

# Molecular phylogenetics of slit-faced bats (Chiroptera: Nycteridae) reveal deeply divergent African lineages

Terrence C. Demos<sup>1</sup>  | Paul W. Webala<sup>2</sup> | Julian C. Kerbis Peterhans<sup>1,3</sup> |  
Steven M. Goodman<sup>1,4</sup> | Michael Bartonjo<sup>5</sup> | Bruce D. Patterson<sup>1</sup> 

<sup>1</sup>Integrative Research Center, Field Museum of Natural History, Chicago, Illinois

<sup>2</sup>Department of Forestry and Wildlife Management, Maasai Mara University, Narok, Kenya

<sup>3</sup>College of Arts and Sciences, Roosevelt University, Chicago, Illinois

<sup>4</sup>Association Vahatra, Antananarivo, Madagascar

<sup>5</sup>Mammalogy Section, National Museums of Kenya, Nairobi, Kenya

## Correspondence

Terrence C. Demos, Integrative Research Center, Field Museum of Natural History, Chicago, IL.

Email: [tdemos@fieldmuseum.org](mailto:tdemos@fieldmuseum.org)

## Funding information

JRS Biodiversity Foundation; WWF Gabon; Bud and Onnolee Trapp; Marshall Field III Fund; Walt and Ellen Newsom; Barbara E. Brown Fund for Mammal Research; Field Museum's Council on Africa; JRS Biodiversity Foundation; John D. and Catherine T. MacArthur Foundation; Department of State; Wildlife Conservation Society; Centers for Disease Control and Prevention

## Abstract

The bat family Nycteridae contains only the genus *Nycteris*, which comprises 13 currently recognized species from Africa and the Arabian Peninsula, one species from Madagascar, and two species restricted to Malaysia and Indonesia in South-East Asia. We investigated genetic variation, clade membership, and phylogenetic relationships in Nycteridae with broad sampling across Africa for most clades. We sequenced mitochondrial cytochrome *b* (*cytb*) and four independent nuclear introns (2,166 bp) from 253 individuals. Although our samples did not include all recognized species, we recovered at least 16 deeply divergent monophyletic lineages using independent mitochondrial and multilocus nuclear datasets in both gene tree and species tree analyses. Mean pairwise uncorrected genetic distances among species-ranked *Nycteris* clades (17% for *cytb* and 4% for concatenated introns) suggest high levels of phylogenetic diversity in Nycteridae. We found a large number of designated clades whose members are distributed wholly or partly in East Africa (10 of 16 clades), indicating that *Nycteris* diversity has been historically underestimated and raising the possibility that additional unsampled and/or undescribed *Nycteris* species occur in more poorly sampled Central and West Africa. Well-resolved mitochondrial, concatenated nuclear, and species trees strongly supported African ancestry for SE Asian species. Species tree analyses strongly support two deeply diverged subclades that have not previously been recognized, and these clades may warrant recognition as subgenera. Our analyses also strongly support four traditionally recognized species groups of *Nycteris*. Mitonuclear discordance regarding geographic population structure in *Nycteris thebaica* appears to result from male-biased dispersal in this species. Our analyses, almost wholly based on museum voucher specimens, serve to identify species-rank clades that can be tested with independent datasets, such as morphology, vocalizations, distributions, and ectoparasites. Our analyses highlight the need for a comprehensive revision of Nycteridae.

Contributing authors: Paul W. Webala ([paul.webala@gmail.com](mailto:paul.webala@gmail.com)), Julian C. Kerbis Peterhans ([jkerbis@fieldmuseum.org](mailto:jkerbis@fieldmuseum.org)), Steven M. Goodman ([sgoodman@fieldmuseum.org](mailto:sgoodman@fieldmuseum.org)), Michael Bartonjo ([abartonjo@yahoo.com](mailto:abartonjo@yahoo.com)), Bruce D. Patterson ([bpatterson@fieldmuseum.org](mailto:bpatterson@fieldmuseum.org))

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2019 The Authors Journal of Zoological Systematics and Evolutionary Research Published by Blackwell Verlag GmbH

## KEYWORDS

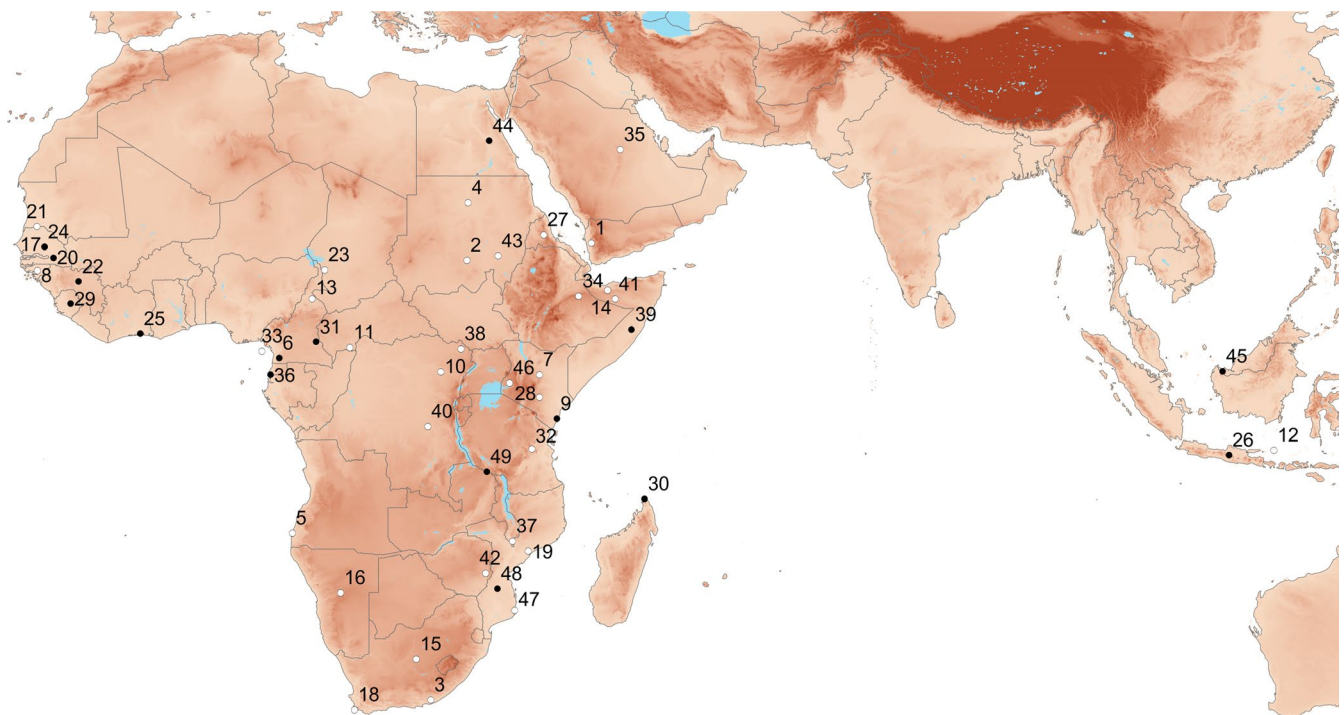
Africa, biodiversity, *Nycteris*, species tree, taxonomy

## 1 | INTRODUCTION

The Palearctic slit-faced bats, family Nycteridae, all belong to the genus *Nycteris* with 13 of 16 recognized species found in continental Africa and offshore islands, one species on Madagascar, and two species endemic to South-East Asia (Mammal Diversity Database, 2019; Simmons, 2005). Members of the Nycteridae are readily recognizable by their nose leaves, which are divided by a deep median furrow running the length of the muzzle, the basis for their common name. They also possess a Y-shaped terminal caudal vertebra that is unique among mammals. Systematic reviews of the family have not been informed by morphological or molecular phylogenetics, and the most recently named species in the family was described a half-century ago (*N. vinsoni*, Dalquest, 1965). To put this taxonomic stasis in context, the number of recognized bat species globally has grown by

26.4% over the last 15 years. In the Palearctic, this has included a 38% increase in the number of species of Rhinolophidae and a >50% increase in species in the genera *Scotophilus* and *Miniopterus* (cf. Simmons, 2005; Mammal Diversity Database, 2019). Here, we use a geographically extensive, multilocus dataset to assay the diversity and infer the evolutionary relationships of Nycteridae in order to establish the foundations for a fuller taxonomic revision.

