UCE-derived mitochondrial phylogeny reveals pervasive mito-nuclear discordances in serotine bats (genus *Eptesicus*) and complex evolutionary history in *Eptesicus* (*Histiotus*)

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Supplementary Materials

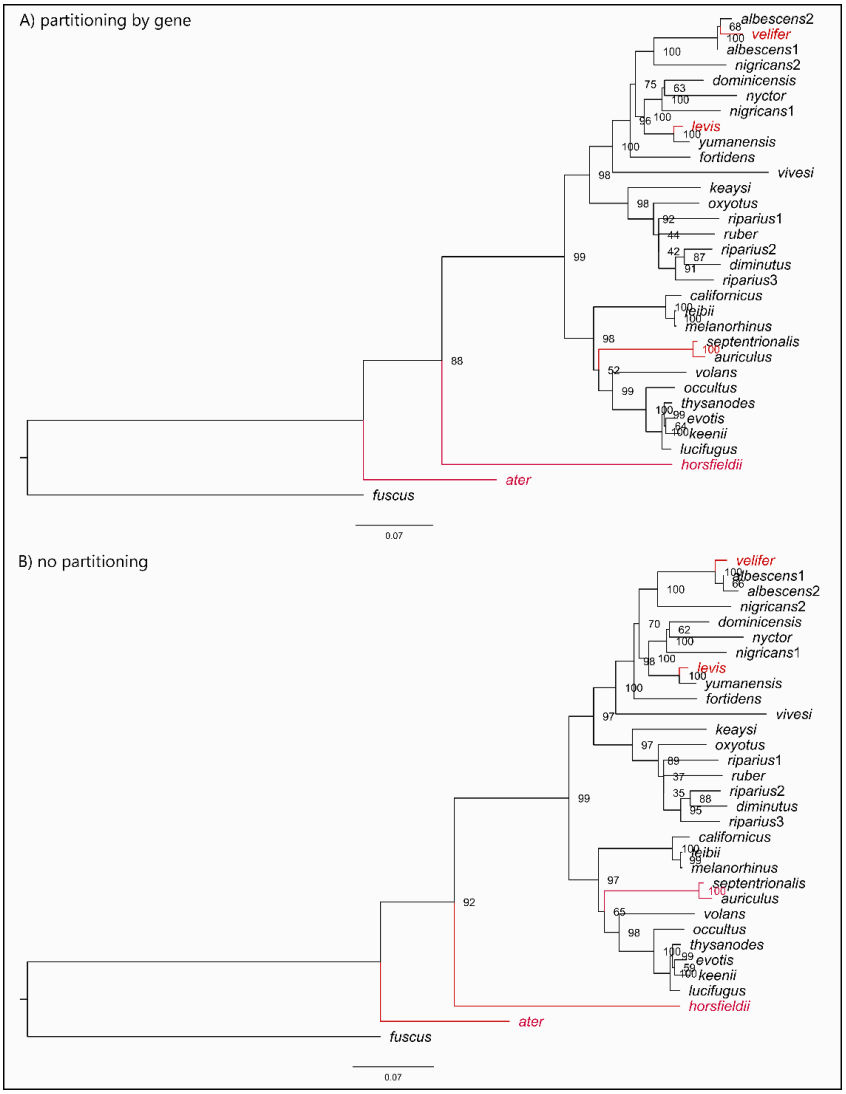
Validating MitoFinder using the *Myotis* UCE libraries

In addition to the *Eptesicus* dataset, we also validated the use of MitoFinder annotation in bats using the UCE libraries of *Myotis* generated by Platt et al. (2018). This dataset provides a good opportunity for validation because whole mitogenomes of the same individuals were also sequenced independently and are available on NCBI. The assembled contigs of 37 UCE libraries in Platt et al. (2018) were downloaded from the Dryad archive (https://doi.org/10.5061/dryad.5g205). We used the same reference genomes in the *Eptesicus* MitoFinder annotations excluding the three mitogenomes generated by Platt et al. (2018), which might generate biased results of better annotations because some individuals would be annotated based on its own data. In addition, the mitogenomes of *E. bottae* and *E. (Histiotus)* were removed because these references were critical for the *Eptesicus* annotation but not that informative for the *Myotis* annotation, and this allowed us to reduce the number of reference genomes (total 42 sequences) for a potentially faster run. MitoFinder v1.4.1 (Allio et al. 2020) annotation (command -a) was run with the vertebrate mitochondrial code (-o 2) and the minimum contig size 50 bp (--min-contig-size 50). All the other parameters were kept as default. The annotated *COI* and *CYTB* sequences were also blasted on NCBI to see if the annotated sequences blast to their corresponding mitogenomes.

