Taxonomic revision of African pipistrelle-like bats with a new species from the West Congolean rainforest

Laura Torrent[1,2,3,4*], Javier Juste[5,6], Inazio Garin[7], Joxerra Aihartza[7], Desiré L. Dalton[8], Mnqobi Mamba[9], Iroro Tanshi[10,11], Luke L. Powell[2,3,4], Sara Padidar[9], Juan Luis Garcia Mudarra[5], Leigh Richards[12], Ara Monadjem[9,13]

1: BiBio (Biodiversity and Bioindicators Research Group), Natural Sciences Museum of Granollers, Granollers, Spain. **2:** CIBIO-InBIO, Research Centre in Biodiversity and Genetic Resources, University of Porto, Campus de Vairão, 4485-661 Vairão, Portugal; **3:** BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal; **4:** Biodiversity Initiative, Houghton, MI 49913, EUA. **5:** Department of Evolutionary Ecology, Estación Biológica de Doñana (CSIC), Sevilla, Spain; **6:** Epidemiology and Public Health, CIBERESP, Madrid, Spain; **7:** University of the Basque Country (UPV/EHU), Sarriena z/g, E48940 Leioa, The Basque Country; **8:** School of Health and Life Sciences, Teesside University, Middlesbrough, TS1 3BX, United Kingdom; **9:** Department of Biological Sciences, University of Eswatini, Private Bag 4, Kwaluseni, Eswatini; **10:** Department of Biology, University of Washington, Seattle, USA; **11:** Small Mammal Conservation Organization, Benin City, Nigeria; **12:** Durban Natural Science Museum, P.O. Box 4085, Durban 4000, South Africa; **13:** Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Private Bag 20, Hatfield 0028, Pretoria, South Africa. Email: ltorrent@mcng.cat

Abstract

The taxonomic conundrum of pipistrelle-like or pipistrelloid bats remains one of the unsolved challenges posed by African Chiroptera. Historically, their cryptic morphology has led to a frequently confused classification and cast doubt on taxonomic arrangements at both genus and species levels. While molecular analyses and extensive reviews of specimens housed in collections worldwide have clarified many systematic relationships among pipistrelloid bats, some species still require validation, leaving gaps in our overall understanding of the systematics of the group. The Congo rainforest, one of Africa's least explored regions, remains underrepresented in systematic studies of pipistrelloid bats. In this study, we combine the use of two mitochondrial genes and cranial morphometric analyses to provide an updated perspective on African pipistrelloid bats, focusing on new material from Equatorial Guinea sampled over multiple years. We confirm the placement of *Af. musciculus* and *Af. crassulus* within the genus *Afropipistrellus*. The former was previously included in *Hypsugo*, while the latter lacked generic confirmation. Additionally, we describe a new species of *Pipistrellus* from Bioko Island, Central Africa, uncovered during systematic bat surveys in the region. Further surveys in the Congo rainforest are needed to unveil African bat diversity and its phylogenetic relationships fully.

Keywords : Africa, baculum, Chiroptera, genus revision, Mammalia, mitochondrial DNA, morphometrics, systematics, taxonomy

Introduction

Understanding bat diversity in Africa is crucial for developing effective conservation strategies and conducting adequate ecological research. In recent years, substantial progress has been made in studying the continent's bat fauna, with new species continuing to be described (Grunwald *et al.* 2023, Monadjem *et al.* 2024, Patterson *et al.* 2024). Nevertheless, large parts of the continent remain understudied, primarily due to its vast and often inaccessible landscapes. Access to remote locations and improved field techniques, such as the use of harp traps (Tanshi and Kingston 2021), have greatly advanced bat research in Africa. Additionally, re-examining museum collections with updated perspectives and modern methods reveals that Africa's bat diversity has been greatly underestimated (Fahr and Kalko 2011). For instance, recent surveys at Mount Nimba, in West Africa, uncovered 10 coexisting pipistrelloid species, of which four were new to science (Monadjem *et al*. 2013, 2021b, Decher *et al*. 2015, Hutterer *et al*. 2019a). These discoveries highlight the relevance of ongoing research and advanced methodologies, while also emphasizing the crucial role of reviewing museum collections in uncovering the true extent of Africa's bat diversity.

Among African bats, the taxonomy of the pipistrelle-like or 'pipistrelloid' bats (Volleth and Heller 1994, Monadjem *et al.* 2013) has been historically complex and subject to extensive revisions (Monadjem *et al.* 2021a, b, Grunwald *et al.* 2023, Demos *et al.* 2024). Researchers have tended to lump together pipistrelle-like species. Most share similar morphological characteristics such as small size, brownish pelage, and similar craniodental shape, which has impeded the recognition of possible cryptic species. Subsequent studies that also incorporated the structure of the baculum and karyology reshaped the classification of the group, recognizing *Hypsugo* Kolenati, 1856, *Neoromicia* Roberts, 1926, and *Pipistrellus* Kaup, 1829 as valid genera (Hill and Harrison 1987, Volleth *et al.* 2001), as well as redefining certain genera like *Eptesicus* (= *Cnephaeus*) by molecular data (Hoofer and Van Den Bussche 2003, Juste *et al.* 2013). In fact, with the contribution of molecular techniques, the overall systematics of the pipistrelloids bats has stabilized somewhat. These molecular results have supported the main separation of the African pipistrelloids established by Volleth and Heller (1994) into two tribes: Vespertilionini, comprising the genera *Afronycteris* Monadjem *et al*., 2021, *Laephotis* Thomas, 1901, *Neoromicia*, *Nycticeinops* Hill and Harrison, 1987, *Pseudoromicia* Monadjem *et al*., 2021, and *Hypsugo*; and Pipistrellini, comprising the genera *Pipistrellus*, *Scotoecus* Thomas, 1901, and *Vansonia* Roberts, 1946 (Amador *et al.* 2018, Monadjem *et al.* 2021a).

Within the Vespertilionini, recent molecular results have shown that *Neoromicia* is sister to *Laephotis* but the relationship between *Afronycteris* and *Pseudoromicia* continues to be unclear (Monadjem *et al.* 2021a, Demos *et al.* 2024). Thus, the systematics of the tribe in Africa remain incompletely resolved, with new species being described periodically. Only within the past decade, five species have been described in the genus *Neoromicia* (Monadjem *et al.* 2013, Decher *et al.* 2015, Goodman *et al.* 2015, 2017, Taylor *et al.* 2022), four more in *Pseudoromicia* (Monadjem *et al.* 2021a, Grunwald *et al.* 2023, Juste *et al.* 2023), two in

Nycticeinops (Hutterer *et al.* 2019a, b), one in *Laephotis* (Monadjem *et al.* 2021b), and one in *Pipistrellus* (Monadjem *et al.* 2021b) (Supporting Information, Table S1). Nevertheless, important gaps remain in the overall understanding of the systematics within the Vespertilionini. For instance, among the 'white-winged' *Pseudoromicia,* only a single cytochrome *c* oxidase subunit I (*COI*) gene sequence is available for the species *Ps. tenuipinnis* (Monadjem *et al.* 2013), despite its wide geographical distribution. Moreover, the distributions of the recently described *Ps. isabella* (Decher *et al.* 2015) in the West and *Ps. nyanza* (Monadjem *et al.* 2021a) in the East remain unclear. This is particularly evident across the Congo rainforest. A comprehensive revision of the genus *Pseudoromicia*, incorporating Central African specimens and additional sequences from different molecular markers and species, is urgently needed to clarify taxonomic units and their distributions. A good example of this is *Ps. principis* from Príncipe Island in the Gulf of Guinea. With the recent availability of comparative genetic sequences, this has been described as a new species (Juste *et al.* 2023) three decades after the initial fieldwork (Juste and Ibáñez 1994a).

Among members of the Vespertilionini tribe, the systematics of the genus *Nycticeinops* has undergone several important changes since its description (Hill and Harrison 1987). Studies published before 2020 placed the Congo members of this clade within the genus *Hypsugo* (e.g. Monadjem *et al*. 2013, Decher *et al*. 2015). However, Hutterer *et al*. (2019a) demonstrated that several species previously included either in *Hypsugo* or in *Pipistrellus* formed a well-differentiated group that they described as the new genus *Parahypsugo*, with *Pa. happoldorum* as the type species. They also showed that *Parahypsugo* comprised only sub-Saharan African species that were only distantly related to the true *Hypsugo* bats, which are found in Europe, North Africa, and the Middle East. Monadjem *et al*. (2021a) synonymized the new genus *Parahypsugo* under *Nycticeinops*, although this is still being debated (Benda *et al.* 2022). Finally, Demos *et al*. (2024) demonstrated, based on nuclear introns' analyses, that all currently known *Nycticeinops*, except *Nycticeinops schlieffenii*, should be recognized in the genus *Afropipistrellus*. Despite these recent insights into the phylogeny of African Vespertilionini, significant uncertainties persist also at the species-level. For instance, the species '*Hypsugo musciculus*' Thomas, 1913, remains yet to be sequenced, leaving its classification within the redefined *Hypsugo* or *Afropipistrellus* unresolved. Similarly, although *Pipistrellus crassulus* Thomas, 1904 has been differentiated both morphologically and geographically from the closely related *Afropipistrellus bellieri*, it has only tentatively been placed in *Afropipistrellus*, since topotypical specimens have not been included in any phylogenetic analysis (Demos *et al*. 2024).

The Pipistrellini tribe is a diverse group across the Palaearctic but is represented by fewer species in sub-Saharan Africa. Nevertheless, new species, such as *Pi. dhofarensis* (Benda *et al.* 2016) and *Pi. simandouensis* (Monadjem *et al.* 2021b), are still being added to the group, and the systematic relationships within the African-described taxa are far from clear. Here, we report specimens and tissues collected from Equatorial Guinea, located in the core of the Congolian rainforest block, that includes pipistrelloid bats from both tribes Vespertilionini and Pipistrellini. Our main objectives are twofold: first, we enhance our understanding of the distribution and phylogenetic relationships among the Vespertilionini pipistrelloids of sub-Saharan Africa. In particular, we address the taxonomic position of the taxa '*Hypsugo musciculus*' and *Afropipistrellus crassulus* based on new molecular and morphological analyses. Second, we describe a new species of *Pipistrellus* using an integrative approach that includes morphological (skull and baculum), molecular and echolocation features.

Materials and methods

Study area

Equatorial Guinea, with an overall land surface of 28 051 km^2, is located in the Gulf of Guinea and consists of two distinct regions: the Atlantic islands of Bioko (2017 km^2), Annobón (17 km^2), and Corisco (15 km^2), and the Mainland Region (26 017 km^2) (Rosas 2022) (Fig. 1). Equatorial Guinea has a typically hot and humid equatorial climate with seasons driven by rainfall regime, which varies between regions. On Bioko Island, there is only one rainy season that lasts from March to October, with the dry season extending from November to February. Mean accumulated precipitation ranges from 1961 mm in the rainy season to 341 mm in the dry season (Rosas 2022). In the Mainland Region, there are two marked rainy seasons: September to December and March to May. The average annual rainfall is 1800–3800 mm and the average temperature is 25°C with an annual variation of +/–5°C (Climate data provided from the Malabo and Bata airport stations. Source: Grupo de Paleoantropología MNCN-CSIC; Rosas 2022.)

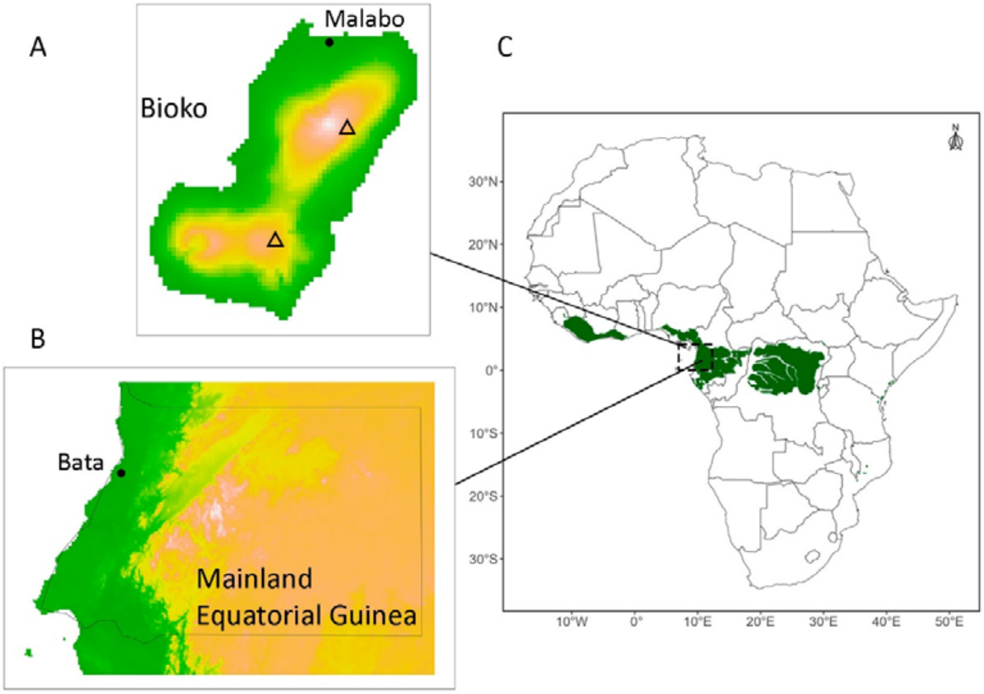

Figure 1. A, inset of Bioko Island showing the two localities (triangles) where *Pipistrellus* sp. nov. was found. B, inset of Mainland Equatorial Guinea. White areas represent the highest elevation points in both maps. C, map of Africa highlighting lowland forests (dark green) with a dashed square indicating the location of Equatorial Guinea.

Data collection

The new bat material was collected during surveys in Equatorial Guinea between 2018 and 2024. One-month bat surveys were conducted on the Mainland Region between January and

February of 2018, 2019, 2020, and in May 2022. A 2-month survey was conducted between January and February 2023 and a 1-week survey in January 2024. Moreover, 1-week and 3-week surveys were conducted on Bioko Island in January 2023 and between January and February 2024. We set mist nets of different lengths, mesh sizes, and filament thickness (Ecotone, Poland) in appropriate locations at each site to maximize our capture success. Mist nets were set either at ground level or up to 7–9 m above ground in suitable bat flyways, such as across small streams or gaps in the forest. Alongside the mist nets, a three-bank and a two-bank homemade harp trap were used in the 2023 and 2024 surveys, respectively. Mist nets and harp traps were set at least 30 min before sunset and kept in place until midnight or when bat activity dropped. Captured bats were sexed and aged (based on ossification of the finger epiphyses) and standard measurements were taken. Bats were released at the same site where they were captured. Some specimens were fixed in 10% formalin, subsequently transferred to 70% ethanol and deposited as vouchers either in the collections of the Doñana Biological Station (EBD-CSIC) in Spain or the Eswatini National Museum of Natural History in Eswatini. Before fixation in formalin, tissue samples were taken from each specimen's lungs, liver, pectoral muscle, or wing membrane, and preserved in 99% ethanol. We followed the guidelines for capturing and handling mammals of Sikes and Animal Care and Use Committee of the American Society of Mammalogists (2016), and had the appropriate collecting permits. Additionally, we studied 107 pipistrelloids from the tribe Vespertilionini and 18 from the tribe Pipistrellini, collected during different expeditions across Equatorial Guinea from the 1960s to the 1990s and housed in the EBD-CSIC collection. These specimens were thoroughly re-examined morphologically and some have been also analysed molecularly.