In the first systematic revision of Nycteridae, Andersen (1912) divided then-known taxa into four species groups: *javanica*, *hispidia*, *aethiopica* [now known as *macrotis*], and *thebaica*. Later, Aellen (1959) divided the *javanica* group into two based on tragus and dental characters: *javanica* (monotypic) and *arge*, which contained both African and Asian species. Using morphometrics and hyoid morphology, respectively, Van Cakenberghe and De Vree (1993a) and Griffiths (1997) later transferred the Asian member of the *arge*



**FIGURE 1** Named taxa of *Nycteris*, showing type localities for recognized species (filled circles) and subspecies or synonyms (open circles). Number codes are as follows: 1 - *adana* K. Andersen, 1912; 2 - *aethiopica* Dobson, 1878; 3 - *affinis* A. Smith, 1829; 4 - *albiventer* Wagner, 1840; 5 - *angolensis* Peters, 1871; 6 - *arge* Thomas, 1903; 7 - *aurantiaca* De Beaux, 1923; 8 - *aurantiaca* Monard, 1939; 9 - *aurita* K. Andersen, 1912; 10 - *avakubia* J. A. Allen, 1917; 11 - *baikii* Gray 1867; 12 - *bastiani* Bergmans & van Bree, 1986; 13 - *benuensis* Aellen, 1952; 14 - *brockmani* K. Andersen, 1912; 15 - *capensis* A. Smith, 1829; 16 - *damarensis* Peters, 1871; 17 - *daubentonii*. Geoffroy, 1813; 18 - *discolor* Wagner, 1840; 19 - *fuliginosa* Peters, 1852; 20 - *gambiensis* K. Andersen, 1912; 21 - *geoffroyi* Desmarest, 1820; 22 - *grandis* Peters, 1865; 23 - *guineensis* Monard, 1939; 24 - *hispidia* Schreber, 1775; 25 - *intermedia* Aellen, 1959; 26 - *javanica*. Geoffroy, 1813; 27 - *labiata* Heuglin, 1861; 28 - *luteola* Thomas, 1901; 29 - *macrotis* Dobson, 1876; 30 - *madagascariensis* G. Grandidier, 1937; 31 - *major* K. Andersen, 1912; 32 - *marica* Kershaw, 1923; 33 - *martini* Fraser, 1843; 34 - *media* K. Andersen, 1912; 35 - *najdiya* Nader & Kock, 1982; 36 - *nana* K. Andersen, 1912; 37 - *oriana* Kershaw, 1922; 38 - *pallida* J. A. Allen, 1917; 39 - *parisii* De Beaux, 1924; 40 - *proxima* Lonnberg & Gyldenstolpe, 1925; 41 - *revollii* Robin, 1881; 42 - *sabiensis* Roberts, 1946; 43 - *senegalensis* Hartmann, 1868; 44 - *thebaica*. Geoffroy, 1818; 45 - *tragata* K. Andersen, 1912; 46 - *tristis* G. M. Allen & Lawrence, 1936; 47 - *villosa* Peters, 1852; 48 - *vinsoni* Dalquest, 1965; and 49 - *woodi* K. Andersen, 1914. An additional name, *pilosa* Gray, 1866 from "Africa," is not shown

group, *N. tragata*, to the *javanica* group. This five-group classification has been widely accepted (e.g., Simmons, 2005), but taxonomic membership in these groups has varied, owing to mosaic character variation. For example, the absence of biometrical differences in teeth measurements suggested the conspecificity of *N. parisii* with *N. woodi* (Van Cakenberghe & de Vree, 1985), but a subsequent study of bacula strongly supported the validity of both species and suggested their assignment to entirely different species groups (Thomas, Harrison, & Bates, 1994). Although qualitative and mensural characters have been used to characterize and differentiate species, external and skull characters are in conflict with other morphological characters (e.g., Happold, 2013a; Monadjem, Taylor, Cotterill, & Schoeman, 2010; Thomas et al., 1994; Van Cakenberghe & de Vree, 1985, 1993a, 1993b, 1998). Except for Griffiths' (1997) analysis of the hyoid apparatus, the morphological characters of the species of Nycteridae have not been subjected to explicit phylogenetic analysis. Figure 1 shows the host of names available for *Nycteris* populations, many of them currently considered synonyms (cf. Simmons, 2005).

Molecular phylogenetic analyses of the Nycteridae are likewise limited, as they included only a handful of species, each represented by a single sample. Shi and Rabosky (2015) used a concatenated supermatrix and included 7 of 16 *Nycteris* species in a time-calibrated analysis of all Chiroptera. They found strong support for the traditional sister relationship between Nycteridae and Emballonuridae (the two families comprising the Emballonuridea of Koopman, 1993). The supermatrix analysis of Amador, Moyers Arévalo, Almeida, Catalano, and Giannini (2018), also based on the same seven *Nycteris* species, found inconsistent evidence for the endemic Malagasy Myzopodidae joining this group. Nevertheless, both studies recovered Nycteridae as monophyletic and a close relative of Emballonuridae, and both studies recovered the two Asian species, *N. tragata* and *N. javanica*, as well-supported sisters. It should be noted, however, that both studies were based on incomplete supermatrices (71% missing data in Amador et al., 2018 and 83% missing in Shi & Rabosky, 2015). Thus, the diversity and phylogenetic relationships of species in Nycteridae remain largely unresolved and the evolutionary independence of *Nycteris* lineages has yet to be established.

Bat surveys across Africa over the last two decades have provided substantial new material for the evaluation of phylogenetic relationships and species limits. In addition, recent studies (Demos, Webala, Bartonjo, & Patterson, 2018; Dool et al., 2016; Patterson et al., 2018) have shown that a multilocus intron system based on different chromosomes and enabling independent representation of the nuclear genome offers clear advantages over analyses based only on mitochondrial data. Advantages include better resolution of earlier divergences (e.g., Demos et al., 2019) and improved detection of instances of mitochondrial introgression (e.g., Dool et al., 2016; Hassanin et al., 2018). Here, we address three key aspects of Nycteridae evolution: (a) recognizing monophyletic lineages within *Nycteris*, focusing on Afrotropical species, and assessing their evolutionary independence using independent nuclear loci

under a coalescent framework; (b) evaluating their phylogenetic relationships using both nuclear and mitochondrial data in gene tree, concatenated, and species tree analyses; and (c) assessing the species-group relationships of *Nycteris* species that had been classified by morphology alone. This study highlights the need for a comprehensive revision of African Nycteridae. Our analyses and discussion serve to identify species-rank clades that need to be tested with independent datasets including morphology, vocalizations, distributions, and ectoparasites.

## 2 | MATERIALS AND METHODS

### 2.1 | Selection of taxa and sampling

The bats newly sequenced for this study ( $n = 249$ ) were collected during recent small mammal surveys across sub-Saharan Africa, with relatively dense sampling in East Africa (see Fig. S1). Initial assignment of individuals to species for East African specimens was determined using meristic, mensural, and qualitative characters presented in the bat keys of Thorn, Kerbis Peterhans, and Baranga (2009) and Patterson and Webala (2012). Field methods followed mammal collecting guidelines (Sikes, 2016) and were approved under Field Museum of Natural History IACUC #2012-003. Tissues were taken from euthanized specimens in the course of preparing voucher specimens following IACUC protocols and the respective national collecting permits. Tissues were variously preserved in ethanol, saturated salt solution (EDTA-DMSO-NaCl), or liquid nitrogen and stored in liquid nitrogen dewars. Four additional *cytochrome b* gene (*cytb*) sequences of *Nycteris* were downloaded from GenBank. *Coleura afra* (Emballonuridae) was included as an out-group. In total, 1–5 genes were analyzed in 253 individuals in this study (see Table S1 for voucher numbers and locality data and Appendix 1 for GenBank accession numbers). To enable subsequent integrative taxonomic revisions, all but four of the individuals analyzed genetically in this study are accompanied by museum voucher specimens suitable for morphological analysis.

In view of the large number of names (many of which are synonyms; Figure 1) and to avoid contributing to current taxonomic confusion in *Nycteris*, we utilized a conservative approach in labeling clades. Where a clade's taxonomic identity was ambiguous or unknown, we referred to it simply as a numbered clade. In some cases, even assignment to equivocal groupings was necessary (e.g., *hispida/aurita* and cf. *hispida/aurita*). Although used as explicit labels in our study, the validity of these names is provisional. Comprehensive morphological assessments of individual specimens making up these clades included in our analyses will be required in order to verify which, if any, existing names may apply to them.

### 2.2 | Amplification and sequencing

We sequenced one mitochondrial protein-coding gene *cytochrome b* (*cytb*) and the nuclear introns *acyl-CoA oxidase 2 intron 3* (ACOX2), *COP9 signalosome subunit 7A intron 4* (COPS7A), *rogdi atypical leucine*

**TABLE 1** Primer information for genes amplified in the current study. References indicated by (a) Salicini, Ibáñez, & Juste, 2011; (b) Eick, Jacobs, & Matthee, 2005; (c) Trujillo, Patton, Schlitter, & Bickham, 2009

Gene	Primers (5'-3')	Amplicon length	References	Thermal profile
ACOX2	ACOX2f CCTSGGCTCDGAGGAGCAGAT ACOX2r GGGCTGTGHAYCACAAACTCT	717 bp	a	3 min at 95°C followed by 10 cycles of 15 s at 95°C, 30 s at 65°C in 1°C decrements from 65°C (64–56°C), and 1 min at 72°C, followed by 36 cycles of 15 s at 95°C, 30 s at 55°C, and 1 min at 72°C, and final 5 min extension at 70°C
COPS7A	COPSf TACAGCATYGGRCGRGACATCCA COPsr TCACYTGCTCCTCRATGCCCKGACA	689 bp	a	Same as ACOX2 above
ROGDI	ROGDI f CTGATGGAYGCGYGTGATGCTGCA ROGDI r CACGGTGAGGCASAGCTTGTGA	505 bp	a	3 min at 95°C followed by 10 cycles of 15 s at 95°C, 30 s at 60°C in 1°C decrements from 60°C (59–51°C), and 1 min at 72°C, followed by 36 cycles of 15 s at 95°C, 30 s at 50°C, and 1 min at 72°C, and final 5 min extension at 70°C
STAT5A	STAT5f CTGCTCATCAACAAGCCCGA STAT5r GGCTTCAGGTTCCACAGTTGC	530 bp	b	Same as <i>ROGDI</i> above
<i>cytb</i>	LGL-765f GAAAAACCAAYCGTTGTWATTCAACT LGL-766r GTTTAATTAGAATYTYAGCTTTGGG		c	3 min at 95°C followed by 36 cycles of 45 s at 95°C, 30 s at 50°C, and 2.5 min at 70°C, and final 5 min extension at 70°C

*zipper intron 7 (ROGDI)*, and *signal transducer and activator of transcription 5A intron (STAT5A)* for specimens of *Nycteris* and the close emballonurid out-group *Coleura afra*. Primers, primer references, and thermocycler conditions are described in Table 1. General methods of DNA extraction, amplification, and sequencing follow Demos et al. (2018) and Patterson et al. (2018). DNA sequences were assembled, aligned, and edited using GENEIOUS PRO v.11.1.5 (Biomatters Ltd.). Alignments were inspected visually and determined to be unambiguous. Several gaps were introduced in the alignments of the four nuclear introns, but their positions were unambiguous. Sequences of *cytb* were translated to amino acids to confirm the absence of premature stop codons and indels. The *cytb* alignment was trimmed to 1,121 nucleotides to minimize missing data. Before phylogenetic analyses using mitochondrial data, we reduced the matrix of 253 individuals to the set of unique sequences, resulting in a final matrix of 164 individuals. The matrix used for calculating *cytb* distances between lineages comprised 250 individuals from the 253 individual alignments. We resolved nuclear DNA to haplotypes with the PHASE program (Stephens, Smith, & Donnelly, 2001) and set the probability threshold to 70%, following Garrick, Sunnucks, and Dyer (2010). PHASE files were formatted and assembled using SeqPhase (Flot, 2010).

