4.0 | 11.00 | | 6.50 | 3.40 | 6.20 | 4.50 | 3.80 | 3.70 | 4.80 | | |
| <i>nanulus</i> | ZWW_96 | | Liberia | M | Adult | 65 | 27 | 8 | 10 | 26.9 | 4.0 | 11.90 | 7.20 | 6.70 | 3.60 | 6.60 | | 3.90 | 3.70 | 4.80 | 8.30 | 4.10 |
| <i>nanulus</i> | ZWW_97 | | Liberia | F | Adult | 65 | 27 | 6 | 12 | 26.2 | 4.0 | 10.80 | 6.70 | 6.60 | 3.20 | 6.20 | 4.50 | 3.70 | 3.40 | 4.80 | 7.60 | 4.00 |
| <i>nanulus</i> | DM13714 | | Sierra Leone | M | Adult | 72 | 25 | 6 | 9 | 28.7 | 4.0 | 11.93 | | 6.62 | 4.06 | 6.59 | 4.55 | 4.21 | 3.84 | 5.00 | 8.61 | 5.08 |
| <i>rusticus</i> | Dom_M_15 | | Eswatini | F | Adult | 72 | 25 | 5 | | 28.0 | 5 | 12.2 | | 7.1 | | | | 4.1 | 3.9 | 5.3 | | |
| <i>rusticus</i> | Mvembili_07 | | Eswatini | M | Adult | 70 | 29 | 5 | 8 | 29.0 | 4 | 12.0 | | | | | | 4.3 | | | | |
| <i>rusticus</i> | Mvembili_08 | | Eswatini | F | Adult | 79 | 32 | 6 | 10 | 29.3 | 5 | 12.6 | | | | | | 4.2 | | | | |
| <i>rusticus</i> | Mvembili_26 | | Eswatini | F | Juvenile | 82 | | | 10 | 29.9 | 4 | 12.55 | 8.10 | 7.30 | 3.55 | 6.65 | 4.35 | 4.25 | 3.75 | 5.25 | 9.25 | 4.25 |
| <i>rusticus</i> | Mvembili_27 | | Eswatini | F | Adult | 76 | 31 | 5 | 9 | 29.0 | 4 | 12.40 | | | | | | 4.10 | | | | |
| <i>rusticus</i> | Mvembili_28 | | Eswatini | F | Juvenile | | | | 10 | 30.1 | 5 | 12.60 | 8.00 | 7.25 | 3.25 | 6.60 | 4.20 | 4.20 | 3.85 | 5.35 | 9.00 | 4.35 |
| <i>rusticus</i> | Mvembili_29 | | Eswatini | M | Juvenile | 76 | 33 | | 9 | 29.5 | 3 | 11.80 | | | | | | 3.90 | | | | |
| <i>rusticus</i> | Mvembili_30 | | Eswatini | M | | 76 | 31 | 6 | 10 | 29.3 | 4 | 12.05 | 8.10 | 7.30 | 3.25 | 6.65 | 4.00 | 4.30 | 3.70 | 5.45 | 9.00 | 4.30 |
| <i>rusticus</i> | Mvembili_31 | | Eswatini | F | | 79 | 33 | | 9 | 30.1 | 5 | 12.45 | 8.30 | 7.30 | 3.35 | 6.90 | 4.20 | 4.20 | 3.90 | 5.50 | 9.10 | 4.40 |
| <i>rusticus</i> | Mvembili_32 | | Eswatini | M | Adult | 72 | 31 | 5 | 11 | 28.0 | 4 | 12.2 | | | | | | 4.2 | | | | |
| <i>rusticus</i> | Mvembili_35 | | Eswatini | M | Adult | 74 | 29 | 6 | 10 | 28.5 | 4 | 12.2 | | | | | | 4.0 | | | | |
| <i>rusticus</i> | Sgcineni_20 | | Eswatini | M | Adult | 74 | 27 | 6 | 9 | 27.0 | 4 | 11.6 | | | | | | 4.1 | | | | |
| <i>rusticus</i> | Sgcineni_21 | | Eswatini | F | Adult | 76 | 31 | 6 | 11 | 29.8 | 4 | | | | | | | 4.3 | | | | |
| <i>rusticus</i> | DM13586 | | South Africa | F | | 79 | 40 | 7.7 | 11 | 28.3 | | 11.9 | 7.9 | 7 | 3.36 | 6.57 | 4.21 | 4.2 | 3.56 | 5.21 | 8.82 | 4.83 |
| <i>rusticus</i> | DM13588 | | South Africa | M | | 79 | | 7 | 10 | 28.1 | 4.3 | 11.8 | 7.9 | 6.81 | 3.34 | 6.42 | 4.29 | 4.2 | 3.88 | 5.2 | 8.68 | 4.52 |
| <i>rusticus</i> | DM14432 | | Angola | | | 80 | 25 | 7.3 | 11 | 29.4 | 4.8 | 11.8 | 7.9 | 7.06 | 3.69 | 6.74 | 4.56 | 4.2 | 3.65 | 5.17 | 8.79 | 4.42 |
| <i>rusticus</i> | DM14439 | | Angola | | | 78 | 21 | | | 31.1 | 4.1 | 11.5 | 7.9 | 6.86 | 3.49 | 6.6 | 4.42 | 4.2 | 3.79 | 5.42 | 8.58 | 4.45 |
| <i>rusticus</i> | DM15059 | | Angola | | | 68 | 23 | | 8.6 | 28.9 | 5.9 | 12.0 | 7.8 | 7.29 | 3.62 | 6.73 | 4.2 | 4.3 | 3.77 | 5.48 | 8.92 | 4.34 |
| <i>rusticus</i> | DM5379 | | South Africa | M | | 74 | 30 | | 10 | 28.1 | 3.98 | 12.3 | 8.1 | 7.15 | 3.42 | 6.72 | 4.02 | 4.3 | 3.9 | 5.38 | 9.06 | 4.5 |
| <i>rusticus</i> | DM5389 | | South Africa | M | | 73 | 30 | | 10 | 28.4 | 3.35 | 11.6 | 7.5 | 6.63 | 3.28 | 6.08 | 3.97 | 4.2 | 3.69 | 4.79 | 8.47 | 4.63 |
| <i>rusticus</i> | DM9127 | | Malawi | M | | | | 6.7 | 10.2 | 30.0 | | 11.9 | 7.3 | 6.66 | 3.61 | 6.44 | 4.32 | 4.2 | 3.76 | 5.26 | 8.55 | 4.52 |
| <i>rusticus</i> | Sgcineni_32 | | Eswatini | M | Adult | 78 | 30 | 5 | 10 | 28.4 | 4 | 12.0 | | | | | | 4.2 | | | | |
| <i>rusticus</i> | Velezizweni_35 | | Eswatini | F | Adult | 77 | 30 | 6 | 10 | 27.8 | 4 | 12.1 | | | | | | 4.2 | | | | |
| <i>inexpectatus</i> | MNHC | Holotype | Cameroon | M | Adult | | | 12 | 12 | 31.5 | | 13.10 | 8.60 | 7.30 | 3.50 | 6.40 | | 4.30 | 4.10 | 5.30 | 9.20 | |
| <i>inexpectatus</i> | ZWW_254 | | Guinea | F | Adult | 77 | 36 | 8 | 12 | 34.0 | 5.0 | 13.00 | | 7.20 | 3.60 | 7.00 | 5.00 | 4.60 | 3.50 | 5.30 | 9.50 | 4.70 |
| <i>muscululus</i> | BMNH13.2.8.1 | Holotype | Cameroon | M | Adult | 64 | 24 | | 10 | 24.4 | | 10.70 | 7.50 | | | 5.50 | | 3.50 | | | | |
| <i>deserti</i> | MNHN_2006-253 | | Algeria | M | Adult | | | | | | | 11.90 | 7.30 | 6.70 | 3.30 | 6.10 | 4.40 | 4.30 | 3.60 | 4.90 | 8.30 | 4.50 |

