ABSTRACT
We sequenced the complete mitochondrial genomes of two bat fly species within the Nycteribiidae (Diptera: Hippoboscoidea) – Dipseliopoda setosa (Cyclopodinae) and Basilia ansifera (Nycteribiinae). Both mitogenomes were complete and contained 13 protein-coding genes, 22 tRNAs, and two rRNAs. Relative to the inferred ancestral gene order of dipteran mitochondrial genomes, no rearrangements were identified in either species. There were large differences in size between the two genomes, with D. setosa having a larger genome (19,164 bp) than B. ansifera (16,964 bp); both species had larger genomes than two previously published Streblidae bat fly species (e.g., Paradysschia parvula and Pararichobius longicrus). The increased genome sizes were due to expansions in the control region and the non-coding region downstream of the light-strand origin of replication. Additional differences between the two mitogenomes included a significantly longer cox3 gene in B. ansifera and a longer nad1 gene in D. setosa. Interestingly, both genomes also had the lowest GC content (D. setosa = 15.9%; B. ansifera = 17.0%) of any available Hippoboscoidea mitochondrial genome (18.8–23.9%). These mitogenomes represent the first sequences from species within the bat fly family Nycteribiidae. The sequence data here will provide a foundation for continued studies of genome evolution more generally within obligate blood-feeding ectoparasites, and specifically for the bat flies as vectors of significant ‘bat-associated’ viruses and microorganisms.
Diptera mitogenomes ranging from 18.8% to 23.9%. Variation in mitochondrial genome sizes reflected significant expansions of different noncoding regions in different species: both the control region and noncoding region downstream of the origin of light-strand replication were expanded by 713 bp and 1385 bp, respectively, in D. setosa as compared to B. ansifera. In addition to these expansions, there were also differences in gene length between the two mitogenomes: B. ansifera had a larger cox3 by 21 amino acids while D. setosa had a longer nad1 by 27 amino acids. All other gene length differences were less than six amino acids, with the exception of nad2 and cox1 genes which are conserved in length in both species.

Mitogenomes from six species within the superfamily Hippoboscoidea were included for phylogenetic analysis, including all hippoboscoids with publicly available sequences on NCBI, as well as mitogenomes for two Glossinidae species – Glossina brevipalpis (SAMN02649554) and Glossina austeni (SAMN02647160) – that were assembled, annotated, and deposited to GenBank for this study. Representative species from Muscoidea and Oestroidea were included for evolutionary context and Ephydroidea species as outgroups. All 13 PCGs from each mitogenome were extracted and aligned using Geneious® v10.2.6 with MAFFT v7.450 (Katoh and Standley 2013) to infer the phylogenetic placement of D. setosa and B. ansifera. We used PartitionFinder2 (Stamatakis 2006; Lanfear 2014; Lanfear et al. 2016) to identify the best partitioning scheme and nucleotide substitution models. We estimated a maximum likelihood phylogeny using RAxML (Stamatakis 2006) based on the partitioned dataset, with 1000 bootstrap replicates using the GTRGAMMAI substitution model for each partition. The phylogenetic tree suggested D. setosa and B. ansifera are monophyletic and that Nycteribiidae are sister to Streblidae (Figure 1).

As the first sequenced Nycteribiidae mitogenomes, the D. setosa and B. ansifera mitogenomes provide a foundation for continued studies of genetic divergence among ecologically and medically important ectoparasites with distinct patterns of diversification tightly coupled with host biology. This is also the first step toward unraveling the systematics of bat flies.

**Ethics statement**

Samples of Dipseliopoda setosa (ex. Stenonycteris lanosus [Chiroptera: Pteropodidae], FMNH232508) and Basilia ansifera (ex. Scotoecus hindei [Chiroptera: Vespertilionidae], FMNH232513) were collected from the Agoro-Agu Forest Reserve [3.81039, 32.92264], Uganda in 2016. Collection permits and material transfer agreements were provided by the Uganda Wildlife Authority (UWA; Ref COD/96/02), Uganda National Council for Science and Technology (UNCST; Ref NS 417).

**Authors’ contributions**

HL conducted field work and collected specimens; MLP and RAC conceived and designed the study; MS generated the data; MS and RAC analyzed the data; MLP, RAC, and MS were involved in data interpretation and writing the paper; all authors revised the manuscript for intellectual content and approved the final version for publication. All authors are accountable for all aspects of the work.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).
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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under the accession nos. MZ826150, MZ826151, MZ826152, and MZ826153. The associated BioProject, Bio-Sample, and SRA experiment numbers are PRJNA772298, SAMN22374446 and SAMN22374447, and SRX13333010 and SRX13333011, respectively.

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