### 2.3 | Gene trees, networks, species trees, and summary statistics

PartitionFinder 2 (Lanfear, Frandsen, Wright, Senfeld, & Calcott, 2016) on CIPRES Science Gateway v.3.1 (Miller, Pfeiffer, & Schwartz, 2010) was used to determine the appropriate model of sequence evolution using the Bayesian information criterion (BIC) for *cytb* and

the four nuclear introns. Interspecific uncorrected sequence divergences (*p*-distances) for *cytb* were calculated for both positions 1, 2, and 3 and positions 1 and 2 only, and intraspecific distances were calculated using positions 1, 2, and 3 using MEGA X 10.0.5 (Kumar, Stecher, Li, Knyaz, & Tamura, 2018).

Maximum-likelihood (ML) inference of *cytb* gene trees and a concatenated alignment using four partitioned nuclear introns were made using the program IQ-TREE version 1.6.0 (Nguyen, Schmidt, von Haeseler, & Minh, 2015) on the CIPRES portal. Gene tree analyses under a Bayesian inference (BI) framework were carried out in MRBAYES v.3.2.6 (Ronquist et al., 2012) on the CIPRES portal to infer gene trees for *cytb* and the partitioned alignment of four nuclear introns. Two replicates were run in MrBayes, and nucleotide substitution models were unlinked across partitions for each nuclear locus in the concatenated alignment. Four Markov chains were run for  $1 \times 10^7$  generations using default heating values and sampled every 1000th generation. Stationarity of the MRBAYES results was assessed in Tracer v1.7 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018). Majority-rule consensus trees were inferred for each Bayesian analysis. PopART (Leigh & Bryant, 2015) was used to construct a median-joining network of cytochrome *b* haplotypes for clades within *Nycteris thebaica*. Pie charts were used to visualize the relative frequencies and relationships of haplotypes in *N. thebaica* clades 1–6.

*Nycteris* taxa were assigned to either species or named clades based on clade support in the analyses of the *cytb* and nuclear intron datasets. As in Demos et al. (2018), results from gene tree analyses were used to identify populations to be used as “candidate species” for the species tree approach implemented in StarBEAST2 (Ogilvie, Bouckaert, & Drummond, 2017), an extension of BEAST v.2.5.1

**TABLE 2** Uncorrected cytb *p*-distances among clades of *Nycteris*: on and below diagonal based on positions 1, 2, and 3; above diagonal, positions 1 and 2. Clades represented by one individual (*N. cf. thebaica 3*, *N. javanica*, *N. nana 1*) not included

Taxon	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]
[1] <i>arge 1</i>	4.3	3.7	3.8	7.6	5.3	3.7	3.6	4.2	4.1	4.9	4.0	6.5	6.6	6.4	6.4	6.5	6.6	3.1
[2] <i>arge 2</i>	15.9	1.1	4.5	7.3	6.3	3.7	3.4	4.5	3.6	4.6	2.6	6.4	6.7	6.5	6.7	6.7	6.5	3.6
[3] <i>cf. hispidaurita</i>	16.1	17.5	0.7	8.0	6.4	3.9	4.0	5.3	4.3	5.3	4.6	7.5	7.4	7.0	7.0	7.5	7.0	4.1
[4] <i>cf. thebaica 1</i>	19.3	19.2	19.5	2.6	6.2	7.4	7.7	8.0	7.1	6.9	7.9	6.7	7.2	7.3	6.8	7.3	7.0	6.0
[5] <i>cf. thebaica 2</i>	17.0	17.8	19.0	14.9	0.1	6.0	6.3	5.8	5.5	6.3	6.1	5.9	6.1	6.0	5.7	6.0	5.8	6.0
[6] <i>grandis</i>	16.3	16.3	17.2	20.3	18.2	1.6	3.7	3.9	4.2	4.9	4.4	7.1	7.2	7.2	7.2	7.0	7.1	3.6
[7] <i>hispidaurita</i>	14.5	15.4	15.0	19.7	18.0	16.1	2.5	5.0	4.5	4.8	4.0	6.9	7.4	7.2	7.0	7.5	7.5	3.2
[8] <i>macrotis 1</i>	17.4	18.1	18.1	20.8	19.5	17.7	17.7	2.2	3.3	4.0	4.9	6.9	7.1	6.9	7.1	6.6	6.8	3.9
[9] <i>macrotis 2</i>	16.3	18.7	17.8	19.4	19.0	18.5	16.3	13.8	0.9	3.7	4.1	7.8	7.5	7.2	7.2	7.1	7.1	3.7
[10] <i>macrotis 3</i>	17.6	19.0	19.2	20.1	20.0	18.7	17.8	14.3	15.0	0.4	5.0	7.4	7.8	8.0	7.7	7.6	7.7	4.4
[11] <i>nana 2</i>	16.1	13.2	17.1	19.3	16.8	17.0	15.3	17.8	17.4	18.0	4.9	6.9	7.1	7.0	7.0	6.9	7.0	4.4
[12] <i>thebaica 1</i>	18.7	18.5	19.1	18.1	17.1	19.4	19.0	19.6	22.2	20.1	18.0	0.4	2.0	1.9	2.0	2.5	1.9	6.4
[13] <i>thebaica 2</i>	18.7	18.4	19.9	18.4	17.8	19.7	19.4	20.1	21.5	20.4	18.2	5.8	0.4	1.3	1.7	2.3	1.4	7.3
[14] <i>thebaica 3</i>	18.9	18.6	19.5	18.2	18.0	19.9	19.6	20.4	21.8	21.1	18.5	5.0	5.0	1.6	1.2	1.6	1.0	7.1
[15] <i>thebaica 4</i>	18.4	18.6	19.7	17.7	17.0	19.9	19.5	19.9	21.6	20.4	17.9	5.1	4.7	3.6	1.6	2.3	1.3	6.9
[16] <i>thebaica 5</i>	18.9	19.2	19.7	18.6	17.5	19.5	20.0	19.6	21.9	20.4	18.4	6.4	6.5	5.6	5.3	0.0	1.4	7.3
[17] <i>thebaica 6</i>	18.2	18.1	19.5	17.4	17.0	18.8	19.4	19.8	21.4	19.7	17.4	5.4	5.2	4.7	4.2	5.4	1.7	7.3
[18] <i>tragata</i>	14.4	17.7	17.2	18.6	18.7	15.8	16.3	18.2	17.9	16.3	17.0	17.0	18.1	17.9	17.9	18.3	17.2	1.3

(Bouckaert et al., 2014). Species tree analyses were carried out using the four nuclear intron alignments with substitution, clock, and tree models unlinked among loci. The lognormal relaxed-clock model was applied to each locus using a Yule tree prior and the linear with constant root population size model. Four replicates were carried out, and the analyses were run for  $2 \times 10^8$  generations with 10% of each run discarded as burn-in. We used Tracer v.1.7 to assess convergence and stationarity of model parameters based on ESS values and examination of trace files.

Sequence alignments used in this study have been deposited on the Figshare data repository (<https://doi.org/10.6084/m9.figshare.8081594.v1>). All newly generated sequences are available on GenBank with accession numbers MK837076–MK837603 (see also Appendix 1).

### 3 | RESULTS

#### 3.1 | Mitochondrial genetic diversity, gene trees, and haplotype network

Sequences were generated and aligned for *cytb* (1,121 bp, 99% coverage), *ACO2* (646 bp, 96% coverage), *COPS7A* (624 bp, 98% coverage), *ROGDI* (450 bp, 98% coverage), and *STAT5A* (523 bp, 98% coverage). The concatenated alignment of four introns for 70 individuals was 97.1% complete (mean sequence length 2,166 bp). Models of sequence evolution inferred by PartitionFinder 2 were as follows: *cytb*, GTR + I+G; *ACO2*, TrN + G; *COPS7A*, TrN + G; *ROGDI*, TrN + G; and *STAT5A*, TrN + G. Uncorrected *cytb* distances for reciprocally monophyletic *Nycteris* lineages in the 250 sequence *cytb* alignment ranged from 3.6% to 22.2% for *cytb* positions 1 + 2 + 3 and 1.0%–8.0% for *cytb* positions 1 + 2 (Table 2). Within-lineage variability for *cytb* positions 1 + 2 + 3 ranged from 0% to 4.9%.