DNA extraction, amplification, and sequencing

The genomic DNA from tissue samples obtained between 2018 and 2023 was extracted following the saline protocol developed by Gemmell and Akiyama (1996). To partially amplify the mitochondrial genes cytochrome *c* oxidase subunit I (*COI*) (520 bp) and cytochrome *b* (*Cytb*) (570 bp), a nested polymerase chain reaction (PCR) was performed using the primers M13BC-FW, BCVRV1/M13, and BCV-RV2 (Alcaide *et al.* 2009) for *COI*, and the primers Molcit-F (Ibáñez *et al.* 2006) and MVZ-16 (Smith and Patton 1993) for *Cytb*. Genomic DNA from the samples obtained in 2024 was isolated using the QIAamp DNA Investigator Kit (Qiagen, Germany). Tissue samples were cut with a scalpel blade and were subsequently digested overnight (20–22 h) in Proteinase K and ATL tissue lysis buffer. Following digestion, DNA was isolated according to the manufacturer's instructions. To partially amplify the mitochondrial genes *COI* (520 bp) and *Cytb* (570 bp), the universal primers BatL5310 and R6036R (Hebert *et al.* 2003) and Cytb-LGL-765-F and Cytb-LGL-766-R were used, respectively (Trujillo *et al.* 2009). Amplifications were performed in separate PCR reactions consisting of 1 × DreamTaq Green PCR Master Mix, 0.4 µM of each primer and approximately 50 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, 45°C for 30 s, and 72°C for 1 min, followed by a final extension at 72°C for 20 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 v.3.1 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 (Thermo Fisher Scientific Inc.).

Specimens stored in the EBD-CSIC collection were preserved with formalin and, therefore, the extraction process followed a specific and slightly modified protocol (Campos and Gilbert 2012). DNA extracts presented a high degradation rate, with low molecular weight DNA. The amplification of a conserved fragment of *Cytb* in these degraded tissues required the design of specific sets of primers every 250 bp, using as scaffolds available homologous sequences from the closest suspected species. Thus, a sequence of *Af. eisentrauti* from Cameroon (ZFMK-MAM-1999_0676) was used to design primers to amplify DNA of old formalin-preserved specimens of *Af.* cf. *eisentrauti* (EBD 19104M) and *Af. happoldorum* (EBD 16827M) housed in the EBD-CSIC collection. Similarly, a sequence from an individual identified as '*Hypsugo musciculus*', on the basis of morphology, from Mainland Equatorial Guinea was used to design primers that amplified two ancient formalin-preserved specimens (EBD 15554M and EBD 20568M) of the same species housed in the EBD-CSIC collection. A sequence of the *Ps. mbamminkom* holotype from Cameroon (FMNH 240714) was used to design primer pairs to amplify an ancient specimen (EBD 19108M) from Bioko Island and housed in the EBD-CSIC collection. Finally, sequences from specimens collected at Basilé Peak on Bioko Island in 2024 were used as a scaffold to design primers to amplify two specimens (EBD 19203M and EBD 19086M) that did not match any known pipistrelloid bats.

Phylogenetic analysis

New *Cytb* sequences for 48 samples of African pipistrelloids from the tribe Vespertilionini were generated in this study and added to 67 ingroup and five outgroup sequences obtained from GenBank (Supporting Information, Table S2). In relation to the Pipistrellini, we generated 14 *Cytb* sequences of African pipistrelloids from the tribe Pipistrellini and added 32 ingroup and five outgroup sequences obtained from GenBank. Lastly, two *COI* sequences were generated and were added to 49 ingroup and two outgroup sequences from GenBank. These sequences were aligned for phylogenetic analysis using MEGA v.7 (Kumar *et al.* 2016). All sequences were manually trimmed and checked for ambiguous peaks. A substitution model of sequence evolution that best fitted the data was estimated in jModelTest v.2.1.10 (Posada 2008). The model selected for *Cytb* and *COI* was general time reversible (GTR) model + Gamma (G) + Invariable sites (I), and this was used for maximum likelihood (ML) analyses (Felsenstein 1981). Branch support values were estimated using a nonparametric bootstrap with 1000 replicates. Inter- and intraspecific corrected *p*-distances between subspecies and lineages were calculated using ML genetic distances in MEGA v.7. We used jModelTest2 v.0.1.10 (Darriba *et al.* 2012) to determine the sequence substitution models that best fit the *Cytb* and *COI* data using the Bayesian information criterion (BIC). Gene tree analyses using a Bayesian inference (BI) model were generated in MrBayes v.3.2.7 (Ronquist *et al.* 2012) for the same alignment as the ML analysis. Two independent runs were conducted in MrBayes using five Markov chains run for 6 000 000 generations under default heating values and sampled every 1000 generations. A conservative 25% burn-in was used, and the majority-rule consensus trees were assembled for each Bayesian analysis.

External and craniodental measurements

External and craniodental measurements were taken from each specimen with a calliper (to the nearest 0.1 mm for external and 0.01 mm for craniodental characters) following Monadjem *et al*. (2021a). The following external measurements were taken in the field: body mass (W, in g) with a 20 g Pesola scale, and forearm length (FA). The cranial measurements were as follows: greatest skull length (GSKL), from the posteriormost point of the occipital to the anteriormost point of the incisors; condylo-incisive length (CIL), from the occipital condyles to the anteriormost point of the incisors; greatest zygomatic breadth (ZYGO), taken as the greatest width across the zygomatic arches; greatest braincase width (GBW), taken in the frontal plane above the zygomatic arches; greatest skull height (GSH), taken from the lowest point of the basioccipital 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 the mastoid processes; and greatest mandible length (MAND), taken from the posteriormost point of the condyles to the anteriormost point of the incisors. The dental measurements included width across the third molars (M^3–M^3), taken across the outermost point of the alveoli of the third molars of the upper jaw; complete upper canine–molar tooth row (C–M^3), taken from the anteriormost point of the alveolus of the canine to the posteriormost point of the alveolus of the third molar; width across upper canines (C–C), taken across the outermost points of the alveoli of the canines; and complete mandibular canine–molar tooth row (c–m_3), taken from the anteriormost point of the alveolus of the canine to the posteriormost point of the third molar. Tooth abbreviations are as follows: I, incisor; C, canine; P, premolar; M, molar; with upper teeth presented in upper case and lower teeth in lower case. More extended definitions of these measurements are found in Monadjem *et al*. (2013). To ensure the accuracy of our dental measurements, we recorded these under a stereo microscope (Zeiss 2 or Euromex) with 1.6–6× magnifications.

Multivariate morphological analyses

We obtained principal components analysis (PCA) of $\log_{10}$-transformed values of craniodental measurements to compare the various genetically confirmed taxa presented in this study. PCAs were conducted on the variance–covariance matrix using the package 'vegan' (Oksanen *et al.* 2013) run in R v.3.6.2 (R Core Team 2021) and plotted using 'ggplot2' (Wickham 2016).

Bacula preparation

The baculum (or os penis) of selected adult male specimens was extracted and photographed following Hill and Harrison (1987) and Kearney *et al*. (2002). First, the penis was measured (total length in mm) and then removed at its base and macerated in a 5% KOH solution at room temperature for a variable amount of time (but usually around two days) (Hill and Harrison 1987, Kearney *et al.* 2002). Next, the baculum was stained with Alizarin Red, which is calcium-specific and aids in distinguishing the bony baculum from the surrounding soft tissue. The specimens deposited at the University of Eswatini were prepared by severing the glans penis and then immersing it in dilute sodium hydroxide that was heated to 85°C for a variable period (*c*. 30 s). The stained bacula were preserved in 100% glycerol after being drawn, measured from the dorsal view, and photographed under a stereo microscope (Zeiss 2). The morphological descriptions of the bacula followed Hill and Harrison (1987).

Acoustic recordings

As part of a broader effort to build a call library of the insectivorous bats of Equatorial Guinea, we recorded echolocation calls of hand-released pipistrelloid bats with either an EchoMeter Touch 2 PRO (Wildlife Acoustics, USA), a Pettersson M500 (Pettersson Elektronik, Sweden), or a BatLogger (Elekon, Switzerland) with a sample rate of 384 kHz. Bats were released in open spaces near the site of capture and followed on foot as they flew away. In 2024, recordings of *Pipistrellus* sp. nov. from Basilé Peak were taken near trapping sites from free-flying individuals and individuals confirmed genetically, and recorded as they were released. While doing the recordings no other vespertilionid bats were flying in the area. Following Jung *et al*. (2014), we characterized echolocation calls by measuring the peak frequency (or frequency with maximum energy) (FME), start frequency (StartF), and end frequency (EndF) using Kaleidoscope PRO software (v.5.6.8, Wildlife Acoustics, USA) at a Fast Fourier Transform (FFT) with a sampling rate of 512. The mean of 10 calls with the best signal-to-noise ratios was calculated for each individual bat.

Results

Tribe Vespertilionini

Phylogenetic analysis of *Cytb* and *COI* identified several well-supported clades, although basal nodes were generally poorly supported. Our phylogeny of Vespertilionini clearly distinguished the seven genera (*Afronycteris*, *Afropipistrellus*, *Hypsugo*, *Laephotis*, *Neoromicia*, *Nycticeinops*, and *Pseudoromicia*) that are currently recognized in Africa. The members of the genera *Laephotis* and *Neoromicia* form monophyletic clades that are sister to each other based on both ML and BI analyses (Fig. 2). Similarly, *Afronycteris* and *Pseudoromicia* genera form monophyletic clades supported by both ML and BI analyses (Fig. 2). However, in the *COI* phylogenies, only monophyly of the genera *Laephotis*, *Neoromicia*, and *Afronycteris* were supported (Supporting Information, Fig. S1). In the ML phylogeny, the members of the genus *Afropipistrellus*, except for *Af. eisentrauti*, formed a monophyletic clade sister to the genus *Hypsugo* '*sensu stricto*' (from North Africa and elsewhere in the Palaearctic and Indo-Malayan regions) (Fig. 2). Interestingly, in the BI phylogeny, *Ny. schlieffenii* and *Ny.* cf. *schlieffenii* were supported as sister to the *Afropipistrellus* clade, whereas *Af. eisentrauti* was not. Moreover, *Af. crassulus* together with a sample of *Af.* cf. *crassulus* from Tanzania were placed in our topology with high bootstrap support as a sister species of *Af. bellieri*. Furthermore, the new sequences from '*Hypsugo musciculus*' from Equatorial Guinea were also placed in the topology within *Afropipistrellus*, showing sister-relationships to *Af. happoldorum* and to *Af. grandidieri*. A final group of samples from Equatorial Guinea corresponded to the species *Af. happoldorum* (Fig. 2).

