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The complete mitochondrial genomes of the Ethiopian endemic shrew *Crocidura yaldeni* s.l. (Mammalia: Soricidae)

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ABSTRACT: Complete mitochondrial genomes of the Ethiopian endemic shrew *Crocidura yaldeni* s.l. were assembled for the first time. We provide data concerning the sequencing, assembly, and annotation of the obtained mitochondrial genomes. The phylogenetic analysis revealed the existence of several lineages within *C. yaldeni* s.l. that was consistent with the previous studies based on mitochondrial gene cytochrome *b.* These data provide a basis for further studies on phylogeny and mechanisms of the presumable mitochondrial DNA introgression of this group.

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Полные митохондриальные геномы эндемичной эфиопской белозубки *Crocidura yaldeni* s.l. (Mammalia: Soricidae)

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РЕЗЮМЕ: Впервые были собраны полные митохондриальные геномы эндемичной эфиопской белозубки *Crocidura yaldeni* s.l. Мы предоставляем данные, касающиеся секвенирования, сборки и аннотирования полученных митохондриальных геномов. Филогенетический анализ показывает существование нескольких митохондриальных линий в пределах *C. yaldeni* s.l., что согласуется с ранними работами, основанными на единственном митохондриальном гене цитохрома *b*. Полученные данные создают основу для дальнейших исследований филогении и механизмов предполагаемой интрогрессии митохондриальной ДНК этой группы.

КЛЮЧЕВЫЕ СЛОВА: белозубка леса Белетта, митохондриальная ДНК, Эфиопия.

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Introduction

The Ethiopian Plateau is an important centre of high diversity and adaptive radiation for the genus *Crocidura*. Thirty species of white-toothed shrews are known from Ethiopia, 13 of them are currently considered to be endemic to the country (Lavrenchenko *et al*., 2016; Craig *et al*., 2020; Konečný *et al*., 2020; Martynov *et al*., 2023; Zemlemerova *et al*., 2024). However, recent work (Dianat *et al.*, 2024) showed that local endemism might be rather higher than it was previously found. Here, we focused on the taxon *Crocidura yaldeni sensu lato* (s.l.) Lavrenchenko, Voyta and Hutterer, 2016 (as defined by Dianat *et al*., 2024) that was described on the basis of four specimens collected in the Beletta forest (Lavrenchenko *et al.*, 2016).

Analysis of morphological data referred *Crocidura yaldeni sensu stricto* (s.s.) to the large-sized shrew group. It was shown that this species is substantially larger in size than some other Ethiopian endemic species such as *C. glassi* Heim de Balsac, 1966, *C. harenna* Hutterer and Yalden, 1990, *C. lucina* Dippenaar, 1980, *C. macmillani* Dollman, 1915, *C. baileyi* Osgood, 1936, etc. (Lavrenchenko *et al*., 2016). Previous phylogenetic analysis based on a mitochondrial gene cytochrome *b* (Konečný *et al*., 2020) revealed three different lineages within *C. yaldeni* s.l. that occupied different habitats from south-western to central-northern Ethiopia. Recently, Dianat *et al.* (2024) found another new mitochondrial lineage of this taxon from Choqa Mountain. Additionally, they showed that the mitochondrial phylogeny of *Crocidura yaldeni* s.l. is incongruent with nuclear phylogeny suggesting a mitochondrial introgression.

As a next step to understand evolutionary history of this complex, we present here the phylogenetic analysis *of Crocidura yaldeni* s.l. based on the complete mitochondrial genomes. These data increase the quantity of available genomic data for this white-toothed shrew group and provide a basis for further studies on phylogeny and mechanisms of the presumable mitochondrial DNA introgression and/or adaptation.

Material and methods

We sequenced 12 mitochondrial genomes of *C. yaldeni* s.l. from different localities in Ethiopia. These specimens were sampled in 2012–2018 and deposited in the Research Facility of the Institute of Vertebrate Biology of the Czech Academy of Sciences (IVB) in Studenec (field labels starting with ETH) and in the Section of Mammalogy at the Zoological Museum of Moscow University (ZMMU). Additionally, we downloaded sequences of complete mitochondrial genomes of the following species from GenBank: *C. yaldeni*, *C. lucina*, *C. baileyi*, *C. harenna*, *C. macmillani* and *C. glassi*. The details on all the material used in the study are given in Tab. 1.

