Online ISSN: 2588-3526
Journal of Wildlife and Biodiversity
Volume 8(2): 179-192 (2024) (http://www.wildlife-biodiversity.com/)
Research Article

New records of the Ethiopian long-eared bat *Plecotus balensis* in central Ethiopia

Ahmed Seid Ahmed[1,2]*, Anagaw Atickem[2], Afework Bekele[2], Diress Tsegaye[3,4], Nils Chr. Stenseth[2,4], David J. Zinner[5], Christian Roos[6], Dietmar Zinner[5,7,8]

[1]Department of Biology, Hawassa University, Hawassa, Ethiopia
[2]Department of Zoological Sciences, Addis Ababa University, Addis Ababa, Ethiopia
[3]Landscape Monitoring, Division of Survey and Statistics, Norwegian Institute of Bioeconomy Research (NIBIO), Ås, Norway
[4]Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biology, University of Oslo, Blindern, Oslo, Norway
[5]Cognitive Ethology Laboratory, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany
[6]Gene Bank of Primates and Primate Genetics Laboratory, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany
[7]Department of Primate Cognition, Georg-August-University of Göttingen, Göttingen, Germany
[8]Leibniz-Science Campus Primate Cognition, Göttingen, Germany
*Email: ahmedse4378@gmail.com
Received: 11 December 2023 / Revised: 29 March 2024 / Accepted: 22 April 2024/ Published online: 01 May 2024.
How to cite: Ahmed, A.S., Atickem, A., Bekele, A., Tsegaye, D., Stenseth, N.C., Zinner, D.J., Roos, C.H., Zinner, D. (2024). New records of the Ethiopian long-eared bat Plecotus balensis in central Ethiopia, Journal of Wildlife and Biodiversity, 8(2), 179-192. **DOI**: https://doi.org/10.5281/zenodo.11099537

Abstract
The diversity of bats in Ethiopia comprises at least 80 species, among them the Ethiopian long-eared bat that was described in 2000. It is most likely endemic to the highlands of Ethiopia. However, knowledge of the distribution of the species is limited. During a bat survey in 12 regions of central Ethiopia stretched over 700 km along the Ethiopian Rift, we trapped long-eared bats at sites in three regions and confirmed the species' identity by molecular analysis. All occurrence sites of *P. balensis* were above 2500 m, confirming this taxon as a high-altitude species. Two of the regions are additions to the known range of *P. balensis* but it is most likely present in more high-altitude areas of Ethiopia than currently known. Additional surveys in so far unsampled areas are therefore indicated.
Keywords: Chiroptera, Vespertilioninae, Horn of Africa, distribution range, molecular species identification, cytochrome *b*

Introduction

Long-eared bats (genus *Plecotus*, Vespertilioninae) are primarily species of the temperate zone of Eurasia but also occur around the Mediterranean Sea and in the Nile Valley up to northern Sudan (Benda et al., 2004; Juste et al., 2004). The internal taxonomy of the genus has changed substantially in recent years due to the application of molecular analyses for species identification (Kruskop et al., 2020). Several species have only recently been described (Mucedda et al., 2002; Benda et al., 2004; Spitzenberger et al., 2006; Dolch et al., 2021). One of these is the Ethiopian long-eared bat *Plecotus balensis* Kruskop & Lavrenchenko, 2000. It is the most southern member of the genus and the only Afrotropical species (Benda et al., 2004). It was first described from the Harenna Forest in the Bale Mountains National Park (Kruskop & Lavrenchenko, 2000). Subsequently, it was confirmed at additional sites in the Ethiopian Highlands including the Simien Mountains, Mount Abune Yosef, the Guassa Community Conservation Area, Chilallo Mountains, and several more sites in the Bale Mountains (Juste et al., 2004; Razgour et al., 2019). It is believed to be endemic to the Ethiopian Highlands, but its exact distribution is unknown so far.

The *Plecotus* species of Ethiopia (Rüppell, 1842, Shewa) and Eritrea (Sordelli, 1902; Senna, 1905; Asmara) have been first recognized as *P. auritus*, but later renamed as *P. austriacus*, most likely belonging to the subspecies *christii* Gray, 1838 (Largen et al., 1974). This subspecies was later elevated to full species *P. christii*. Yalden et al. (1996; specimen in the World Museum, Liverpool) reported *P. austriacus* from two sites in the Bale Mountains which most likely are *P. balensis* (Juste et al., 2004). Whether the specimen from Eritrea belongs to *P. balensis* or *P. christii* needs to be investigated (Kruskop & Lavrenchenko, 2000). Benda et al. (2011) recognize the Eritrean form as *P. balensis* and they tentatively assigned *Plecotus* specimens from Yemen as *Plecotus* cf. *balensis*. During a bat survey in central Ethiopia, we were able to trap several species, among them phenotypically *P. balensis*. The aim of our study was to verify the species identification with molecular methods and to verify their position within the *Plecotus* phylogeny.

Material and methods

Ethical statement

We obtained ethical approval from the Review Board of Addis Ababa University Institutes (IRB) for trapping bats and sacrificing and depositing a few individuals as voucher specimens in the Genetics Laboratory of the Department of Zoological Science at the University of Addis Ababa (Reference number: CNCSDO/181/14/2021).

Trapping

During a bat survey along the Ethiopian Rift Valley and adjacent highlands, we trapped bats at 55 sites in 12 regions (Fig. 1). We set up mist nets in and around roosting sites, such as caves, churches, and lodge sites. We used five mist nets, each 12 m long and 3 m wide. Mist netting was conducted between 7:00 pm and 9:00 pm for 7 to 13 nights per site. We tentatively identified the trapped bats in the field based on phenotype (Fig. 2) and morphological measurements. After measuring the bats, we immediately released them into their natural environment, except for some voucher specimens. From these specimens, we took tissue samples for a molecular analysis to verify phenotypic identification. We preserved the fresh tissue (liver, kidney, and heart) in 18 ml plastic tubes with 97% ethanol for further processing. The samples were shipped to the genetics lab of the German Primates Center for molecular species identification.

