Mammalian Biology
https://doi.org/10.1007/s42991-021-00120-7

ORIGINAL ARTICLE

The phylogenetic relationships within the Eastern Afromontane clade of *Crocidura* based on mitochondrial and nuclear data

Anna A. Bannikova[1] · Elena D. Zemlemerova[2] · Vladimir S. Lebedev[3] · Leonid A. Lavrenchenko[2]

Received: 24 December 2020 / Accepted: 22 March 2021

Abstract

Eastern Afromontane region is a well-known hotspot of biodiversity and endemism; however, the relationships between groups of organisms inhabiting different highland areas are still poorly understood. Herein, the phylogenetic relationship between endemic *Crocidura* from Ethiopia and Tanzania was assessed using mitochondrial and nuclear data. At the genus scale, all analyses supported the monophyly of the Eastern Afromontane clade. Within this clade, most of the Ethiopian endemics form a group, which is paraphyletic relative to a more compact Tanzanian clade. The Ethiopian *C. macmillani* was found to be closely related to Tanzanian species. In fact, according to the mitochondrial DNA data, it may be a descendant of the *C. montis–C. luna* clade. The molecular dating results suggest that the Ethiopian–Tanzanian clade diverged at ca. 3.4 Mya while the onset of radiation within the clade corresponds to Early Pleistocene (ca. 2 Mya). The inferred phylogenetic pattern is consistent with the scenario that has Ethiopia as the primary centre of diversification for the Eastern Afromontane clade. The areas southwards from Ethiopia were found to be colonized through a single dispersal event at 1.3–0.7 Mya; the distribution of *C. macmillani* might be explained by a secondary re-colonization of Ethiopia. Collectively, the nuclear and mitochondrial data revealed a low divergence between morphologically distinct and elevationally parapatric *C. thalia* and *C. glassi*, thereby aligning with the gradient model of speciation.

Keywords Eastern Afromontane region · White-toothed shrew · *Crocidura* · Phylogeny · Molecular clock

Introduction

The Eastern Afromontane biodiversity hotspot (EABH) is one of the globally important centres of species richness and endemism. In fact, it has the second highest number of endemic species of higher vertebrates on Earth, after Madagascar (Mittermeier et al. 2011). The EABH is composed of a discontinuous and divided chain of several mountain ranges, spreading from Saudi Arabia and Yemen down to Mozambique and Zimbabwe. The largest block of the EABH is formed by the Ethiopian Plateau (EP), which is divided by the Great Ethiopian Rift Valley into a main north-western massif (western plateau) and a smaller south-eastern mountain range (eastern plateau). The north-western massif is further divided by several deep river valleys, such as the Blue Nile Valley. The diversity and uniqueness of the Ethiopian fauna can be connected to specific features of the EP, including its isolated position, pronounced altitudinal zonation, extremely diverse geomorphology, and drastic environmental changes in the past. In accordance with the complex relief and environmental heterogeneity,

Handling editor: Allan McDevitt.

✉ Anna A. Bannikova
hylomys@mail.ru

Elena D. Zemlemerova
zemlemerovalena@ya.ru

Vladimir S. Lebedev
wslebedev@gmail.com

Leonid A. Lavrenchenko
llavrenchenko@gmail.com

[1] Lomonosov Moscow State University, Leninskie Gory 1, 119991 Moscow, Russia

[2] Severtsov Institute of Ecology and Evolution, RAS, Leninskii pr. 33, Moscow 119071, Russia

[3] Zoological Museum of Moscow State University, B. Nikitskaya 6, Moscow 125009, Russia

Published online: 05 April 2021

the fauna of the country is characterized by a high level of endemism. Currently, 55 mammalian species are considered to be endemic to Ethiopia; however, this list is by no means exhaustive (Lavrenchenko and Bekele 2017). The small mammal fauna is particularly unique. For example, the proportion of rodent species occurring only in Ethiopia is 41% if the entire country is considered but 90% if only the taxa living in the Afromontane ecosystems of the EP are considered (Bryja et al. 2019a).

The small mammals from the EP are particularly suitable for the studies focusing on the modes and tempo of speciation. Based on the fast habitat destruction in Ethiopia, taxonomic and evolutionary studies on Ethiopian small mammals are especially important and urgent. Recently, assessments of the phylogenetic structure of some species groups of small mammals that are endemic to Ethiopia were carried out (Lavrenchenko and Verheyen 2005; Lavrenchenko et al. 2004, 2014, 2017; Šumbera et al. 2018; Bryja et al. 2019b; Konečný et al. 2020; Mizerovská et al. 2020). The results of these studies demonstrated that although most diversification events can be explained by a classical allopatric model, there are indications of ecological (gradient) speciation at an elevational gradient (Lavrenchenko 2011; Bryja et al. 2018). The latter model proceeds from the possibility of diversifying forms (without complete interruption of the gene flow between them), leading up to complete speciation induced by different directions of selection along a strong environmental gradient (Smith et al. 1997; Orr and Smith 1998; Moritz et al. 2000; Nosil 2012; Linck et al. 2020a, b; Couvreur et al. 2020). The EP was also identified as the cradle for some groups of small mammals (*Lophuromys flavopunctatus* species complex, genera *Dendromus* and *Tachyoryctes*), where they radiated. These groups could also colonize other parts of the EABH from the EP (Lavrenchenko et al. 2007, 2017; Šumbera et al. 2018).

