Supplementary Materials

Supplementary Text. Detailed protocols on downloading and manipulating GenBank sequences and generating gene matrix, as implemented in Geneious R11.

Data manipulation

We used Geneious R11 (Geneious, Auckland, New Zealand) (Olsen, 2014) to download, manipulate, and align sequences, as described below. We performed a NCBI nucleotide search using the keyword *Cryptotis*. We downloaded all available cyt *b*, 16S rRNA, *ApoB*, and *Brca1* sequences of *Cryptotis* spp. from GenBank. Only nine samples had the *COI* gene, so this gene was not used in our study. For outgroup species used in mitogenome phylogenetic analysis, we also carefully selected reliable vouchers and downloaded the nuclear *ApoB* and *Brca1* genes for the same voucher when possible. All downloaded sequences were grouped by gene to facilitate the following manipulation.

We modified sequence names to facilitate concatenation and analysis. Firstly, we renamed each sequence by combining the species names and vouchers and used "-" as a separator to separate the two terms. We removed anything in brackets in the sequence names (if existing). We then used an underscore to replace any space and colon in the sequence names to avoid any incompatibility issues (many programs/packages/software cannot recognize spaces and colons in sequence names). For specimen vouchers, we performed the following modifications before analyses.

1. Remove all brackets "**\(.*\)**" using Java regex.
2. Remove "**holotype**".
3. Remove "**LACM:**".
4. Remove "**:MAMM:**".
5. Remove "**Mammalogy:74166-**".
6. Remove "**ROM:MAM:96535-**".
7. Remove space if it is followed by a number "**\s(?=[0-9])**" using java regex.
8. Remove any colon if it is followed by a number "**:(?=[0-9])**" using java regex.
9. Remove any space at the end of the voucher "**\s+$**" using Java regex.
10. Remove any space at the beginning "^**\s+**" using Java regex.
11. Change any remaining colon to underscore.
12. Change any remaining space to underscore.
13. Remove any underscore at the end of the voucher.

Sample identifications (voucher and isolate) were carefully examined by cross-checking previously published papers and supplementary files therein (Baird et al., 2018; Dubey et al., 2007; He et al., 2015; Ohdachi et al., 2006). For example, *C. magnus* voucher LAF1515 (Ohdachi et al., 2006) and *C. magnus* isolate X4 (Dubey et al., 2007) are the same specimen, and *C. parvus* isolate CRPI and *C. parvus* voucher ANSHC8192 are the same specimen. Sequences representing these samples were concatenated. There were also a few spelling mistakes, which were corrected by eye.

The *C. oreoryctes* voucher CMNH SP10885 was removed because it lacked cyt *b* data, which could result in unreasonable phylogenetic positioning of the sample close to the root rather than a well-supported clade (Springer & Douzery, 1996). For the same reason, we removed the *C. oreoryctes* voucher CMNH SP10904, which had *Brca1* but no *ApoB* gene sequence.

We aligned each nuclear gene using MAFFT and trimmed both ends of the alignments. MAFFT cannot correctly align complete mitogenomes (~13 kb) with short genes (16S rRNA and cyt *b*) because it can result in incorrect mismatches. Thus, we first split the mitogenomes into four partitions, i.e., i) *12S*, ii) *16S*, iii) *Nd1* through *Nd5* (11 genes), and iv) cyt *b*, and aligned each partition individually using MAFFT, followed by concatenation to obtain full alignment. For the sake of safety, we manually checked alignments for all genes by eye, and replaced any suspicious bases with N.

Taxonomy

Noguera-Urbano et al. (2019) (Appendix I) identified UIS-MZ-1594 as *Cryptotis* sp. Considering the locality and its close genetic relationship to *C. thomasi*, it may be *C. tamensis* **(Fig. 2)**. Noguera-Urbano et al. (2019) (Appendix I) identified MHNUC-1572 as *Cryptotis squamipes*.

REFERENCES

Baird AB, McCarthy TJ, Trujillo RG, Kang YY, Esmaeiliyan M, Valdez J, et al. 2018. Molecular systematics and biodiversity of the *Cryptotis mexicanus* group (Eulipotyphla: Soricidae): two new species from Honduras supported. *Systematics and Biodiversity*, **16**(2): 108–117.