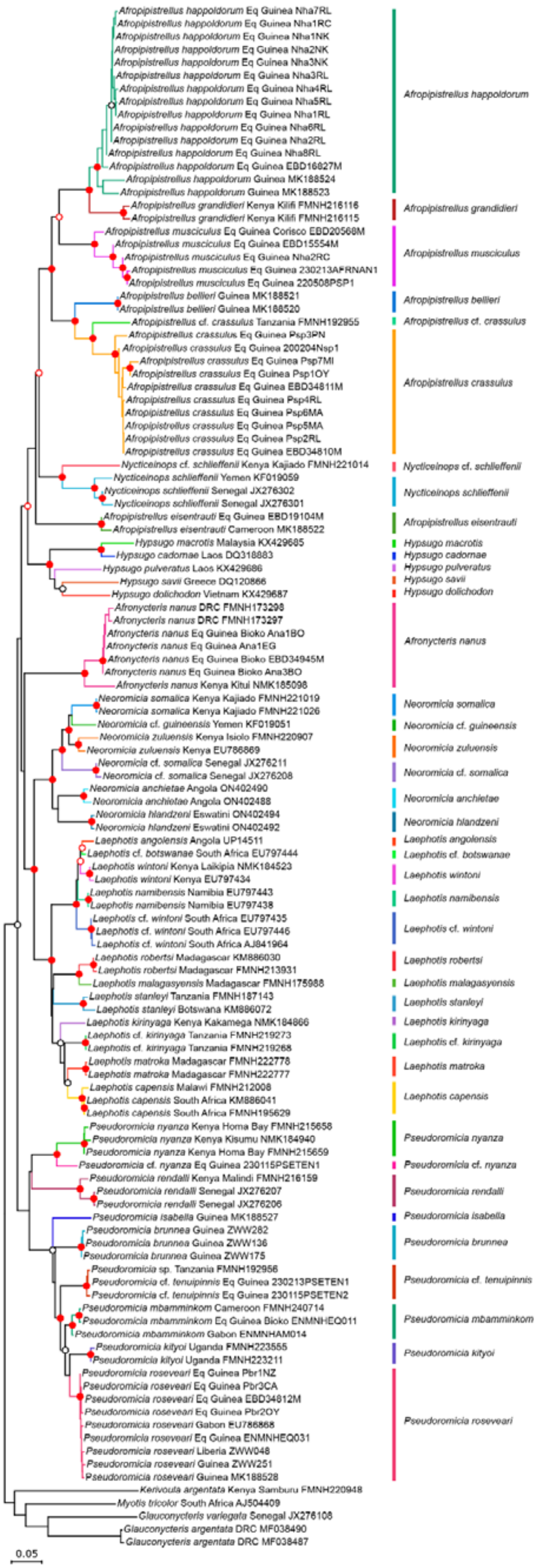
Afropipistrellus happoldorum Eq Guinea Nha7RL
Afropipistrellus happoldorum Eq Guinea Nha1RC
Afropipistrellus happoldorum Eq Guinea Nha1NK
Afropipistrellus happoldorum Eq Guinea Nha2NK
Afropipistrellus happoldorum Eq Guinea Nha3NK
Afropipistrellus happoldorum Eq Guinea Nha3RL
Afropipistrellus happoldorum Eq Guinea Nha4RL
Afropipistrellus happoldorum Eq Guinea Nha5RL
Afropipistrellus happoldorum Eq Guinea Nha1RL
Afropipistrellus happoldorum Eq Guinea Nha6RL
Afropipistrellus happoldorum Eq Guinea Nha2RL
Afropipistrellus happoldorum Eq Guinea Nha8RL
Afropipistrellus happoldorum Eq Guinea EBD16827M
Afropipistrellus happoldorum Guinea MK188524
Afropipistrellus happoldorum Guinea MK188523
Afropipistrellus grandidieri Kenya Kilifi FMNH216116
Afropipistrellus grandidieri Kenya Kilifi FMNH216115
Afropipistrellus musciculus Eq Guinea Corisco EBD20568M
Afropipistrellus musciculus Eq Guinea EBD15554M
Afropipistrellus musciculus Eq Guinea Nha2RC
Afropipistrellus musciculus Eq Guinea 230213AFRNAN1
Afropipistrellus musciculus Eq Guinea 220508PSP1
Afropipistrellus bellieri Guinea MK188521
Afropipistrellus bellieri Guinea MK188520
Afropipistrellus cf. crassulus Tanzania FMNH192955
Afropipistrellus crassulus Eq Guinea Psp3PN
Afropipistrellus crassulus Eq Guinea 200204Nsp1
Afropipistrellus crassulus Eq Guinea Psp7MI
Afropipistrellus crassulus Eq Guinea Psp1OY
Afropipistrellus crassulus Eq Guinea EBD34811M
Afropipistrellus crassulus Eq Guinea Psp4RL
Afropipistrellus crassulus Eq Guinea Psp6MA
Afropipistrellus crassulus Eq Guinea Psp5MA
Afropipistrellus crassulus Eq Guinea Psp2RL
Afropipistrellus crassulus Eq Guinea EBD34810M
Nycticeinops cf. schlieffenii Kenya Kajiado FMNH221014
Nycticeinops schlieffenii Yemen KF019059
Nycticeinops schlieffenii Senegal JX276302
Nycticeinops schlieffenii Senegal JX276301
Afropipistrellus eisentrauti Eq Guinea EBD19104M
Afropipistrellus eisentrauti Cameroon MK188522
Hypsugo macrotis Malaysia KX429685
Hypsugo cadornae Laos DQ318883
Hypsugo pulveratus Laos KX429686
Hypsugo savii Greece DQ120866
Hypsugo dolichodon Vietnam KX429687
Afronycteris nanus DRC FMNH173298
Afronycteris nanus DRC FMNH173297
Afronycteris nanus Eq Guinea Bioko Ana1BO
Afronycteris nanus Eq Guinea Ana1EG
Afronycteris nanus Eq Guinea Bioko EBD34945M
Afronycteris nanus Eq Guinea Bioko Ana3BO
Afronycteris nanus Kenya Kitui NMK185098
Neoromicia somalica Kenya Kajiado FMNH221019
Neoromicia somalica Kenya Kajiado FMNH221026
Neoromicia cf. guineensis Yemen KF019051
Neoromicia zuluensis Kenya Isiolo FMNH220907
Neoromicia zuluensis Kenya EU786869
Neoromicia cf. somalica Senegal JX276211
Neoromicia cf. somalica Senegal JX276208
Neoromicia anchietae Angola ON402490
Neoromicia anchietae Angola ON402488
Neoromicia hlandzeni Eswatini ON402494
Neoromicia hlandzeni Eswatini ON402492
Laephotis angolensis Angola UP14511
Laephotis cf. botswanae South Africa EU797444
Laephotis wintoni Kenya Laikipia NMK184523
Laephotis wintoni Kenya EU797434
Laephotis namibensis Namibia EU797443
Laephotis namibensis Namibia EU797438
Laephotis cf. wintoni South Africa EU797435
Laephotis cf. wintoni South Africa EU797446
Laephotis cf. wintoni South Africa AJ841964
Laephotis robertsi Madagascar KM886030
Laephotis robertsi Madagascar FMNH213931
Laephotis malagasyensis Madagascar FMNH175988
Laephotis stanleyi Tanzania FMNH187143
Laephotis stanleyi Botswana KM886072
Laephotis kirinyaga Kenya Kakamega NMK184866
Laephotis cf. kirinyaga Tanzania FMNH219273
Laephotis cf. kirinyaga Tanzania FMNH219268
Laephotis matroka Madagascar FMNH222778
Laephotis matroka Madagascar FMNH222777
Laephotis capensis Malawi FMNH212008
Laephotis capensis South Africa KM886041
Laephotis capensis South Africa FMNH195629
Pseudoromicia nyanza Kenya Homa Bay FMNH215658
Pseudoromicia nyanza Kenya Kisumu NMK184940
Pseudoromicia nyanza Kenya Homa Bay FMNH215659
Pseudoromicia cf. nyanza Eq Guinea 230115PSETEN1
Pseudoromicia rendalli Kenya Malindi FMNH216159
Pseudoromicia rendalli Senegal JX276207
Pseudoromicia rendalli Senegal JX276206
Pseudoromicia isabella Guinea MK188527
Pseudoromicia brunnea Guinea ZWW282
Pseudoromicia brunnea Guinea ZWW136
Pseudoromicia brunnea Guinea ZWW175
Pseudoromicia sp. Tanzania FMNH192956
Pseudoromicia cf. tenuipinnis Eq Guinea 230213PSETEN1
Pseudoromicia cf. tenuipinnis Eq Guinea 230115PSETEN2
Pseudoromicia mbamminkom Cameroon FMNH240714
Pseudoromicia mbamminkom Eq Guinea Bioko ENMNHEQ011
Pseudoromicia mbamminkom Gabon ENMNHAM014
Pseudoromicia kityoi Uganda FMNH223555
Pseudoromicia kityoi Uganda FMNH223211
Pseudoromicia roseveari Eq Guinea Pbr1NZ
Pseudoromicia roseveari Eq Guinea Pbr3CA
Pseudoromicia roseveari Eq Guinea EBD34812M
Pseudoromicia roseveari Eq Guinea Pbr2OY
Pseudoromicia roseveari Gabon EU786868
Pseudoromicia roseveari Eq Guinea ENMNHEQ031
Pseudoromicia roseveari Liberia ZWW048
Pseudoromicia roseveari Guinea ZWW251
Pseudoromicia roseveari Guinea MK188528
Kerivoula argentata Kenya Samburu FMNH220948
Myotis tricolor South Africa AJ504409
Glauconycteris variegata Senegal JX276108
Glauconycteris argentata DRC MF038490
Glauconycteris argentata DRC MF038487
Afropipistrellus happoldorum
Afropipistrellus grandidieri
Afropipistrellus musciculus
Afropipistrellus bellieri
Afropipistrellus cf. crassulus
Afropipistrellus crassulus
Nycticeinops cf. schlieffenii
Nycticeinops schlieffenii
Afropipistrellus eisentrauti
Hypsugo macrotis
Hypsugo cadornae
Hypsugo pulveratus
Hypsugo savii
Hypsugo dolichodon
Afronycteris nanus
Neoromicia somalica
Neoromicia cf. guineensis
Neoromicia zuluensis
Neoromicia cf. somalica
Neoromicia anchietae
Neoromicia hlandzeni
Laephotis angolensis
Laephotis cf. botswanae
Laephotis wintoni
Laephotis namibensis
Laephotis cf. wintoni
Laephotis robertsi
Laephotis malagasyensis
Laephotis stanleyi
Laephotis kirinyaga
Laephotis cf. kirinyaga
Laephotis matroka
Laephotis capensis
Pseudoromicia nyanza
Pseudoromicia cf. nyanza
Pseudoromicia rendalli
Pseudoromicia isabella
Pseudoromicia brunnea
Pseudoromicia cf. tenuipinnis
Pseudoromicia mbamminkom
Pseudoromicia kityoi
Pseudoromicia roseveari
0.05

Figure 2. Results of maximum likelihood (ML) and Bayesian inference (BI) analyses combined on a ML tree of mitochondrial cytochrome b sequences of African pipistrelloids from the tribe Vespertilionini incorporating data from Equatorial Guinea based on the General Time Reversal + Gamma (G) + Invariable sites (I) model. Filled red circles on nodes denote bootstrap (BS) values ≥ 70% and Bayesian posterior probabilities (PP) ≥ 0.90. Open circles outlined in black indicate BS ≥ 70% and PP < 0.90, and open circles outlined in red indicate BS < 70% and PP > 0.90. DRC refers to the Democratic Republic of the Congo. Labels include species name, GenBank accession number or specimen/sample number, and country of collection (Supporting Information, Table S2). Branch colours indicate individual species/clade membership.

Within the genus *Pseudoromicia*, both ML and BI analyses recovered a clade formed by *Ps. brunnea*, *Ps. isabella*, *Ps. kityoi*, *Ps. mbamminkom*, *Ps. roseveari*, and *Ps.* cf. *tenuipinnis* (Fig. 2). The three new sequences from Equatorial Guinea were obtained from individuals of white-winged bats, identified morphologically as *Ps. tenuipinnis* in the field and confirmed based only on the genetic material. Two of these sequences grouped with a *Ps.* cf. *tenuipinnis* sample from Tanzania in a group formed by *Ps. mbamminkom*, *Ps. kityoi*, *Ps. roseveari*, and *Ps.* sp. from Tanzania. However, one sequence was placed within the species *Ps. nyanza* (Fig. 2). The remaining samples from Equatorial Guinea belonging to the tribe were included within the clades that defined the species *Afronycteris nanus*, *Ps. mbamminkom*, and *Ps. roseveari* for both ML and BI analyses (Fig. 2).

K2P genetic divergence between *Afropipistrellus* species ranged from 7.4% between *Af. happoldorum* and *Af. grandidieri*, to 28.3% between *Af. crassulus* and *Af. eisentrauti*. Divergence of *Af. musciculus* from *Hypsugo* species ranged from 16.5% to 19.7% (Supporting Information, Table S3). The divergence between *Nycticeinops* and *Afropipistrellus* species ranged from 12.6% between *Ny.* cf. *schlieffenii* and *Af. bellieri*, to 27.8% between *Ny. schlieffenii* and *Af. crassulus*. The genetic divergence between *Ps. nyanza* and *Ps.* cf. *nyanza* was 10.5%, and between these two taxa and *Ps.* cf. *tenuipinnis* was 21.3% and 21.5%, respectively. Moreover, the divergence between *Ps. brunnea* and *Ps. mbamminkom*, and *Ps. roseveari* was 8.1% and 6.4% respectively (Supporting Information, Table S3).

The PCA ordination of craniodental variables for African *Afropipistrellus* and *Nycticeinops* species accounted for 85% of the variation, within the two first axes. The first principal component (PC1) represented a size gradient with positive loadings for all measurements (Table 1). The ordination allocated *Af. musciculus* in a distinct region of the morphospace in which the remaining species were distributed along the size axis with some degree of overlap among them (Fig. 3). *Afropipistrellus musciculus* appears on the left of the graph, thus being the smallest *Afropipistrellus* of the species studied. The second principal component (PC2) had a distinct high positive loading (0.927) for the variable GSH, indicating that the main difference in shape among the studied samples was the skull height (Table 1). The samples of *Af. musciculus* presented the most inflated skull of all *Afropipistrellus* and *Nycticeinops* studied (Fig. 3). The complete set of body and craniodental measurements of all *Afropipistrellus* specimens from Equatorial Guinea used in this study is reported in Supporting Information Tables S4–S6.

Table 1. Factor loadings for PC1 and PC2 of the principal components analysis (PCA) based on standardised craniodental measurements of *Afropipistrellus bellieri*, *Af.* cf. *crassulus*, *Af. crassulus*, *Af. eisentrauti*, *Af. grandidieri*, *Af. happoldorum*, *Af. musciculus*, and *Nycticeinops schlieffenii*.

Character	**PC1**	**PC2**
GLS	0.385	-0.158
MAST	0.360	-0.226
POB	0.299	0.170
GSH	0.218	0.927
$C\text{-}M^3$	0.381	-0.047
C-C	0.377	-0.114
$M^3\text{-}M^3$	0.386	-0.142
$c\text{-}m_3$	0.385	0.015
Cumulative total variation explained	76%	85%

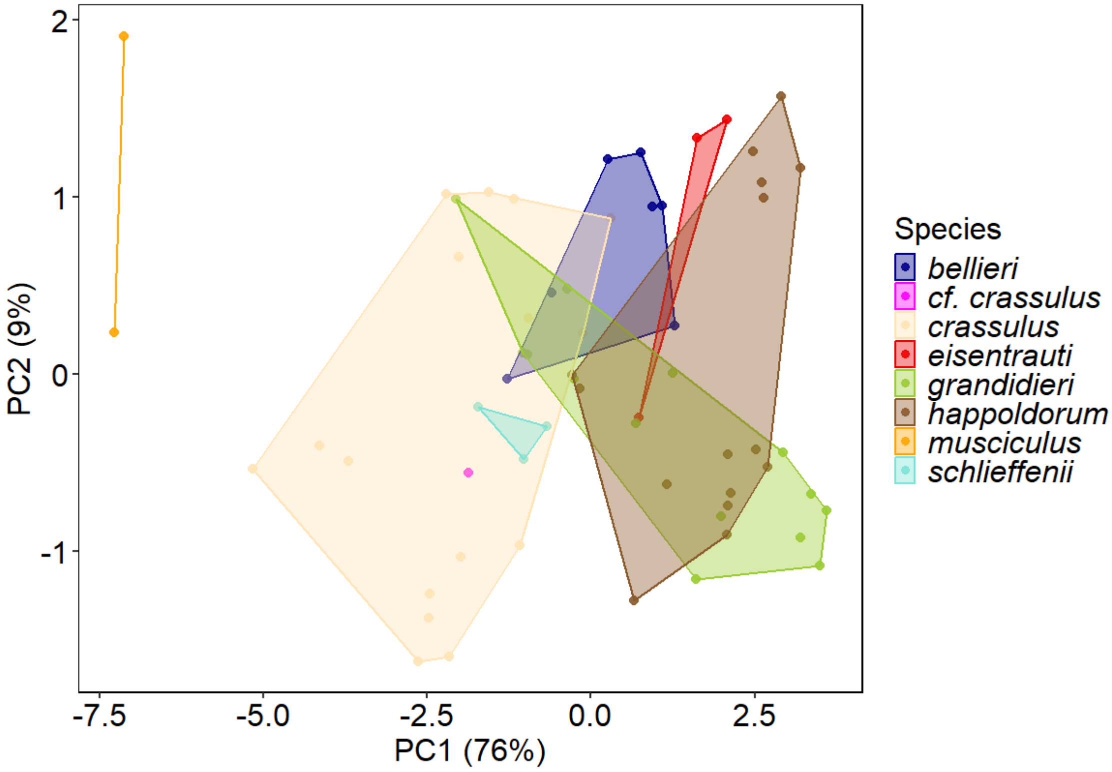

Figure 3. A principal components analysis plotting the first two components for craniodental measurements of African *Afropipistrellus* and *Nycticeinops* species. See Table 1 for the variables used in this analysis and the loadings on PC1 and PC2.

The bacula of *Afropipistrellus* showed remarkable variation in shape among species. The baculum of *Af. eisentrauti* was small and lacked the long, slender, and tapered shaft of other species within the genus (Supporting Information, Figs S2, S3, Table S7). Unlike the *Af. eisentrauti* baculum drawing presented in Hill and Harrison (1987: 69), the *Af. eisentrauti* specimen (EBD 19104M) had a slightly pointed tip (Supporting Information, Fig. S2). The bacula of *Af. musciculus* and *Af. happoldorum* were long and slender with a narrow tip and two basal lobes, *Af. musciculus* was *c*. 1 mm shorter in length, the base conspicuously bifurcated and overall thinner than *Af. happoldorum* (Supporting Information, Fig. S2, Table S7). Moreover, the baculum of *Af. happoldorum* had the thickest shaft of the five *Afropipistrellus* species. The baculum of *Af. crassulus* was the thinnest of all *Afropipistrellus* species, only comparable to *Af. bellieri*, with an overall long shape and a narrow tip, and short but conspicuous basal lobes (Supporting Information, Figs S2, S3, Table S7).

The mean echolocation call parameters of *Af. crassulus* (FME 46.65, StartF 90.94, and EndF 40.15 kHz) were higher than for *Af. happoldorum* (FME 40.55, StartF 78.87, and EndF 35.20 kHz) (Table 2). The echolocation calls of *Ps.* cf. *mbamminkom*, *Ps. roseveari*, *Ps.* cf. *nyanza*, and *Ps.* cf. *tenuipinnis* from Equatorial Guinea overlapped in the three call parameters measured with a FME between 39.21 and 40.40 kHz, StartF between 60.33 and 65.98 kHz, and EndF between 30.37 and 35.15 kHz (Table 2).

Table 2. Echolocation call parameters of pipistrelloid bats recorded from Equatorial Guinea. FME, frequency of maximum energy; StartF, start frequency; EndF, end frequency; *N*, individuals. Values represent mean, standard deviation (SD) and range for each measurement, between brackets. Ten pulses per file were measured.

