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MITOGENOME REPORT

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The complete mitogenomes of all four *Cryptospiza* species (Aves: Estrildidae)

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ABSTRACT

Crimsonwings are estrildid finches found in the understory of montane rainforests of sub-Saharan Africa. The genus includes four species: *Cryptospiza jacksoni* Sharpe 1902, *C. shelleyi* Sharpe 1902, *C. reichenovii* (Hartlaub 1874), and *C. salvadorii* Reichenow 1892. The first two are endemic to the Albertine Rift, while the latter two are more widespread. Despite being well-represented in museum collections, genetic resources are scarce. Here we provide complete mitogenomes for all four species, each containing the standard 37 avian genes. Analyses showed *C. shelleyi* as sister to the other three species, with *C. reichenovii* and *C. salvadorii* being highly similar (99.2%). Further research is needed to explore their evolutionary history.

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Introduction

The genus *Cryptospiza* comprises four species of estrildid finches (Aves: Estrildidae) that inhabit the understory of montane regions of sub-Saharan Africa (Figure 1). All four species co-occur in some parts of the Albertine Rift in Central Africa (e.g. Willard et al. 1996). *Cryptospiza shelleyi* Sharpe 1902 and

Figure 1. Specimens used in the present study, from left to right, *C. jacksoni* (FM356462), *C. reichenovii* (FM356444), *C. salvadorii* (FM356449), *C. shelleyi* (FM356487). Photo by J.D. Maddox.

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Cryptospiza jacksoni Sharpe 1902 are Albertine Rift endemics with *C. shelleyi* being very locally distributed and listed as endangered by the International Union for Conservation of Nature (BirdLife International 2017). The other two species, *Cryptospiza reichenovii* (Hartlaub 1874) and *Cryptospiza salvadorii* Reichenow 1892, occur in broadly dispersed montane regions across the continent from southern Ethiopia, to the Cameroonian highlands, to the Malawian highlands and Angolan highlands. These species are not known to migrate, so their evolutionary history may track the historical connectivity of the montane forests in which they occur. Genetic resources for the genus are scarce even though approximately 1000 specimens currently reside in natural history museums. Here, we provide the complete, annotated mitochondrial genome for each of the four species in the genus *Cryptospiza* from specimens collected in the Rwenzori mountains of Uganda.

Methods and materials

We requested and obtained permission to use frozen tissue samples from vouchered museum specimens (Table 1) from the Field Museum of Natural History Bird Collection (Ben Marks, Collection Manager, bmarks@fieldmuseum.org) and extracted DNA using Qiagen's DNeasy Blood and Tissue Kit following the manufacturer's instructions. We prepared libraries using Illumina's Nextera Mate Pair Library Preparation Kit and sequenced them on a MiSeq using a 500 cycle v2 reagent kit. Mitogenomes were assembled from raw reads using GetOrganelle v1.7.7.0 (Jin et al. 2020) with default settings. We then mapped the raw reads to their respective draft references in Geneious Prime v2022.2.2 (Kearse et al. 2012) to confirm the accuracy of each assembly. To annotate assembled mitogenomes, we used MITOS2 (Donath et al. 2019) to identify protein-coding genes, ribosomal RNA (rRNA) genes, and control

Table 1. Information of vouchered specimens used in this study.

Species	Voucher #	Date	Country	Location	Elevation (m)
C. jacksoni	FM356462	14 Nov 1990	Uganda	Choha, 6 km NW Ibanda, Mubuku Valley, Rwenzori Mts	1960
C. reichenovii	FM356444	13 Nov 1990	Uganda	Choha, 6 km NW Ibanda, Mubuku Valley, Rwenzori Mts	1960
C. salvadorii	FM356449	8 Dec 1990	Uganda	Nyabitaba, 10 km NW Ibanda, Mubuku Valley, Rwenzori Mts	2700
C. shelleyi	FM356487	8 Apr 1991	Uganda	Choha, 6 km NW Ibanda, Mubuku Valley, Rwenzori Mts	1960

All individuals were females.