Our results showed that MitoFinder annotated all 15 mitochondrial genes using the 37 *Myotis* UCE libraries from Platt et al. (2018). A total of 32 samples were annotated with at least one gene, and four of the failed samples were UCEs enriched from nuclear reference genomes which thus do not contain mitochondrial genes. The absence of mitochondrial gene annotation in nuclear genomes suggests that MitoFinder annotations are not biased by nuclear copies of mitochondrial genes. A total of 24 samples were annotated with *CYTB* sequences and 22 with *COI* sequences. One sample was blasted to a different but closely related species and three samples were blasted to different species using *COI* but correct

species using *CYTB* (Table S4). All other sequences were blasted to the respective mitogenome reference of the specimen sequenced and submitted by Platt et al. (2018; Table S4). Accordingly, the BLAST results indicated high accuracy of our MitoFinder annotations on bat UCE libraries.

All mitochondrial genes annotated from the 32 *Myotis* UCE libraries (Platt et al. 2018) were concatenated to construct maximum likelihood phylogeny in RAxML (Stamatakis, 2014) with the GTRGAMMA model, a rapid ML searching (-f a), and 1000 rapid bootstraps (-# 1000). The ML trees with no partitioning or partitioning by genes were almost identical to each other and showed very similar topology to the mitogenome phylogeny in Platt et al. (2018), except for a few branches (highlighted below). These minor differences might be due to the less complete gene sequences from MitoFinder annotation compared to the whole mitogenome sequences used in the previous study. Therefore, the phylogeny results also indicated good efficiency as well as accuracy of MitoFinder annotations on bat UCE libraries.



The ML mitochondrial phylogenetic trees using the annotated UCE libraries from Platt et al. (2018) with A) the partitioning scheme by gene or B) no partitioning. Each tip represents one individual and is named by species names according to Platt et al. (2018). All species belong to the genus *Myotis* except for the outgroup *Eptesicus fuscus*. Bootstrap supports are shown on the nodes. Red tips and branches highlight differences from the mitogenome phylogeny in Platt et al. (2018).

Table S1. MitoFinder annotation on the 96 UCE libraries generated in Yi and Latch (2022). The mitochondrial sequences of COI and CYTB genes were submitted (accessions listed) using the corrected species names (listed) when the original sample was misidentified.

* *Note: Table S1 is attached as a separate excel file.*

Table S2. The 91 mitochondrial reference genomes used in MitoFinder annotation. All are mitochondrion complete genomes except for one partial genome MF143474.

Accession	Species	Length (bp)	DNA
NC_034227	*Myotis bechsteinii*	17151	circular
KX757757	*Myotis bechsteinii*	17151	circular
NC_025308	*Myotis brandtii*	17470	circular
KM199849	*Myotis brandtii*	17470	circular
NC_015828	*Myotis formosus*	17159	circular
HQ184048	*Myotis formosus*	17159	circular
NC_056773	*Myotis petax*	17299	circular
KT199100	*Myotis petax*	17299	circular
NC_056111	*Myotis ricketti*	17098	circular
MN245054	*Myotis ricketti*	17098	circular
CM024383	*Myotis myotis*	17320	circular
NC_041638	*Myotis frater*	17089	circular
MH177276	*Myotis frater*	17089	circular
KT901455	*Myotis myotis*	17213	circular
NC_029849	*Myotis lucifugus*	17038	circular
KP273591	*Myotis lucifugus*	17038	circular
NC_029422	*Myotis muricola*	17224	circular
NC_029346	*Myotis myotis*	17213	circular
KT213444	*Myotis muricola*	17224	circular
KT199099	*Myotis petax*	17297	circular
KT199102	*Myotis petax*	17296	circular
KT199101	*Myotis petax*	17299	circular
NC_022698	*Myotis ikonnikovi*	16584	circular
NC_022694	*Myotis macrodactylus*	17562	circular
KF111724	*Myotis ikonnikovi*	16584	circular
KF440685	*Myotis macrodactylus*	17562	linear
NC_061569	*Kerivoula minuta*	16699	circular
NC_061567	*Kerivoula papillosa*	16671	circular
OK274261	*Kerivoula papillosa*	16774	circular
OK274260	*Kerivoula minuta*	16699	circular
OK274258	*Kerivoula papillosa*	16671	circular
NC_050995	*Lasionycteris noctivagans*	16175	circular
MT774151	*Lasionycteris noctivagans*	16175	circular
MT774150	*Lasionycteris noctivagans*	16175	circular
MT774149	*Lasionycteris noctivagans*	16175	circular
NC_049871	*Myotis septentrionalis*	17150	circular
MK547202	*Myotis septentrionalis*	17150	circular
MT588108	*Myotis blythii*	16752	circular
NC_044489	*Miniopterus fuliginosus*	16961	circular
MH523628	*Miniopterus fuliginosus*	16961	circular