The ML phylogeny for Nycteridae based on *cytb* shows division of the family into four deeply diverged subclades (labeled as clades 1A, 1B, 2A, and 2B in Figure 2a). The topology of the maximum clade credibility tree is substantially similar in topology to the maximum-likelihood tree presented here. The monophyly of all named clades was strongly supported with the exception of *Nycteris thebaica* clade 6. Relationships among clades were generally well supported with the exception of the position of (a) the relationships of the geographically delimited clades within *N. thebaica*, (b) *N. cf. thebaica* clade 3, and (c) the relationship of *N. arge* clade 1 and *N. tragata* + *N. javanica*. Two nodes had equivocal support (bootstrap (BS)  $\geq 70\%$ , posterior probability (PP)  $< 0.95$ ): the node uniting *N. thebaica* clades 1–6 and *N. cf. thebaica* clades 1 + 2 and the node uniting *N. arge* clade 2 and *N. nana* clade 1.

Several clades with broad geographic sampling showed relatively high levels of within-clade genetic variation (i.e., *N. hispida/aurita*, *N. grandis*, and *N. macrotis* clade 1). For those clades with limited geographic sampling, we recovered high levels of divergence among populations in *N. cf. thebaica* 1 and *N. nana* clade 2. Both ML and BI analyses strongly supported *N. arge* clade 1 (Central African Republic [CAR], Democratic Republic of Congo [DRC], Gabon, Uganda) + *N. tragata* (Malaysia) + *N. javanica* (Borneo) as nested well within the other African *Nycteris* clades. The ML and BI trees support multiple deeply divergent clades separated by  $>10\%$  *cytb* distances. The number of deeply diverged clades that include individuals from East Africa (Kenya, Tanzania, and Uganda) is high: 10 of 16 clades in the trees include individuals from this region.

The median-joining network of *cytb* haplotype diversity for the six allopatric populations within *N. thebaica* showed no shared alleles among clades (Figure 3). The haplotype network revealed the existence of six well-differentiated clades (minimum separation of clades was 19 substitutions), although *N. thebaica* clade 4 (coastal Kenya) clusters ambiguously between *N. thebaica* clade 5 (Mozambique) and *N. thebaica* clade 2 (Tanzania and Zanzibar).

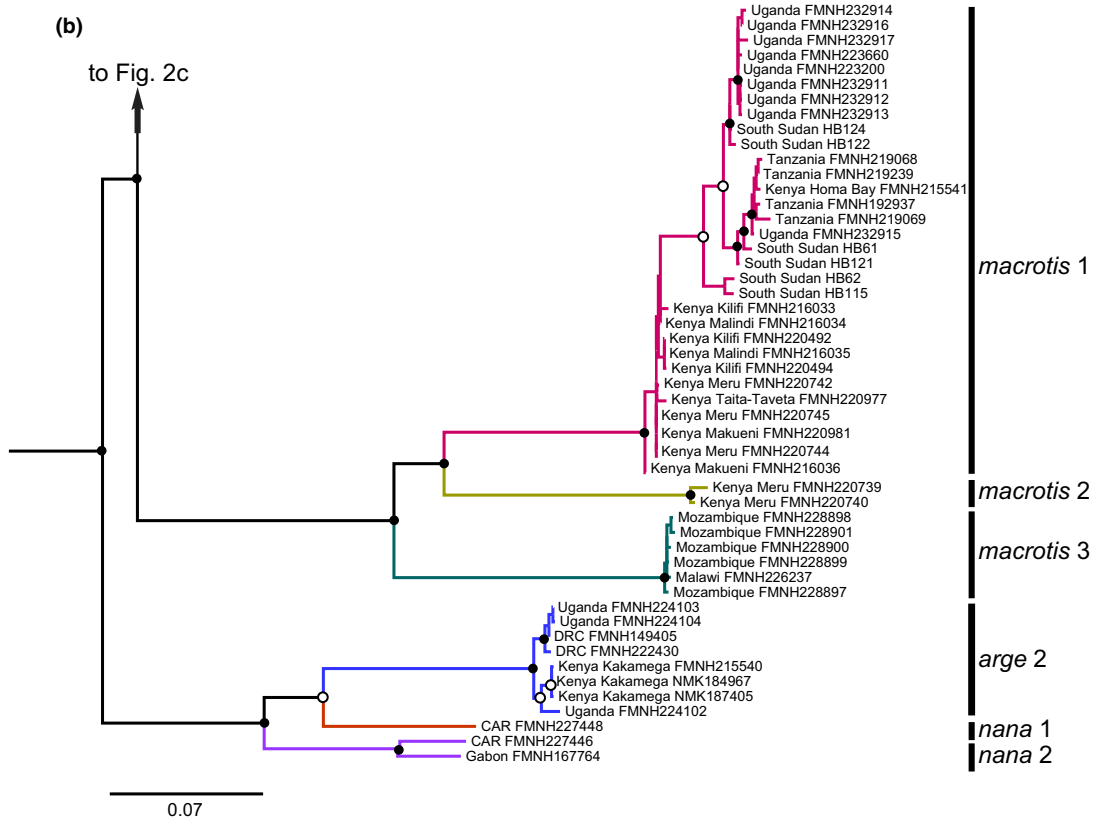
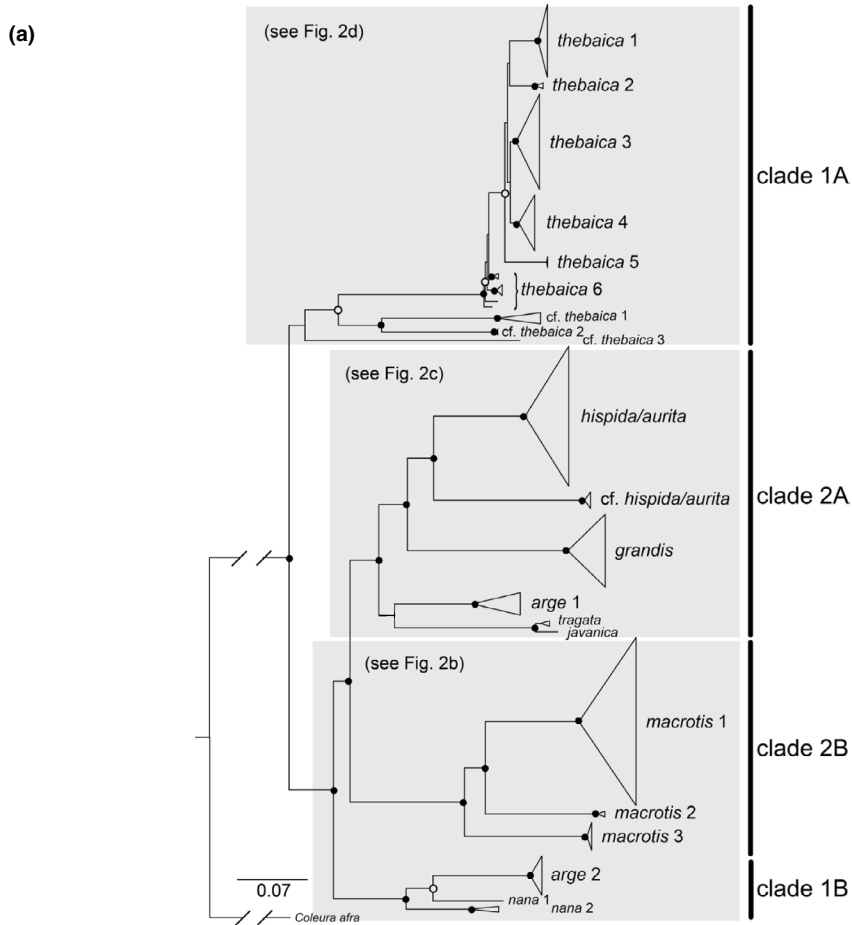
#### 3.2 | Concatenated nuclear gene trees

The ML gene tree inferred from the concatenated nuclear genes *ACO2*, *COPS7A*, *ROGDI*, and *STAT5A* (70 individuals; matrix  $> 97\%$  complete) is shown in Figure 4. This tree was similar to the BI tree with strong support for 22 of 25 major nodes. All of the named clades are strongly supported as monophyletic. Unlike the *cytb* gene trees, the position of *N. arge* clade 2 + *N. nana* clade 1 + *N. nana* clade 2 is ambiguous, while *N. cf. thebaica* clade 3 is strongly supported as part of the *N. thebaica* group. *Nycteris tragata* from SE Asia is strongly supported as nested within African *Nycteris* clades but is not sister to *N. arge* clade 1 as in the *cytb* gene trees. The most striking difference between the concatenated nuclear trees and the mitochondrial gene trees is the absence of support for genetic structure among the numbered lineages of *N. thebaica*. None of the clades named as *N. thebaica* 1–6 are supported as monophyletic, and relationships among individuals are poorly supported.