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He K, Woodman N, Boaglio S, Roberts M, Supekar S, Maldonado JE. 2015. Molecular phylogeny supports repeated adaptation to burrowing within small-eared shrews genus of *Cryptotis* (Eulipotyphla, Soricidae). *PLoS One*, **10**(10): e0140280.

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Springer MS, Douzery E. 1996. Secondary structure and patterns of evolution among mammalian mitochondrial 12S rRNA molecules. *Journal of Molecular Evolution*, **43**(4): 357–373.

Supplementary Tables and Figures

Supplementary Table S1. Species groups of *Cryptotis* according to Woodman (2019).

Supplementary Table S2. Samples and sequences used in mitogenome tree estimation.

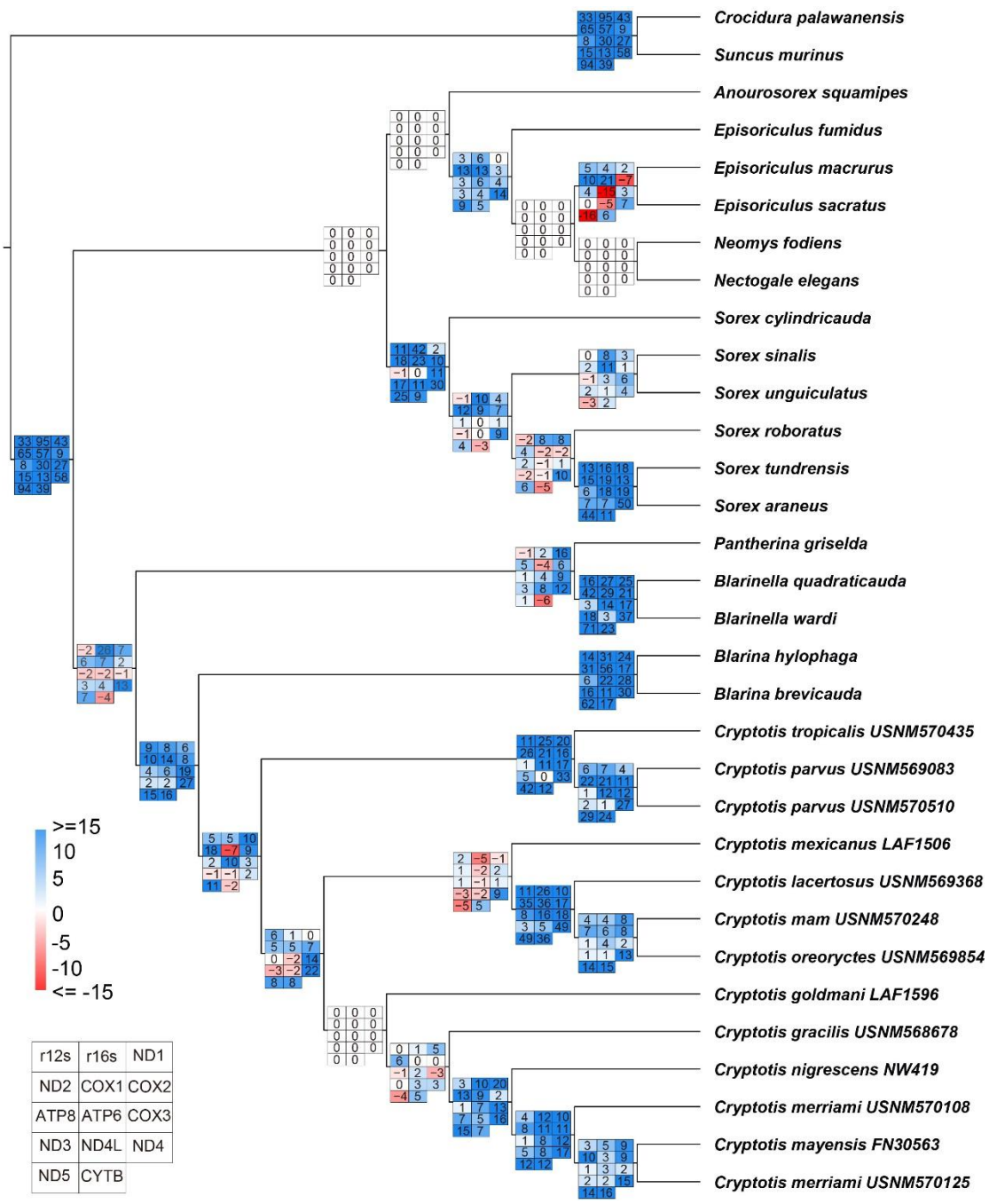
Supplementary Table S3. Partitioning schemes used in mitogenome RAxML analyses.