Thirty of the 32 shrew species of the family Soricidae, which is known to exist in Ethiopia, belong to the genus, *Crocidura*. Among them, 12 species (40% of the total) are currently considered to be endemic to the country and all are forest (7 species) or Afroalpine (5 species) inhabitants (Lavrenchenko et al. 2016; Konečný et al. 2020). Therefore, the Ethiopian endemic shrew fauna consists of forest and montane *Crocidura* species known to occur only within the altitudinal range of 1200–4050 m a.s.l. Accordingly, local endemism may be rather high. In fact, of the six *Crocidura* species known from the Bale Massif (eastern plateau), four are endemic to the south-eastern highlands (Hutterer and Yalden 1990). The remarkable number of endemic *Crocidura* species clearly demonstrates that the EP is an important centre of high diversity and adaptive radiation for this genus.

Available cytogenetic data suggest that most Ethiopian endemic *Crocidura*, possessing a diploid number of 36 chromosomes (Lavrenchenko et al. 1997; Bannikova et al. 2001), are derived from an ancestral Palaearctic branch of the genus. Several studies based on repetitive DNA elements (Bannikova et al. 2001, 2005) and mitochondrial cytochrome *b* gene sequences (Lavrenchenko et al. 2009) revealed that 36-chromosomal species that are endemic to Ethiopia form a monophyletic group of relatively recent origin. Moreover, this analysis revealed a high degree of similarity (only 0.5% of nucleotide substitutions in the cytochrome *b* gene) between the mitochondrial genomes of endemic *Crocidura glassi* Heim de Balsac, 1966, and *C. thalia* Dippenaar, 1980, which can be explained by the ecological (gradient) model of speciation (Lavrenchenko 2011). Currently, there are no studies based on sequences of nuclear genes for Ethiopian *Crocidura* species. Furthermore, the relationship between *Crocidura* species endemic to Ethiopia and congeners inhabiting other mountains of the EABH, south of the EP, remains unclear.

The aims of this study were (a) to reconstruct the phylogenetic relationships between *Crocidura* species of Eastern Afromontane region with an emphasis on the species endemic to Ethiopia and Tanzania based on the most comprehensive multi-locus dataset of DNA sequences to date; (b) to estimate the time of divergence for the main lineages; (c) to prove the hypothesis that the EP is a cradle for the *Crocidura* species group endemic to the EABH; and (d) to estimate the level of genetic divergence between *C. glassi* and *C. thalia*, morphologically distinct species with elevational parapatry.

Materials and methods

Taxon sampling

The original sample used in our phylogenetic study included 41 specimens of nine species (seven of which are endemic to the EP) of the genus *Crocidura*, which were collected from 25 sites in 9 localities throughout Ethiopia and an adjacent area after extensive trapping during the Joint Ethio-Russian Biological Expedition (JERBE) held from 1995 to 2017. Seven specimens of five species from Eurasia were also included in the study (Fig. 1, Table 1). All voucher specimens were deposited in the Zoological Museum of Moscow (Lomonosov) State University (ZMMU). Standard external body measurements (head and body length, tail length, hind foot length, and weight) were recorded in the field. The intial species identification was performed based on the morphological criteria and then using the barcoding method derived from a comparative analysis of sequences of *cytb* with the GenBank database.

The dataset consisted mainly of species from the Eastern Afromontane region including *C. bottegi* Thomas, 1898, *C. macmillani* Dollman, 1915, *C. baileyi* Osgood, 1936, *C.*