Table S2. Average genetic distances within and between clades of *Neoromicia*, *Pipistrellus* and *Parahypsugo* species as determined with Cyt b in MEGA v.7 (Kumar *et al.*, 2016). Values in parentheses indicate within species distance.

| | <i>Laephotis botswanae</i> | <i>Neoromicia isabella</i> | <i>Neoromicia nana</i> | <i>Neoromicia rendalli</i> | <i>Neoromicia capensis</i> | <i>Neoromicia brunnea</i> | <i>Neoromicia roseveari</i> | <i>Neoromicia somalica</i> | <i>Neoromicia zuluensis</i> | <i>Nycticeinops schlieffeni</i> | <i>Parahypsugo bellieri</i> | <i>Parahypsugo happoldorum</i> | <i>Pipistrellus cf. hesperidus</i> | <i>Pipistrellus deserti</i> | <i>Pipistrellus grandidieri</i> | <i>Pipistrellus hesperidus</i> | <i>Pipistrellus kuhlii</i> | <i>Pipistrellus nanulus</i> | <i>Pipistrellus nathusii</i> | <i>Pipistrellus pipistrellus</i> | <i>Pipistrellus pygmaeus</i> | <i>Pipistrellus rusticus</i> | <i>Pipistrellus simandouensis sp. nov.</i> | Outgroup |
|--|----------------------------|----------------------------|------------------------|----------------------------|----------------------------|---------------------------|-----------------------------|----------------------------|-----------------------------|---------------------------------|-----------------------------|--------------------------------|------------------------------------|-----------------------------|---------------------------------|--------------------------------|----------------------------|-----------------------------|------------------------------|----------------------------------|------------------------------|------------------------------|--|----------|
| <i>Laephotis botswanae</i> | 0.004 | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Neoromicia isabella</i> | 0.171 | - | | | | | | | | | | | | | | | | | | | | | | |
| <i>Neoromicia nana</i> | 0.192 | 0.172 | 0.001 | | | | | | | | | | | | | | | | | | | | | |
| <i>Neoromicia rendalli</i> | 0.187 | 0.158 | 0.197 | 0.004 | | | | | | | | | | | | | | | | | | | | |
| <i>Neoromicia capensis</i> | 0.105 | 0.161 | 0.187 | 0.186 | 0.005 | | | | | | | | | | | | | | | | | | | |
| <i>Neoromicia brunnea</i> | 0.169 | 0.104 | 0.213 | 0.139 | 0.139 | 0.000 | | | | | | | | | | | | | | | | | | |
| <i>Neoromicia roseveari</i> | 0.186 | 0.100 | 0.206 | 0.150 | 0.175 | 0.081 | 0.005 | | | | | | | | | | | | | | | | | |
| <i>Neoromicia somalica</i> | 0.161 | 0.171 | 0.203 | 0.189 | 0.180 | 0.184 | 0.176 | 0.049 | | | | | | | | | | | | | | | | |
| <i>Neoromicia zuluensis</i> | 0.164 | 0.164 | 0.232 | 0.205 | 0.178 | 0.164 | 0.152 | 0.078 | - | | | | | | | | | | | | | | | |
| <i>Nycticeinops schlieffeni</i> | 0.180 | 0.163 | 0.201 | 0.199 | 0.178 | 0.163 | 0.172 | 0.180 | 0.173 | 0.005 | | | | | | | | | | | | | | |
| <i>Parahypsugo bellieri</i> | 0.164 | 0.202 | 0.217 | 0.191 | 0.196 | 0.196 | 0.212 | 0.201 | 0.189 | 0.185 | 0.000 | | | | | | | | | | | | | |
| <i>Parahypsugo happoldorum</i> | 0.161 | 0.182 | 0.202 | 0.182 | 0.160 | 0.183 | 0.187 | 0.171 | 0.172 | 0.159 | 0.155 | 0.007 | | | | | | | | | | | | |
| <i>Pipistrellus cf. hesperidus</i> | 0.211 | 0.191 | 0.226 | 0.222 | 0.220 | 0.202 | 0.211 | 0.200 | 0.170 | 0.208 | 0.197 | 0.193 | 0.002 | | | | | | | | | | | |
| <i>Pipistrellus deserti</i> | 0.193 | 0.188 | 0.217 | 0.239 | 0.191 | 0.197 | 0.193 | 0.196 | 0.183 | 0.178 | 0.204 | 0.189 | 0.161 | 0.000 | | | | | | | | | | |
| <i>Pipistrellus grandidieri</i> | 0.170 | 0.189 | 0.205 | 0.182 | 0.168 | 0.185 | 0.184 | 0.184 | 0.192 | 0.183 | 0.172 | 0.084 | 0.204 | 0.187 | 0.006 | | | | | | | | | |
| <i>Pipistrellus hesperidus</i> | 0.201 | 0.196 | 0.247 | 0.200 | 0.216 | 0.182 | 0.213 | 0.204 | 0.177 | 0.202 | 0.206 | 0.222 | 0.124 | 0.158 | 0.209 | 0.013 | | | | | | | | |
| <i>Pipistrellus kuhlii</i> | 0.185 | 0.191 | 0.226 | 0.244 | 0.205 | 0.195 | 0.191 | 0.195 | 0.178 | 0.174 | 0.204 | 0.196 | 0.165 | 0.013 | 0.197 | 0.153 | 0.004 | | | | | | | |
| <i>Pipistrellus nanulus</i> | 0.229 | 0.218 | 0.239 | 0.236 | 0.232 | 0.199 | 0.190 | 0.243 | 0.246 | 0.190 | 0.212 | 0.221 | 0.176 | 0.161 | 0.207 | 0.188 | 0.167 | 0.012 | | | | | | |
| <i>Pipistrellus nathusii</i> | 0.186 | 0.244 | 0.216 | 0.239 | 0.192 | 0.201 | 0.230 | 0.215 | 0.195 | 0.227 | 0.200 | 0.218 | 0.194 | 0.143 | 0.227 | 0.175 | 0.148 | 0.193 | 0.000 | | | | | |
| <i>Pipistrellus pipistrellus</i> | 0.171 | 0.207 | 0.248 | 0.207 | 0.193 | 0.202 | 0.193 | 0.239 | 0.222 | 0.195 | 0.216 | 0.228 | 0.186 | 0.173 | 0.238 | 0.142 | 0.168 | 0.155 | 0.175 | NA | | | | |
| <i>Pipistrellus pygmaeus</i> | 0.160 | 0.216 | 0.220 | 0.225 | 0.182 | 0.221 | 0.215 | 0.216 | 0.170 | 0.175 | 0.211 | 0.206 | 0.180 | 0.158 | 0.207 | 0.153 | 0.157 | 0.176 | 0.175 | 0.114 | NA | | | |
| <i>Pipistrellus rusticus</i> | 0.202 | 0.202 | 0.247 | 0.218 | 0.252 | 0.186 | 0.215 | 0.219 | 0.228 | 0.176 | 0.262 | 0.241 | 0.128 | 0.183 | 0.197 | 0.086 | 0.165 | 0.169 | 0.183 | 0.146 | 0.146 | NA | | |
| <i>Pipistrellus simandouensis sp. nov.</i> | 0.189 | 0.166 | 0.215 | 0.205 | 0.182 | 0.188 | 0.181 | 0.201 | 0.185 | 0.177 | 0.175 | 0.197 | 0.112 | 0.136 | 0.195 | 0.079 | 0.135 | 0.128 | 0.155 | 0.132 | 0.147 | 0.116 | NA | |
| Outgroup | 0.272 | 0.256 | 0.288 | 0.269 | 0.264 | 0.261 | 0.260 | 0.277 | 0.265 | 0.254 | 0.274 | 0.261 | 0.254 | 0.245 | 0.264 | 0.265 | 0.242 | 0.242 | 0.276 | 0.266 | 0.248 | 0.262 | 0.237 | |