Species	**N**	**FME ± SD**	**StartF ± SD**	**EndF ± SD**
Pipistrellus sp. nov.	3	40.1 ± 1.32 (37.5 – 42.0)	101.3 ± 6.19 (83.0 – 114.9)	31.5 ± 2.94 (24.2 – 35.3)
Afropipistrellus happoldorum	2	40.6 ± 2.02 (38.0 – 43.5)	78.9 ± 10.97 (62.8 – 95.7)	35.2 ± 1.34 (32.9 – 37.5)
Afropipistrellus crassulus	2	46.7 ± 0.95 (44.9 – 48.8)	90.9 ± 4.22 (83.7 – 99.7)	40.2 ± 1.04 (38.0 – 41.7)
Pseudoromicia cf. *mbamminkom*	2	39.4 ± 0.60 (39.0 – 41.0)	60.3 ± 2.83 (54.7 – 64.5)	33.5 ± 1.07 (32.1 – 35.3)
Pseudoromicia roseveari	2	40.4 ± 1.77 (39.0 – 43.5)	65.3 ± 5.88 (56.2 – 74.5)	35.2 ± 1.15 (32.4 – 37.1)
Pseudoromicia cf. *nyanza*	1	39.2 ± 0.44	66.0 ± 5.50	30.4 ± 1.53
Pseudoromicia cf. *tenuipinnis*	3	39.8 ± 1.31 (38.0 – 42.0)	63.0 ± 11.31 (47.0 – 80.9)	32.9 ± 2.04 (29.0 – 37.1)

Tribe Pipistrellini

For the tribe Pipistrellini, our reconstructions, under both ML and BI criteria, recovered the three genera currently recognized in Africa (*Pipistrellus*, *Scotoecus*, and *Vansonia*) (Fig. 4). In the *Cytb* phylogeny, within the genus *Pipistrellus*, a strongly supported clade comprising specimens captured on Bioko Island stood out as a distinct group, sister to *Pi. hesperidus* and *Pi. simandouensis* but clearly differentiated from these two species (Fig. 4). Likewise, a *COI* phylogenetic tree incorporating sequences from both tribes also reaffirms the monophyly of the group. However, the relationship between the three species must be interpreted carefully because the topology from the *COI* phylogeny did not support the *Cytb* hypothesis (Fig. 4; Supporting Information, Fig. S1). Moreover, both ML and BI analyses placed *Pi.* cf. *hesperidus* from Senegal apart from the other sequences identified as *Pi. hesperidus* from Kenya and showing sister-relationships with a separate clade representing *Pi. rusticus* from Namibia (Fig. 4).

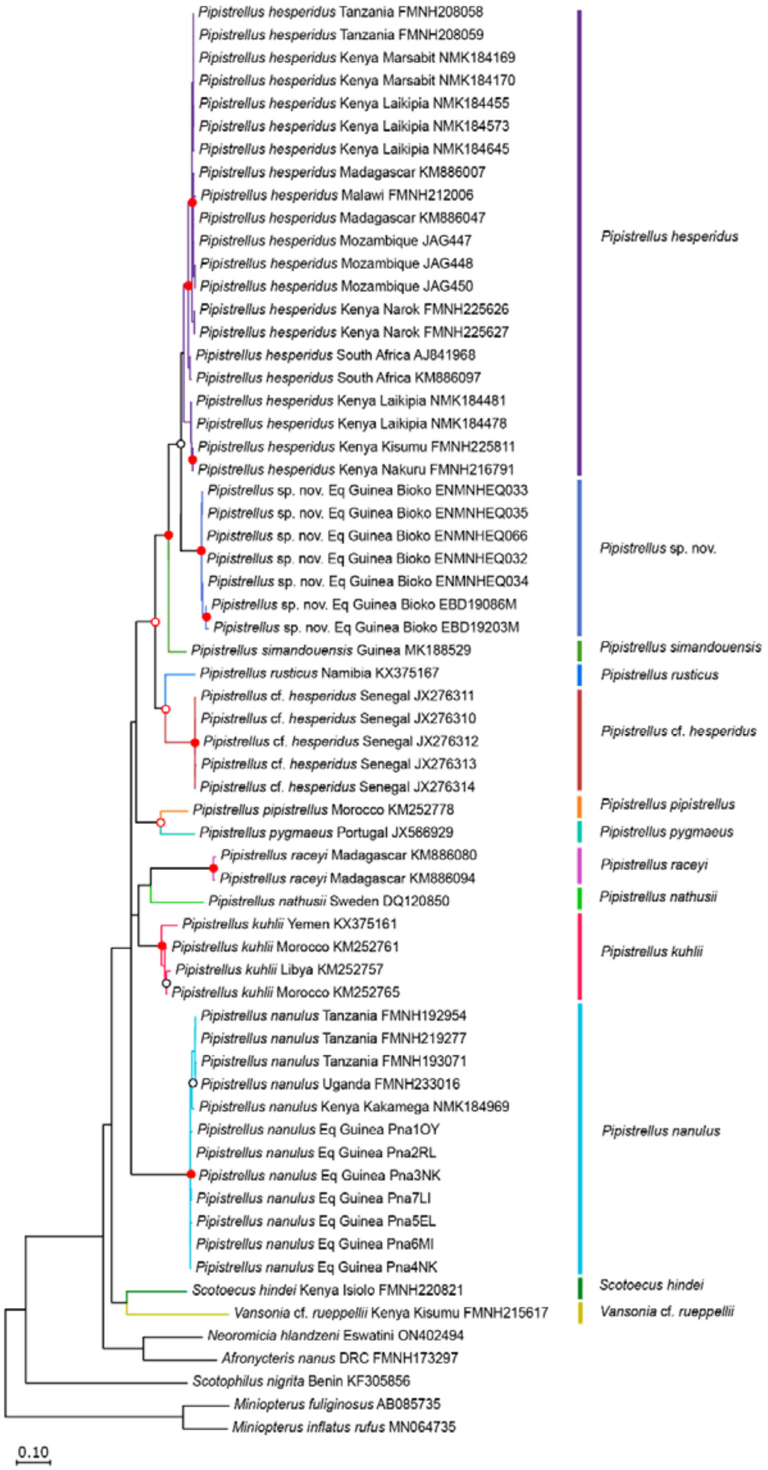

Figure 4. Results of maximum likelihood (ML) and Bayesian inference (BI) analyses combined on a ML tree of mitochondrial cytochrome b sequences of African pipistrelloids from the tribe Pipistrellini incorporating data from Equatorial Guinea based on the General Time Reversal + Gamma (G) + Invariable sites (I) model. Filled red circles on nodes denote bootstrap (BS) values ≥ 70% and Bayesian

posterior probabilities (PP) ≥ 0.90. Open circles outlined in black indicate BS≥70% and PP < 0.90, and open circles outlined in red indicate BS < 70% and PP > 0.90. DRC refers to the Democratic Republic of the Congo. Labels include species name, GenBank accession number or specimen/sample number, and country of collection (Supporting Information, Table S2). Branch colours indicate individual species/clade membership.

K2P genetic divergence between the undescribed lineage from Bioko and *Pi. hesperidus* and *Pi. simandouensis* were 6.4% and 11.1%, respectively. Genetic divergence between this new lineage and the other *Pipistrellus* varied from 10.3% to 26.3% (Table 3).

Table 3. Estimates of evolutionary divergence were determined for the Pipistrellini tribe based on the average number of base substitutions between groups of species. Analyses were conducted using the Kimura 2-parameter model (Tamura *et al.* 2021). The rate variation among sites was modelled with a gamma distribution (shape parameter = 1). The analysis involved 59 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + noncoding. All positions containing gaps and missing data were eliminated. There was a total of 191 positions in the final dataset.

	Pipistrellus hesperidus	*Pipistrellus* sp. nov.	*Pipistrellus simandouensis*	*Pipistrellus rusticus*	*Pipistrellus* cf. *hesperidus*	*Pipistrellus pipistrellus*	*Pipistrellus pygmaeus*	*Pipistrellus raceyi*	*Pipistrellus nathusii*	*Pipistrellus kuhlii*	*Pipistrellus nanulus*	*Vansonia* cf. *rueppellii*
Pipistrellus hesperidus												
Pipistrellus sp. nov.	0.064											
Pipistrellus simandouensis	0.106	0.111										
Pipistrellus rusticus	0.075	0.103	0.128									
Pipistrellus cf. *hesperidus*	0.086	0.146	0.121	0.139								
Pipistrellus pipistrellus	0.200	0.179	0.155	0.153	0.223							
Pipistrellus pygmaeus	0.165	0.157	0.216	0.143	0.200	0.136						
Pipistrellus raceyi	0.192	0.189	0.151	0.216	0.131	0.156	0.202					
Pipistrellus nathusii	0.203	0.198	0.175	0.190	0.198	0.188	0.150	0.165				
Pipistrellus kuhlii	0.142	0.128	0.128	0.166	0.173	0.182	0.157	0.158	0.117			
Pipistrellus nanulus	0.198	0.139	0.095	0.176	0.194	0.136	0.197	0.192	0.170	0.137		
Vansonia cf. *rueppellii*	0.273	0.239	0.267	0.318	0.296	0.245	0.247	0.251	0.284	0.254	0.240	

The ordination of the samples for the two main axes of a PCA multivariate analysis on the craniodental measurements of the African *Pipistrellus* species shows that most taxa are distributed along the morphospace's first axis with little overlap (Fig. 5). The first two principal axes accounted for 87% of the total variation, and PC1 represented a size variation with negative loadings for all craniodental measurements (Table 4). Hence, the smallest species (*Pi. nanulus*) appeared on the right side of PC1. In contrast, the new lineage from Bioko is located on the left side of the gradient (Fig. 5), indicating that this group represents the largest of the series studied. PC2, which can be interpreted as summarizing the variation in shape, had two high negative loadings; the most significant negative value (–0.781) corresponded to skull height (GSH), and the second most significant negative value (–0.428) corresponded to postorbital width (POB) (Table 4). Species with higher projections on PC2 had narrower interorbital regions and flatter skulls. *Pipistrellus simandouensis* from West Africa scarcely overlaps with the lineage found on Bioko Island (Fig. 5).

Table 4. Factor loadings for PC1 and PC2 of the PCA based on standardised craniodental measurements of *Pipistrellus* sp. nov., *Pi. simandouensis*, *Pi. hesperidus*, *Pi. nanulus*, and *Pi. rusticus*.

Character	**PC1**	**PC2**
GLS	-0.382	0.150
MAST	-0.331	0.312
POB	-0.324	-0.428
GSH	-0.268	-0.781
C-M^3	-0.386	0.143
C-C	-0.373	0.134
M^3-M^3	-0.374	0.222
c-m$_3$	-0.374	-0.002
Cumulative total variation explained	78%	87%

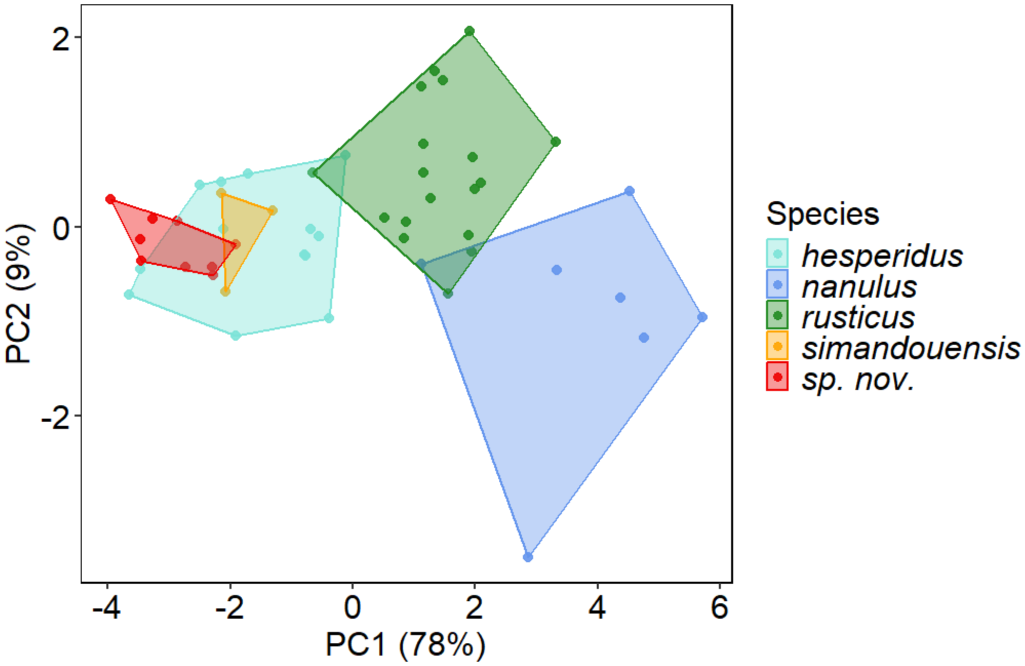

Figure 5. A principal components analysis ordination plotting the first two principal components of craniodental measurements of African *Pipistrellus* species. See Table 4 for the variables used in this analysis and the loadings on PC1 and PC2.

The penises and bacula of the *Pipistrellus* taxa from Bioko, as well as *Pi. simandouensis*, *Pi. hesperidus*, *Pi. rusticus*, and *Pi. nanulus*, are presented in Figures 6 and 7 and Table 5. Overall, the shape of the penises was similar, with a swollen glans and long whitish hairs, except for *Pi. nanulus* that presented the same thickness from the base to the glans of the penis (Fig. 6). The bacula of the five taxa had elongated shafts, bifurcated tips, wide bases, and are of similar size, except for *Pi. nanulus*, which shows a distinctly larger and sturdier baculum than the other species (Fig. 7).

Table 5. Penis and baculum measurements for five African *Pipistrellus* spp. Measurements were taken from digital images of the dorsal and lateral baculum views of *Pipistrellus* sp. nov., *Pi. simandouensis*, *Pi. hesperidus*, *Pi. nanulus*, and *Pi. rusticus*.

Species	Penis total length (mm)	Baculum total length (mm)	Baculum basal width (mm)	Baculum tip width (mm)
Pipistrellus sp. nov.	8.21 (ENMNH EQ034)	1.80 (holotype) and 1.53 (EBD 19086M)	0.32 (holotype) and 0.23 (EBD 19086M)	0.09 (holotype) and 0.07 (EBD 19086M)
Pi. simandouensis	8.40 (holotype)	1.32 (holotype)	0.32 (holotype)	0.11 (holotype)
Pi. hesperidus	7.20 (ENMNH Jilobi 12)	1.47 (JAG 447)	0.23 (JAG 447)	0.10 (JAG 447)
Pi. nanulus	6.38 (EMNH ZWW272)	2.98 (DM 13230) and 3.03 (EBD 20501M)	0.68 (DM 13230) and 0.84 (EBD 20501M)	0.51 (DM 13230) and 0.70 (EBD 20501M)
Pi. rusticus	5.43 (EMNH DOM-M-18)	1.52 (DM 16085)	0.35 (DM 16085)	0.13 (DM 16085)

Figure 6. Frontal and lateral views of the penis of (A, B) *Pipistrellus* sp. nov. (ENMNH EQ034), (C, D) *Pi. hesperidus* (ENMNH Jilobi 12), (E, F) *Pi. rusticus* (ENMNH DOM-M-18), and (G, H) *Pi. nanulus* (ENMNH ZWW272).

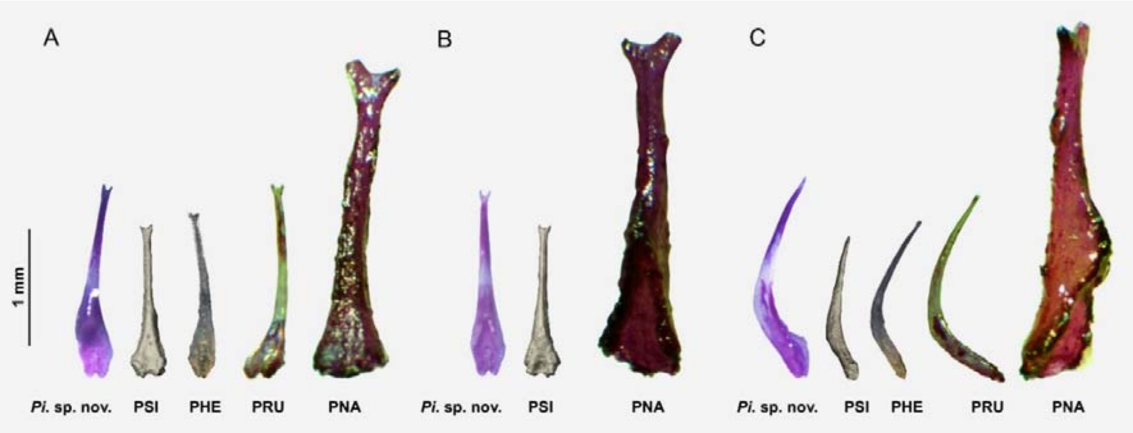

Figure 7. Dorsal (A), ventral (B) and lateral (C) views of the baculum of five species examined in this study: *Pi.* sp. nov.—Pi. sp. nov. holotype (EBD 19203M), PSI—*Pi. simandouensis* (ZMFK-MAM-2008.0302), PHE—*Pi. hesperidus* (JAG 447), PRU—*Pi. rusticus* (DM 16085), and PNA—*Pi. nanulus* (DM 13230). For PHE and PRU the ventral view was not available. Scale = 1 mm.