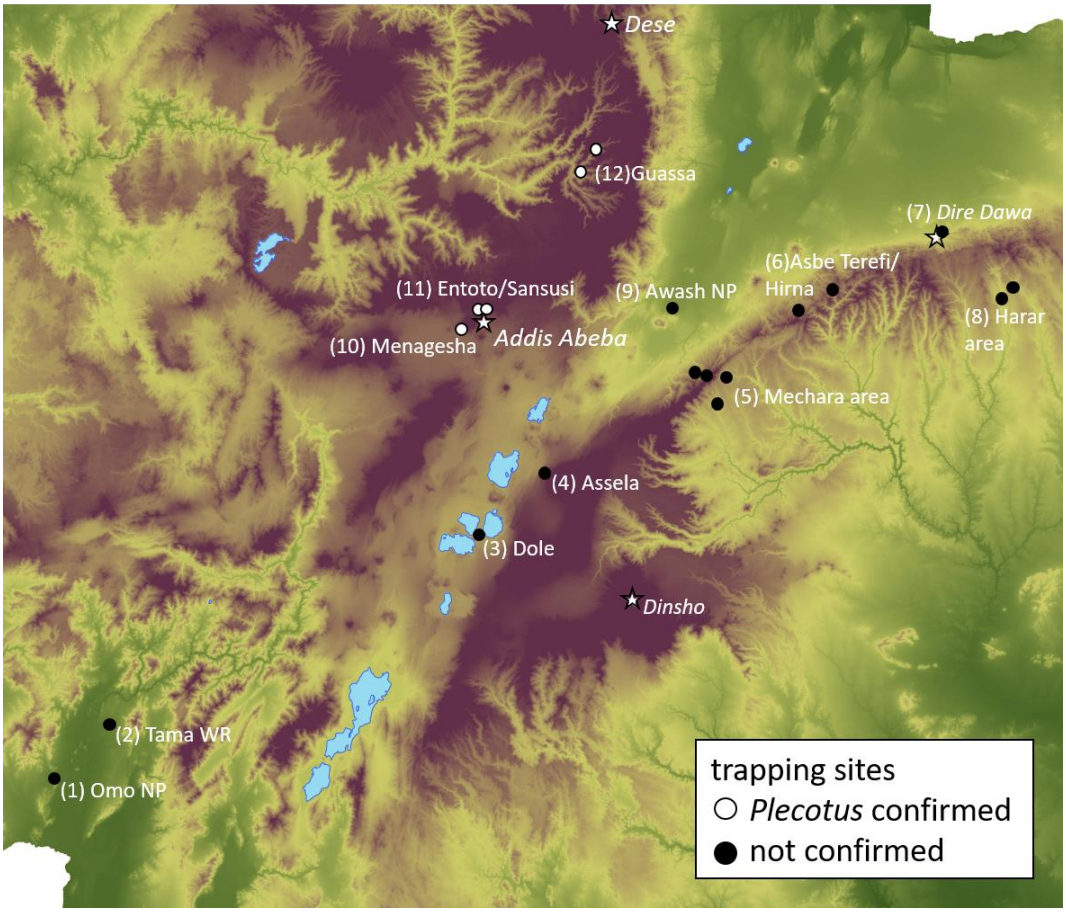

Figure 1. Bat trapping sites in 12 regions in central Ethiopia. *Plecotus balensis* was only trapped in regions 10-12. (Green colours indicate lower, brown colours higher altitude).

Figure 2. Pictures of the Ethiopian long-eared bat *Plecotus balensis* (a–f face views; g and h ventral views; i dorsal view; j lateral view). Voucher specimens have been deposited in the Genetics Laboratory of the Department of Zoological Science at the University of Addis Ababa, Ethiopia) (Photos by Ahmed Seid Ahmed)

Morphometrics

After capturing the bats, we placed them individually in cotton bags. We took morphometric measurements of the external morphology from those individuals that were sacrificed using rulers, ensuring measurements with adjustments made to the closest 0.1 cm. Body mass was taken with a Pesola spring scale.

DNA extraction and sequencing

We extracted DNA from tissue samples using the NucleoSpin Tissue Mini Kit (Macherey-Nagel) following the standard protocol of the supplier including the recommended sample digestion overnight at 56°C and 900 rpm (Thermomixer Comfort; Eppendorf). We amplified the complete mitochondrial cytochrome b gene (cytb, 1140 bp) with primers 5'-

AAAAAYCACCGTTGAYTTCAAC-3' and 5'-ATTACACTGGTCTTGTAAACCA-3' via polymerase chain reaction (PCR). The PCR reaction with a total volume of 30 µL contained 3 µL buffer (10× with 18 mM $MgCl_2$), 0.6 µL dNTPs (10 mM each), 1 µL of each primer (1 µM), 0.3 µL (1 U) FastStart HighFidelity Taq DNA polymerase (Roche), and 50 ng genomic DNA. Amplification was performed in a laboratory cycler (SensoQuest) with a pre-denaturation step at 94°C for 2 min, followed by 40 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 1.5 min, and terminated with a final extension step at 72°C for 5 min. PCR products were size-separated on 2% agarose gels, excised from the gel, purified with the Monarch DNA Gel Extraction Kit (New England Biolabs), and sent to Eurofins Genomics for Sanger sequencing using both amplification primers. Obtained sequence electropherograms were checked and manually corrected with SOFTWARE (Logsdon et al., 2011). Newly generated sequences have been deposited in GenBank (Accession Numbers PP212955 - PP212962).