Table S3. Eigenvector loadings of the principal components analysis (PCA) for PC1, PC2, and PC3 based on standardized craniodental measurements of *Pipistrellus simandouensis* **sp. nov.**, *Pip. hesperidus*, *Pip. inexpectatus*, *Pip. nanulus*, *Pip. rusticus* and *Pip. (Par.) grandidieri*.

| Character | PC1 | PC2 | PC3 |
|--------------------------------------|--------|--------|--------|
| GSKL | -0.332 | 0.095 | -0.140 |
| POS | -0.286 | 0.041 | -0.339 |
| MAST | -0.327 | -0.386 | 0.702 |
| GSW | -0.323 | 0.137 | -0.229 |
| GSH | -0.246 | -0.839 | -0.276 |
| C-M ³ | -0.331 | 0.205 | 0.057 |
| C-C | -0.328 | 0.107 | 0.041 |
| M ³ -M ³ | -0.332 | 0.094 | -0.112 |
| MAND | -0.333 | 0.076 | -0.118 |
| c-m ³ | -0.311 | 0.220 | 0.461 |
| Cumulative total variation explained | 86.3% | 92.4% | 95.6 |

Table S4. Bacula measurements for five pipistrelloid bat species from West Africa. Measurements were taken from digital images of the dorsal bacula views of *Pipistrellus simandouensis* **sp. nov.**, *Pip. grandidieri*, *Pip. hesperidus*, *Pip. nanulus* and *Pip. rusticus*.

| Species | Total length (mm) | Basal width (mm) | Tip width (mm) | Tip notch height (mm) |
|---|----------------------|---------------------|----------------|--------------------------|
| <i>Pip. simandouensis</i> sp. nov. | 1.32 | 0.32 | 0.11 | 0.03 |
| <i>Pip. grandidieri</i> | 2.00 | 0.38 | 0.07 | N/A |
| <i>Pip. hesperidus</i> | 1.47 | 0.23 | 0.10 | 0.03 |
| <i>Pip. nanulus</i> | 2.98 | 0.68 | 0.51 | 0.13 |
| <i>Pip. rusticus</i> | 1.52 | 0.35 | 0.13 | 0.04 |

Figure 1. Map showing the distribution of the newly described species, *Pipistrellus simandouensis* **sp. nov.**, as well as other *Pipistrellus* species in West Africa; an arrow points to the type locality. The inset is a map of Africa showing all the African *Pipistrellus* specimens used in this study.