Based on the molecular and morphological differentiation presented above, we conclude that the new lineage from Bioko Island, Equatorial Guinea, represents an unknown *Pipistrellus* species. We first reassess the taxonomy of *Af. crassulus* and *Af. musciculus*, and then describe the new species of *Pipistrellus*.

Taxonomy
Family Vespertilionidae Gray, 1821
Tribe Vespertilionini Gray, 1821
Genus *Afropipistrellus* Thorn *et al.,* 2007
Afropipistrellus crassulus (Thomas, 1904)
Broad-headed pipistrelle
Synonymy:
*Pipistrellus crassulus*Thomas (1904),
Pipistrellus (*Pipistrellus*) *crassulus*Hill and Harrison (1987),
*Hypsugo crassulus*Heller *et al*. (1994),
Pipistrellus (*Vansonia*) *crassulus*Koopman (1994),
*Parahypsugo crassulus*Hutterer *et al.* (2019a),
*Nycticeinops crassulus*Monadjem *et al*. (2021a)

Material examined

EBD 13937M, male, Mainland Equatorial Guinea, collected by Javier Juste on 24 September 1985; EBD 17653M, female, Mainland Equatorial Guinea, collected by Javier Juste on 26 February 1988; EBD 17743M, female, Mainland Equatorial Guinea, collected by Javier Juste on 25 May 1988; EBD 34810M, female, and EBD 34811M, male, Mainland Equatorial Guinea, collected by Laura Torrent, Inazio Garin, Joxerra Aihartza, Anton Ayong Nguema and Esther Abeme Nguema Alene on 15 May 2022; ENMNH EQ013 female from Hormigas Camp, Bioko Island, collected by Ara Monadjem, Iroro Tanshi and Mnqobi Mamba 15 January 2024; and

ENMNH EQ023 female, from Mainland Equatorial Guinea, collected by Ara Monadjem, Laura Torrent and Mnqobi Mamba on 23 January 2024.

Description and remarks

Afropipistrellus crassulus is distinguished from *Af. bellieri* from West Africa by molecular and morphological characters. It is, on average, smaller and with a more flattened skull than *bellieri* (Fig. 3). Sagittal and lambdoid crests are little developed in *Af. crassulus*, whereas in *Af. bellieri* they form a weak occipital helmet. In *Af. crassulus*, P^2 is minute and displaced lingually, barely visible above the gum, whereas in *Af. bellieri* it is larger and more visible. Moreover, P^4 and C^1 are usually in contact in *Af. crassulus* but separated in *Af. bellieri*. The baculum of *Af. crassulus* is longer with a narrow tip, and the base is divided into two lobes by a deep cleavage, while in *Af. bellieri* the tip is inflated and the base is rounded (Supporting Information, Figs S2, S3) (Heller *et al.* 1994, Bates *et al.* 2013, Hutterer *et al.* 2019a, Moratelli *et al.* 2019).

While *Af. bellieri* is only known from West African rainforests (Guinea, Liberia, and Ivory Coast) (Monadjem *et al.* 2013, 2016, Decher *et al.* 2015), *Af. crassulus* is known from a wider diversity of habitats (rainforests, swamps, montane and coastal forests to savannas) from Equatorial Guinea, Cameroon, Republic of Congo, Democratic Republic of the Congo (DRC), Nigeria, Sudan, Uganda, Kenya, and Angola (Bates *et al.* 2013, Fahr 2013, Hutterer *et al.* 2019a, Tanshi *et al.* 2021) (Supporting Information, Fig. S4A).

Family Vespertilionidae Gray, 1821
Tribe Vespertilionini Gray, 1821
Genus *Afropipistrellus* Thorn *et al*., 2007
Afropipistrellus musciculus (Thomas, 1913)
Mouse-like pipistrelle
Synonymy:
*Pipistrellus musciculus*Thomas (1913),
Pipistrellus (*Pipistrellus*) *musciculus*Hayman *et al*. (1966),
Pipistrellus (*Hypsugo*) *musciculus*Hill and Harrison (1987),
*Hypsugo musciculus*Simmons (2005)

Material examined

EBD 15554M, male, Mainland Equatorial Guinea, collected by Javier Juste on 8 May 1987; EBD 20568M, male, Mainland Equatorial Guinea, collected by Javier Juste on 16 May 1990.

Description and remarks

This is a small bat with a total length between 64.0 and 69.0 mm, a forearm between 25.5 and 26.1 mm, and a body mass of *c*. 3.4 to 4.0 g (Supporting Information, Table S4). The pelage is dark brown dorsally and ventrally and the hair is unicoloured. The skull is comparatively small and the braincase is high with a mean GSKL of 11.53 mm and GSH of 5.09 mm (Supporting Information, Table S5). I^2 is bicuspid and I^3 is smaller; the anterior upper premolar is minute and displaced lingually; lower molars are myotodont. It presents the smallest dental measurements among all *Afropipistrellus* examined with a C–M^3 mean value of 3.81 mm, C–C 3.45 mm, M^3–M^3 4.90 mm, and c–m_3 4.00 mm (Supporting Information, Table S6). The baculum of *Af. musciculus* is long and slender, with a pointed tip and two conspicuous and thin lobes at the base (Supporting Information, Fig. S2, Table S7). *Afropipistrellus musciculus r*anges from West Sierra Leone to West DRC, with records from Ghana, Cameroon, Equatorial Guinea, and Gabon (Supporting Information, Fig. S4D). It is mainly known from the lowland rainforest in Central Africa but West Africa records are from savanna habitats (Monadjem *et al.* 2024). A post-lactating female was captured in February in Mainland Equatorial Guinea.

Family Vespertilionidae Gray, 1821
Tribe Pipistrellini Tate, 1942
Genus *Pipistrellus* Kaup, 1829
Pipistrellus etula Torrent *et al*., sp. nov.
Bioko pipistrelle
Synonymy:
*Pipistrellus kuhlii*Hill (1968)?
*Pipistrellus kuhlii*Hutterer *et al*. (1992)?
*Pipistrellus kuhlii*Juste and Ibáñez (1994b)

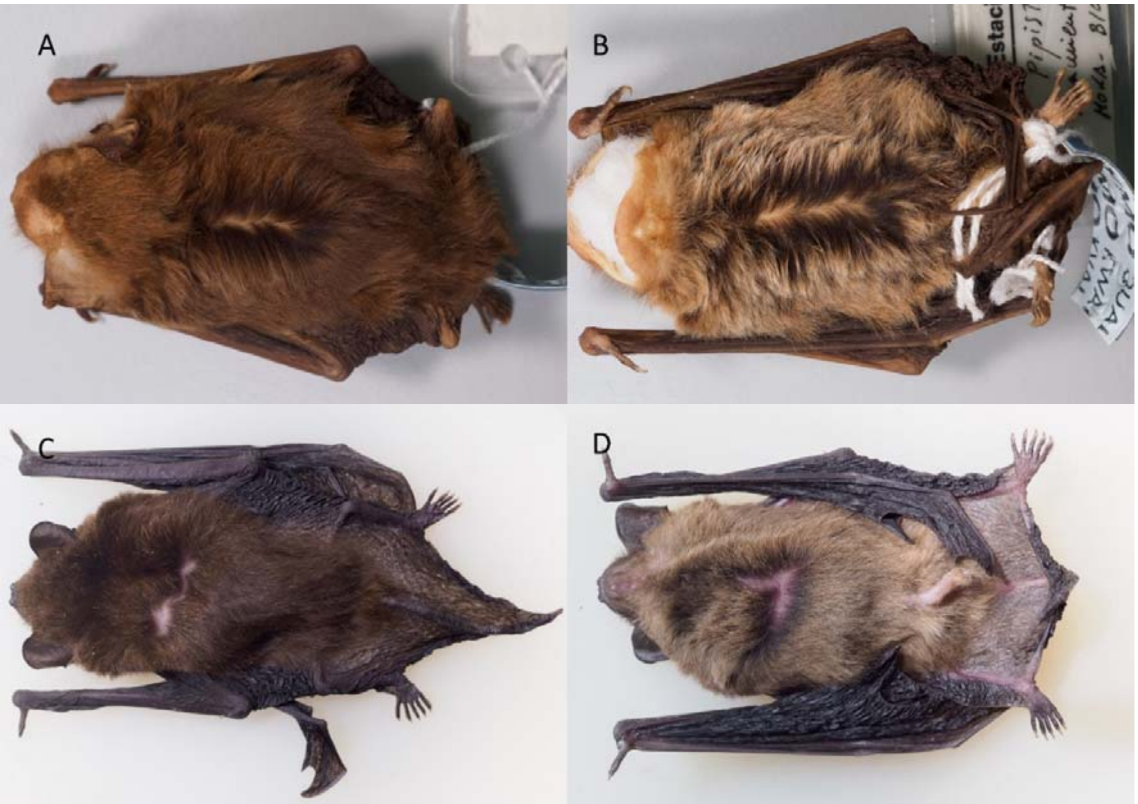

Figure 8. Dorsal (left) and ventral (right) views of (A, B) *Pipistellus etula* sp. nov. holotype EBD 19203M and (C, D) *Pi. etula* sp. nov. specimen ENMNH EQ034.

Holotype:

EBD 19203M. The bat was collected by Javier Juste. It is an adult male fixed in formalin and currently preserved in 70% alcohol, with the skull and baculum extracted and cleaned. Photographs of the fur, tragus, rhinarium, and thumb are illustrated in Figures 8 and 9, while the skull and mandible are illustrated in Figure 10. The penis and the baculum are presented in Figures 6 and 7.

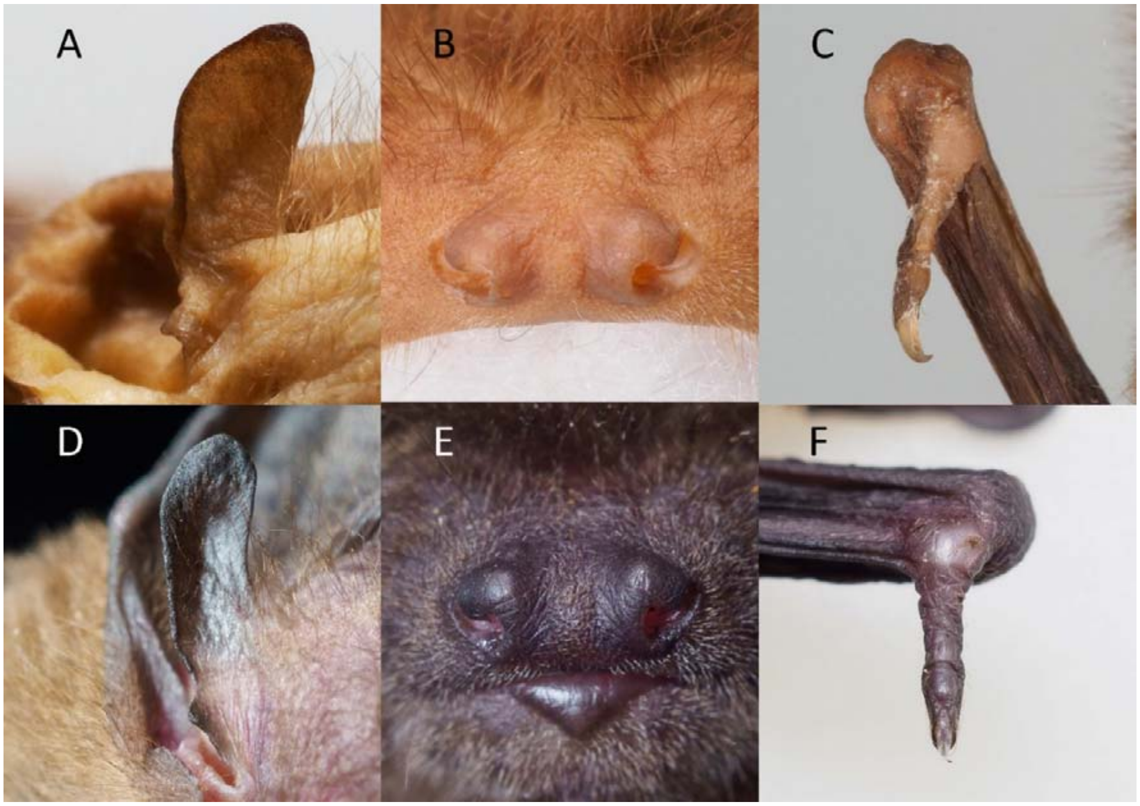

Figure 9. Tragus, rhinarium, and thumb pad of (A, B, C) *Pipistellus etula* sp. nov. holotype EBD 19203M and (D, E, F) *Pi. etula* sp. nov. specimen ENMNH EQ034.

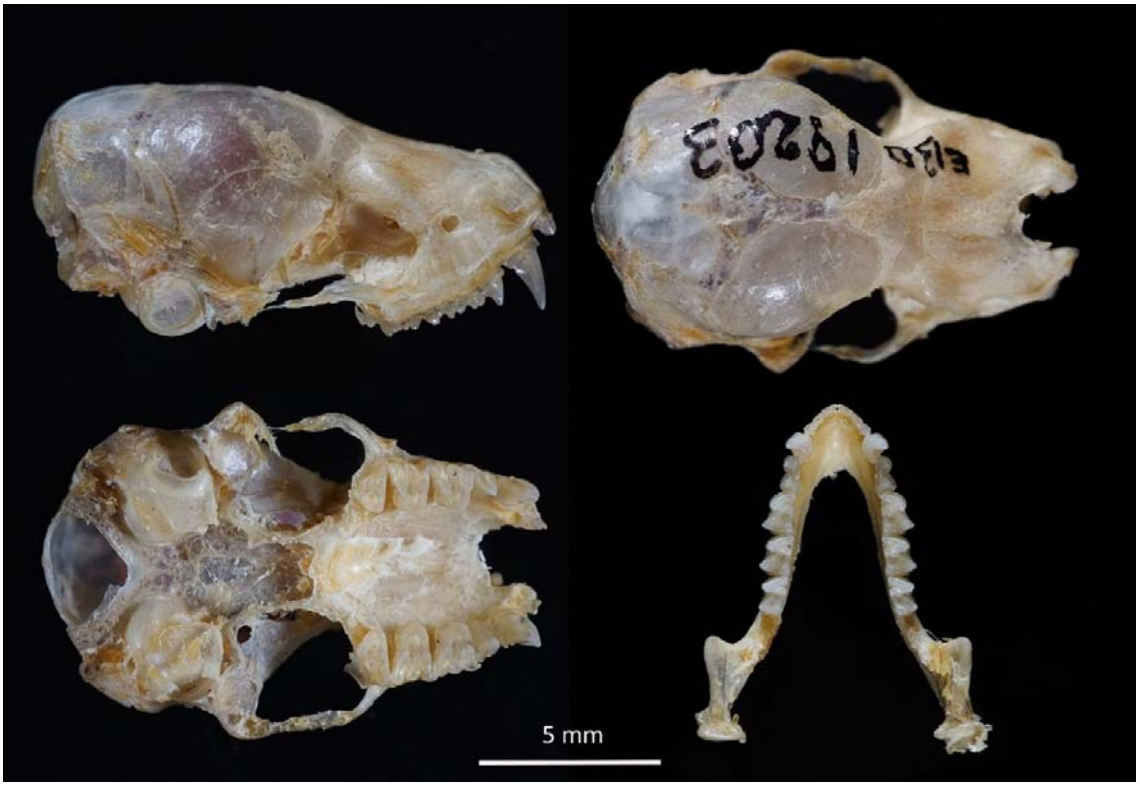

Figure 10. Lateral, dorsal, and ventral views of the skull and mandible of *Pipistrellus etula* sp. nov. holotype (EBD 19203M).