Molecular species identification

For the molecular species identification, we compared our newly generated cytb haplotypes with sequences deposited in GenBank using the nucleotide Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi) of the National Library of Medicine (NCBI). Among the sequences with the closest matches, we found 33 unique haplotypes of *P. balensis*. To explore the phylogenetic affinities of the newly detected *P. balensis* specimens, we downloaded orthologous sequences of *P. balensis* and other *Plecotus* species from GenBank. A drawback of the phylogenetic reconstruction was the fact that for most closely related species, the available data consisted of only partial cytb sequences (e.g., *P. balensis* 650 bp, *P. christii* 522 bp), which even did not overlap completely. Therefore, the length of the resulting alignment was only 492bp. As an outgroup, we used closely related species of the Vespertilioninae *Barbastella leucomelas*, *Glauconycteris variegate*, and *Scotoecus hirundo* (Table 1). The complete alignment consisted of 68 sequences.

Table 1. Origin, sequence lengths (bp), and GenBank accession numbers of bat samples.

Taxon	Group	Length	Id / haplo	Provenance	Lat	Long	AccNum	Source
P. balensis	*austriacus*	1140	GU1 / 01	Guassa	10.29119	39.78599	PP212955	this study
P. balensis	*austriacus*	1140	ET59 / 03	Entoto	9.08922	38.76447	PP212956	this study
P. balensis	*austriacus*	1140	MF37 / 04	Menagesha	8.96730	38.54892	PP212957	this study
P. balensis	*austriacus*	1140	MF36 / 04	Menagesha	8.96730	38.54892	PP212958	this study
P. balensis	*austriacus*	1140	SF46 / 02	Sansusi	9.08155	38.69510	PP212959	this study
P. balensis	*austriacus*	1140	SF47 / 03	Sansusi	9.08155	38.69510	PP212960	this study
P. balensis	*austriacus*	1140	SF48 / 03	Sansusi	9.08071	38.69455	PP212961	this study
P. balensis	*austriacus*	1140	SF49 / 03	Sansusi	9.08071	38.69455	PP212962	this study
P. balensis	*austriacus*	650		Bale Mts			MW166392	[1]
P. balensis	*austriacus*	650		Bale Mts			MW166395	[1]
P. balensis	*austriacus*	650		Bale Mts			MW166396	[1]
P. balensis	*austriacus*	650		Bale Mts			MW166397	[1]
P. balensis	*austriacus*	650		Bale Mts			MW166398	[1]
P. balensis	*austriacus*	650		Bale Mts			MW166393	[1]
P. balensis	*austriacus*	650		Bale Mts			MW166394	[1]
P. balensis	*austriacus*	650		Bale Mts			MW166384	[1]
P. balensis	*austriacus*	650		Bale Mts			MW166383	[1]
P. balensis	*austriacus*	650		Bale Mts			MW166385	[1]
P. balensis	*austriacus*	650		Bale Mts			MW166386	[1]
P. balensis	*austriacus*	650		Bale Mts			MW166432	[1]
P. balensis	*austriacus*	650		Mt Chilalo			MW166402	[1]
P. balensis	*austriacus*	650		Guassa			MW166425	[1]
P. balensis	*austriacus*	650		Guassa			MW166430	[1]
P. balensis	*austriacus*	650		Guassa			MW166427	[1]
P. balensis	*austriacus*	650		Guassa			MW166426	[1]
P. balensis	*austriacus*	650		Guassa			MW166433	[1]
P. balensis	*austriacus*	650		Mt Abune			MW166417	[1]
P. balensis	*austriacus*	650		Mt Abune			MW166416	[1]
P. balensis	*austriacus*	650		Mt Abune			MW166420	[1]
P. balensis	*austriacus*	650		Mt Abune			MW166422	[1]
P. balensis	*austriacus*	650		Mt Abune			MW166423	[1]
P. balensis	*austriacus*	650		Mt Abune			MW166418	[1]
P. balensis	*austriacus*	680		Mt Abune			AF413798	[2]
P. balensis	*austriacus*	680		Mt Abune			AF413799	[2]
P. balensis	*austriacus*	650		Simien Mts			MW166405	[1]
P. balensis	*austriacus*	650		Simien Mts			MW166410	[1]
P. balensis	*austriacus*	650		Simien Mts			MW166409	[1]
P. balensis	*austriacus*	650		Simien Mts			MW166413	[1]
P. balensis	*austriacus*	650		Simien Mts			MW166408	[1]
P. balensis	*austriacus*	650		Simien Mts			MW166415	[1]
P. balensis	*austriacus*	650		Simien Mts			MW166404	[1]
P. balensis	*austriacus*	650		Simien Mts			MW166406	[1]
P. christii	*austriacus*	522		Egypt, Sinai			EU743799	[3]
P. christii	*austriacus*	522		Libya			EU743800	[3]
P. christii	*austriacus*	522		Jordan			EU743801	[3]
P. austriacus	*austriacus*	680		Spain			AF513788	[2]

P. austriacus	*austriacus*	680	Portugal	AF513786	[2]
P. kolombatovici	*austriacus*	680	Turkey	AF513785	[2]
P. teneriffae	*austriacus*	680	Canary Is.	EU360701	[4]
P. auritus	*auritus*	1140	Denmark	MT410875	[5]
P. auritus	*auritus*	1140	Denmark	MN122881	[5]
P. auritus	*auritus*	1140	Switzerland	OQ885414	[6]
P. auritus	*auritus*	1140	Switzerland	OQ885415	[6]
P. auritus	*auritus*	1140	?	OY734038	[7]
P. macrobullaris	*auritus*	1140	Italy	KR134353	[8]
P. macrobullaris	*auritus*	1140	Turkey	KR134407	[8]
P. macrobullaris	*auritus*	1140	Syria	KR134406	[8]
P. turkmenicus	*auritus*	1140	Mongolia	MT583351	[9]
P. kozlovi	*auritus*	1140	Mongolia	MT583349	[9]
P. ognevi	*auritus*	1140	Mongolia	MT583350	[9]
P. (*auritus*) *ognevi*	*auritus*	1140	Korea	HM164052	[10]
P. homochrous	*auritus*	1140	Vietnam	MN160089	[11]
P. homochrous	*auritus*	1140	Vietnam	MN160086	[11]
P. sacrimontis	*auritus*	1140	Japan	LC036641	[12]
P. (*auritus*) *sacrimontis*	*auritus*	1140	Japan	AB085734	[13]
Barbastella leucomelas		1140	China	KU922958	[14]
Glauconycteris variegata		1140	Senegal	JX276108	[15]
Scotoecus hirundo		1140		JX276317	[15]