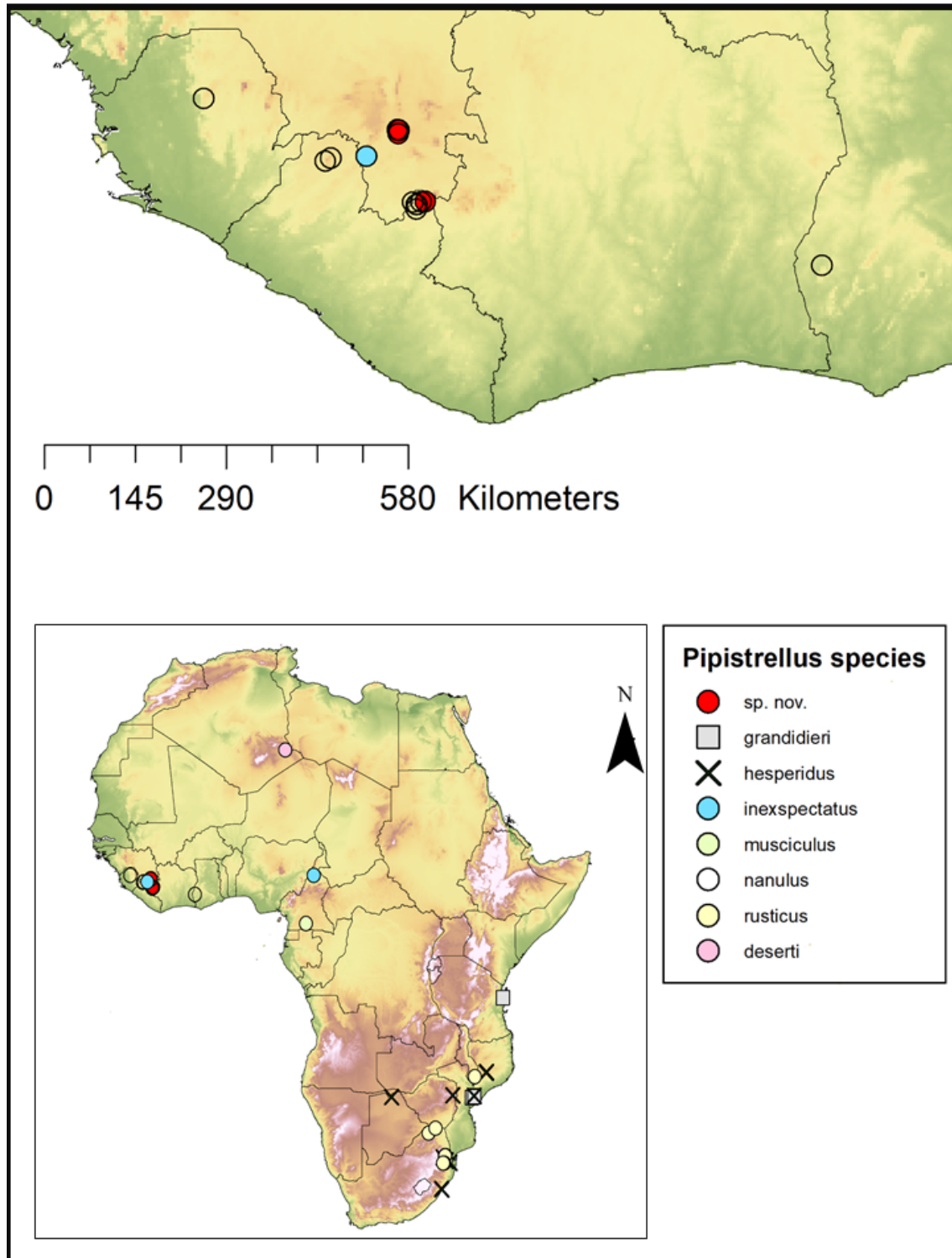


Figure 2. (A) Maximum likelihood tree of COI sequences based on the Hasegawa-Kishino-Yano model conducted in MEGA7. The tree with the highest log likelihood (-5569.83) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. (B) Maximum likelihood tree of Cyt *b* based on the Tamura-Nei substitution model conducted in MEGA7. The tree with the highest log likelihood (-5599.78) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches.