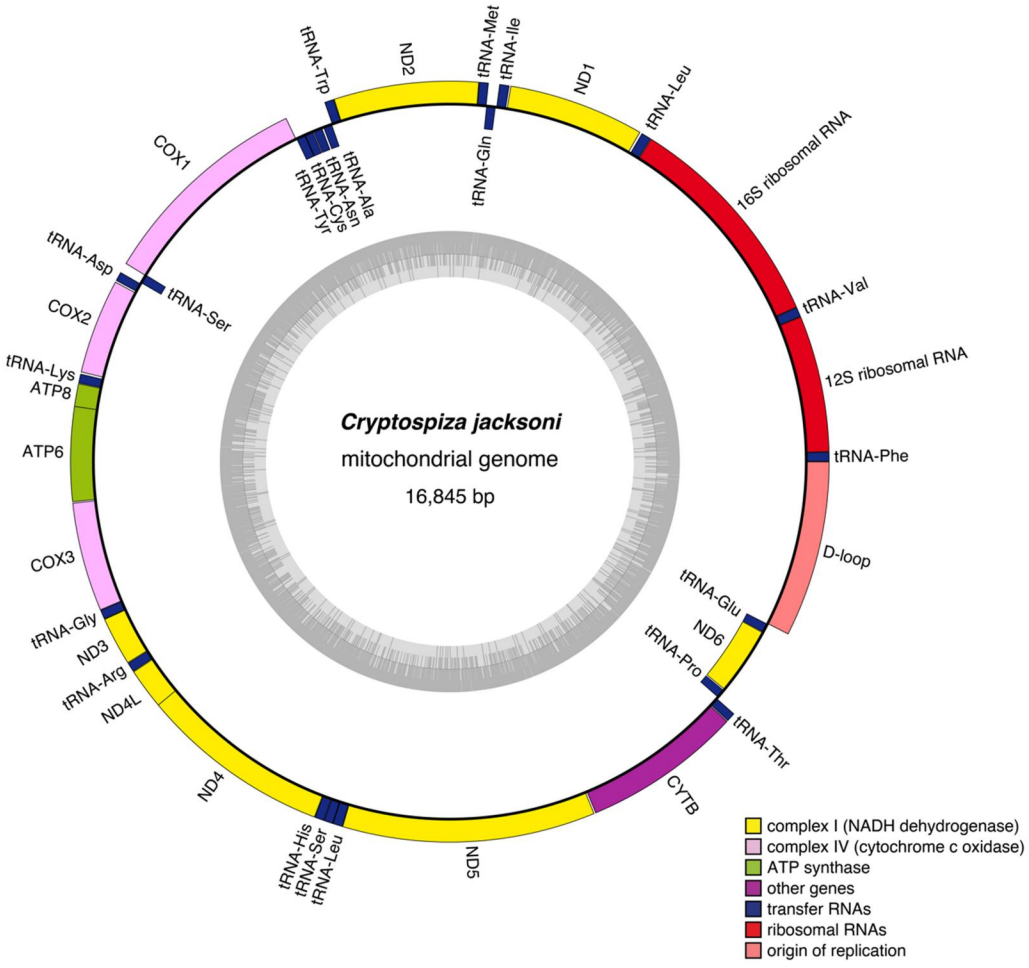

Figure 2. Annotated mitogenome of *C. jacksoni* PQ213854. The annotated features of the outer circle are colored by their functional categories as shown in the legend (bottom right), and the inner circle indicates the GC content.

Table 2. Pairwise similarities (%) of the four *Cryptospiza* mitochondrial genomes.