1 Razgour et al., 2021; 2 Juste et al., 2004; 3 Benda et al., 2008; 4 Garcia-Mudarra et al., 2009; 5 Margaryan 2020, unpubl.; 6 Ruedi et al., 2023; 7 2023, unpubl.; 8 Alberdi et al., 2015; 9 Kruskop et al., 2020; 10 Hwang & Kim 2016, unpubl.; 11 Fukui et al., 2020; 12 Kawai 2015, unpubl.; 13 Sakai et al., 2003; 14 Liu 2017, unpupl.; 15 Koubínová et al., 2013

In a second approach, we reconstructed a phylogeny based on the complete cytb (1140 bp). However, due to the limited number of full-length cytb sequences, the alignment comprised only 27 sequences with our *P. balensis* sequences as the only representatives of the *austriacus* group (see supplement). Maximum-likelihood trees were reconstructed with IQ-tree 2.2.0 (Minh et al., 2020) using the best-fit model as obtained by ModelFinder (Kalyaanamoorthy et al., 2017) in IQ-tree under the Bayesian Information Criterion (BIC) and 1000 ultrafast bootstrap replications (Hoang et al., 2018). Best-fit models for the alignments containing 492 bp and 1140 bp were TPM2+F+I+G4 and K3Pu+F+I+G4, respectively. Trees were visualized and edited with FigTree 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/).

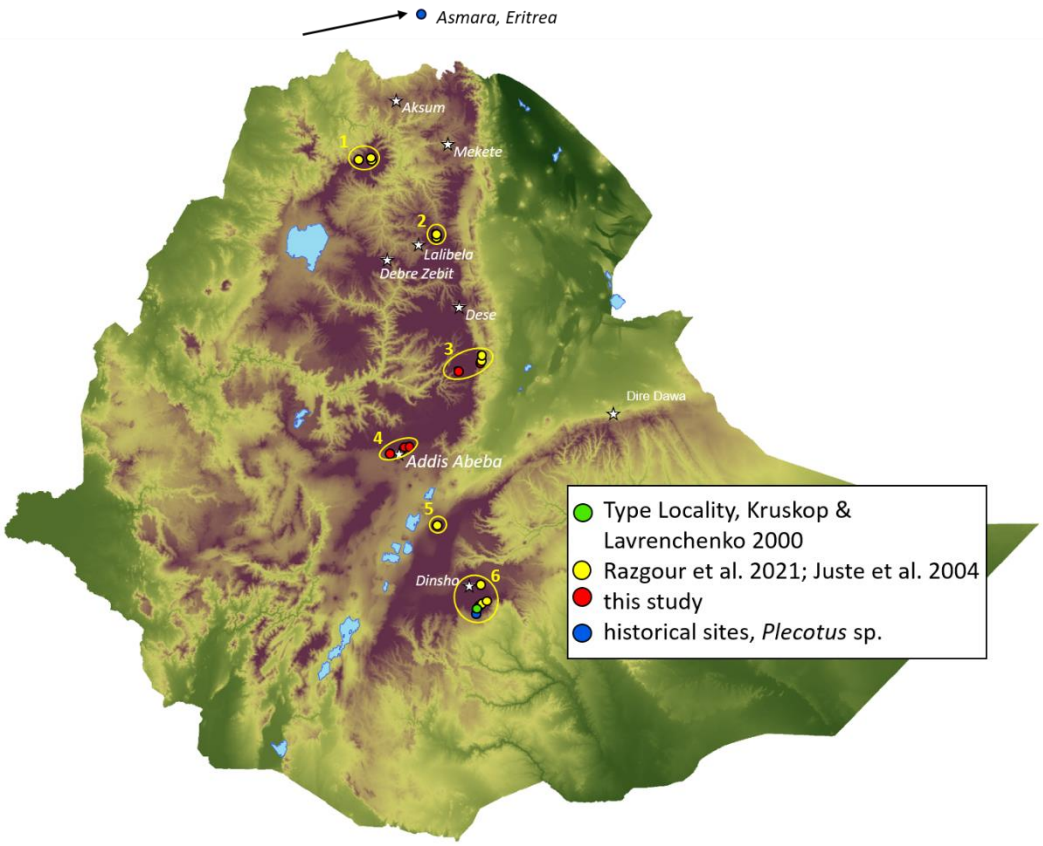

Figure 3. Distribution of Plecotus balensis in the Ethiopian highlands. The taxa at the historical sites have been listed as *Plecotus auritus*, *P. austriacus,* or *P. christii* (Sordelli 1902; Senna 1905; Largen et al., 1974; Yalden et al., 1996). (1) Simien Mountains; (2) Mount Abune Yosef; (3) Guassa Community Conservation Area; (4) Menagesha, Entoto, Sansusi; (5) Chilallo Mountains; (6) Bale Mountains. (Green colours indicate lower, brown colours higher altitude).

Results

In three of the 12 trapping areas, we caught *P. balensis*. These areas were in the central highlands of Ethiopia at altitudes between 2500 m and 3300 m [Menagasha (region 10), Entoto/Sansusi (region 11), Guassa CCA (region 12)] (Fig. 3). The average body size of our ten measured individuals tends to be slightly larger than that of the holotype (Table 2).

Table 2. Geographic coordinates (decimal degree) of origin and morphological measurements of ten *Plecotus balensis* individuals and the holotype (Kruskop and Lavrenchenko, 2000).