Figure 2A

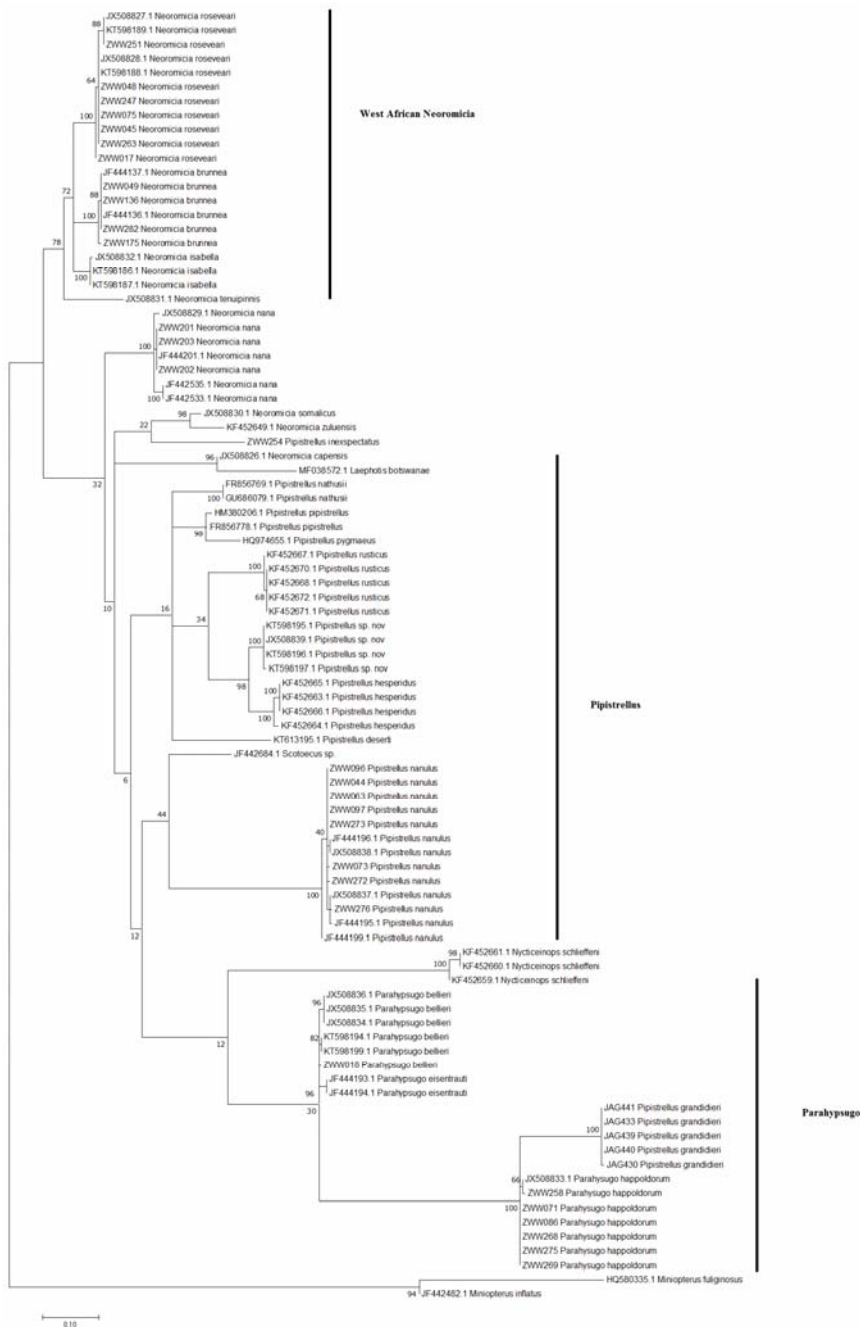


Figure 2B

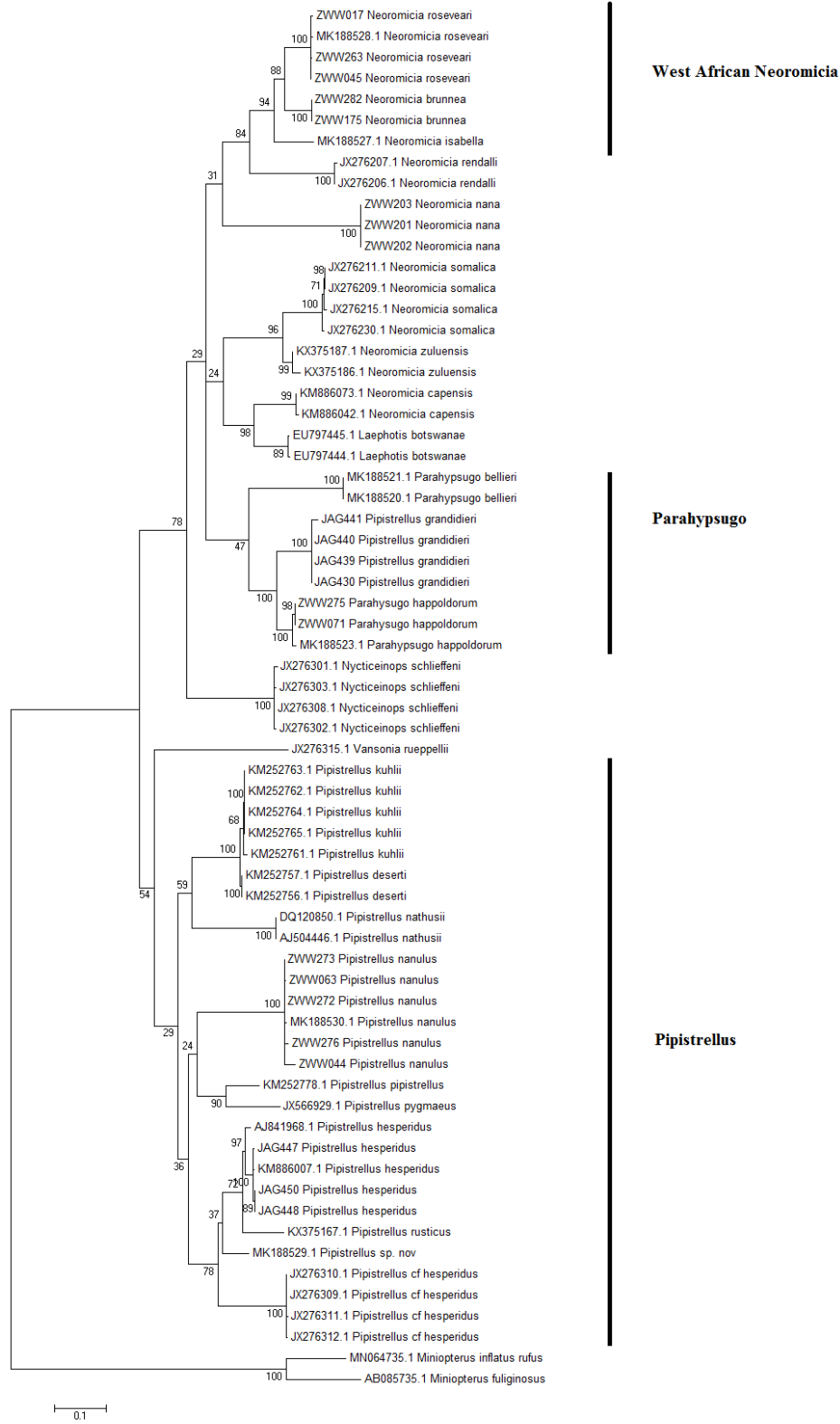


Figure 3. A Principal Components Analysis (PCA) graph plotting the first two components for craniodental measurements of African *Pipistrellus* species including *Parahypsugo grandidieri*. See Table S3 for the variables used in this analysis and the loadings on PC1 and PC2.

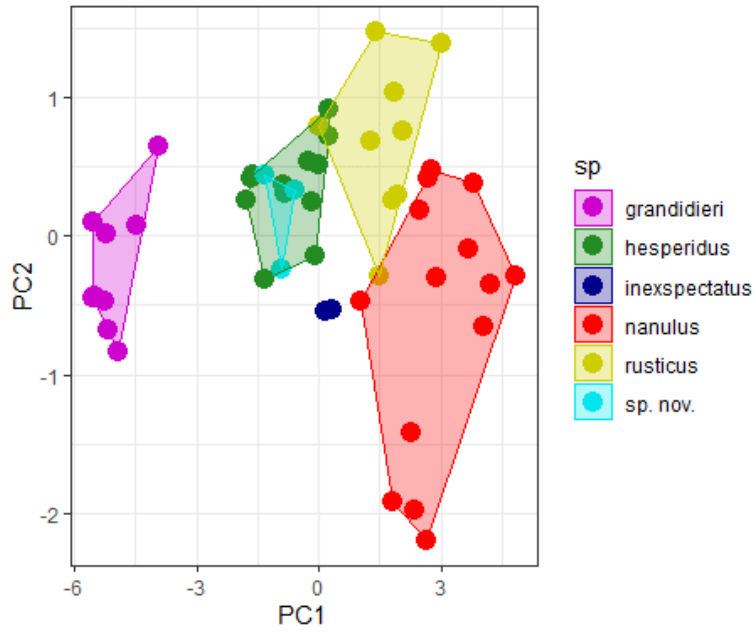


Figure 4. Portraits (A) and (B) of holotype (ZFMK2008-0302), (C) of specimen from Liberia (DM13220) of *Pipistrellus simandouensis* **sp. nov.** showing unicoloured fur, and (D) of *Pip. hesperidus* for comparison showing the bicoloured fur of this species (photographs A-B by Jan Decher, C-D by Ara Monadjem).