	C. jacksoni	*C. reichenovii*	*C. salvadorii*	*C. shelleyi*
C. jacksoni	–	95.4	95.4	93.7
C. reichenovii	95.4	–	99.2	93.6
C. salvadorii	95.4	99.2	–	93.6
C. shelleyi	93.7	93.6	93.6	–

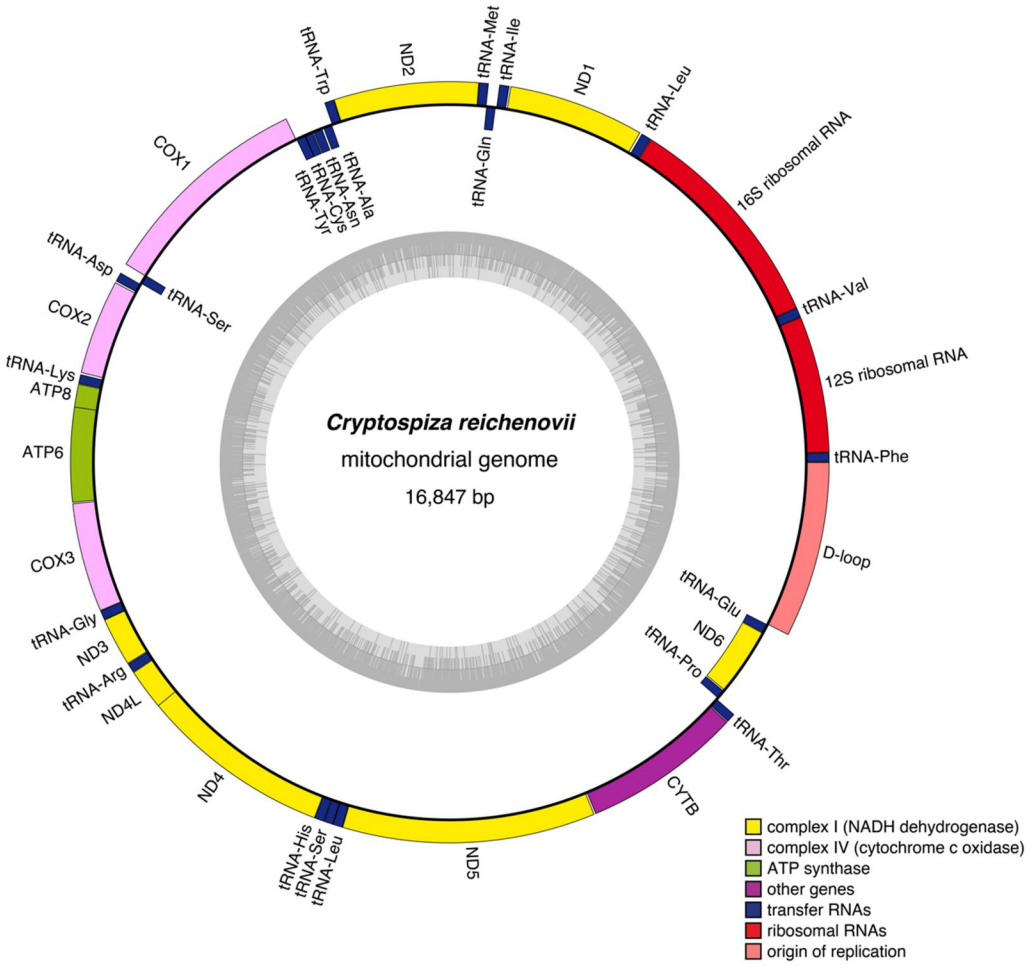

Figure 3. Annotated mitogenome of *C. reichenovii* PQ213855. The annotated features of the outer circle are colored by their functional categories as shown in the legend (bottom right), and the inner circle indicates the GC content.