Genomic DNA from ethanol-preserved tissues was extracted using the Jena Bioscience PP-208 DNA extraction kit (Jena Bioscience, Germany). Total-DNA samples were fragmented using a Covaris M220 ultrasonicator (Covaris LLC, USA). DNA library preparation was carried out using the NEBNext Ultra II kit (NEB, E7645S) and NEBNext Multiplex Library Kit (Dual Index Set2) (NEB, E7780S). Libraries were sequenced on an Illumina HiSeq 2000 platform at Novogene (UK). Quality of obtained Illumina reads was assessed in FastQC (Andrews *et al.*, 2010). Assembly and annotation of complete mitochondrial genomes were performed in MitoZ 3.4 (Meng *et al.*, 2019). Newly obtained mitogenomes' sequences were uploaded to GenBank (Tab. 1).

Phylogenetic trees were generated for the dataset of 13 mitochondrial protein-coding genes (PCGs), two ribosomal RNA (rRNA) genes, and 22 transfer RNA (tRNA) genes by maximum likelihood (ML) and Bayesian inference (BI) methods. ML analysis was conducted in IQTREE v.1.6 (Nguyen *et al.*, 2015) using 1000 bootstrap replications. Bayesian tree reconstruction was performed in MrBayes 3.2 (Ronquist *et al*., 2012). Models suitable for sequence evolution were selected in PartitionFinder v. 2.1.1. (Lanfear *et al.*, 2012). Models with either two or six rate matrix parameters were selected for each partition on the basis of the results obtained in PartitionFinder. The analysis included two independent runs of four chains. The chain length was set to 5 000 000 generations, with sampling every 2 000 generations. Pairwise genetic distances were estimated using the Kimura-2 parameter model (Kimura, 1980) implemented in MEGA7 (Kumar *et al.*, 2016).

Results and discussion

The final alignment of mitogenomes contained 15 471 bp from 18 specimens including outgroup. Maximum likelihood and Bayesian analyses produced similar topologies; therefore, only the ML tree is shown in Fig. 1. Our results revealed the existence of two deeply divergent clades. The first one was found on Choqa Mt. and the second was distributed in the Central Ethiopian highlands and in the forests in the south of the Abyssinian plateau. The genetic distance (K2P) between them was approximately 3.64±0.16%. The latter clade was divided into three groups. One of these groups contained individuals from Afroalpine localities in Ankober and Guwasa and from all localities from south-western forests (lineage "yaldeni I" sensu Konečný *et al.*, 2020). The second clade was represented by one individual from Saja village (Kafa zone) from south-western forests (ETH2086, lineage "yaldeni II"). The third one was formed by the individuals from an Afroalpine habitat in the Borena Saynt NP (lineage "yaldeni III"). The genetic distance between them ranged from 0.47% to 0.73%. This result was consistent with the studies of Konečný *et al.* (2020) (except specimens from Choqa Mt.) and Dianat *et al.* (2024) using a single mitochondrial gene, *cytb*. However, some differences in tree topology compared to the latter study were revealed. According to Dianat *et al.* (2024) lineage "yaldeni II" appears as the sister group

Table. 1 The list of the materials used in the study.
Bold marks newly obtained mitogenomes' sequences. Sequences 14–18 were used as outgroup.