MF143491*	*Myotis lucifugus*	17066	circular
MF143489*	*Myotis melanorhinus*	17018	circular
MF362599	*Myotis lucifugus*	17006	linear
KT210199	*Myotis brandtii*	16808	circular
MF143474*	*Eptesicus fuscus*	16716	linear
OP328298 *	*Eptesicus bottae*	16816	circular
OP328299 *	*Eptesicus bottae*	16840	circular
OP328300 *	*Eptesicus bottae*	16864	circular
OP345298 *	*Eptesicus (Histiotus) macrotus*	16798	circular
OP345299 *	*Eptesicus (Histiotus) macrotus*	16800	circular
OP345300 *	*Eptesicus (Histiotus) macrotus*	16963	circular
OP345301 *	*Eptesicus (Histiotus) montanus*	16618	circular
OP345302 *	*Eptesicus (Histiotus) montanus*	16617	circular
OP345303 *	*Eptesicus (Histiotus) magellanicus*	16620	circular
OP345304 *	*Eptesicus (Histiotus) macrotus*	16634	circular
OP345305 *	*Eptesicus (Histiotus) montanus*	16618	circular
OP345306 *	*Eptesicus (Histiotus) montanus*	16570	circular
OP345307 *	*Eptesicus (Histiotus) montanus*	16618	circular
OP345308 *	*Eptesicus (Histiotus) macrotus*	16799	circular
OP345309 *	*Eptesicus (Histiotus) magellanicus*	16612	circular
OP345310 *	*Eptesicus (Histiotus) magellanicus*	16618	circular
OP345311 *	*Eptesicus (Histiotus) macrotus*	16799	circular
OP345312 *	*Eptesicus (Histiotus) montanus*	16618	circular
OP345313 *	*Eptesicus (Histiotus) montanus*	16814	circular
OP345314 *	*Eptesicus (Histiotus) macrotus*	16799	circular
OP345315 *	*Eptesicus (Histiotus) macrotus*	16799	circular
OP345316 *	*Eptesicus (Histiotus) montanus*	16618	circular
OP345317 *	*Eptesicus (Histiotus) montanus*	16618	circular
OP345318 *	*Eptesicus (Histiotus) magellanicus*	16618	circular
OP345319 *	*Eptesicus (Histiotus) magellanicus*	16619	circular
OP345320 *	*Eptesicus (Histiotus) magellanicus*	16618	circular
OP345321 *	*Eptesicus (Histiotus) magellanicus*	16618	circular
OP345322 *	*Eptesicus (Histiotus) magellanicus*	16618	circular
OP345323 *	*Eptesicus (Histiotus) magellanicus*	16618	circular
OP345324 *	*Eptesicus (Histiotus) magellanicus*	16618	circular
OP345325 *	*Eptesicus (Histiotus) magellanicus*	16618	circular
OP345326 *	*Eptesicus (Histiotus) magellanicus*	16618	circular
OP345327 *	*Eptesicus (Histiotus) magellanicus*	16618	circular
OP345328 *	*Eptesicus (Histiotus) magellanicus*	16618	circular
OP345329 *	*Eptesicus (Histiotus) magellanicus*	16606	circular
OP345330 *	*Eptesicus (Histiotus) montanus*	16576	circular
OP345331 *	*Eptesicus (Histiotus) macrotus*	16798	circular
OP345332 *	*Eptesicus (Histiotus) montanus*	16618	circular
OP345333 *	*Eptesicus (Histiotus) montanus*	16618	circular
OP345334 *	*Eptesicus (Histiotus) macrotus*	16800	circular
OP345335 *	*Eptesicus (Histiotus) macrotus*	16798	circular
OP345336 *	*Eptesicus (Histiotus) macrotus*	16800	circular
OP345337 *	*Eptesicus (Histiotus) montanus*	16617	circular
OP345338 *	*Eptesicus (Histiotus) montanus*	16617	circular
OP345339 *	*Eptesicus (Histiotus) macrotus*	16800	circular
OP345340 *	*Eptesicus (Histiotus) macrotus*	16799	circular

* The genomes not included in MitoFinder annotation on the UCE libraries from Platt et al. (2018).