regions. In addition, we used tRNAscan-SE v2.0.11 (Lowe and Chan 2016; Chan et al. 2021) to identify transfer RNAs (tRNA). We then manually inspected and curated all annotations in Geneious Prime v2024.24.05 and visualized mitogenomes in OGDRAW v1.3.1 (Greiner et al. 2019). To estimate genetic distances between species, we aligned all four mitogenomes using MAFFT v7.490 (Katoh and Standley 2013) with default settings. Finally, to confirm the placement of *Cryptospiza* with previous studies, we selected 5 estrildid species with available whole mitogenomes in GenBank, representing three Estrildidae subfamilies: *Erythrura gouldiae* (NC_050680; Xue et al. 2020) from Erythrurinae, *Lonchura castaneothorax* (MF770340; Stryjewski and Sorenson 2017) and *Lonchura punctulata* (NC_028036; Bao et al. 2016) from Lonchurinae, and *Taeniopygia guttata* (NC_007897; Mossman et al. 2006) and *Poephila acuticauda* (PP372650; McDiarmid et al. 2024) from Estrildinae. While Fjeldså et al. (2020) and Olsson and Alström (2020) recognize 6 subfamilies, whole mitogenomes are currently available for only three. We constructed maximum-likelihood phylogenies using RAxML v8.2.11 (Stamatakis 2014) with the GTR+G model of nucleotide substitution model and 1000 bootstrap replicates. *Vidua chalbeata* (MN356437; Feng et al. 2020) and *Vidua macroura* (NC_053065; Feng et al. 2020) were used as an outgroup.

Results

Each of the four assemblies produced a single, circular contig with an average sequence coverage of 157x for *C. jacksoni*,