Figure 4A



Figure 4B



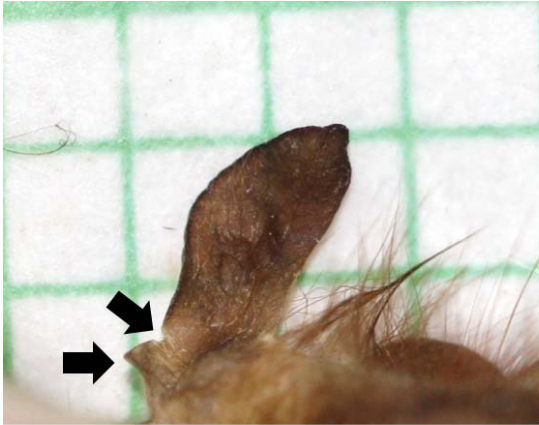
Figure 4C



Figure 4D



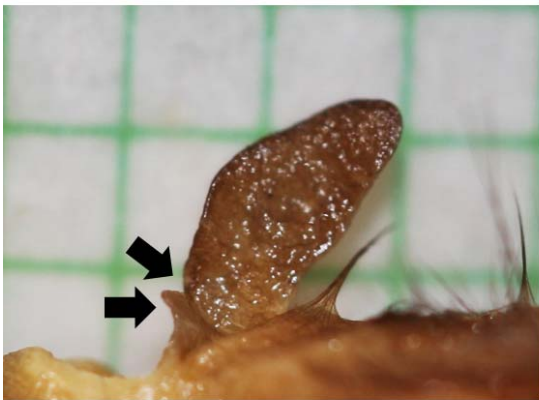
Figure 5. Tragi of *Pipistrellus simandouensis* sp. nov., *P. nanulus*, *P. hesperidus* and *P. rusticus*. Arrows indicate the position of the indentation/notch and basal projection of the outer margin in each species. The museum number of each specimen photographed is provided below the name.



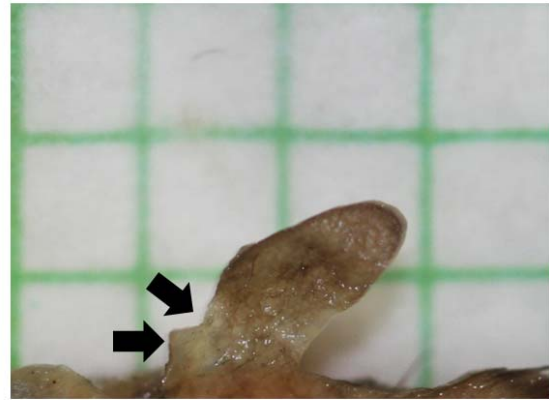
Pipistrellus sp. nov
DM 13220



Pipistrellus nanulus
DM 14196



Pipistrellus hesperidus
DM 8477



Pipistrellus rusticus
DM 13587

Figure 6. The cranium of *Pipistrellus simandouensis* **sp. nov.** (holotype, ZFMK2008-0302) showing lateral, ventral, and dorsal views of the neurocranium; and lateral view of the mandible (photographs by D. Rohwedder and R. Hutterer). The black scale bar on the bottom of the image = 10 mm.