Type locality:

Biao Lake, Bioko Island, Equatorial Guinea (Figs 1, 11A). It was netted on 14 April 1989 in the montane forest of the slopes of Biao Peak, which shelters the crater that currently forms Biao Lake (3.3554666N; 8.6211751E) at an elevation of 1826 m above sea level (a.s.l.).

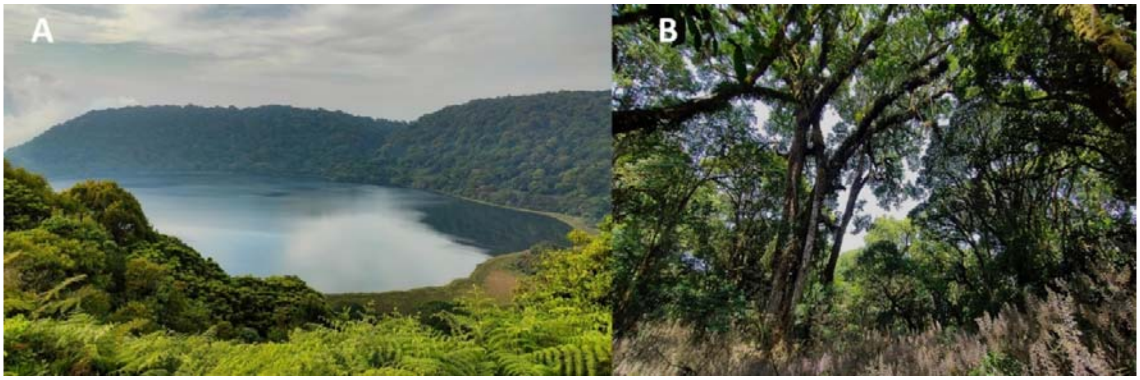

Figure 11. Type locality of *Pipistrellus etula* sp. nov. holotype and paratypes, Biao Lake (A) and Basilé Peak montane forest (B), Bioko Island, Equatorial Guinea. Photo credit: Laura Torrent.

Etymology:

'etula' means island or nation in the language of the original Bubi people of Bioko Island.

Paratypes:

Three additional bats, identified as belonging to this new species, were captured at the type locality on 13 April 1989 (EBD 19086M, male) and 15 April 1989 (EBD 19087M, male and EBD 19088M, female). The individual EBD 19086M was sequenced and grouped with the holotype in the *Cytb* phylogeny (Fig. 4).

Diagnosis

A medium-sized pipistrelloid bat, assigned to the genus *Pipistrellus* based on the presence of a small anterior upper premolar, the baculum shape (Hill and Harrison 1987) and *Cytb* and *COI* phylogenies (Fig. 4; Supporting Information, Fig. S1). The cranium is more inflated in ***Pipistrellus etula* sp. nov.** than in most *Afropipistrellus*, except *Af. eisentrauti* and *Af. happoldorum*. *Pipistrellus etula* sp. nov. is separated from *Pi. simandouensis* by its lighter bicoloured pelage (Fig. 8), the shape of the penis and baculum, and the tragus notch (Figs 6, 7; Supporting Information, Fig. S5), and the lingual position of the upper anterior premolar (Fig. 10). The penis of *Pi. etula* sp. nov. has a white protuberance located on the extremity of the prepuce, which is absent in *Pi. simandouensis* (Fig. 6), and the baculum of *Pi. etula* sp. nov. is longer, the bilobed base is more elongated, and the tip is narrower and sharper than in *Pi. simandouensis* (Fig. 7; Table 5). *Pipistrellus etula* sp. nov. can be distinguished from *Pi. hesperidus* by its tragus notch, and the shape of the penis and baculum (Figs 6, 7; Supporting Information, Fig. S5). The baculum of *Pi. etula* sp. nov. is longer, and the tip is more pointed than in *Pi. hesperidus* (Fig. 7; Table 5). However, these three species widely overlap in craniodental and external measurements (Tables 6–8). *Pipistrellus etula* sp. nov. is larger in external and cranial features than *Pi. nanulus* Thomas, 1904, *Pi. rusticus* Tomes 1861, *Pi. aero* Heller, 1912, and *Pi. inexspectatus* Aellen, 1959. It is easily distinguished from *Vansonia rueppellii*, which has pure white underparts (Moratelli *et al.* 2019, Monadjem *et al.* 2021a).

Table 6. External measurements (mm) and mass (g) of *Pipistrellusetula* sp. nov. from Bioko Island, Equatorial Guinea. Measurements are presented as mean ± standard deviation, range, and sample size (*N*). Measurements of the holotype and other individuals of the new species and other species of *Pipistrellus* occurring in sub-Saharan Africa are shown for comparative purposes.

Species or taxon	Body mass	Forearm length	Total length	Tail length	Ear length	Hindfoot length
Pipistrellus etula sp. nov. holotype EBD 19203M	9.5	34.4	NA	NA	NA	NA
Pipistrellus etula sp. nov. all specimens - mean	7.8 ± 1.23 (6.0-9.5), N = 9	35.1 ± 0.75 (34.4-37.0), N = 9	84.0 ± 1.41 (82.0-85.0), N = 4	38.3 ± 1.26 (37.0-40.0), N = 4	10.8 ± 0.50 (10.0-11.0), N = 4	8.8 ± 0.50 (8.0-9.0), N = 4
Pipistrellus simandouensis	6.5 ± 2.12 (5.0 and 8.0), N = 2	33.3 ± 0.55 (32.7-33.7), N = 3	88.7 ± 7.37 (83.0-97.0), N = 3	32.5 ± 0.71 (32.0 and 33.0), N = 2	9.5 ± 2.12 (8.0 and 11.0), N = 2	6.5 ± 0.71 (6.0 and 7.0), N = 2
Pipistrellus hesperidus	5.3 ± 0.87 (4.2-7.0), N = 17	32.0 ± 1.78 (29.0-35.0), N = 22	81.2 ± 5.15 (73.0-	31.6 ± 4.66 (24.0-	10.6 ± 1.46 (8.0-	7.5 ± 0.64 (6.5-9.0), N = 17

			90.0), N = 19	38.0), N = 19	13.0), N = 21	
Pipistrellus nanulus	3.7 ± 0.56 (2.0-4.8), N = 35	26.7 ± 1.16 (25.2-31.6), N = 42	68.6 ± 4.10 (62.0-80.0), N = 41	26.5 ± 2.52 (20.0-32.0), N = 36	9.5 ± 1.20 (7.0-12.0), N = 39	6.0 ± 1.03 (4.0-8.0), N = 34
Pipistrellus rusticus	4.4 ± 0.83 (3.0-6.0), N = 25	29.1 ± 1.00 (27.0-31.1), N = 28	76.6 ± 4.25 (68.0-88.0), N = 25	30.6 ± 4.75 (21.0-44.0), N = 25	9.1 ± 1.80 (5.0-11.0), N = 25	6.2 ± 0.91 (5.0-7.7), N = 19

Table 7. Cranial measurements (mm) of *Pipistrellusetula* sp. nov. from Bioko Island, Equatorial Guinea. Measurements are presented as mean ± standard deviation, range and sample size (*N*). Measurements of the holotype and other individuals of the new species and other species of *Pipistrellus* occurring in sub-Saharan Africa are shown for comparative purposes.

Species or taxon	GSKL	ZYGO	MAST	POB	GSH
Pipistrellus etula sp. nov. holotype EBD 19203M	13.12	8.65	7.30	3.92	4.88
Pipistrellus etula sp. nov. all specimens - mean	13.37 ± 0.31 (13.02-13.79), N = 9	8.58 ± 0.22 (8.27-8.99), N = 9	7.40 ± 0.17 (7.19-7.68), N = 9	3.91 ± 0.05 (3.83-3.97), N = 9	4.86 ± 0.11 (4.71-5.06), N = 9
Pipistrellus simandouensis	13.06 ± 0.41 (12.60-13.38), N = 3	8.53 ± 0.32 (8.30-8.90), N = 3	7.35 ± 0.17 (7.16-7.50), N = 3	3.86 ± 0.11 (3.80-3.99), N = 3	4.67 ± 0.11 (4.58-4.79), N = 3
Pipistrellus hesperidus	12.98 ± 0.38 (12.50-13.84), N = 31	8.32 ± 0.41 (7.50-9.28), N = 29	7.37 ± 0.25 (6.96-7.90), N = 31	3.82 ± 0.22 (3.46-4.20), N = 31	4.69 ± 0.24 (4.39-5.14), N = 31
Pipistrellus nanulus	11.43 ± 0.41 (10.20-12.16), N = 46	7.32 ± 0.38 (6.60-8.41), N = 36	6.66 ± 0.23 (6.19-7.28), N = 46	3.51 ± 0.19(3.20-4.06), N = 46	4.45 ± 0.29 (3.97-5.24), N = 43
Pipistrellus rusticus	12.03 ± 0.41 (10.90-12.82), N = 30	7.83 ± 0.34 (7.0-8.3), N = 19	7.01 ± 0.29 (6.20-7.30), N = 20	3.49 ± 0.15 (3.25-3.69), N = 19	4.28 ± 0.17 (3.97-4.56), N = 17

Table 8. Dental measurements (mm) of *Pipistrellusetula* sp. nov. from Bioko Island, Equatorial Guinea. Measurements are presented as mean ± standard deviation, range and sample size (*N*). Measurements of the holotype and other individuals of the new species and other species of *Pipistrellus* occurring in sub-Saharan Africa are shown for comparative purposes.

Species or taxon	C-M^3	C-C	M^3-M^3	c–m_3
Pipistrellus etula sp. nov. holotype EBD 19203M	4.74	4.20	5.91	5.04
Pipistrellus etula sp. nov. all specimens - mean	4.83 ± 0.12 (4.68-5.01), N = 9	4.32 ± 0.11 (4.12-4.48), N = 9	6.04 ± 0.21 (5.81-6.56), N = 9	5.20 ± 0.18 (4.95-5.43), N = 9
Pipistrellus simandouensis	4.76 ± 0.06 (4.70-4.80), N = 3	4.13 ± 0.05 (4.09-4.20), N = 3	5.64 ± 0.15 (5.50-5.80), N = 3	5.12 ± 0.13 (4.97-5.23), N = 3
Pipistrellus hesperidus	4.66 ± 0.18 (4.25-4.95), N = 31	4.15 ± 0.18 (3.80-4.60), N = 31	5.66 ± 0.22 (5.30-6.12), N = 30	4.98 ± 0.18 (4.55-5.27), N = 30
Pipistrellus nanulus	3.89 ± 0.17 (3.60-4.34), N = 46	3.61 ± 0.22 (3.20-4.17), N = 46	4.91 ± 0.22 (4.40-5.30), N = 46	4.15 ± 0.30 (3.70-5.08), N = 43
Pipistrellus rusticus	4.18 ± 0.13 (3.80-4.57), N = 28	3.83 ± 0.16 (3.56-4.25), N = 19	5.27 ± 0.19 (4.79-5.59), N = 19	4.49 ± 0.18 (4.25-4.98), N = 17

Description

External characters

***Pipistrellus etula* sp. nov.** is a medium-sized pipistrelloid bat (similar in size to *Pi. simandouensis* and *Pi. hesperidus*), but large for the genus *Pipistrellus*. It is the largest known species within the genus in Africa (all measurements except total length are greater than in *Pi. simandouensis* and is larger than *Pi. hesperidus*), with a total length of 82.0–85.0 mm and a forearm length of 34.4–37.0 mm (Table 6). The dorsal pelage is light brown (cinnamon colour), while the ventral pelage is paler, with the individual hairs being bicoloured and tipped lighter than the bases (Fig. 8). The patagium and uropatagium are both dark brown. There is a distinct, but small, pad at the base of the thumb (Fig. 9C, F). The ears and the tragus are typical for the genus *Pipistrellus* (Figs 9A, D, 12; Supporting Information, Fig. S5). The shape of the ears is subtriangular, rounded at the tip, and dark brown (Fig. 12). The tragus (Fig. 9A, D) is moderately long but does not reach half of the length of the ear (Fig. 12). It is relatively broad with a straight leading edge; the outer edge is convex above, with a well-defined notch below and a pointed projection above the base (Supporting Information, Fig. S5). The rhinarium fits the shape described for the genus *Pipistrellus* (Hutterer *et al.* 2019a), with terminal parts well-defined and the nostrils projecting from the snout (Fig. 9B, E). The external measurements of the holotype, paratypes, and other specimens of *Pipistrellus etula* sp. nov. are shown in Table 6.

Figure 12. Portrait of a *Pipistrellus etula* sp. nov. female captured in Basilé Peak, Bioko Island, Equatorial Guinea. Photo credit: Gerard Carbonell.

The penis of *Pi. etula* sp. nov. is relatively long (8.21 mm in ENMNH EQ034), wider in the prepuce than at the base, and entirely covered with short, whitish hairs (Fig. 6; Table 5). The prepuce shows a white protuberance, contrasting with its otherwise yellowish coloration. The total length of the penis is about 10% of the total body length (Table 5). The baculum of *Pi. etula* sp. nov. is slightly longer and overall larger than the bacula of *Pi. simandouensis* and *Pi. hesperidus*, with a total length of 1.80 mm (Fig. 7; Table 5). In *Pi. etula* sp. nov., the tip is bifurcate and the base is distinctly bilobed with a deep ridge (Fig. 7).

Craniodental characters

The skull is relatively robust for a *Pipistrellus*, while the rostrum is neither particularly broad nor narrow. The braincase is moderately inflated and rises above the level of the rostrum (Fig. 10), in contrast to the relatively flatter skulls of *Afropipistrellus* (except for *Af. eisentrauti* and

Af. happoldorum) (Table 7; Supporting Information, Table S5). Cranial measurements for the holotype, the paratypes, and other specimens of *Pi. etula* sp. nov. are shown in Table 7. The dentition of *Pi. etula* sp. nov. is I 2/3, C 1/1, P 2/3, M 2/3, which is typical of the genus *Pipistrellus*. In the upper tooth row, I^1 is not bifid, and I^2 is relatively small, not reaching half the height of I^1. P^1 is small, displaced lingually in the toothrow, and not visible laterally (Fig. 10). Lower incisors are trifid, which is typical of the genus *Pipistrellus*. Dental measurements for the holotype, the paratypes, and other specimens of *Pi. etula* sp. nov. are shown in Table 8.

Biology

Pipistrellus etula sp. nov. is currently known only from two localities on Bioko Island, based on seven sequenced specimens (appearing in Fig. 4): two specimens from Biao Lake, and five specimens from Basilé Peak. Moreover, tissue samples and acoustic recordings of five more individuals (captured and released) were collected from Basilé Peak. Both localities are old volcanos with summits from 2007 to 3011 m a.s.l.; thus, we expect this is a species closely associated with montane forested habitats (Fig. 11A, B).

The *Pi. etula* sp. nov. specimen ENMNH EQ033 echolocated at a FME (StartF and EndF) of 40.2 kHz (100.3 and 32.5 kHz). The mean (± SD) FME (StartF and EndF) for two other *Pi. etula* sp. nov. individuals (ENMNH EQ032 and 240130P3) at Basilé Peak locality was 40.0 ± 1.31 kHz (101.8 ± 3.73 and 30.9 ± 3.28 kHz) (Table 2).