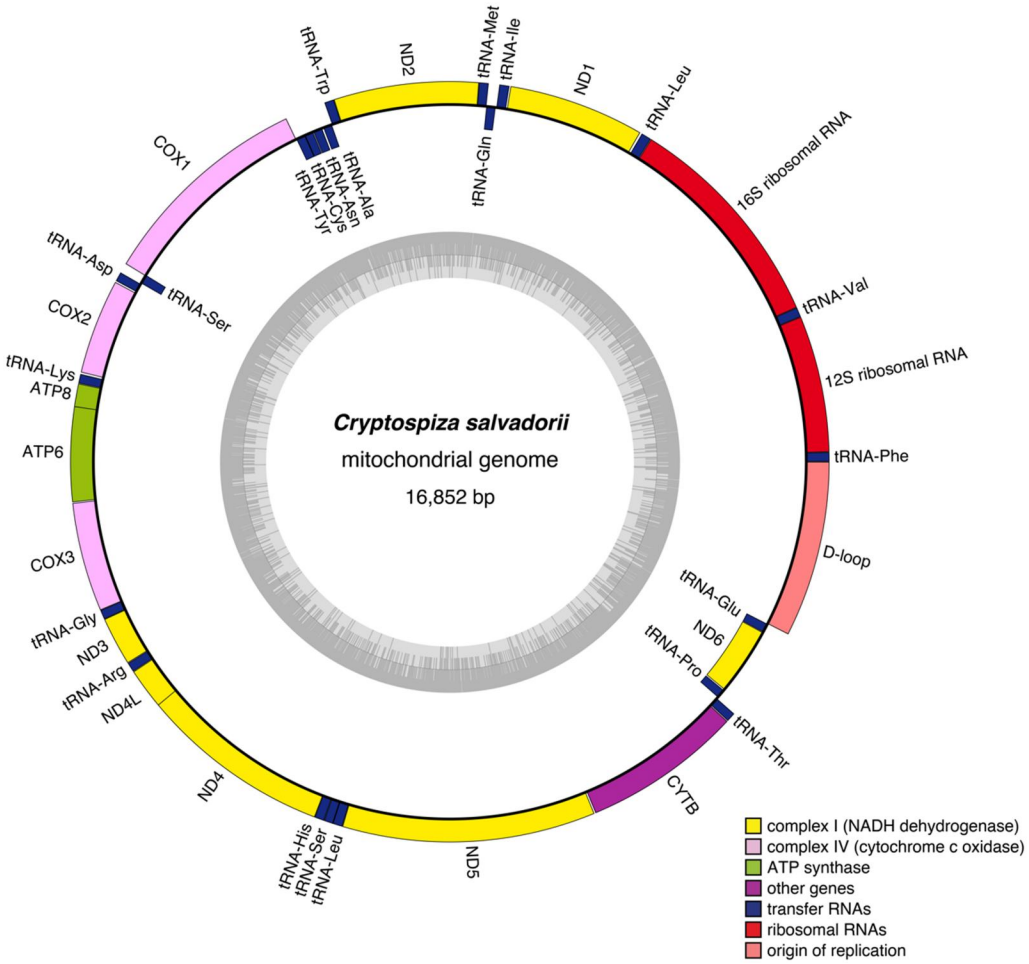

Figure 4. Annotated mitogenome of *C. salvadorii* PQ213856. The annotated features of the outer circle are colored by their functional categories as shown in the legend (bottom right), and the inner circle indicates the GC content.

306x for *C. reichenovii*, 568x for *C. shelleyi*, and 648x for *C. salvadorii* (Figure S1). Mitogenome length was similar among the four species, ranging from 16,836 (*C. shelleyi*) to 16,852 bp (*C. salvadorii*), and each contained the same order of 22 tRNAs, 2 rRNAs, 13 protein-coding genes, and a D-loop control region (Figures 2–5). GC content was also similar among species ranging from 46.0% for *C. salvadorii* to 46.5% for *C. shelleyi*. Pairwise similarities of the four species ranged from 93.6 to 99.2% (Table 2), with *C. shelleyi* being the most genetically divergent. Phylogenetic analysis revealed the genus *Cryptospiza* as a well-supported, monophyletic group with *C. reichenovii* and *C. salvadorii* as sister species that together are sister to *C. jacksoni* and *C. shelleyi* (Figure 6). The annotated mitogenomes have been deposited in GenBank with the accession numbers PQ213854.1 (*C. jacksoni*), PQ213855.1 (*C. reichenovii*), PQ213856.1 (*C. salvadorii*), and PQ213857.1 (*C. shelleyi*).

Discussion and conclusions

The especially low divergence between the morphologically similar *C. reichenovii* and *C. salvadorii* is consistent with the findings of Bowie (2003) and Olsson and Alström (2020). Olsson and Alström (2020) suggested the two species may have either split recently or that gene flow between the two is now occurring. Bowie (2003) did not find any geographical structure throughout the ranges of either species or evidence of hybridization where they are sympatric. Therefore, Bowie postulated that either dispersal is extensive, which contradicts known behavioral data, or that the two species have recently diverged, and mitochondrial DNA is insufficient to identify potential differences. In the Rwenzori mountains of Uganda, where all the samples are from, there appears to be elevational segregation between *C. reichenovii* and *C. salvadorii*. Willard et al. (1996) conducted surveys at 1960 m, 2075 m, 2700 m, and 3400 m in the Mbukuku and Bujuku river valleys, Rwenzori Mountains, Uganda, and found *C. reichenovii* occurred only at 1960 m and *C. salvadorii* only at 2700 m. In contrast, *C. jacksoni* was found from 1960 to 2700 m and *C. shelleyi* from 1960 to 3400 m. Moreover, all *C. salvadorii* captured in mistnets were found in bamboo thickets. Given the collections of *Cryptospiza* in natural history museums, the opportunity is ripe for future research to elucidate the evolutionary history of the genus.