ID	Site	Latitude	Longitude	Sex	FA mm	TB mm	FL mm	EL mm	TL mm	BL mm	TR mm	WS mm	BM g	cytb
holo	Harenna	6.75000	39.73333	M	36.3	14.4	7.1/8.3	37	48	45	15.2			
ET59	Entoto	9.08922	38.76447	M	41.5	18	8.5	40	46	47	19	290	7.5	yes
MF36	Menagesha	8.96730	38.54892	F	40	17.5	9	39	51	49	19	285	8	yes
MF37	Menagesha	8.96730	38.54892	M	41	18	8	41	50	48.5	18.5	284	7.5	yes
MF39	Menagesha	8.96730	38.54892	M	40	18	8	40	47	51	19	284	7.7	no
SF46	Sansusi	9.08155	38.69510	M	41	19	9	37	41	53	18	282	8	yes
SF47	Sansusi	9.08155	38.69510	M	40	18.5	8.7	39	50	49	19	285	8.5	yes
SF48	Sansusi	9.08071	38.69455	F	39.5	17.5	8	41	48.5	48	18	287	7.5	yes
SF49	Sansusi	9.08071	38.69455	M	41	18.4	8.3	41.5	49	50	19	284	7.9	yes
GU1	Guassa	10.29119	39.78599	M	40	19	9	38	42	52	18.5	283	7.5	yes
GU3	Guassa	10.17490	39.47450	F	38	17.5	7.5	36.5	42	46	18	279	7	no
Mean					40.2	18.1	8.4	39.3	46.7	49.4	18.6	284.3	7.7	
SD					1.00	0.57	0.52	1.72	3.74	2.19	0.14	2.76	0.39	

FA = forearm length; TB = tibia length; FL = foot length; EL = ear length; TL = tail length: BL = body length; TR = tragus; WS = wingspan; BM = body mass.

Genetic taxon identification

We sequenced the complete cytb gene from eight individuals from which we retrieved four haplotypes. Using BLAST search, the greatest matches (99.5 and 99.6% identity, query cover 57% and 59%, accession length 650 bp and 680 bp, respectively) were found with the partial cytb sequences of *P. balensis* provided by Razgour et al. (2021) and Juste et al. (2004). The phylogenetic tree based on 492 bp (Fig. 4) confirms a well-supported monophyly for *P. balensis* and suggests *P. christii* as the sister taxon. As expected, *P. balensis* falls in the *austriacus* species group. Due to the short sequence lengths, our eight *P. balensis* sequences comprised only two haplotypes of which one (GU1) from Guassa was identical to haplotype Guassa02 of Razgour et al. (2021), whereas the second haplotype found at Sansusi, Entoto (region 11) and Menagesha (region 10) was distinct. The mitochondrial intra-specific phylogenetic relationships of *P. balensis* seem to be geographically ordered with a division into a southern, central, and northern deme. However, the statistical support for most of the nodes in the tree is weak.