Discussion

In this study, we have enhanced our understanding of the systematics of the African pipistrelloids by incorporating newly collected bat specimens from Equatorial Guinea along with museum specimens from the same region. This dataset includes representatives from both tribes, Vespertilionini and Pipistrellini, which encompass the African pipistrelloids (Monadjem *et al.* 2021a, b, Taylor *et al.* 2022). Our results using *Cytb* and *COI* molecular markers concur with previous studies for African pipistrelloids, in recognizing the following monophyletic genera: *Afronycteris* (for *nanus*), *Laephotis* (for *angolensis*, cf. *botswanae*, *botswanae*, *capensis*, *kirinyaga*, cf. *kirinyaga*, *malagasyensis*, *matroka*, *namibensis*, *robertsi*, *stanleyi*, *wintoni*, cf. *wintoni*), *Neoromicia* (for *anchietae*, cf. *guineensis*, *hlandzeni*, *somalica*, cf. *somalica*, *zuluensis*), *Pipistrellus* (for *Pipistrellus etula* sp. nov., *hesperidus*, cf. *hesperidus*, *kuhlii*, *nanulus*, *nathusii*, *pipistrellus*, *pygmaeus*, *raceyi*, *rusticus*, *simandouensis*), and *Pseudoromicia* (for *brunnea*, *isabella*, *kityoi*, *mbamminkom*, *nyanza*, cf. *nyanza*, *rendalli*, *roseveari*, *tenuipinnis*, cf. *tenuipinnis*) (Figs 2, 4; Supporting Information, Fig. S1). Concerning the Vespertilionini, our new material provides genetic and morphological evidence to keep *crassulus* in the genus *Afropipistrellus* as Demos *et al*. (2024) predicted. In the topologies, *Af. crassulus* is sister to *Af. bellieri* and *Af.* cf. *crassulus* from Tanzania (FMNH 192955). Moreover, our topologies reveal two distinct lineages of *Af. crassulus* separated by a gap in central Congo (Supporting Information, Fig. S4A). It is still uncertain whether this gap indicates a genuine biogeographic separation or if the eastern African records (from Kenya, Uganda, South Sudan, and eastern DRC) represent a new species. Additional material is required to resolve this issue.

Our study has also clarified the taxonomic position of '*musciculus'*, which we demonstrate belongs to the genus *Afropipistrellus* (Fig. 2). The position of this taxon remained uncertain for years due to the lack of genetic material and the limited availability of specimens. It was considered closely related to *Pi. hesperidus* by Koopman (1975); later, it was tentatively placed with *Pi. nanus* by Hill and Harrison (1987), although they could not study its baculum. Simmons (2005) placed it within *Hypsugo*, a classification that was maintained in later revisions (Simmons and Cirranello 2025). This placement persisted because the species could not be incorporated into recent molecular revisions of African taxa due to the absence of sequenced material (Monadjem *et al.* 2021a, b). Our study finally excludes the genus *Hypsugo* from sub-Saharan Africa. The morphological comparisons using the new material indicate that *Af. musciculus* is distinguished from other forest *Afropipistrellus* species by its small size and inflated skull (Fig. 3; Supporting Information, Tables S4, S5). Moreover, this study also expands the known distribution of *Af. happoldorum* from West Africa to Central Africa (Supporting Information, Fig. S4C).

The species *Af. eisentrauti* has been another enigmatic pipistrelloid, because of the scarcity of specimens and the confusion created by two misidentified specimens from Ivory Coast that were uploaded to GenBank as *Af. eisentrauti* (see: Lim and Van Coeverden de Groot 1997, Fahr 2013); these specimens actually represents *Af. bellieri*. True *Af. eisentrauti* is only known from montane areas of Cameroon and now also from Monte Alen mountain range in Equatorial Guinea. The small differentiation in mitochondrial DNA between the two individuals from each mountain locality (Cameroon and Equatorial Guinea), now isolated by a large area of lowland rainforest and transformed ecosystems, could be due to a recent colonization event, or more likely, to a post-glacial split (Supporting Information, Fig. S4B). Nevertheless, the evolutionary relationship of these lineages remain unsolved and its clarification will require the analysis of future markers and specimens.

Within *Pseudoromicia*, we report four individuals of *Ps. roseveari* from Equatorial Guinea, which represent the extension of the known distribution of this species to Central Africa. One individual was taken as a voucher and kept in EBD-CSIC collections (EBD 34812M) and the rest were released after examination and sampling (Supporting Information, Fig. S4F). Two other pipistrelloids, one from Mainland Equatorial Guinea and the other from Bioko Island, were genetically identified as belonging to the related species *Ps. mbamminkom*. These two new records extend southwards the distribution of this recently described bat, known previously only from its type locality on Mount Mbam Minkom, in the Central Region of Cameroon (Grunwald *et al.* 2023) (Supporting Information, Fig. S4E). Finally, we collected tissue samples of several white-winged bats morphologically similar and belonging to the *tenuipinnis* group, although none was kept as vouchers. Nevertheless, the resulting phylogenetic position of the *Cytb* sequences of these *Ps.* cf. *tenuipinnis* from Equatorial Guinea was inconclusive in the topologies and as a consequence their molecular identification is unresolved (Fig. 2). In fact, while two of the *Ps.* cf. *tenuipinnis* sequences match with *Pseudoromicia* sp. from Tanzania (FMNH 192956), one aligns in a different group as a sister lineage of *Ps. nyanza* from Kenya and is thus considered as *Ps.* cf. *nyanza*. Moreover, the five specimens at EBD-CSIC are provisionally identified as *Ps.* cf. *tenuipinnis* pending further research. Twenty-three additional specimens housed at the EBD-CSIC collections were identified morphologically as *Pseudoromicia*, but they could not be conclusively assigned to either *Ps. brunnea* or *Ps. mbamminkom* without genetic support. To conclude, the new material of *Pseudoromicia* pipistrelloids provided in this study highlights the need for further research to clarify the taxonomy and distribution of the lineages *Ps. brunnea*, *Ps. mbamminkom*, *Ps. nyanza*, and *Ps. tenuipinnis* in Central Africa and their evolutionary relationships.

Within the Pipistrellini tribe, our description of *Pipistrellus etula* sp. nov. fills in a geographical gap existing between the species *Pi. simandouensis* found in West Africa, and the closely related *P. hesperidus*, in eastern and south-eastern Africa (Fig. 13). These three large *Pipistrellus* appear to be 'paramontane' species, as their distributions include montane forests along slopes and plateaux at mid and high altitudes (see: Taylor *et al*. 2024). It seems that the three species, which share a similar morphology, are isolated in highland forested habitats (between 450 and 3000 m a.s.l.) and are currently separated by large expanses of lowland rainforest, which may have contributed to their speciation process. Moreover, we suspect that the specimens collected in the Cameronian and Nigerian highlands (recorded above 2200 m a.s.l.) by Hill (1968) and Hutterer *et al*. (1992), identified as *Pi. kuhlii*, may also refer to *Pi. etula* sp. nov.. Therefore, the species' distribution may actually extend from Bioko Island to the Cameroon Volcanic line (see red dots within the circle in Fig. 13), although currently it should be considered as an endemic from Bioko Island. The lineage *Pi.* cf. *hesperidus*, described by Koubínová *et al*. (2013) from Senegal, clusters with *Pi. rusticus*, and it is unrelated to *Pi. hesperidus* '*sensu stricto*' (Fig. 2). An available name that may pertain to *Pi.* cf. *hesperidus* is *Pipistrellus marrensis* Thomas and Hinton, 1923, from 'Jebel Marra' in south-western Sudan. However, further analysis must be conducted to solve this taxonomic conundrum.

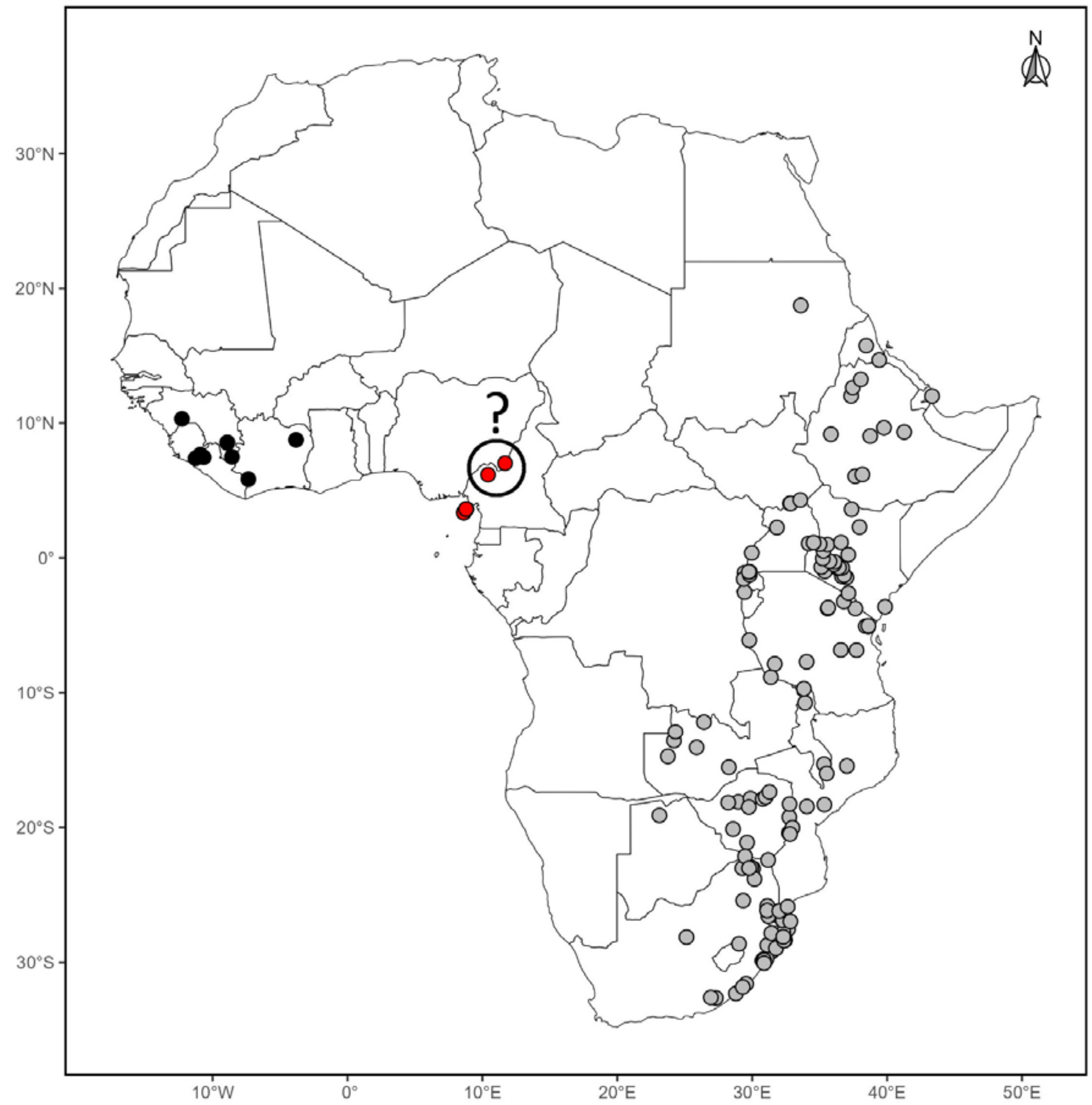

Figure 13. Distribution map of *Pipistrellus etula* sp. nov. (red dots), *Pi. simandouensis* (black), and *Pi. hesperidus* (grey). The Mainland records (red dots within the circle) represent speculative individuals of *Pi. etula* sp. nov. based only on morphological identification.

Despite the improvements in our understanding of the taxonomy and systematics of the African pipistrelloids, there are still important questions to be clarified within both the Vespertilionini and Pipistrellini tribes. The taxonomy of the genus *Afropipistrellus* remains unresolved. The addition of new mitochondrial DNA sequences from Equatorial Guinea has not yet clarified the phylogenetic relationships between *Af. eisentrauti* and the remainder of the genus (Fig. 2; Supporting Information Fig. S1). Similarly among the Pipistrellini, the relationships between the genera *Pipistrellus*, *Vansonia*, and *Scotoecus* also need to be clarified (Fig. 4). It seems clear that further studies on African pipistrelloids should include more sequences, particularly from nuclear markers, to resolve their internal relationships.

Finally, this work expands to Central Africa the distributions of two pipistrelloid species (*Af. happoldorum* and *Ps. roseveari*) previously known only from West Africa. Moreover, it provides new localities in Central Africa for four poorly documented species, namely, *Af. eisentrauti*, *Af. musciculus*, *Af. crassulus*, and *Ps. mbamminkom*. The *Pipistrellus etula* sp. nov. represents the 13th pipistrelle species identified in the Lower Guinea forests, underscoring the region's significance as a hotspot for pipistrelloid diversity. This is comparable to the Upper Guinea forests, which also harbours 13 species of pipistrelloids (Monadjem *et al.* 2024). Furthermore, *Pi. etula* sp. nov. is the first documented endemic bat species on Bioko Island, as previously only the shrew *Myosorex eisentrauti* had been identified as an endemic (Hutterer 2013). Nonetheless, these numbers could rise further with the confirmation of additional species yet to be verified in the area, such as *Ps. tenuipinnis* and *Ps. nyanza*. Moreover, most of the recently described sub-Saharan pipistrelloid species were found outside Central Africa, except *Ps. mbamminkom* and *Ps. principis*. As this study has shown, they could also expand through the vast Congolian rainforests, which remain largely unsampled. Given the relatively extensive research conducted in West, East, and southern Africa, future efforts should prioritize the Congolian rainforests. Investigating the biodiversity of this area will provide crucial data to address ongoing taxonomic challenges and support the development of effective conservation strategies.

Conclusion

Based on our new pipistrelloids bat material from Equatorial Guinea, we describe a new species of *Pipistrellus* for the Congolian rainforests, present an updated phylogeny for the tribes Pipistrellini and Vespertilionini, and confirm the generic positions of *Af. musciculus* and *Af. crassulus*.

[Version of Record, first published online 13 June 2025, with fixed content and layout in compliance with Art. 8.1.3.2 ICZN. http://zoobank.org/urn:lsid:zoobank.org:pub:285694AA-51CA-40B7-A695-271EBAE6CD3B]

FUNDING

This study was funded by the Bat Conservation International (grant SS2212), the Barcelona Zoo Foundation (grant EGBATS), the Rufford Foundation (grant 38597-1), Global Challenges Research Funds and Basque Government (grants IT1169-19 and IT1571-22) have supported our recent field expeditions in Equatorial Guinea. The Kate Barlow Award from Bat Conservation Trust partially supported sequencing expenses. Luke L. Powell and Laura Torrent have been supported by European Union's Horizon 2020 research and innovation programme under grant agreement No 854248.

Acknowledgements

The EBD-CSIC collections were gathered as part of the Environmental Cooperation Programme in Equatorial Guinea, which was conducted by the Spanish Cooperation Agency (AECI, formerly OCGE) from 1984 to 1991. Some of the specimens were collected with the support of Conservation International while carrying out a rapid biological assessment under the Rapid Assessment Program (RAP), in partnership with Bioko Biodiversity Protection

Program (BBPP), Equatorial Guinea's National Institute of Forestry Development and Management of the System of Protected Areas (INDEFOR-AP), University of Equatorial Guinea (UNGE) and Biodiversity Initiative (BI). Special thanks to Fidel Esono Mba Eyon, Maximilliano Fero Meñe, David Montgomery, Francisco Mitogo Micha, Caridad Ayong, and Trond Larsen for providing us with all the permits to collect and export samples (permit number 0096, issued: 20 May 2022; permit number 20/2024, issued: 1 February 2024) to the Eswatini Zoological Museum at the University of Eswatini and the Doñana Biological Station (EBD-CSIC) in Spain. We deeply thank Jennifer Leonard, Noelia Pérez Hernández, and Anna Cornellas, and the molecular facilities of the EBD-CSIC (LEM-EBD) for their rigorous lab work. We appreciate the support and assistance of Carlos Urdiales, curator, and Manolo López Ribera, taxidermist, of the EBD-CSIC collections. Special thanks to Adrià López-Baucells from the BiBio Research Group. L.T. would like to thank her field team members Esther Abeme Nguema Alene, Diogo F. Ferreira, Alex Hall, Claire Pooley, Miguel Angel Fuentes, and Obamba Bibang Nsehe, as well as Sandra Juste Monayong and Anton Ayong Nguema and his family for their kind hospitality every time we visited Equatorial Guinea. We also acknowledge the help of many local field assistants and forest ecoguides.