#### 3.3 | Species trees

Samples from parameter values of the four StarBEAST analyses had ESS values  $>200$ , with the exception of the five tree-height parameters which all had values  $>100$ . We discarded the first 10%

**FIGURE 2** (a) Maximum-likelihood phylogeny of 163 *Nycteris* specimens based on cytochrome *b*. The phylogeny was inferred in IQ-TREE and its topology closely resembled the phylogeny calculated in MrBayes under a Bayesian framework. Filled circles on nodes denote bootstrap values (BS)  $\geq 70\%$  and Bayesian posterior probabilities (PP)  $\geq 0.95$ , open circles outlined in black indicate BS  $\geq 70\%$  and PP  $< 0.95$ , and unmarked nodes indicate BS  $< 70\%$  and PP  $< 0.95$ . Support values for most minor clades are not shown. Species names assigned on basis of preliminary field identifications or examination of museum specimens. (b–d) enlarged sections of the complete *cytb* tree showing individual relationships. Specimen localities include counties for densely sampled Kenya. CAR refers to Central African Republic and DRC to Democratic Republic of the Congo. Museum acronyms are defined in Appendix 1



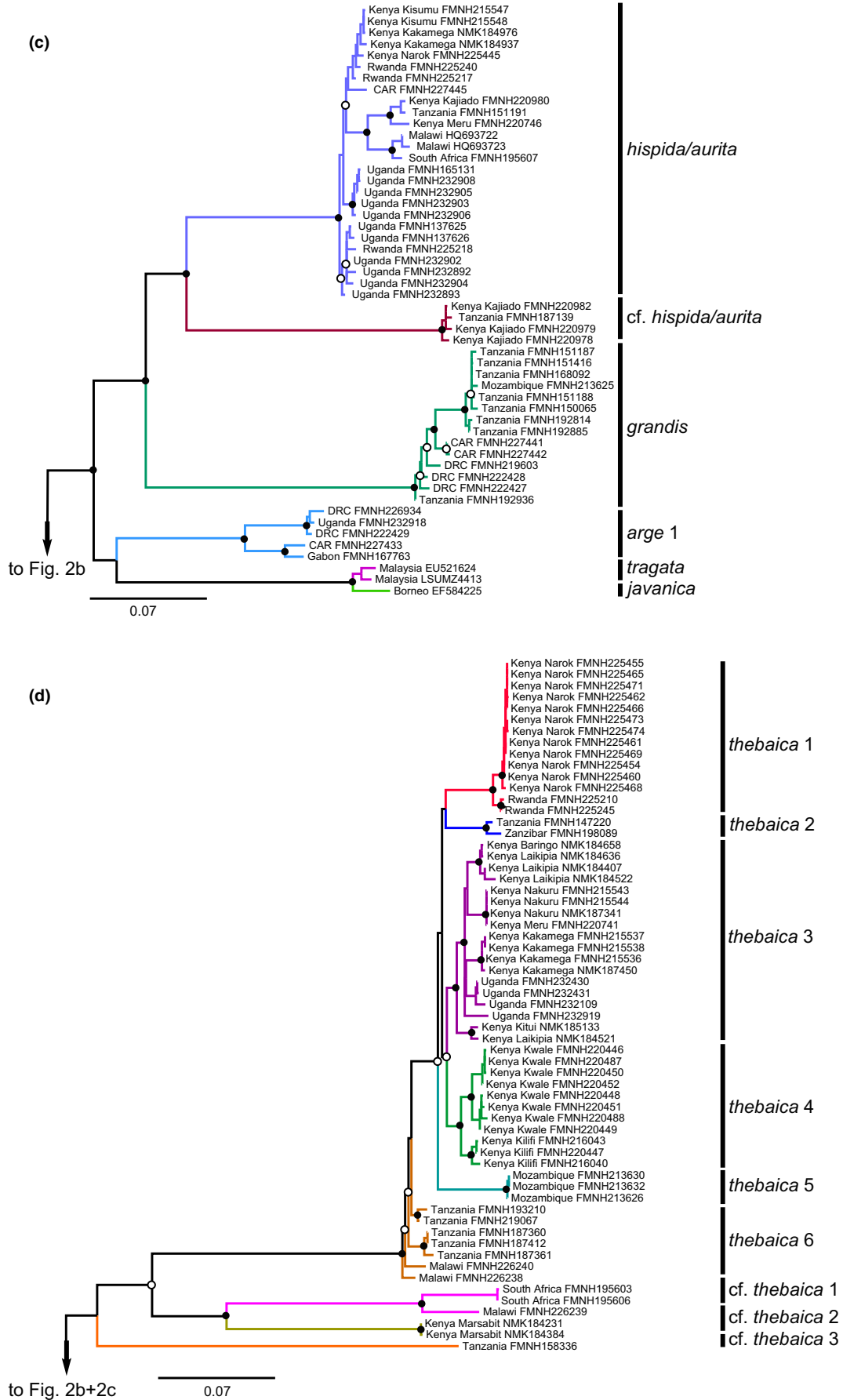
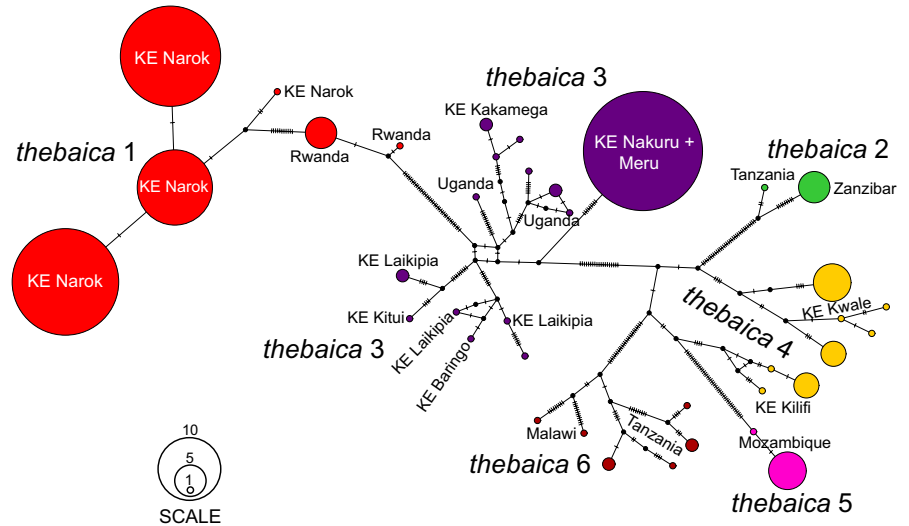


FIGURE 2 (Continued)



**FIGURE 3** PopART network median-joining analysis of cytochrome *b* haplotypes for 127 individuals representing *Nycteris thebaica* clades 1 to 6. Colored circles represent different sampled haplotypes, and black circles represent inferred missing or unsampled states. Hatch marks each denote a mutational step between haplotypes. CAR refers to Central African Republic, DRC to Democratic Republic of the Congo, and KE to Kenya



of each run, leaving 18,000 species trees in the posterior distributions that were then merged using LogCombiner. The topology of the maximum clade credibility tree (Figure 5) was identical across all four replicates. Species tree analysis using StarBEAST resulted in a topology that is strongly supported, with 12 of 13 nodes having  $PP \geq 0.95$ . As in the concatenated nuclear gene trees, but unlike the *cytb* gene trees, *Nycteris* cf. *thebaica* 3 is strongly supported as sister to the other *N. thebaica* clades. There is strong support for the node uniting *N. arge* 2 + *N. nana* 1 + *N. nana* 2 with the *N. thebaica* clades, resolving a relationship that was poorly supported in all of the gene tree analyses. Most relationships among *N. thebaica* clades 1–6 are poorly supported and minimally diverged, consistent with the assignment of individuals from all six clades to *N. thebaica* (Figure S1). *N. arge* 1 is weakly supported as sister to the strongly supported grouping *N. hispida/aurita* + *N. cf. hispida/aurita* + *N. grandis* + *N. tragata*. *Nycteris tragata*, the only Asian species tested, is well supported within the African clades.