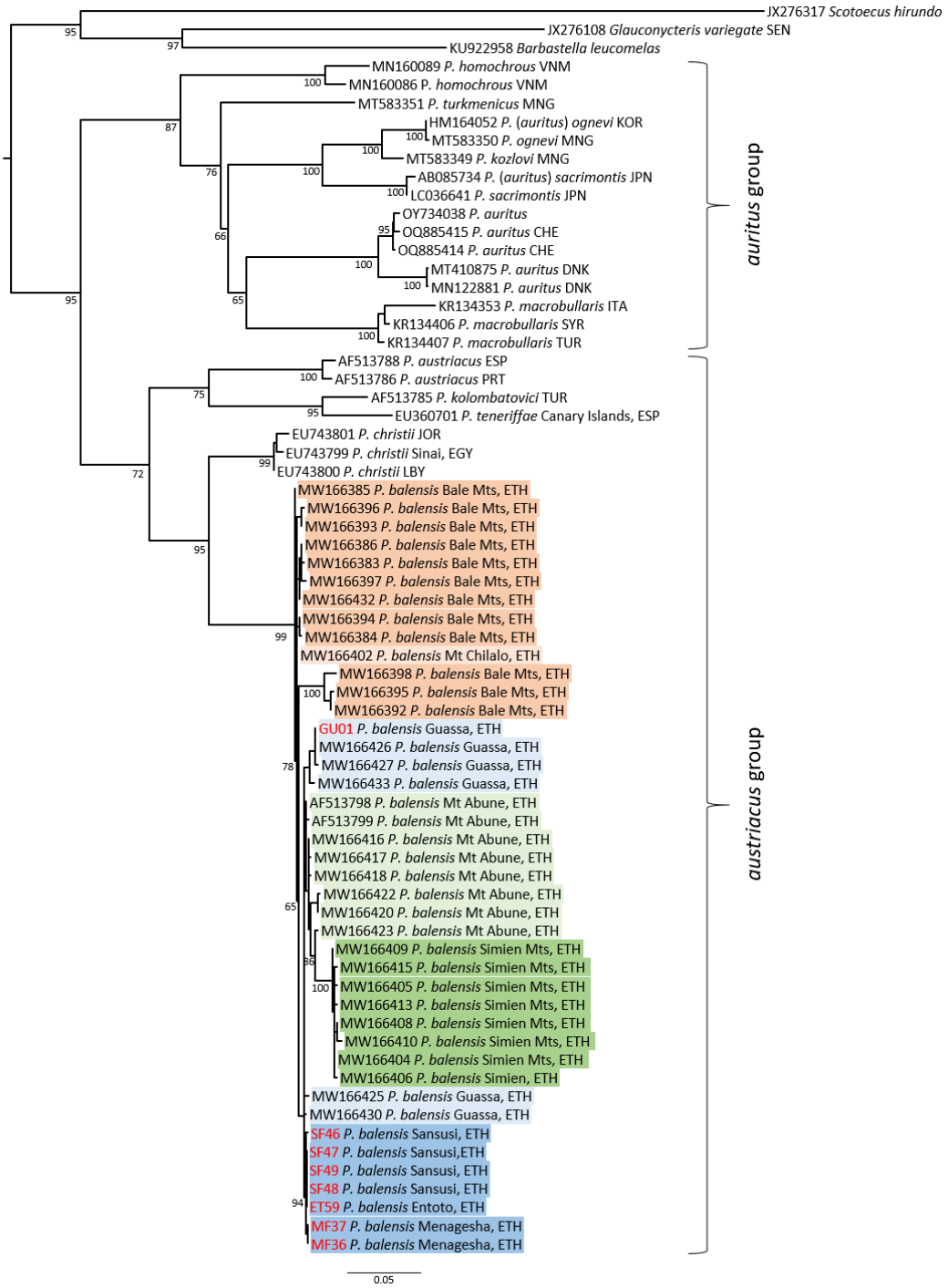

Figure 4. Phylogenetic relationships of the newly generated *Plecotus balensis* haplotypes (marked red) based on partial cytb sequences (492 bp). Node labels refer to ML bootstrap support values. Orange colours indicate northern samples, blue colours indicate central samples and green colours indicate southern samples. (CHE = Switzerland; DNK = Denmark; EGY = Egypt; ETH = Ethiopia; ESP = Spain; ITA = Italia; JOR = Jordan; JPN = Japan; KOR = South Korea; LBY = Libya; MNG = Mongolia; PRT = Portugal; SEN = Senegal; SYR = Syria; TUR = Turkey)

For the complete cytb sequences (1140 bp) we retrieved four haplotypes which form together a well-supported clade within the genus *Plecotus* (Fig. 5). In this phylogeny, the major division was between *P. balensis* as a representative of the *austriacus* group and taxa of the *auritus* group. Within the *auritus* group, we found four similarly well-supported clades. One consisted

of *Plecotus* taxa from central and northern Europe (*P. auritus*), a second from the Mediterranean and Near East region (*P. macrobullaris*), a third (*P. sacrimontis*), and fourth (*P. kozlovi*, *P. ognevi*) from East Asia. However, the deeper splits and the relationships of *P. turkmenicus* and *P. homochrous* within the clade are not well supported.

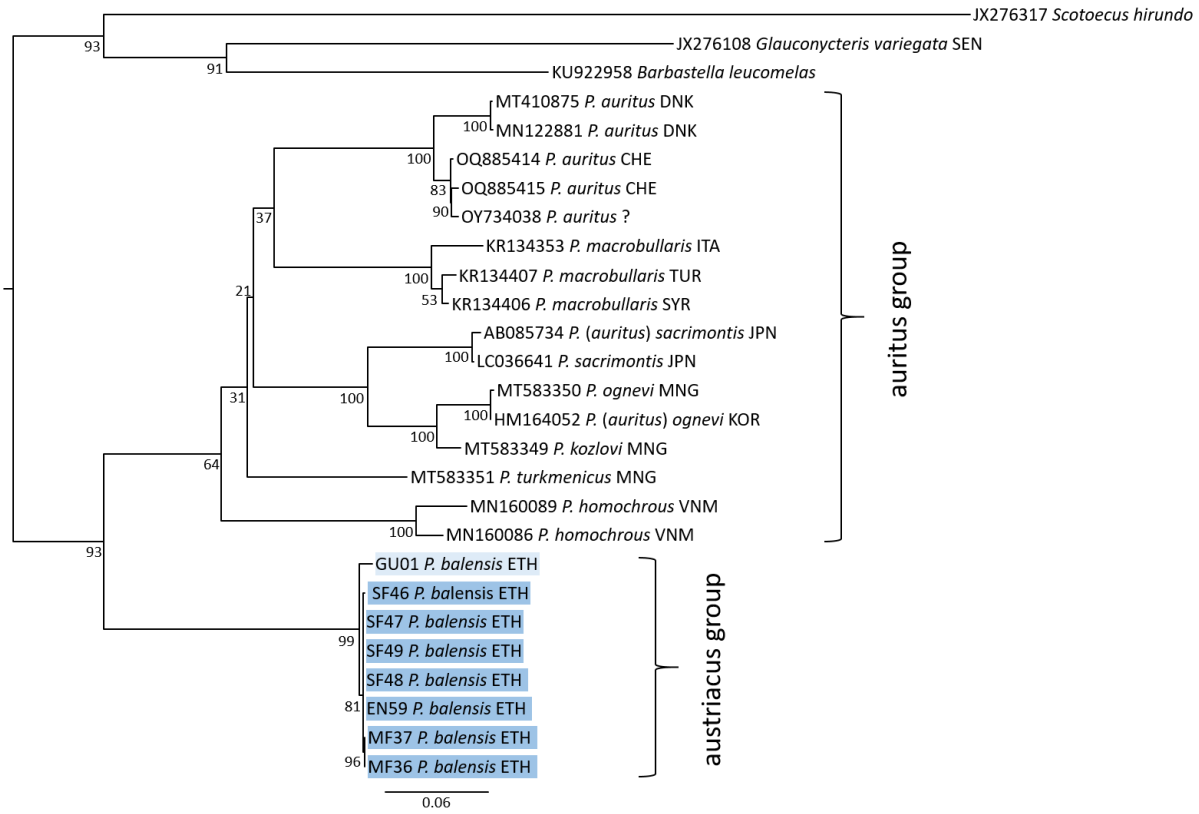

Figure 5. Phylogenetic relationships of *Plecotus* spp. mtDNA lineages based on complete cytb sequences (1140 bp). *Plecotus balensis* (marked blue) forms a strongly supported clade within the genus *Plecotus* but the relationships among the Plecotus clades are only weakly supported. Node labels refer to ML bootstrap support values. (CHE =Switzerland; DNK = Denmark; ETH = Ethiopia; ITA = Italia; JPN = Japan; KOR = South Korea; MNG = Mongolia; SEN = Senegal; SYR = Syria; TUR = Turkey; VNM = Vietnam)