Supplementary Table S4. Partitioned branch support for each gene at internodes of best ML tree.

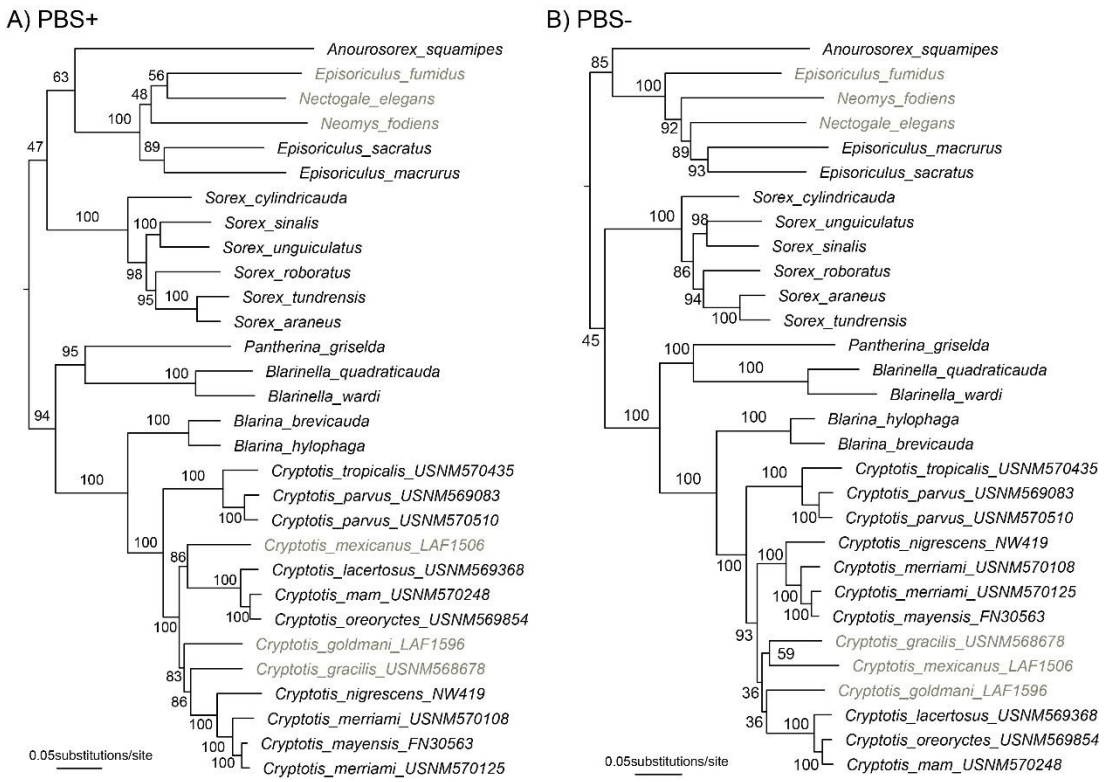
Supplementary Table S5. SH, KH, and AU tests using CONSEL to compare best ML tree and alternative phylogenies using mitogenome data. Tree topologies are given and also presented in Supplementary Figure S3.

Supplementary Table S6. SH, KH, and AU tests using CONSEL to test alternative hypotheses using comprehensive concatenation data.