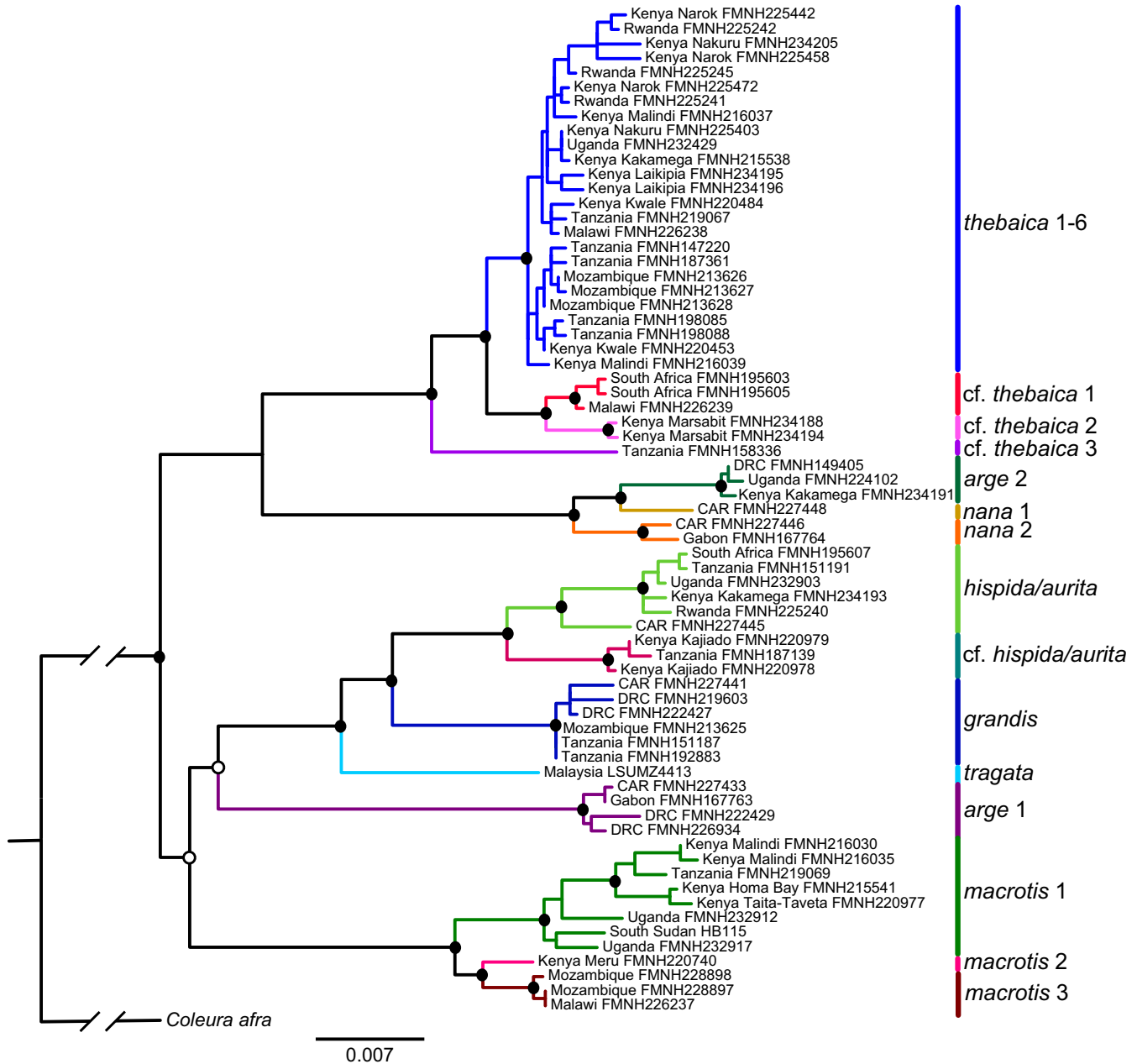
## 4 | DISCUSSION

### 4.1 | Multiple deeply diverged lineages

The monogeneric Nycteridae has been estimated to have diverged from Emballonuridae 51–53 Mya (Amador et al., 2018; Shi & Rabosky, 2015), and the most recent common ancestor age for the family has been placed variously at 18 mya (Shi & Rabosky, 2015) to 33.9 mya (Amador et al., 2018); Nycteridae ranks as a relatively ancient lineage among Chiroptera. Ours is the most taxonomically and geographically comprehensive phylogenetic study of Nycteridae to date. We recovered multiple instances of deep lineage divergence at both the inter- and intra-clade levels. Mean pairwise uncorrected genetic distances among species-ranked *Nycteris* clades for *cytb* were 0.17. In comparison, and in equivalent systematic surveys, overall *cytb* distances in *Scotophilus* (0.10; Demos et al., 2018) and *Rhinolophus* (0.10; Demos et al., in review) were less than that of *Nycteris*. Overall mean

genetic distances for concatenated intron datasets showed parallel variation: The mean distance of *Nycteris* was 0.04, *Rhinolophus* was 0.02, and *Scotophilus* was 0.01. As elaborated below, two deeply diverged multispecies clades are apparent in all of the phylogenetic analyses that we executed.

One of the most striking contrasts between the *cytb* gene tree (Figure 2d) and both the concatenated nuclear tree and species tree (Figures 4 and S2) is the pattern of fine-scale geographic structure for *N. thebaica* apparent only in the mitochondrial tree: There is strong support for monophyly of 5 of 6 labeled *N. thebaica* clades. Population-level sampling recovered well-supported and geographically restricted clades in (1) Kenya + Rwanda, (2) Tanzania, (3) Kenya + Uganda, (4) Kenya, and (5) Mozambique (Figure 3). The most divergent of these clades, *N. thebaica* clade 5 from Mozambique, is >5% *cytb* diverged from sister *N. thebaica* clades (Figure 2a, d). However, little population structure is present in either the concatenated nuclear analyses (Figure 4) or in the alternate species tree analysis where individuals were assigned to “species” based on clade membership in the mitochondrial tree (Figure S2). Although incomplete lineage sorting may be expected to play a role in mitonuclear discordance at this phylogenetic level, we note that other haplogroups did not exhibit such discordance at similar levels of divergence (e.g., *N. arge* 1 with subclades in West-Central vs. East-Central Africa, and *N. tragata* + *N. javanica*). This raises the possibility that the pattern results from sex-biased dispersal within the *N. thebaica* species group. Monadjem (2005) longitudinal study of *N. thebaica* survivorship in Swaziland offers robust evidence for female philopatry and male-biased dispersal. Of 39 females he banded as adults, nearly a quarter were living in the same culverts 4.5 years later, whereas only one of the 29 banded males was recaptured. Although other *Nycteris* dispersal studies are lacking, his observations are compatible with the strongly contrasting mitochondrial and nuclear population structures inferred here and warrant further life-history studies of other *Nycteris* species.



**FIGURE 4** Concatenated Bayesian phylogeny of four independent nuclear introns of *Nycteris*. Filled circles at nodes denote ML bootstrap values (BS)  $\geq 70\%$  and Bayesian posterior probabilities (PP)  $\geq 0.95$ , open circles outlined in black indicate BS  $\geq 70\%$  and PP  $< 0.95$ , and unmarked nodes indicate BS  $< 70\%$  and PP  $< 0.95$ . Support values for most minor clades are not shown. Specimen localities include counties for Kenya. CAR refers to Central African Republic and DRC to Democratic Republic of the Congo. Museum acronyms are defined in Appendix 1

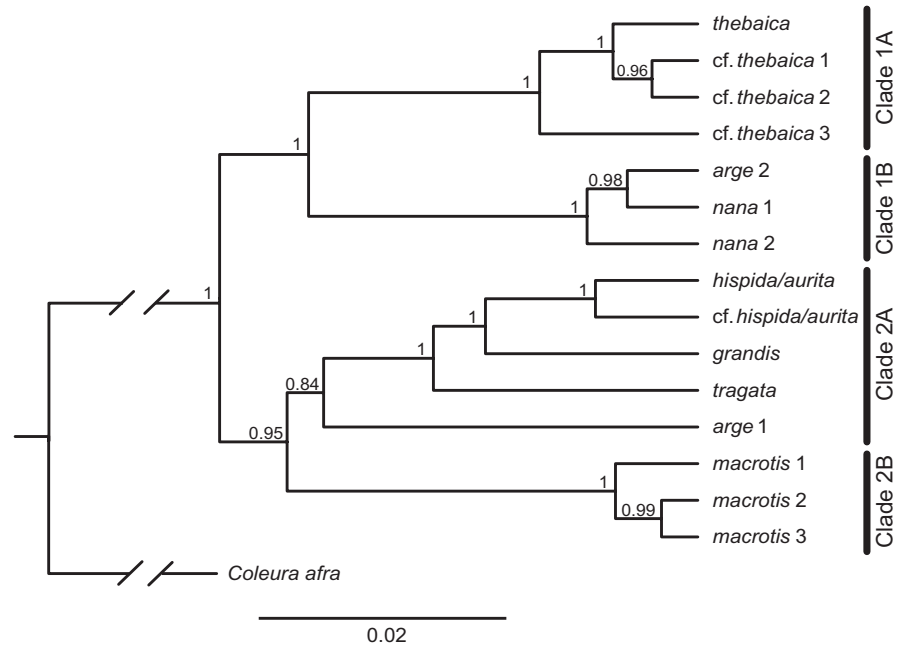
However, analyses using microsatellites or SNPs to exclude other possible explanations for this mitonuclear discordance would be necessary to establish this.

#### 4.2 | Phylogenetic relationships

Our analyses conflict with earlier efforts to resolve the phylogenetic relationships of *Nycteris*. The tree of Shi and Rabosky (2015) recovered the pair *N. hispida* and *N. thebaica* as sister to all *Nycteris* species; the remainder were arranged as *N. javanica* + *N. tragata* as

sister to *N. grandis* + *N. arge*, with *N. macrotis* subtending this group. In contrast, Amador et al. (2018) recovered *N. macrotis* as the earliest diverging lineage of *Nycteris*, which was sister to a pair of clades, one containing the Asian species *N. tragata* and *N. javanica* and the other containing the African species *N. grandis* and *N. arge* as sisters, joined successively by *N. hispida* and *N. thebaica*. The two studies used the same 7 *Nycteris* species (*arge*, *grandis*, *hispida*, *javanica*, *macrotis*, *thebaica*, and *tragata*), but Amador et al. (2018) partitioned *cytb* and the two nuclear genes included in their analysis (vWF and BRCA) by codon position, whereas Shi and Rabosky partitioned their dataset

**FIGURE 5** Species tree for *Nycteris* inferred using four nuclear loci in StarBEAST. Nodes are labeled with posterior probabilities



by gene. All 7 *Nycteris* species in the concatenated ML analysis of Shi and Rabosky had BS support  $\geq 70\%$ , whereas the concatenated ML tree of Amador et al. (2018) more weakly supported *N. macrotis* as sister to the remaining *Nycteris* clades at 60%.

In contrast to both studies, we found strong support (PP 1.0) for two major subclades within the genus (Figures 4 and 5), each comprised of two groups of species. In the first subclade, *N. thebaica* and the three *N. cf. thebaica* clades form one group (Clade 1A), while *N. arge* clade 2 and the two *N. nana* clades comprise their sister (Clade 1B). In the second subclade, three *N. macrotis* clades comprise one group (Clade 2B) and *N. tragata*, *N. grandis*, *N. hispida/aurita*, and *N. cf. hispida/aurita* comprise the other (Clade 2A). Less securely placed in the latter group is *N. arge* 1 (PP = 0.84). Additional highly informative nuclear markers for bats (e.g., Dool et al., 2016; Demos et al., 2018) are likely responsible for improved resolution although better taxonomic and geographic sampling in this study may also contribute. To some extent, comparisons with these earlier investigations are limited by our conservative approach in withholding species assignment for specimens deemed cryptic and/or subtly differentiated from named taxa. That said, expanded taxonomic coverage alone, regardless of names assigned to terminals in the study, could be expected to result in conflicting topologies, as would possible incorrect species identifications from previous studies that relied on GenBank data.