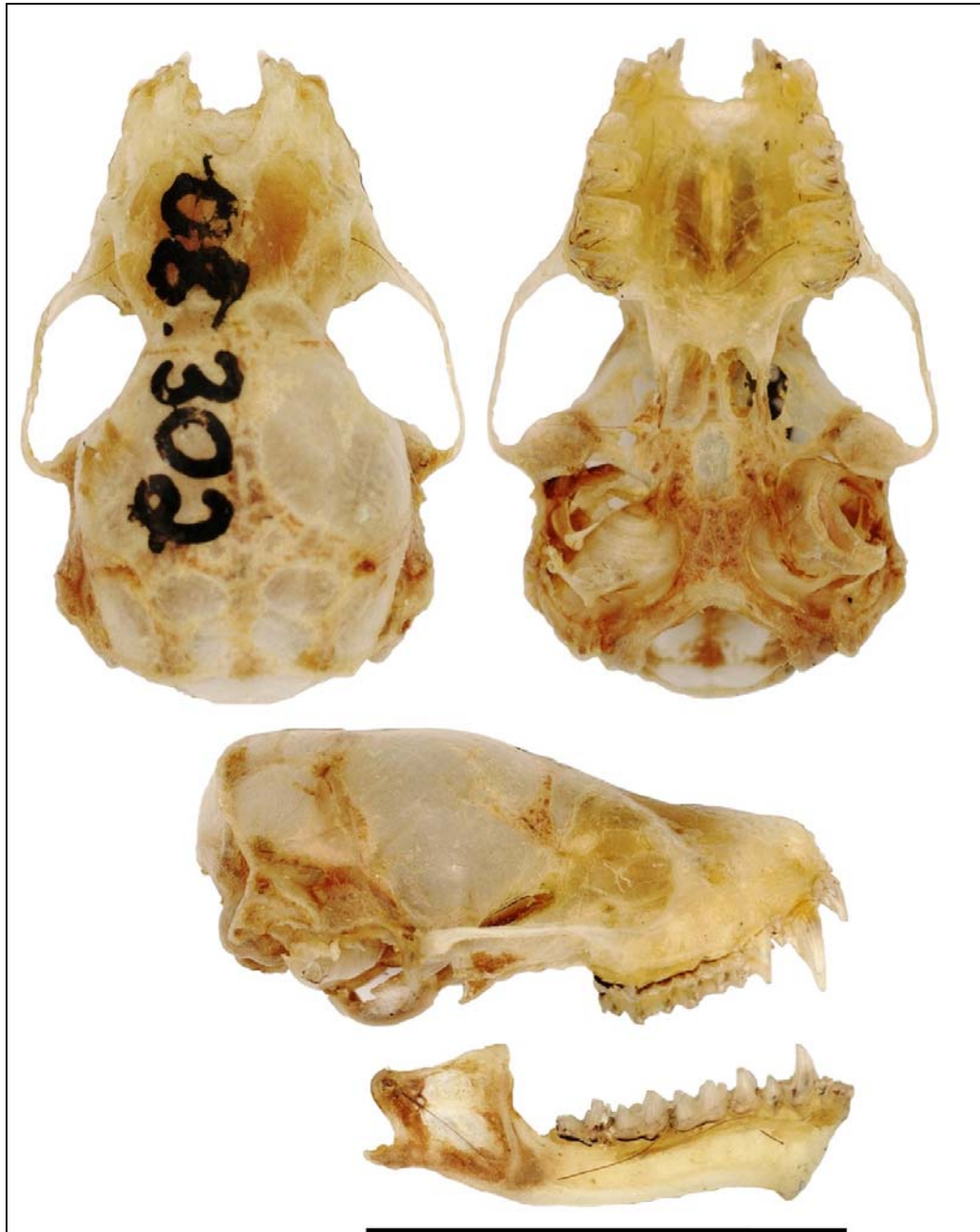


Figure 7. Upper teeth of *Pipistrellus simandouensis* **sp. nov.** (holotype, ZFMK2008-0302) showing: (A) the relatively small-sized outer incisors which are less than half the length of the inner incisors; and (B) the moderately sized anterior premolar which is situated in the toothrow and hence creating a small gap between C and P² (photographs © Jan Decher).

Figure 7A



Figure 7B



Figure 8. A line drawing of the penis of *Pipistrellus simandouensis* **sp. nov.** (holotype, ZFMK2008-0302). Scale bar = 4 mm.

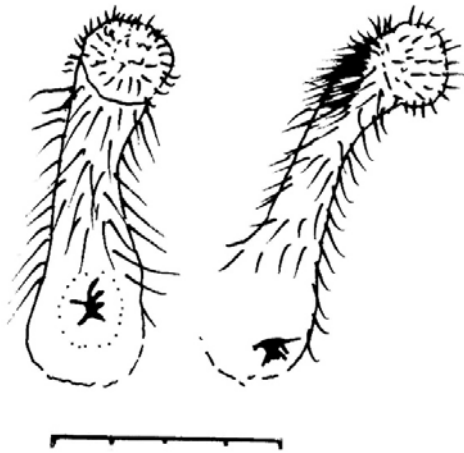


Figure 9. Dorsal (a), ventral (b) and lateral (c) views of bacula of five species examined in this study: P.sp nov. - *Pipistrellus sp. nov.*, PGR - *Pipistrellus grandidieri*, PHE - *Pipistrellus hesperidus*, PNA - *Pipistrellus nanulus*, PRU - *Pipistrellus rusticus*. Scale bars indicate 1 mm.

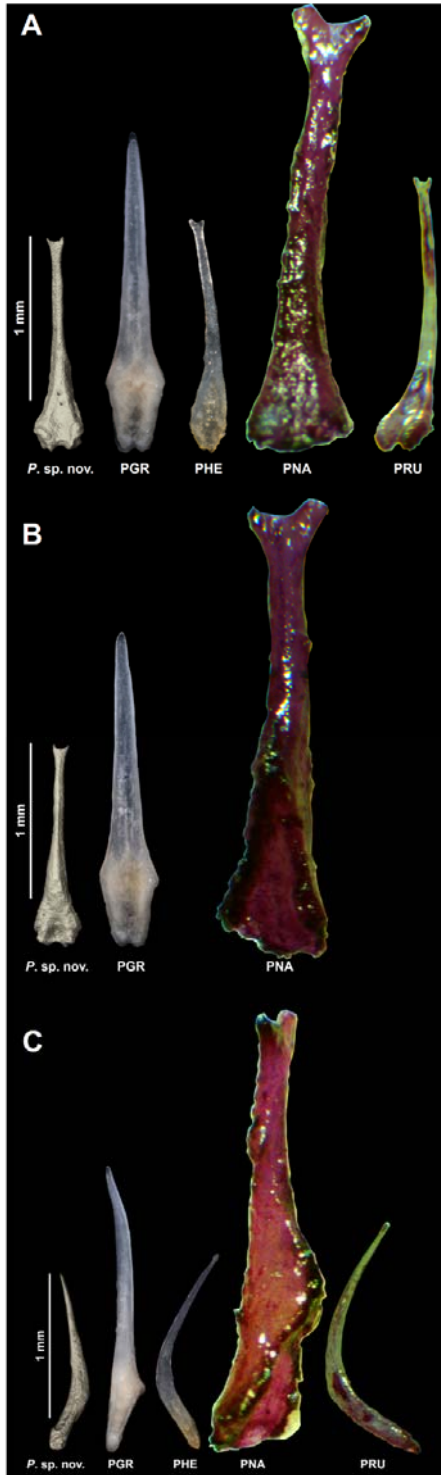


Figure S1. The post-cranial skeleton of *Pipistrellus simandouensis* sp. nov. (holotype, ZFMK2008-0302) showing ventral and lateral views (3D reconstruction based on μ -Ct scan).