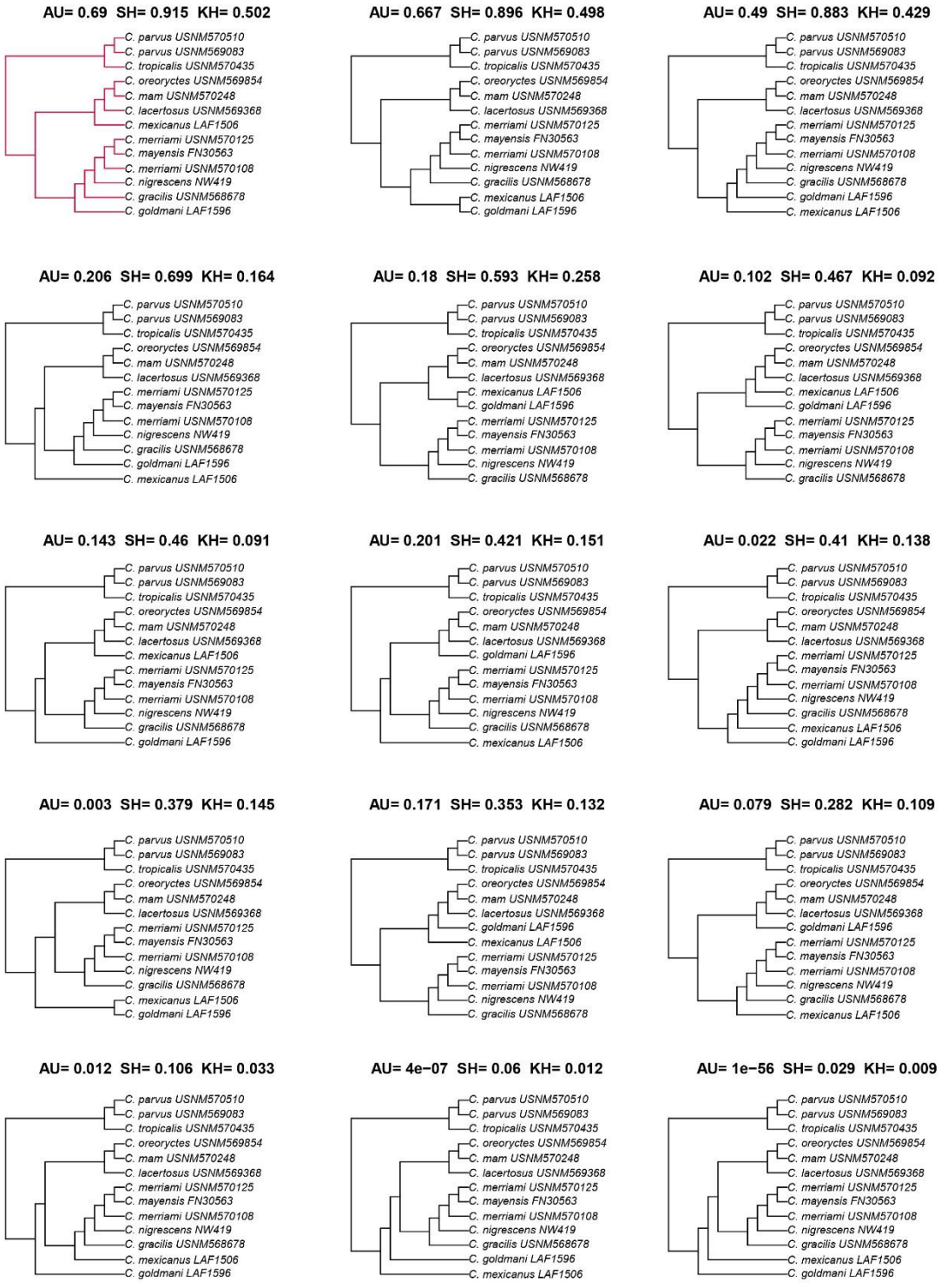
Supplementary Tables S1–**S6** are listed as a separate excel file due to their large size.