Comparing the mitochondrial (Figure 2a), concatenated nuclear (Figure 4), and species trees (Figure 5) in our analyses, the only major inconsistency concerns the position of *N. arge* 2 + *N. nana* 1 + *N. nana* 2. The *cytb* gene tree analyses strongly support this clade as sister to *N. macrotis*, but the high genetic distances in this dataset raise the specter of substitutional saturation. In turn, the concatenated gene tree analyses infer poor support for the clade as sister to *N. thebaica*, whereas the species tree analyses strongly support

the clade as sister to the *N. thebaica* group (PP = 1.0). Examination of relationships in both the concatenated nuclear and species trees, along with their substantial branch lengths, provide strong support for two major and four subordinate clades of species within *Nycteris*. The subordinate groupings represent species groups, as discussed below. The major clades have not previously been recognized, and the use of subgenera for these clades may be appropriate. As discussed by Teta (2019), there are several advantages of applying the category of subgenus to well-supported clades. The category is recognized in zoological nomenclature at a rank intermediate between genus and species and regulated by the zoological code. Its use preserves binomial usage, and thus nomenclatural stability, and by joining closely related species it can be used to generate phylogenetic predictions (e.g., Teta, Cañón, Patterson, & Pardiñas, 2017; Voss, Gutiérrez, Solari, Rossi, & Jansa, 2014). Proposals to formally name these groups of *Nycteris* species should include the compilation of comprehensive morphological diagnoses, which is outside the purview of this study.

### 4.3 | Species groups of *Nycteris*

The four subordinate clusters in the two subclades have been recognized since Andersen's (1912) first generic synopsis. Except for the position of the Asian taxa, they roughly correspond to his four species groups as they are currently defined (e.g., Happold, 2013b). All are separated by *cytb* distances of at least 16%, and their clade membership is strongly supported in the species tree. First, the cluster comprising *Nycteris thebaica* + *N. cf. thebaica* 1–3 (Clade 1A) is strongly supported as monophyletic in the species tree and is  $>17\%$  *cytb* diverged from its sister. This group is distributed in northeastern, eastern, and southern Africa and, by definition, corresponds to the *N. thebaica* species group, although other assigned group

members *N. gambiensis* and *N. vinsoni* were not explicitly included in our analyses. Second, and sister to the *N. thebaica* species group, is a cluster comprising *N. arge* 2 + *N. nana* 1 and 2 (Clade 1B), which is strongly supported as monophyletic and genetically distant (>17% *cytb*) from all other *Nycteris*. Distributed across western, Central, and eastern Africa, this grouping corresponds to the *arge* species group, although our analyses failed to include other group members *N. intermedia* and *N. major* (unless the former is in fact represented but mislabeled as *N. nana* 1 or *N. nana* 2). Third, the cluster comprising *N. hispida/aurita*, *N. cf. hispida/aurita*, *N. grandis*, and *N. tragata* (Clade 2A) is strongly supported as monophyletic and is >16% *cytb* diverged from the *N. macrotis* lineages that comprise its sister. This group is widely distributed; its African members correspond to the *N. hispida* species group but there is strong support for the additional membership of *N. tragata* from SE Asia. Although we did not sequence *N. javanica* for nuclear loci, the close relationship of *N. javanica* to *N. tragata* is well established (Amador et al., 2018; Shi & Rabosky, 2015; Figure 2a). Previous morphological indications that *N. javanica* and *N. tragata* were sister to the *N. thebaica*, *N. hispida*, and *N. macrotis* species groups (Griffiths, 1997) were clearly homoplasious. The relationship of *N. arge* 1 is uncertain, although it is weakly supported as sister to clade 2A in the species tree. Fourth, a final cluster comprises *N. macrotis* clades 1–3 (Clade 2B) and is strongly supported as monophyletic. It is >16% *cytb* diverged from its sister clade and includes members from South Sudan to Malawi and Mozambique east of the Albertine Rift and Congo Basin. It corresponds to the *macrotis* group, although our samples did not include identified representatives of *N. madagascariensis*, *N. parisi*, and *N. woodi*.

The fact that every newly sequenced *Nycteris* is associated with an identifiable museum voucher specimen means that forging linkages between genetic and morphological patterns is possible and because *Nycteris* taxa were all proposed on morphological grounds, this linkage enables sound nomenclature. Had the same genetic work been accomplished with biopsies from bats that were subsequently released, which is now technically possible, it would be impossible to confirm the identities and characterize the distinctive features of these lineages. As a case in point, lineages designated *N. arge* clades 1 and 2 (Figures 4 and 5) were each identified as *N. arge* in the field but clearly represent distinct lineages that likely belong to different species groups. Resolving the relationships of cryptic lineages is greatly expedited by comprehensive voucher material that preserves a broad array of biological characters, in the case of bats including skeletal and soft-part anatomy, genitalia, vocalizations, and parasites, in addition to their genetic attributes (Gippoliti, 2018). Currently, 16 species of *Nycteris* are accepted as valid species, but several of these lack tissue samples in repositories or GenBank accessions and many lack vouchers with genetic material from near their type localities, hindering efforts to specify names (see Fig. S1). Based on the number of well-supported and deeply diverged lineages inferred here using multiple datasets and phylogenetic inference methods, it is likely that our analyses have uncovered several undescribed taxa.

The next steps in elucidating Nycteridae relationships will be in reconciling the phylogenetic patterns described in this paper with the

extensive morphological analyses developed around *Nycteris* types and throughout their geographic distributions by Van Cakenberghe and de Vree (1985, 1993a, 1993b, 1998). Only then will it be possible to replace the various annotations on our figures with a robust binomial nomenclature.

## ACKNOWLEDGEMENTS

Our analysis was strengthened with samples collected by the late W. T. Stanley, Carl Dick, Ruth Makena, David Wechuli, Richard Yego, and Aziza Zuhura. We acknowledge with special thanks the assistance of Jake Esselstyn and Donna Dittmann (LSUMNS), Caleb Phillips and Heath Garner (Museum of Texas Tech University), Mark Omura and Hopi Hoekstra (Harvard Museum of Comparative Zoology), and Jacqui Miller and Burton Lim (ROM) for loans of material. We thank Erwin Lagadec, Gildas Le Minter, Ara Monadjem, and Corrie Schoeman for collaboration in collecting specimens from Mozambique. We also salute the efforts of curators and collection managers in all the institutions cited in the Appendix 1 for maintaining the museum voucher specimens that enable subsequent integrative taxonomic studies needed to assign names confidently. We thank the Grainger Bioinformatics Center for partial funding of this study. Field collections in eastern and southern Africa were funded by a variety of agencies in cooperation with the Field Museum of Natural History, especially the JRS Biodiversity Foundation. Field Museum's Council on Africa, Marshall Field III Fund, and Barbara E. Brown Fund for Mammal Research were critical to fieldwork and analyses, as was the support of Bud and Onnolee Trapp and Walt and Ellen Newsom. Thanks to the John D. and Catherine T. MacArthur Foundation, Fulbright Program of US Department of State, Wildlife Conservation Society, and the Centers for Disease Control and Prevention sponsored and assisted in providing samples from DRC, Malawi, Mozambique, and Uganda. WWF Gabon supported fieldwork in Gabon, as did the Partenariat Mozambique-Réunion dans la recherche en santé: pour une approche intégrée d'étude des maladies infectieuses à risque épidémique (MoZaR; Fond Européen de Développement Régional, Programme Opérationnel de Coopération Territoriale) in Mozambique. Comments from the reviewers, one of them exceptionally helpful on details, helped us improve the final draft of the manuscript.

## ORCID

Terrence C. Demos  <https://orcid.org/0000-0003-0522-3708>

Bruce D. Patterson  <http://orcid.org/0000-0002-2249-7260>

## REFERENCES

- Aellen, V. (1959). Chiroptères nouveaux d'Afrique. *Archives Des Sciences, Genève*, 12(2), 217–235.
- Amador, L. I., Moyers Arévalo, R. L., Almeida, F. C., Catalano, S. A., & Giannini, N. P. (2018). Bat systematics in the light of unconstrained