№	Species	Field/ tissue code	Museum collection code	GenBank code	Collecting site
1	*C. yaldeni*	3224	ZMMU S-202814	**PV296116**	Western Plateau, Choqa Mountain (N 10.705; E 37.845; 3961 m a.s.l.)
2		3364	ZMMU S-202951	**PV472533**	Western Plateau, Choqa Mountain (N 10.633; E 37.826; 3645 m a.s.l.)
3		30	ZMMU S-165342	PQ664586	Western Plateau, Beletta Forest (N 7.547; E 36.564; 1900 m a.s.l.)
4		ETH0733		**PV472534**	Western Plateau, Bonga, Chara (N 7.294; E 36.376; 2407 m a.s.l.)
5		ETH1480		**PV472535**	Western Plateau, Geche (N 7.558; E 35.400; 2102 m a.s.l.)
6		ETH1529		**PV472536**	Western Plateau, Chingawa forest (N 7.421; E 35.4; 2333 m a.s.l.)
7		ETH1862		**PV472537**	Western Plateau, Guwasa (N 10.294; E 39.800; 3320 m a.s.l.)
8		ETH1885		**PV472538**	Western Plateau, Ankober (N 9.657; E 39.747; 3194 m a.s.l.)
9		ETH1908		**PV472539**	Western Plateau, Ankober (N 9.650; E 39.746; 3165 m a.s.l.)
10		ETH2086		**PV472540**	Western Plateau, Kafa zone (N 7.512; E 36.057; 2010 m a.s.l.)
11		ETH1308		**PV472541**	Western Plateau, Borena Saynt NP (N 10.874; E 38.786; 3121 m a.s.l.)
12		ETH1682		**PV472542**	Western Plateau, Borena Saynt NP (N 10.878; E 38.816; 3514 m a.s.l.)
13		ETH1683		**PV472543**	
14	*C. baileyi*	1089	ZMMU S-172690	PQ863111	Mount Guna near Yitba (N 11.717; E 38.250; 3800 m a.s.l.)
15	*C. harenna*	2295	ZMMU S-192705	PQ863112	Eastern Plateau, Bale Mountains, Katcha area (N 6.700; E 39.773; 2190 m a.s.l.)
16	*C. lucina*	1905	ZMMU S-189286	PQ863110	Western Plateau, Debre Sina (N 9.826; E 39.735; 3233 m a.s.l.)
17	*C. macmillani*	933	ZMMU S-167293	PQ863113	Western Plateau, Sheko Forest (N 7.04; E 35.30; 1930 m a.s.l.)
18	*C. glassi*	2324	ZMMU S-192708	PQ863107	Eastern Plateau, Bale Mountains, Sanetti Plateau (N 6.788; E 39.766; 3730 m a.s.l.)

of the lineage "yaldeni I", whereas our results based on the complete mitochondrial genomes showed the sister relationship between lineages "yaldeni II" and "yaldeni III" (albeit without high support).

The previous study (Dianat *et al.*, 2024) revealed the multiple discrepancies between the *cytb* and nuclear phylogenies of *C. yaldeni* s.l. Based on the analysis of the genome-wide single nucleotide polymorphisms (SNPs) (Fig. 1) (Dianat *et al.*, 2024), *C. yaldeni* s.l. from high elevations (3121–3514 m a.s.l.) in central-northern Ethiopia (Guwasa, Ankober and Borena Saynt NP) was shown to be different from *C. yaldeni* s.s., which inhabit low elevation (2010–2407 m a.s.l.) forests of south-western Ethiopia. Generally, nuclear data clearly divided *C. yaldeni* s.l. according to the distributional and altitudinal ranges while mitochondrial data revealed the admixture. Presumably, this discordance combining with our results of the complete mitochondrial genomes might confirm the hypothesis for the historical introgression of mtDNA of *C. yaldeni* from lower-elevation forests to the Afroalpine regions previously hypothesized by Konečný *et al.* (2020). Besides the different elevations and habitat types, these groups have morphological differences: the forest group is very similar to the type series of this species; the high elevation group is smaller and resembles *C. thalia* Dippenaar, 1980 (Konečný *et al.*, 2020). In agreement with that, recent studies revealed the existence of past introgressive hybridization in multiple rodent genera in central Ethiopia (*Stenocephalemys* — Bryja *et al.*, 2018, Mizerovská *et al.*, 2020; *Arvicanthis* — Bryja *et al.*, 2019, *Lophuromys* — Komarova *et al.*, 2021).

Adaptive mitochondrial introgression appears to be a relatively common phenomenon (Toews & Belsford,