CRediT statement

Laura Torrent (data collection, molecular analyses, writing, morphometrics), Javier Juste (data collection, molecular analyses, writing), Inazio Garin (data collection), Joxerra Aihartza (data collection), Desiré L. Dalton (molecular analysis), Mnqobi Mamba (data collection), Iroro Tanshi (data collection), Luke L. Powell (data collection, funding, logistics, editing), Sara Padidar (molecular analysis), Juan Luis Garcia Mudarra (molecular analysis), Leigh Richards (specimens information), and Ara Monadjem (data collection, morphometrics, writing). All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest regarding the publication of this article.

DATA AVAILABILITY

The data underlying this article are available in the GenBank Nucleotide Database at https://www.ncbi.nlm.nih.gov/genbank/

References

Alcaide M, Rico C, Ruiz S, Soriguer R, Muñoz J, Figuerola J. **2009**. Disentangling Vector-Borne Transmission Networks: A Universal DNA Barcoding Method to Identify Vertebrate Hosts from Arthropod Bloodmeals. *Plos ONE* 4: 1–6.

Amador LI, Moyers Arévalo RL, Almeida FC, Catalano SA, Giannini NP. **2018**. Bat Systematics in the Light of Unconstrained Analyses of a Comprehensive Molecular Supermatrix. *Journal of Mammalian Evolution* 25: 37–70.

Bates PJJ, Cameron K, Pearch MJ, Hayes B. **2013**. A review of the bats (Chiroptera) of the Republic of Congo, including eight species new to the country. *Acta Chiropterologica* 15: 313–340.

Benda P, Reiter A, Uhrin M, Varadínová Z. **2016**. A New Species of Pipistrelle Bat (Chiroptera: Vespertilionidae) from Southern Arabia. *Acta Chiropterologica* 18: 301–323.

Benda P, Uvizl M, Mazoch V, Šklíba J, Červený J. **2022**. African bats in the collection of the National Museum, Prague (Chiroptera). I. Bats from Zambia. *Lynx, new series* 53: 291–332.

Campos PF, Gilbert TMP. **2012**. DNA Extraction from Formalin-Fixed Material. In: Shapiro B, Hofreiter M, eds. *Ancient DNA. Methods in Molecular Biology*. New York: Humana Press, 81–85.

Darriba D, Taboada GL, Doallo R, Posada D. **2012**. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772–772.

Decher J, Hoffmann A, Schaer J, Norris RW, Kadjo B, Astrin J, Monadjem A, Hutterer R. **2015**. Bat Diversity in the Simandou Mountain Range of Guinea, with the Description of a New White-Winged Vespertilionid. *Acta Chiropterologica* 17: 255–282.

Demos TC, Webala PW, Monadjem A, Patterson BD. 2024. Nuclear introns support the subtribe Laephotina and recently proposed genera of African Vespertilionidae. *Acta Chiropterologica* 26: 143-152.

Fahr J. **2013**. *Pipistrellus crassulus* Broad-Headed Pipistrelle. In: Happold M, Happold D, eds. *Mammals of Africa. Volume IV. Hedgehogs, shrews and bats*. London: Bloosmbury Publishing, 617–619.

Fahr J, Kalko EKV. **2011**. Biome transitions as centres of diversity: Habitat heterogeneity and diversity patterns of West African bat assemblages across spatial scales. *Ecography* 34: 177–195.

Felsenstein J. **1981**. Evolutionary Trees from DNA Sequences: A Maximum Likelihood Approach. *Journal of Molecular Evolution* 17: 368–376.

Gemmell NJ, Akiyama S. **1996**. An efficient method for the extraction of DNA from vertebrate tissues. *Trends in Genetics* 12: 338–339.

Goodman SM, Kearney T, Ratsimbazafy MM, Hassanin A. **2017**. Description of a new species of *Neoromicia* (Chiroptera: Vespertilionidae) from southern Africa: A name for ‘n. cf. *melckorum*’. *Zootaxa* 4236: 351–374.

Goodman SM, Rakotondramanana CF, Ramasindrazana B, Kearney T, Monadjem A, Schoeman MC, Taylor PJ, Naughton K, Appleton B. **2015**. An integrative approach to characterize Malagasy bats of the subfamily Vespertilioninae Gray, 1821, with the description of a new species of *Hypsugo*. *Zoological Journal of the Linnean Society* 173: 988–1018.

Grunwald AL, Demos TC, Nguéagni Y, Tchamba MN, Monadjem A, Webala PW, Peterhans JCK, Patterson BD, Ruedas LA. **2023**. A review of bats of the genus *Pseudoromicia* (Chiroptera: Vespertilionidae) with the description of a new species. *Systematics and Biodiversity* 21: 1–18.

Hayman RW, Misonne X, Verheyen WN. 1966. The Bats of the Congo and Rwanda and Burundi. *Annalen van het Koninklijk Museum voor Midden Afrika: Zoologische Wetenschappen* 154: 69–71.

Hebert PDN, Cywinska A, Ball SL, DeWaard JR. **2003**. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* 270: 313–321.

Heller KG, Volleth M, Kock D. **1994**. Notes on some vespertilionid bats from the Kivu region, Central Africa (Mammalia: Chiroptera). *Senckenbergiana Biologica* 74: 1–8.

Hill JE. **1968**. Bats from the Cameroons, with the description of a new species of *Pipistrellus*. *Bonner Zoologische Beiträge* 19: 43–48.

Hill J, Harrison DL. **1987**. The baculum in the Vespertilioninae (Chiroptera: Vespertilionidae) with a systematic review, a synopsis of *Pipistrellus* and *Eptesicus*, and the descriptions of a new genus and subgenus. *Bulletin of the British Museum (Natural History) Zoology* 52: 225–305.

Hoofer SR, Van Den Bussche RA. **2003**. Molecular phylogenetics of the chiropteran family Vespertilionidae. *Acta Chiropterologica* 5: 1–63.

Hutterer R. **2013**. *Myosorex eisentrauti* Eisentraut's Mouse Shrew. In: Happold M, Happold D, eds. *Mammals of Africa, Volume IV: Hedgehogs, Shrews and Bats.* London: Bloomsbury Publishing, 153–154.

Hutterer R, Decher J, Monadjem A, Astrin J. **2019a**. A New Genus and Species of Vesper Bat from West Africa, with Notes on *Hypsugo*, *Neoromicia*, and *Pipistrellus* (Chiroptera: Vespertilionidae). *Acta Chiropterologica* 21: 1–22.

Hutterer R, Dieterlen F, Nikolaus G. **1992**. Small mammals from forest islands of eastern Nigeria and adjacent Cameroon, with systematical and biogeographical notes. *Bonner Zoologische Beiträge* 43: 393–414.

Hutterer R, Koenig A, Kerbis J. **2019b**. A further new species of vesper bat from Central Africa (Chiroptera: Vespertilionidae). *Lynx* 50: 51–59.

Ibáñez C, García-Mudarra JL, Ruedi M, Stadelmann B, Juste J. **2006**. The Iberian contribution to cryptic diversity in European bats. *Acta Chiropterologica* 8: 277–297.

Jung K, Molinari J, Kalko EKV. **2014**. Driving factors for the evolution of species-specific echolocation call design in new world free-tailed bats (molossidae). *PLoS ONE* 9: e85279.

Juste J, Benda P, Garcia-Mudarra JL, Ibáñez C. **2013**. Phylogeny and systematics of Old World serotine bats (genus *Eptesicus*, Vespertilionidae, Chiroptera): An integrative approach. *Zoologica Scripta* 42: 441–457.

Juste J, Ibáñez C. **1994a**. Bats of the Gulf of Guinea islands: faunal composition and origins. *Biodiversity and Conservation* 3: 837–850.

Juste J, Ibáñez C. **1994b**. Contribution to the knowledge of the bat fauna of Bioko Island, Equatorial Guinea (Central Africa). *Zeitschrift fur Saugetierkunde* 59: 274-281

Juste J, Torrent L, Méndez-Rodríguez A, Howard K, García-Mudarra JL, Nogueras J, Ibáñez C. **2023**. A new Pipistrelle bat from the oceanic Island of Príncipe (Western Central Africa). *Journal of Mammalogy* 104: 361–371.

Kearney T, Volleth M, Contrafatto G, Taylor PJ. **2002**. Systematic implications of chromosome GTG-band and bacula morphology for Southern African *Eptesicus* and *Pipistrellus* and several other species of Vespertilioninae (Chiroptera: Vespertilionidae). *Acta Chiropterologica* 4: 1–22.

Koopman KF. **1975**. Bats of the Sudan. *Bulletin of the American Museum of Natural History* 154: 353–444.

Koopman KF. 1994. Chiroptera: Systematics. In: Niethammer J, Schliemann H, Starck D. eds. *Handbook of Zoology, Volume 8*. Berlin: Walter de Gruyter Press, 1–217.

Koubínová D, Irwin N, Hulva P, Koubek P, Zima J. **2013**. Hidden diversity in Senegalese bats and associated findings in the systematics of the family Vespertilionidae. *Frontiers in Zoology* 10: 1–16.

Kumar S, Stecher G, Tamura K. **2016**. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874.

Lim BK, Van Coeverden de Groot J. **1997**. Taxonomic report of small mammals from Cote d'Ivoire. *Journal of African Zoology* 111: 261–279.

Monadjem A, Demos TC, Dalton DL, Webala PW, Musila S, Kerbis Peterhans JC, Patterson BD. **2021a**. A revision of pipistrelle-like bats (Mammalia: Chiroptera: Vespertilionidae) in East Africa with the description of new genera and species. *Zoological Journal of the Linnean Society* 191: 1–33.

Monadjem A, Montauban C, Webala PW, Laverty TM, Bakwo-Fils EM, Torrent L, Tanshi I, Kane A, Rutrough AL, Waldien DL, Taylor PJ. **2024**. African bat database: curated data of occurrences, distributions and conservation metrics for sub-Saharan bats. *Scientific Data* 11: 1309.

Monadjem A, Richards LR, Decher J, Hutterer R, Mamba ML, Guyton J, Naskrecki P, Wipfler B, Kropff AS, Dalton DL. **2021b**. A phylogeny for African Pipistrellus species with the description of a new species from West Africa (Mammalia: Chiroptera). *Zoological Journal of the Linnean Society* 191: 548–574.

Monadjem A, Richards L, Denys C. **2016**. An African Bat Hotspot: The Exceptional Importance of Mount Nimba for Bat Diversity. *Acta Chiropterologica* 18: 359–375.

Monadjem A, Richards L, Taylor PJ, Stoffberg S. **2013**. High diversity of pipistrelloid bats (Vespertilionidae: *Hypsugo*, *Neoromicia*, and *Pipistrellus*) in a West African rainforest with the description of a new species. *Zoological Journal of the Linnean Society* 167: 191–207.

Moratelli R, Burgin C, Cláudio V, Novaes R, López-Baucells A, Haslauer R. **2019**. Family Vespertilionidae. In: Wilson DE, Mittermeier RA, eds. *Handbook of the Mammals of the World. Vol. 9. Bats*. Barcelona: Lynx Edicions.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Oksanen MJ. **2013**. Package 'vegan'. *Community Ecology Package* 2: 1–295.

Patterson BD, Demos TC, Torrent L, Grunwald AL, Montauban C, Kerbis Peterhans JC, McDonough MM, Dick CW, Bartonjo M, Schoeman MC, Ruedas LA, Juste J. 2024. Systematics of the *Rhinolophus landeri* complex, with evidence for 3 additional Afrotropical bat species. *Journal of Mammalogy* 106: 187–201.

Posada D. **2008**. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256.

R Core Team. **2021**. R: A Language and Environment for Statistical Computing. Viena: *R Foundation for Statistical Computing*. https://www.R-project.org

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Huelsenbeck JP. **2012**. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.

Rosas A. **2022**. *Tesoros naturales de Guinea Ecuatorial. Consejo Superior de Investigaciones Científicas* (A Rosas, Ed.). Madrid: Editorial CSIC.

Sikes RS and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy* 97: 663–688.

Simmons NB. **2005**. Order Chiroptera. In: Wilson DE, Reeder DM, eds. *Mammal Species of the World: A taxonomic and geographic reference.* Baltimore: The John Hopkins University Press.

Simmons NB, Cirranello AL. **2025**. Bat Species of the World: A taxonomic and geographic database. https://batnames.org (09 January 2025, date last accessed).

Smith MF, Patton JL. **1993**. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. *Biological Journal of the Linnean Society* 50: 149–177.

Tamura K, Stecher G, Kumar S. **2021**. MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution* 38: 3022–3027.

Tanshi I, Kingston T. **2021**. Introduction and implementation of harp traps signal a new era in bat research. *50 Years of Bat Research*. Springer, Cham., 255–270.

Tanshi I, Obitte BC, Monadjem A, Kingston T. **2021**. Hidden Afrotropical bat diversity in Nigeria: ten new country records from a biodiversity hotspot. *Acta Chiropterologica* 23: 313–343.

Taylor PJ, Kearney TC, Clark VR, Howard A, Mdluli M V., Markotter W, Geldenhuys M, Richards LR, Rakotoarivelo AR, Watson J, Balona J, Monadjem A. **2024**. Southern Africa's Great Escarpment as an amphitheater of climate-driven diversification and a buffer against future climate change in bats. *Global Change Biology* 30, e17344.

Taylor PJ, Strydom E, Richards L, Markotter W, Toussaint DC, Kearney T, Cotterill FPD (Woody), Howard A, Weier SM, Keith M, Neef G, Mamba ML, Magagula S, Monadjem A. **2022**. Integrative taxonomic analysis of new collections from the central Angolan highlands resolves the taxonomy of African pipistrelloid bats on a continental scale. *Zoological Journal of the Linnean Society* 196: 1570–1590.

Thomas O. 1904. XXII. New bats from British East Africa collected by Mrs. Hinde, and from the Cameroons by Mr. G.L. Bates. *Annals and Magazine of Natural History* 13: 206–210.

Thomas O. 1913. On African bats and shrews. *Annals and Magazine of Natural History* 11: 314–321.

Trujillo RG, Patton JC, Schlitter DA, Bickham JW. **2009**. Molecular phylogenetics of the bat genus *Scotophilus* (Chiroptera: Vespertilionidae): Perspectives from paternally and maternally inherited genomes. *Journal of Mammalogy* 90: 548–560.

Volleth M, Bronner G, Göpfert MC, Heller KG, Helversen O V., Yong HS. **2001**. Karyotype comparison and phylogenetic relationships of Pipistrellus-like bats (Vespertilionidae; Chiroptera; Mammalia). *Chromosome Research* 9: 25–46.

Volleth M, Heller KG. **1994**. Phylogenetic relationships of vespertilionid genera (Mammalia: Chiroptera) as revealed by karyological analysis. *Zeitschrift für zoologische Systematik und Evolutionsforschung* 32: 11–34.

Wickham H. **2016**. Ggplot 2: elegant graphics for data analysis. New York: Springer.