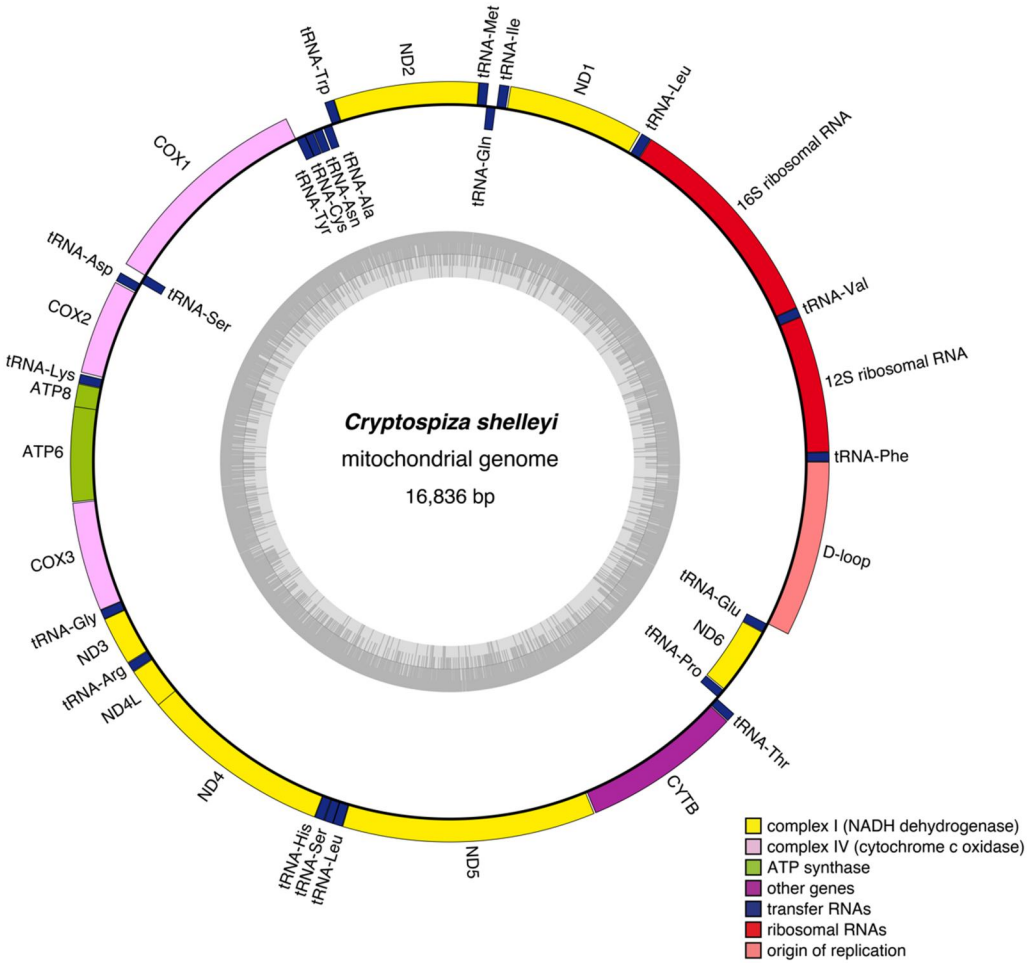

Figure 5. Annotated mitogenome of *C. shelleyi* PQ213857. The annotated features of the outer circle are colored by their functional categories as shown in the legend (bottom right), and the inner circle indicates the GC content.