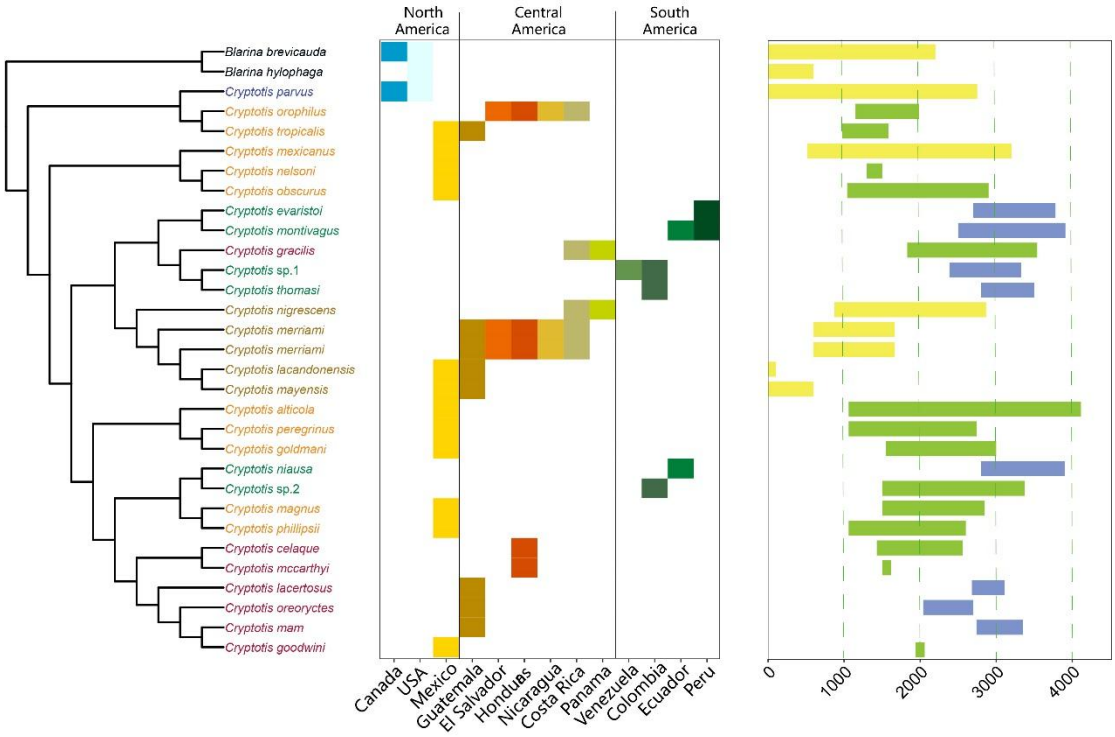
Supplementary Figure S1. Partitioned Bremer support on internodes of best RAxML mitogenome tree. Boxes at internodes show partitioned Bremer support values for each mitochondrial gene. Colors indicate whether gene supports (blue) or rejects (red) internode relationship.



Supplementary Figure S2. RAxML phylogenetic tree estimated using two subsets of complete mitogenomes. Branch lengths represent substitutions/site. Branch numbers refer to BS. RAxML tree estimated using (A) *12S*, *ND2*, *COX2*, *ATP8*, *COX3*, *ND4*, and cyt *b*, which all supported the best ML tree (denoted as PBS+, see **Supplementary Table S4**), and tree estimated using B) *16S*, *ND1*, *COX1*, *ATP6*, *ND3*, *ND4L*, and *ND5*, which mostly rejected the best ML tree (denoted as PBS−). Taxa whose phylogenetic positions differed between trees are in gray.



Supplementary Figure S3. Comparison of best ML tree and 14 alternative hypotheses regarding phylogenetic positions of *C. mexicanus* and *C. goldmani* using AU, SH, and KH tests. Only three phylogenies were rejected by AU, and only two phylogenies supporting *C. mexicanus* and *C. goldmani* as basal branches after the *C. parvus*-group were rejected by AU, SH, and KH. Thus, the mitogenome data could not reject most alternative phylogenetic hypotheses or a rapid diversification scenario.



Supplementary Figure S4. Continental and elevational distributions of *Cryptotis* species in this study. Left panel shows modified tree from comprehensive gene tree (kept only one tip per species, except *C. merriami*, for which two distinct lineages were retained). Middle panel shows continental and national distributions for each species. Right panel shows elevational distribution of each species, colors represent lower limits of distribution (yellow: <1 000 m; green: 1 000–2 000 m; blue: >2 000 m). National and elevational data are from Woodman (2019) and IUCN red list of threatened species website (https://www.iucnredlist.org/, last accessed April 2021). We used national and elevational data for *C. tamensis* and *C. squamipes* for *Cryptotis* sp. 1 and *Cryptotis* sp. 2, respectively. See **Supplementary Text** for explanation.