Discussion

Our results correspond well with the previous studies on long-eared bats from Ethiopia and other regions. We confirmed the presence of *P. balensis* in areas 25 km west and 10 km north of the centre of Ethiopia's capital, Addis Abeba. This area is about 180 km and 200 km, respectively, southwest of the closest known site, Guassa CCA (Razgour et al. 2021). Rüppel (1842) collected *Plecotus auritus* (most likely *P. balensis*) in the historical "Shoa" (Shewa) region and our new *Plecotus* sites close to Addis Abeba are within this region. It would be interesting to obtain genetic information from the Rüppell specimen and the Bale Mountains specimen deposited at the museum in Liverpool (Yalden et al. 1996). In general, our samples add new mitochondrial haplotypes to the genetic diversity of *P. balensis* and support a

geographic pattern of haplotype distribution. It is most likely that the Ethiopian population divides into a southern, central, and northern deme. This pattern reminds us of the distribution of the genetic diversity of another Ethiopian high-altitude species, *Theropithecus gelada* (Zinner et al., 2018; Trede et al., 2020). However, since only relatively few mountain areas have been surveyed for the presence of *P. balensis*, this pattern might change and turn into a clinal genetic distribution. At least the ecological niche model by Razgour et al. (2021) suggests suitable habitats in several other mountainous areas of Ethiopia, e.g., Gojjam and Ahmar Mountains, and a much wider distribution of the species during the last glacial maximum. It remains to be investigated whether the distribution range of *P. balensis* extends further north into Eritrea and whether an area of sympatry of the two species exists.

Our *Plecotus* specimens tend to be slightly larger than the holotype, which comes from the Bale Mountains. Whether the *Plecotus* demes actually differ in body size needs to be further explored. On the other side, we took the morphometric measurement with a ruler, which is most likely more error-prone than other methods. Although our current analysis is mainly based on a relatively short part of the cytb gene, it nevertheless strongly supports the identification of our samples as *P. balensis*. Our phylogenetic reconstruction indicates a sister relationship with *P. christii*, which is not surprising, given that it is geographically the closest species. However, here we need to be cautious, because of the limited genetic information. The Ethiopian long-eared bat is currently classified as Data Deficient by the IUCN (Lavrenchenko et al. 2019). A denser sampling of *Plecosus* in Ethiopia would improve the information on the status of this and other species of the genus. Additionally, sampling in Eritrea, northeastern Sudan, and the western mountains of the Arabian Peninsula and the inclusion of additional genetic markers would be important, and essential to answer open questions about the phylogeny of long-eared bats in the region around the Red Sea.

Acknowledgements

We are grateful to the Ethiopian Wildlife Conservation Authority (EWCA) for granting permission to conduct this research. We also thank the joint Ethiopian-Russian Biological Expedition team who provided support during the first phase field in the Menagesha Forest and the scouts, officers, and personnel at each national park and trapping site for their valuable assistance in data collection. The research was supported by the Addis Ababa University Department of Zoological Sciences and the Hawassa University NORAD Project (Ref. No. AVP/05177/15). We also thank two anonymous reviewers.

References

Alberdi, A., Gilbert, M. T. P., Razgour, O., Aizpurua, O., Aihartza, J., & Garin, I. (2015). Contrasting population-level responses to Pleistocene climatic oscillations in an alpine bat revealed by complete mitochondrial genomes and evolutionary history inference. Journal of Biogeography. 42(9): 1689-1700. https://doi.org/10.1111/jbi.12535

Benda, P., Kiefer, A., Hanák, V., & Veith, M. (2004). Systematic status of African populations of long-eared bats, genus *Plecotus* (Mammalia: Chiroptera). Folia Zoologica. 53(1): 2-47. https://doi.org/10.3161/001.006.0202

Benda, P., Dietz, C., Andreas, M., Hotový, J., Lučan, R. K., Maltby, A., Truscot, J., & Vallo, P. (2008). Bats (Mammalia: Chiroptera) of the Eastern Mediterranean and Middle East. Part 6. Bats of Sinai (Egypt) with some taxonomic, ecological and echolocation data on that fauna. Acta Societatis Zoologicae Bohemicae. 72: 1–103.

Benda, P., Al-Jumaily, M.M., Reiter, A., & Nasher, A.K. (2011). Noteworthy records of bats from Yemen with description of a new species from Socotra. Hystrix, the Italian Journal of Mammalogy. 22(1). https://doi.org/10.4404/hystrix-22.1-4473

Dolch, D., Stubbe, M., Gärtner, B., Thiele, K., Ariunbold, J., Batsaikhan, N., Lkhagvasuren, D., Stubbe, A., & Steinhauser, D. (2021). Phylogenie, Morphologie und Ökologie mongolischer Langohrfledermäuse der Gattung *Plecotus* (Mammalia, Chiroptera, Vespertilionidae). Erforschung biologischer Ressourcen der Mongolei / Exploration into the Biological Resources of Mongolia. 14: 123-185.

Fukui, D., Tu, V. T., Thanh, H. T., Arai, S., Harada, M., Csorba, G., & Son, N. T. (2020). First record of the genus *Plecotus* from Southeast Asia with notes on the taxonomy, karyology and echolocation call of *P. homochrous* from Vietnam. Acta Chiropterologica. 22(1): 57-74. https://doi.org/10.3161/15081109ACC2020.22.1.006

García-Mudarra, J. L., Ibáñez, C., & Juste, J. (2009). The Straits of Gibraltar: barrier or bridge to Ibero-Moroccan bat diversity? Biological Journal of the Linnean Society. 96(2): 434-450. https://doi.org/10.1111/j.1095-8312.2008.01128.x

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., & Vinh, L.S. (2018). UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution. 35(2): 518–522. https://doi.org/10.1093/molbev/msx281