Table S3. The 13 newly sequenced individuals of *Eptesicus (Histiotus)*. ROM represents the Royal Ontario Museum and UFPB represents Universidade Federal da Paraíba, Brazil.

Museum catalogue	ID	Gene	Species	Country	Province/ State	Location
UFPB 9492	Bat20	*COI, CYTB*	*E. (Histiotus) diaphanopterus*	Brazil	Bahia	Boqueirão da Onça, village of São Pedro do Lago, Sento Sé
UFPB 9493	Bat21	*COI*	*E. (Histiotus) diaphanopterus*	Brazil	Bahia	Boqueirão da Onça, village of São Pedro do Lago, Sento Sé
-	Bat77	*COI, CYTB*	*E. (Histiotus) sp.*	Brazil	São Paulo	Ipero
-	Bat78	*COI, CYTB*	*E. (Histiotus) sp.*	Brazil	São Paulo	Ipero
UFPB 9497	Bat79	*COI, CYTB*	*E. (Histiotus) diaphanopterus*	Brazil	Bahia	Boqueirao da Onca, Santo Se
UFPB 9494	Bat80	*COI, CYTB*	*E. (Histiotus) diaphanopterus*	Brazil	Bahia	Boqueirao da Onca, Santo Se
ROM125161	F58359	*COI, CYTB*	*E. (Histiotus) mochica*	Peru	Piura	Los Peroles, 4 km SW of Cascapamba
ROM125162	F58360	*COI, CYTB*	*E. (Histiotus) mochica*	Peru	Piura	Los Peroles, 4 km SW of Cascapamba
ROM125578	F58737	*COI, CYTB*	*E. (Histiotus) mochica*	Peru	Piura	Quebrada Parinas, 10 km NE of Talara
ROM125579	F58738	*COI*	*E. (Histiotus) mochica*	Peru	Piura	Quebrada Parinas, 10 km NE of Talara
ROM125580	F58739	*COI*	*E. (Histiotus) mochica*	Peru	Piura	Quebrada Parinas, 10 km NE of Talara
ROM125581	F58740	*COI*	*E. (Histiotus) mochica*	Peru	Piura	Quebrada Parinas, 10 km NE of Talara
ROM125582	F58741	*COI, CYTB*	*E. (Histiotus) mochica*	Peru	Piura	Quebrada Parinas, 10 km NE of Talara

Table S4. BLAST of the 24 *CYTB* and 22 *COI* sequences annotated using UCE libraries from Platt et al. (2018). The sequence ID, species name, museum catalogues, and mitogenome accessions are from Platt et al. (2018). Most sequences were blasted to the mitogenome reference of the same specimen, except for the few cases where the blast sequence accessions are shown in parentheses. All blast results are *Myotis* species and thus the genus name is not shown. Samples blasted to different species are bolded. The BLAST runs were carried out in January 2023.

Sequence_Dryad_ Platt.et.al.2018_	**species_ Platt.et.al.2018**	**museum_ Platt.et.al.2018**	**mitogenome_ Platt.et.al.2018**	**BLAST_COI**	**BLAST_ CYTB**
myotis-albescens2-TK135124	Myotis albescens	QCAZ 9157	MF143497	albescens	albescens
myotis-atacamensis-M4430	Myotis ater *	M4430	MF143493	atacamensis	atacamensis
myotis-austroriparius-RDS7705	**Myotis septentrionalis**	**TK 194420**	**MF143487**	**auriculus (MF143486)**	septentrionalis
myotis-dominicensis-TK15624	Myotis dominicensis	TK 15624	MF143467	dominicensis	dominicensis
myotis-evotis-MSB47323	Myotis evotis	MSB 47323	MF143468	evotis	evotis
myotis-fortidens-MSB54941	**Myotis fortidens**	**MSB 54941**	**MF143483**	**yumanensis (MF143485)**	fortidens (KC747690)
myotis-horsefeldi-1926039	Myotis horsfieldii	MHNG 1926.039	MF143494	horsfieldii	horsfieldii
myotis-keaysi-TK13525	Myotis keaysi	TK 13525	MF143477	keaysi	keaysi
myotis-keeni-AF74075	Myotis keenii	UAM 113849	MF143472	NA	keenii
myotis-leibi-TK24872	Myotis leibii	TK 24872	MF143488	leibii	leibii
myotis-lucifugus-MSB46679	Myotis lucifugus	MSB 46679	MF143491	lucifugus	lucifugus
myotis-martinequensis-TK151413	Myotis nyctor *	TK 151413	MF143498	martiniquensis	martiniquensis
myotis-melanhorinus-M8944	**Myotis melanorhinus**	**TK 193888**	**MF143489**	**leibii (MF143488)**	melanorhinus
myotis-nigricans1-TK135514	Myotis nigricans	QCAZ 9601	MF143495	nigricans	nigricans
myotis-nigricans2-RDS7791	Myotis nigricans	TK 194145	MF143484	nigricans	nigricans
myotis-nigricans3-TK134759	Myotis diminutus*	QCAZ 9168	MF143481	nigricans	nigricans
myotis-occultus-MSB121995	**Myotis occultus**	**MSB 121995**	**MF143490**	**lucifugus (OP345295)**	**lucifugus (OP345297)**
myotis-oxyotus-RCO1013	Myotis oxyotus	UMMZ RCO1013	MF143479	oxyotus	oxyotus
myotis-riparius-TK145199	Myotis riparius	TK 145199	MF143480	riparius	riparius
myotis-ruber-MVZ185692	Myotis ruber	MVZ 185692	MF143478	NA	ruber (NC036315)
myotis-thysanodes-07LEP	Myotis thysanodes	07LEP	MF143492	thysanodes	thysanodes
myotis-vivesi-MSB42658	Myotis vivesi	MSB 42658	MF143476	vivesi (HQ966967)	vivesi (AJ504406)
myotis-volans-MSB40886	Myotis volans	MSB 40886	MF143496	volans	volans
myotis-yumanensis-RDS7734	Myotis yumanensis	TK 194144	MF143485	yumanensis	yumanensis