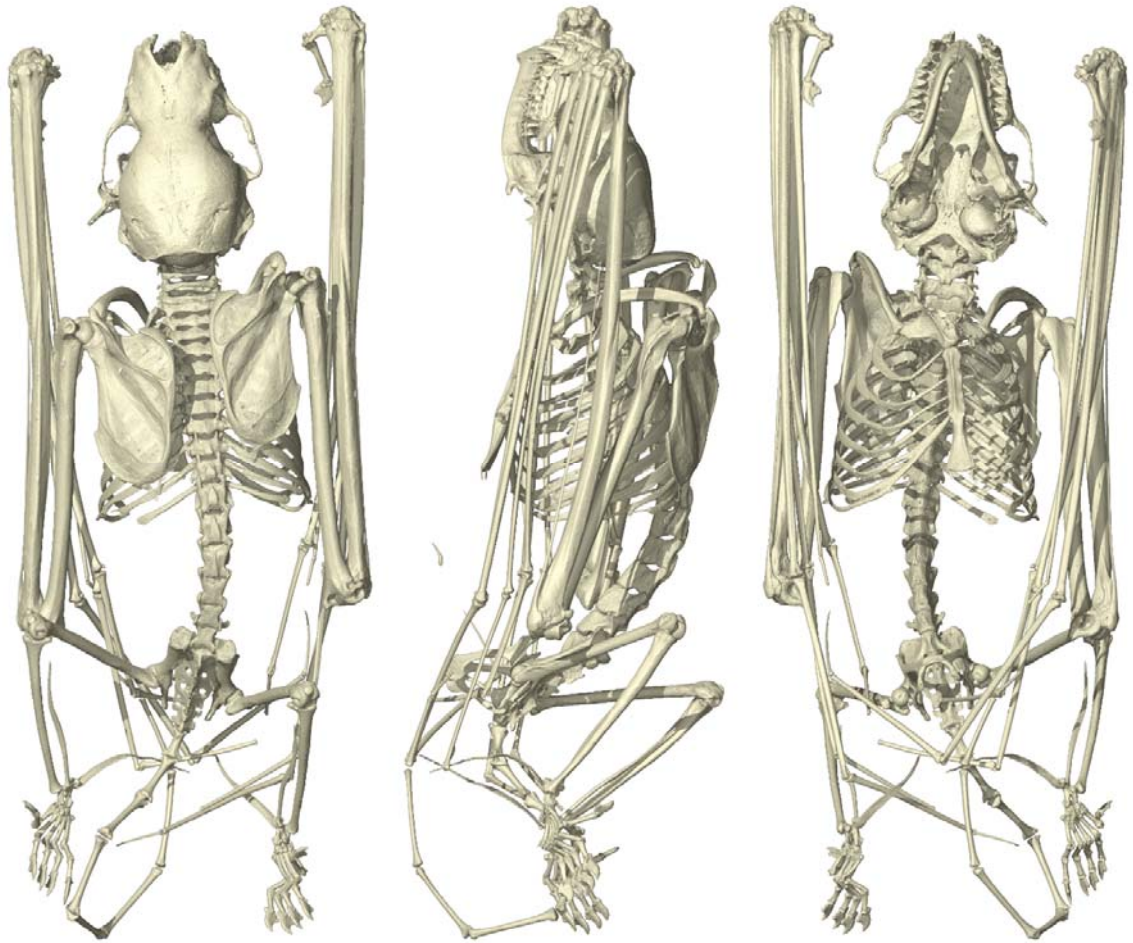


Figure S2. Bayesian Inference (BI) of the COI dataset. Values at the nodes are posterior probability values.

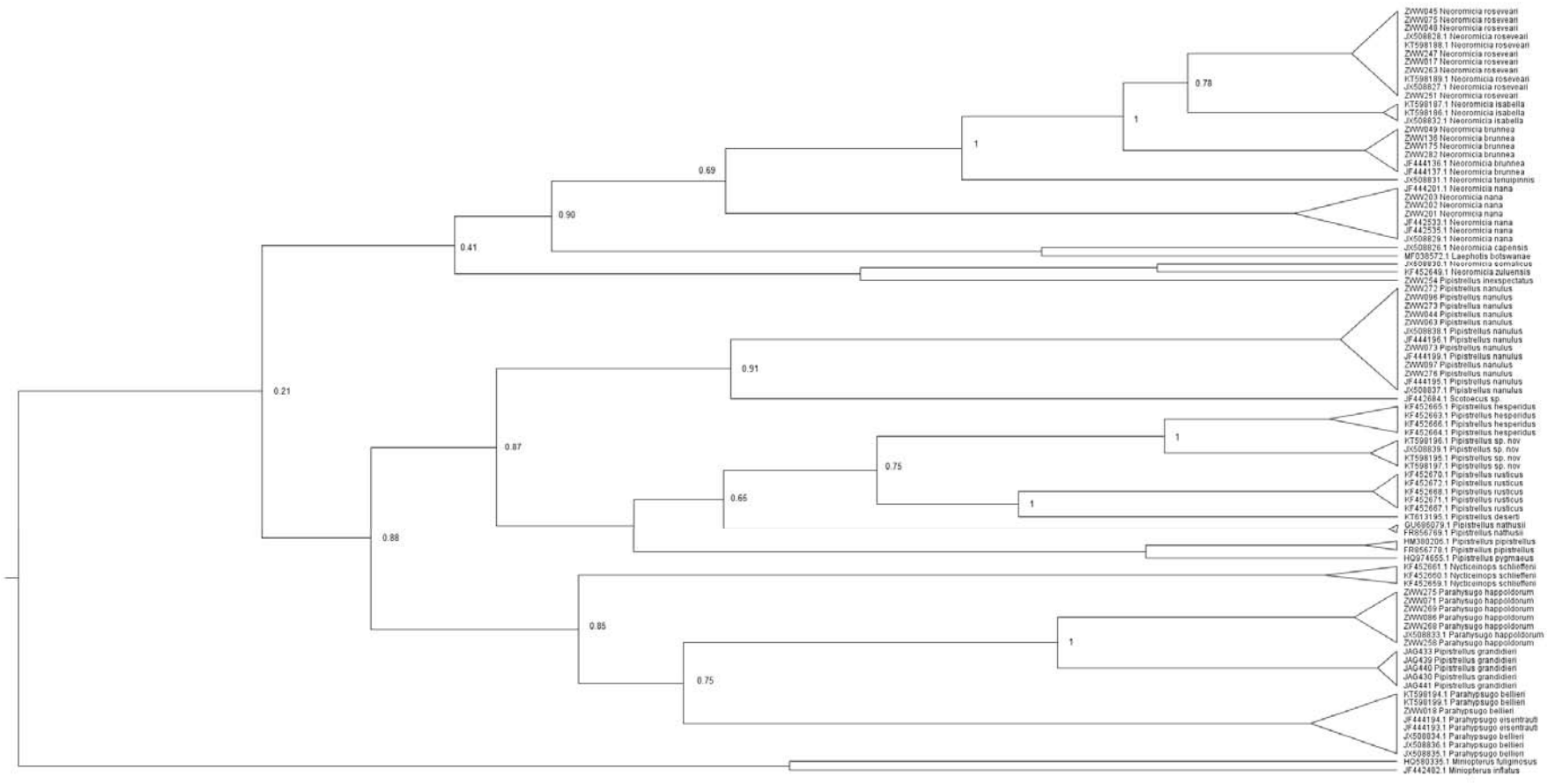


Figure S3. Bayesian Inference (BI) of the *Cyt b* dataset. Values at the nodes are posterior probability values.