- analyses of a comprehensive molecular supermatrix. *Journal of Mammalian Evolution*, 25(1), 37–70. <https://doi.org/10.1007/s10914-016-9363-8>
- Andersen, K. (1912). Brief diagnoses of eight new *Petalia*, with a list of the known forms of the genus. *Annals & Magazine of Natural History, Series*, 8(10), 546–550.
- Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., ... Drummond, A. J. (2014). BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, 10(4), e1003537. <https://doi.org/10.1371/journal.pcbi.1003537>
- Dalquest, W. W. (1965). Mammals from the Save River, Mozambique, with descriptions of two new bats. *Journal of Mammalogy*, 46(2), 254–264. <https://doi.org/10.2307/1377845>
- Demos, T. C., Webala, P. W., Bartonjo, M., & Patterson, B. D. (2018). Hidden diversity of African Yellow house bats (Vespertilionidae, *Scotophilus*): Insights from multilocus phylogenetics and lineage delimitation. *Frontiers in Ecology and Evolution*, 6, 86. <https://doi.org/10.3389/fevo.2018.00086>
- Demos, T. C., Webala, P. W., Goodman, S. M., Kerbis Peterhans, J. C., Bartonjo, M., & Patterson, B. D. (2019). Molecular phylogenetics of the African horseshoe bats (Chiroptera: Rhinolophidae): expanded geographic and taxonomic sampling of the Afrotropics. *BMC Evolutionary Biology*. <https://doi.org/10.1186/s12862-019-1485-1>
- Dool, S. E., Puechmaile, S. J., Foley, N. M., Allegrini, B., Bastian, A., Mutumi, G. L., ... Jacobs, D. S. (2016). Nuclear introns outperform mitochondrial DNA in inter-specific phylogenetic reconstruction: Lessons from horseshoe bats (Rhinolophidae: Chiroptera). *Molecular Phylogenetics and Evolution*, 97, 196–212. <https://doi.org/10.1016/j.ympev.2016.01.003>
- Eick, G. N., Jacobs, D. S., & Matthee, C. A. (2005). A nuclear DNA phylogenetic perspective on the evolution of echolocation and historical biogeography of extant bats (Chiroptera). *Molecular Biology and Evolution*, 22, 1869–1886. <https://doi.org/10.1093/molbev/msi180>
- Flot, J. F. (2010). SEQUENCEPHASE: A web tool for interconverting PHASE input/output files and FASTA sequence alignments. *Molecular Ecology Resources*, 10(1), 162–166. <https://doi.org/10.1111/j.1755-0998.2009.02732.x>
- Garrick, R. C., Sunnucks, P., & Dyer, R. J. (2010). Nuclear gene phylogeography using PHASE: Dealing with unresolved genotypes, lost alleles, and systematic bias in parameter estimation. *BMC Evolutionary Biology*, 10(1), 118. <https://doi.org/10.1186/1471-2148-10-118>
- Gippoliti, S. (2018). Natural history collecting and the arrogance of the modern Ark researcher. *Bionomina*, 13(1), 69–73. <http://dx.doi.org/10.11646/bionomina.13.1.6>
- Griffiths, T. A. (1997). Phylogenetic position of the bat *Nycteris javanica* (Chiroptera: Nycteridae). *Journal of Mammalogy*, 78(1), 106–116. <https://doi.org/10.2307/1382644>
- Happold, M. (2013a). Family Nycteridae: Slit-faced bats. In M. Happold, & D. C. D. Happold (Eds.), *The Mammals of Africa, Vol. IV: Hedgehogs, Shrews and Bats* (pp. 438–439). London, UK: Bloomsbury Publishing.
- Happold, M. (2013b). Genus *Nycteris*: Slit-faced bats. In M. Happold, & D. C. D. Happold (Eds.), *The Mammals of Africa, Vol. IV: Hedgehogs, Shrews and Bats* (pp. 440–442). London, UK: Bloomsbury Publishing.
- Hassanin, A., Colombo, R., Gembu, G. C., Merle, M., Tu, V. T., Görföl, T., ... Ing, R. K. (2018). Multilocus phylogeny and species delimitation within the genus *Glauconycteris* (Chiroptera, Vespertilionidae), with the description of a new bat species from the Tshopo Province of the Democratic Republic of the Congo. *Journal of Zoological Systematics and Evolutionary Research*, 56(1), 1–22. <https://doi.org/10.1111/jzs.12176>
- Koopman, K. F. (1993). Order Chiroptera. In D. E. Wilson, & D. A. M. Reeder (Eds.), *Mammal species of the World: A taxonomic and geographic reference*, 2nd ed. (pp. 137–242). Washington, DC: Smithsonian Institution Press.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2016). PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, 34(3), 772–773. <https://doi.org/10.1093/molbev/msw260>
- Leigh, J. W., & Bryant, D. (2015). POPART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6(9), 1110–1116. <https://doi.org/10.1111/2041-210X.12410>
- Mammal Diversity Database (2019). *American Society of Mammalogists*. Retrieved from [www.mammaldiversity.org](http://www.mammaldiversity.org). Accessed 2019–01–10.
- Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). *Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Gateway Computing Environments Workshop (GCE)*. In New Orleans, LA: IEEE. doi: <http://dx.doi.org/10.1109/GCE.2010.5676129>
- Monadjem, A. (2005). Survival and roost-site selection in the African bat *Nycteris thebaica* (Chiroptera : Nycteridae) in Swaziland. *Belgian Journal of Zoology*, 135(Suppl.), 103–107.
- Monadjem, A., Taylor, P. J., Cotterill, F. P. D., & Schoeman, M. C. (2010). *Bats of Southern and Central Africa: A biogeographic and taxonomic synthesis*. Johannesburg, South Africa: Wits University Press: i-xii; 1–596.
- Nguyen, L.-T., Schmidt, H. A., von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, 32(1), 268–274. <https://doi.org/10.1093/molbev/msu300>
- Ogilvie, H. A., Bouckaert, R. R., & Drummond, A. J. (2017). StarBEAST2 brings faster species tree inference and accurate estimates of substitution rates. *Molecular Biology and Evolution*, 34(8), 2101–2114. <https://doi.org/10.1093/molbev/msx126>
- Patterson, B. D., & Webala, P. W. (2012). Keys to the bats (Mammalia: Chiroptera) of East Africa. *Fieldiana: Life and Earth Sciences*, 6, 1–60. <https://doi.org/10.3158/2158-5520-12.6.1>
- Patterson, B. D., Webala, P. W., Bartonjo, M., Nziza, J., Dick, C. W., & Demos, T. C. (2018). On the taxonomic status and distribution of African species of *Otomops* (Chiroptera: Molossidae). *PeerJ*, 6, e4864. <https://doi.org/10.7717/peerj.4864>
- Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67(5), 901–904. <https://doi.org/10.1093/sysbio/syy032>
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., ... Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61(3), 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Salicini, I., Ibáñez, C., & Juste, J. (2011). Multilocus phylogeny and species delimitation within the Natterer's bat species complex in the Western Palearctic. *Molecular Phylogenetics and Evolution*, 61(3), 888–898. <https://doi.org/10.1016/j.ympev.2011.08.010>
- Shi, J. J., & Rabosky, D. L. (2015). Speciation dynamics during the global radiation of extant bats. *Evolution*, 69(6), 1528–1545. <https://doi.org/10.1111/evo.12681>
- Sikes, R. S. (2016). 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy*, 97(3), 663–688. <https://doi.org/10.1093/jmammal/gyw078>
- Simmons, N. B. (2005). Chiroptera. In D. E. Wilson, & D. A. M. Reeder (Eds.), *Mammal species of the world: A taxonomic and geographic reference*, Vol. 1, 3rd ed. (pp. 312–529). Baltimore, MD: Johns Hopkins University Press.

- Stephens, M., Smith, N. J., & Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data. *The American Journal of Human Genetics*, 68(4), 978–989. <https://doi.org/10.1086/319501>
- Teta, P. (2019). The usage of subgenera in mammalian taxonomy. *Mammalia*, 83(3), 209–211. <https://doi.org/10.1515/mammalia-2018-0059>.
- Teta, P., Cañón, C., Patterson, B. D., & Pardiñas, U. F. J. (2017). Phylogeny of the tribe Abrotrichini (Cricetidae, Sigmodontinae): Integrating morphological and molecular evidence into a new classification. *Cladistics*, 33(2), 153–182. <https://doi.org/10.1111/cla.12164>
- Thomas, N. M., Harrison, D. L., & Bates, P. J. J. (1994). A study of the baculum in the genus *Nycteris* (Mammalia, Chiroptera, Nycteridae) with consideration of its taxonomic importance. *Bonner Zoologische Beiträge*, 45(1), 17–31.
- Thorn, E., Kerbis Peterhans, J. C., & Baranga, J. (2009). Chiroptera. In E. Thorn, & J. C. K. Peterhans (Eds.), *Small Mammals of Uganda*. *Bonner Zoologische Monographien* 55 (pp. 12–75). Bonn, Germany: Museum Alexander Koenig.
- Trujillo, R. G., Patton, J. C., Schlitter, D. A., & Bickham, J. W. (2009). Molecular phylogenetics of the bat genus *Scotophilus* (Chiroptera: Vespertilionidae): Perspectives from paternally and maternally inherited genomes. *Journal of Mammalogy*, 90(3), 548–560. <https://doi.org/10.1644/08-MAMM-A-239R2.1>
- Van Cakenberghe, V., & de Vree, F. (1985). Systematics of African *Nycteris* (Mammalia: Chiroptera). In K.-L. Schuchmann (Ed.), *Proceedings of the International Symposium on African Vertebrates* (pp. 53–90). Berlin, Germany: Museum Alexander Koenig.
- Van Cakenberghe, V., & De Vree, F. (1993a). The systematic status of Southeast Asian *Nycteris* (Chiroptera: Nycteridae). *Mammalia*, 57(2), 227–244. <https://doi.org/10.1515/mamm.1993.57.2.227>
- Van Cakenberghe, V., & de Vree, F. (1993b). Systematics of African *Nycteris* (Mammalia: Chiroptera) Part II. The *Nycteris hispida* group. *Bonner Zoologische Beiträge*, 44(3–4), 299–332.
- Van Cakenberghe, V., & de Vree, F. (1998). Systematics of African *Nycteris* (Mammalia: Chiroptera) Part III. The *Nycteris thebaica* group. *Bonner Zoologische Beiträge*, 48(2), 123–166.
- Voss, R. S., Gutiérrez, E. E., Solari, S., Rossi, R. V., & Jansa, S. A. (2014). Phylogenetic relationships of mouse opossums (Didelphidae, *Marmosa*) with a revised subgeneric classification and notes on sympatric diversity. *American Museum Novitates*, 3817, 1–27. <https://doi.org/10.1206/3817.1>

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Demos TC, Webala PW, Kerbis Peterhans JC, Goodman SM, Bartonjo M, Patterson BD. Molecular phylogenetics of slit-faced bats (Chiroptera: Nycteridae) reveal deeply divergent African lineages. *J Zool Syst Evol Res*. 2019;00:1–14. <https://doi.org/10.1111/jzs.12313>