* Species name of the specimen was updated in Platt et al. (2018).

Table S5. Sample information of all *Eptesicus (Histiotus)* individuals included in the analyses. Source species names and sample localities were based on cited references. Species names given in this study were listed for each individual sample.

* *Note: Table S5 is attached as a separate excel file.*

Figure S1. The mitochondrial phylogenetic trees using the annotated UCE libraries from Yi and Latch (2022). Each tip represents one individual and is named by source species identity followed by sample source and the country of sample location (same as the Fig2 in Yi and Latch 2022). Red tips are individuals with misidentified species names. Note that *Eptesicus nasutus* has been renamed as *Rhyneptesicus nasutus* (Juste et al. 2013), *Eptesicus matroka* has been renamed as *Laephotis matroka* (Simmons & Cir-ranello 2020), and the Old World *Eptesicus* species names have been updated based on Juste et al. (2013). **A) The maximum likelihood tree using no partitioning.** Bootstrap supports are shown on the nodes. **B) The maximum likelihood tree using the partitioning scheme by gene. C) The Bayesian Informative tree using no partitioning.** Numbers on the nodes are posterior probabilities. Red branches highlight the *Eptesicus (Histiotus)* paraphyly. **D) The Bayesian Informative tree using the partitioning scheme by gene.**

Figure S2. The Maximum Likelihood *COI* tree of *Eptesicus*. Each tip represents one individual and is named by the GenBank accession number (if available) followed by the GenBank or source species identity and museum catalogue or source sample code. Red tips indicate potentially mislabeled *Eptesicus* samples. Samples collected and sequenced in this study are labeled with asterisks. Bootstrap supports are shown on the nodes.

Figure S3. The Maximum Likelihood *CYTB* tree of *Eptesicus*. Each tip represents one individual and is named by the GenBank accession number (if available) followed by the GenBank or source species identity and museum catalogue or source sample code. Red tips highlight the four samples previously identified as *Eptesicus (Histiotus) colombiae* but clustered with Neotropical *Eptesicus*. Samples collected and sequenced in this study are labeled with asterisks. Bootstrap supports are shown on the nodes.

Figure S4. The Maximum Likelihood tree using concatenated *COI* and *CYTB* sequences using A) partitioning scheme by gene or B) no partitioning. Each tip represents one individual and is named in the same way as the above trees. Red tips highlight the mislabeled samples. Bootstrap supports are shown on the nodes.

** Figures S1-S4 are attached as separate PDF files.*

Supplementary files

my82-notrim-concatenate.phylip: the alignment of all mitochondrial sequences annotated from the *Eptesicus* UCE libraries. Sequences are named by the source species identity followed by sample ID (Table S1; same in the other alignments).
mafft.COI363_clean.phylip: the alignment of all 363 *COI* sequences including MitoFinder annotations, newly collected sequences, and online references.
mafft.cytb426_clean.phylip: the alignment of all 426 *CYTB* sequences including MitoFinder annotations, newly collected sequences, and online references.