Juste, J., Ibáñez, C., Muñoz, J., Trujillo, D., Benda, P., Karataş, A., & Ruedi, M. (2004). Mitochondrial phylogeography of the long-eared bats (*Plecotus*) in the Mediterranean Palaearctic and Atlantic Islands. Molecular Phylogenetics and Evolution. 31(3): 1114-1126. https://doi.org/10.1016/j.ympev.2003.10.005

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., & Jermiin, L.S. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods. 14(6): 587-589. https://doi.org/10.1038/nmeth.4285

Koubínová, D., Irwin, N., Hulva, P., Koubek, P., & Zima, J. (2013). Hidden diversity in Senegalese bats and associated findings in the systematics of the family Vespertilionidae. Frontiers in Zoology. 10(1): 48. https://doi.org/10.1186/1742-9994-10-48

Kruskop, S.V., & Lavrenchenko, L.A. (2000). A new species of long-eared bat (*Plecotus*; Vespertilionidae, Mammalia) from Ethiopia. Myotis. 38: 5-19.

Kruskop, S.V., Artyushin, I.V., Yuzefovich, A.P., Undrakhbayar, E., Speranskya, A.S., Lisenkova, A.A., Bannikova, A.A., & Lebedev, V.S. (2020). Genetic diversity of Mongolian long-eared bats (Plecotus; Vespertilionidae; Chiroptera). Acta Chiropterologica., 22(2):, 243-255. https://doi.org/10.3161/15081109ACC2020.22.2.002

Largen, M.J., Kock, D., & Yalden, D.W, (1974). Catalogue of the mammals of Ethiopia. 1. Chiroptera. Monitore Zoologico Italiano (Ital. J. Zool.) NS, Suppl. 5, 16: 221-298.

Lavrenchenko, L., Juste, J. and Benda, P. 2019. *Plecotus balensis*. The IUCN Red List of Threatened Species 2019: e.T44930A22045794.

https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T44930A22045794.en. Accessed on 12 February 2024.

Logsdon, M. E., Trounstine, M. C., & Zianni, M. R. (2011). Analysis of the DNA sequencing quality and efficiency of the Apollo100 robotic microcycler in a core facility setting. Journal of Biomolecular Techniques: JBT: 22(2): 53.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler A., & Lanfear R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution. 37(5): 1530-1534. https://doi.org/10.1093/molbev/msaa015

Mucedda, M., Kiefer, A., Pidinchedda, E., & Veith, M. (2002). A new species of long-eared bat (Chiroptera, Vespertilionidae) from Sardinia (Italy). Acta Chiropterologica. 4(2): 121-135. https://doi.org/10.3161/001.004.0202

Razgour, O., Kasso, M., Santos, H., & Juste, J. (2021). Up in the air: Threats to Afromontane biodiversity from climate change and habitat loss revealed by genetic monitoring of the Ethiopian Highlands bat. Evolutionary Applications. 14(3): 794-806. https://doi.org/10.1111/eva.13161

Ruedi, M., Manzinalli, J., Dietrich, A., & Vinciguerra, L. (2023). Shortcomings of DNA barcodes: a perspective from the mammal fauna of Switzerland. Hystrix, the Italian Journal of Mammalogy. 34(1): 54-61. https://doi.org/10.4404/hystrix-00628-2023

Rüppell, E. (1842). Verzeichnis der in dem Museum der Senckenbergischen naturforschenden Gesellschaft aufgestellten Sammlungen. Erste Abtheilung: Säugethiere und deren Skelette. Museum Senckenberg. 3: 145-196.

Sakai, T., Kikkawa, Y., Tsuchiya, K., Harada, M., Kanoe, M., Yoshiyuki, M., & Yonekawa, H. (2003). Molecular phylogeny of Japanese Rhinolophidae based on variations in the complete sequence of the mitochondrial cytochrome b gene. Genes & Genetic Systems. 78(2): 179-189. https://doi.org/10.1266/ggs.78.179

Senna, A. (1905). Contributo alla conoscenza dei Chirotteri Eritrei. Archivio zoologico: pubblicato sotto gli auspicii della unione zoologica italiana. 2(3): 249-308.

Sordelli, F. (1902). Materiali per Ia conoscenza della fauna Eritrea, raccolti dal Dott. Paolo Magretti. Atti della Societá italiana di Scienze naturali. 41: 49-99.

Spitzenberger, F., Strelkov, P.P., Winkler, H., & Haring, E. (2006). A preliminary revision of the genus *Plecotus* (Chiroptera, Vespertilionidae) based on genetic and morphological results. Zoologica Scripta. 35(3): 187-230. https://doi.org/10.1111/j.1463-6409.2006.00224.x

Trede, F., Lemkul, A., Atickem, A., Beehner, J.C., Bergman, T.J., Burke, R., Fashing, P.J., Knauf, S., Mekonnen, A., Moges, A., Nguyen, N., Roos, C., & Zinner, D. (2020). Geographic distribution of microsatellite alleles in geladas (Primates, Cercopithecidae): Evidence for three evolutionary units. Zoologica Scripta. 46(6): 659-667. https://doi.org/10.1111/zsc.12451

Yalden, D.W., Largen, M.J., Kock, D., & Hillman, J.C. (1996). Catalogue of the mammals of Ethiopia and Eritrea. 7 revised checklist, zoogeography and conservation. Tropical Zoology. 9: 73-164. https://doi.org/10.1080/03946975.1996.10539304

Zinner, D., Atickem, A., Beehner, J.C., Bekele, A., Bergman, T.J., Burke, R., Dolotovskaya, S., Fashing, P.J., Gippoliti, S., Knauf, S., Knauf, Y., Mekonnen, A., Moges, A., Nguyen, N., Stenseth, N.C., & Roos, C. (2018). Phylogeography, mitochondrial DNA diversity, and demographic history of geladas (*Theropithecus gelada*). PLoS ONE. 13(8): e0202303. https://doi.org/10.1371/journal.pone.0202303