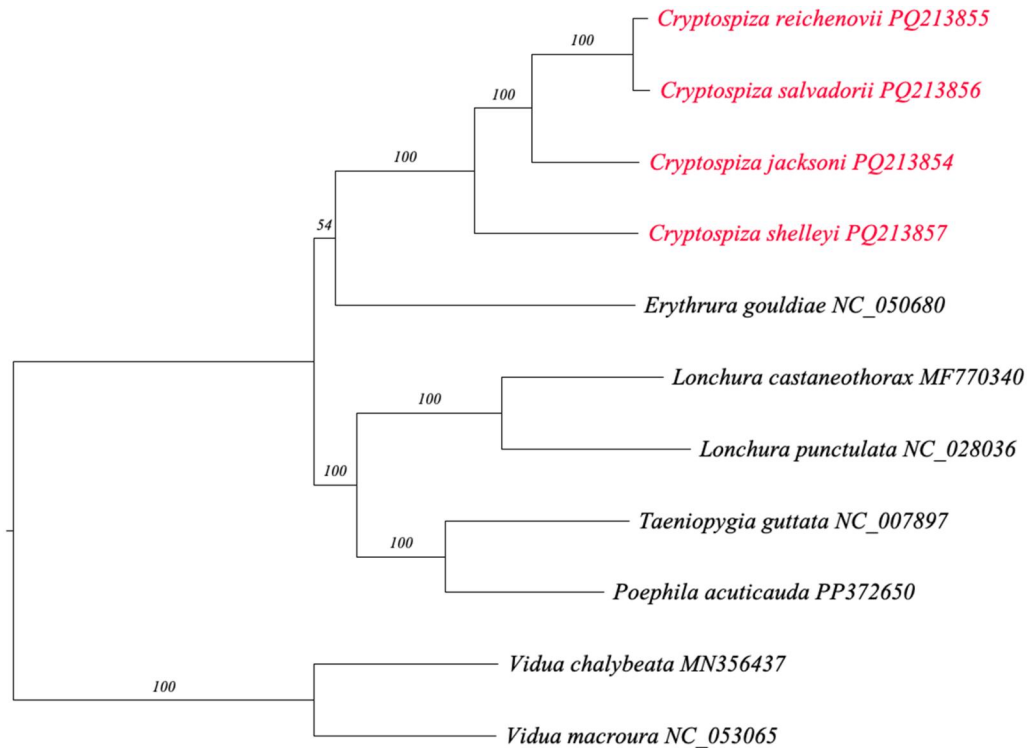

Figure 6. Maximum likelihood phylogeny of the four *Cryptospiza* species and select estrildid finches based on whole mitogenomes. Values indicate bootstrapping percentages. *Vidua* spp. were selected as an outgroup. The following sequences were used: *C. jacksoni* (this study; PQ213854), *C. reichenovii* (this study; PQ213855), *C. salvadorii* (this study; PQ213856), *C. shelleyi* (this study; PQ213857), *Erythrura gouldiae* (NC_050680; Xue et al. 2020), *Lonchura castaneothorax* (MF770340; Stryjewski and Sorenson 2017), *Lonchura punctulata* (NC_028036; Bao et al. 2016), *Taeniopygia guttata* (NC_007897; Mossman et al. 2006), *Poephila acuticauda* (PP372650; McDiarmid et al. 2024), *Vidua chalybeata* (MN356437; Feng et al. 2020), *Vidua macroura* (NC_053065; Feng et al. 2020).

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Authors contributions

JDM: Data analysis and interpretation, drafting and revising manuscript. **EZ:** Data collection and analysis. **KM:** Data collection and analysis. **FG:** Data interpretation, revising manuscript. **TPG**: Data collection, revising manuscript. **JMB:** Project conception and design, drafting and revising manuscript. **SJH:** Project conception and design, revising manuscript. All authors critically reviewed and approved the final manuscript.

Ethical approval

C. shelleyi is listed as an endangered species in the IUCN Red List of Threatened Species (Birdlife International 2017). However, the specimen (FM356487) from which we obtained the tissue used in this study was collected in 1990 when *C. shelleyi* was listed as near threatened. The other three species are listed as least concern. None of the species are CITES listed. All four specimens were collected with the appropriate permits from Uganda and the U.S.A. and in accordance with scientific guidelines established by the IUCN, The Convention on Biological Diversity, and CITES. No animal experiments or sampling was conducted in this study.

Disclosure statement

No potential conflict of interest was reported by the authors.

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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 (https://www.ncbi.nlm.nih.gov/) under the accession numbers PQ213854, PQ213855, PQ213856, PQ213857. The associated BioProject is PRJNA1124982; the Sequence Read Archive (SRA) data numbers are SRR29437166, SRR29437167, SRR29437168, SRR29437169; and the Bio-Sample numbers are SAMN41878406, SAMN41878407, SAMN41878408, SAMN41878409.

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