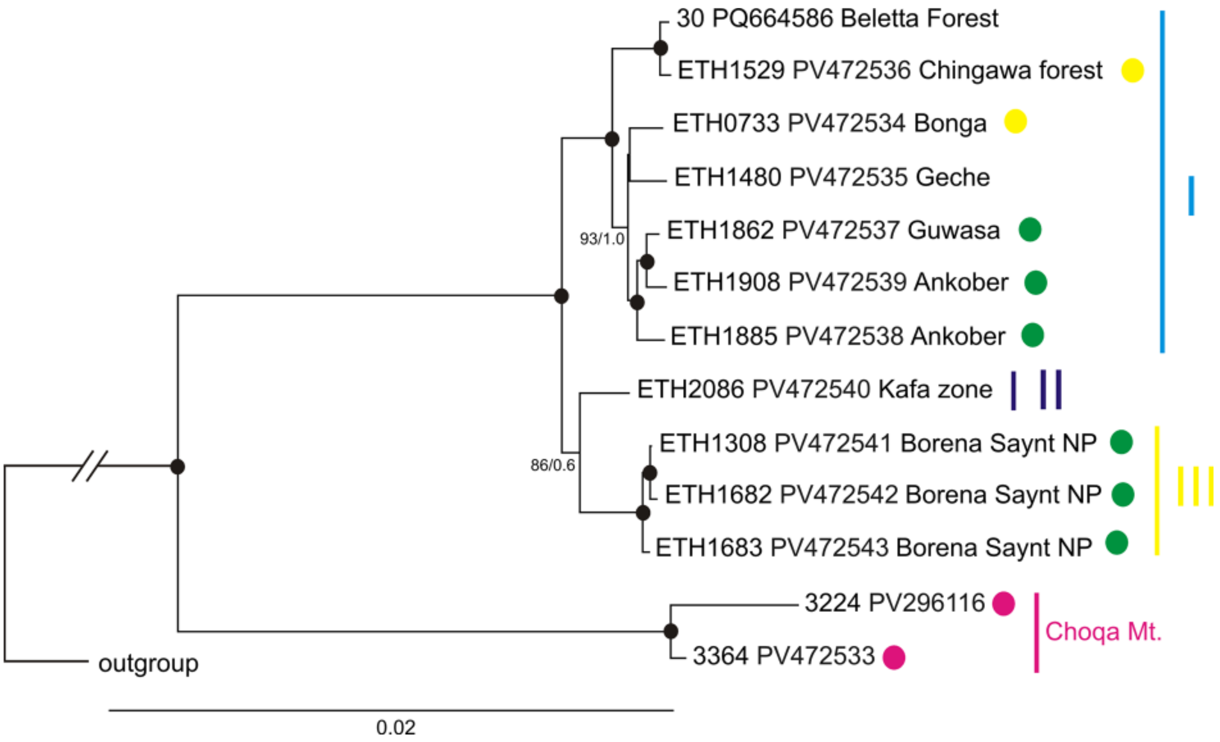

Fig. 1 The ML tree of *Crocidura yaldeni* s.l. based on the complete mitochondrial genomes. Black filled circles at tree nodes indicate support in ML ≥ 97 and BI = 1.0 analyses. Lineages within *C. yaldeni* s.l are marked according to Konečný *et al.* (2020). Color filled circles within *C. yaldeni* s.l correspond to the samples used in SNP analysis and are marked according to Dianat *et al.* (2024). Outgroup: *C. lucina*, *C. baileyi*, *C. harenna*, *C. macmillani* and *C. glassi.*

2012). As mtDNA encodes 13 genes that are involved in production of up to 95% of cellular energy (Da Fonseca *et al.*, 2008), interspecific introgression of mitochondrial genome can provide a relatively rapid way for adaptation to environments where specific modifications to oxidative phosphorylation system are beneficial (Yannic *et al.*, 2010; Melo-Ferreira *et al.*, 2012). Despite the extensive research focused on the adaptive introgression of mtDNA (Toews & Brelsford, 2012), only a limited number of studies offer empirical evidence for the mechanisms that underlie its proposed adaptive significance (Boratynski *et al.*, 2014; Kostin & Lavrenchenko, 2018). As mentioned above, *C. yaldeni* s.l. is able to inhabit various environments, from low-elevation humid montane forests to high-elevation *Erica* bush and grasslands with *Lobelia* that makes it highly variable and thus highly adaptable to different niches and habitats. The distinctive distribution characteristics of *C. yaldeni* s.l. position this group as a suitable model for future empirical verifications of the adaptive introgression hypothesis, as well as for exploring the co-evolutionary mechanisms between mitochondrial and nuclear genes that encode proteins involved in cellular respiration. Our results represent the first recovery of the complete mitochondrial genomes for *C. yaldeni* s.l., marking an initial step for the future research of this area.

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