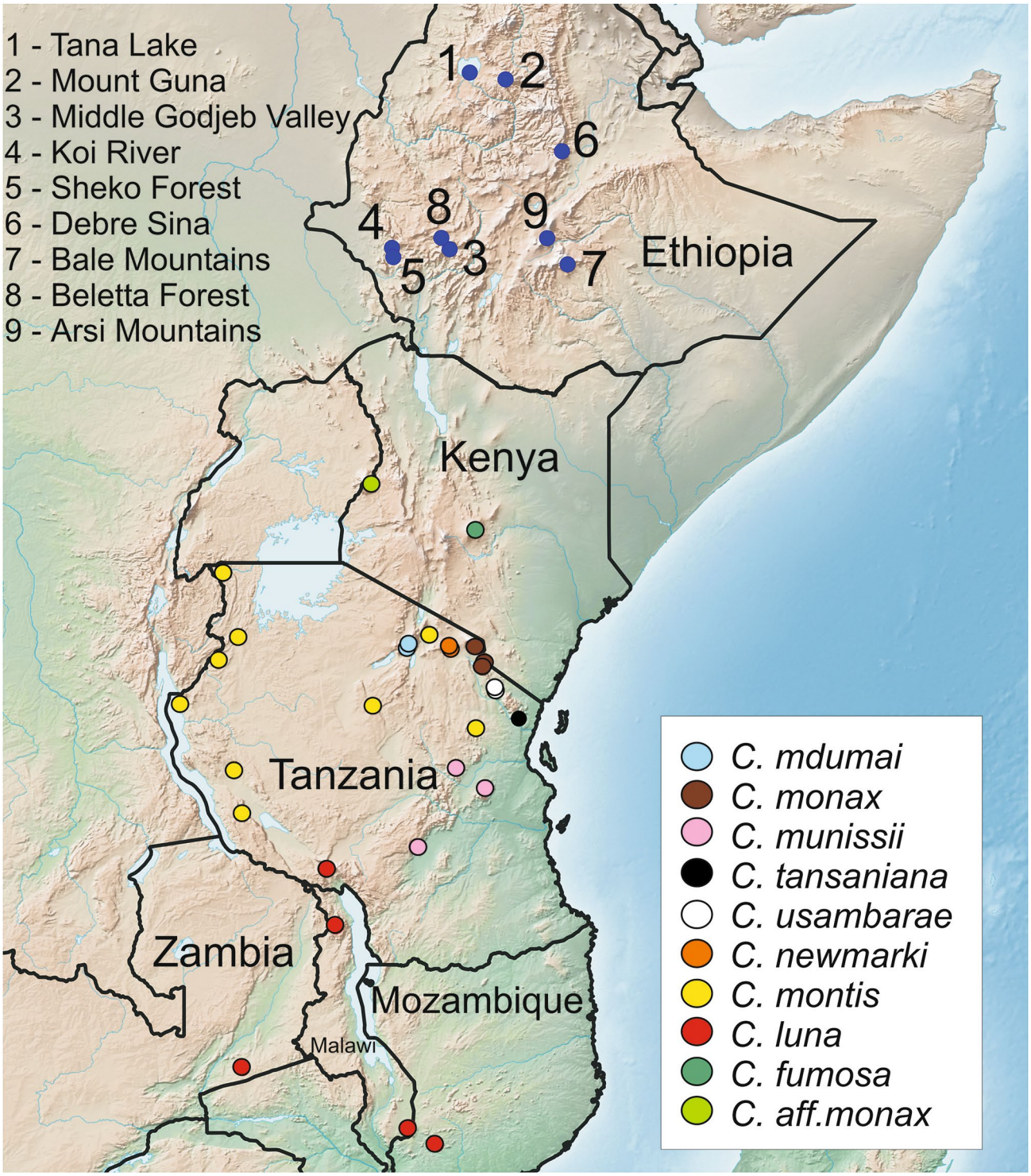

Fig. 1 The geographic distribution of sampling localities in Ethiopia (our material), Tanzania (by Stanley et al. 2015), and other countries of Eastern Afromontane regions (by Sabuni et al. 2018). The locality names and detailed geographic information for Ethiopian species are presented in Table 1

harenna Hutterer and Yalden, 1990, *C. lucina* Dippenaar, 1980, *C. yaldeni* Lavrenchenko, Voyta and Hutterer, 2016, *C. thalia* Dippenaar, 1980, *C. glassi* Heim de Balsac, 1966, *C. montis* Thomas, 1906, *C. usambarae* Dippenaar, 1980, *C. tanzaniana* Hutterer, 1986, *C. monax* Thomas, 1910, *C. newmarki* Stanley, Hutterer, Giarla and Esselstyn 2015, *C.*

Table 1 List of original material used in the study

No.	Species	Field/tissue and Figs. 2, 3, S1 code	Museum collection code	Collecting site
1	*C. olivieri*	oliv 1253	S-176006	(1) Tana Lake, Dega-Istefanos (N 11.896; E 37.310; 1802 m ASL)
2		oliv 1126	S-172813	(1) Vanzaye, shore of the Gumara River (N 11.717; E 38.250; 1750 m ASL)
3	*C. parvipes*	parv 733	S-166027	(3) Western Plateau, Middle Godjeb Valley (N 7.250; E 36.783; 1220 m ASL)
4		parv 740	S-166030	
5		parv 742	S-166032	
6		parv 898	S-167212	(4) Koi River, 37 km SW of the Bebeka Coffee Farm (N 7.283; E 35.266; 1130 m ASL)
7		parv 899	S-167213	
8	*C. macmillani*	macmill 734	S-166029	(3)Western Plateau, Middle Godjeb Valley (N 7.250; E 36.783; 1220 m ASL)
9		macmill 933	S-167293	(5) Western Plateau, Sheko Forest (N 7.04; E 35.30; 1930 m ASL)
10		macmill 741	S-166031	(3) Western Plateau, Middle Godjeb Valley (N 7.250; E 36.783; 1220 m ASL)
11	*C. lucina*	Clu 1905	S-189286	(6) Western Plateau, Debre Sina (N 9.826; E 39.735; 3233 m ASL)
12		Clu 2379	S-192709	(7a) Eastern Plateau, Bale Mountains, Sanetti Plateau (N 6.851; E 39.883; 4110 m ASL)
13		Clu 2393	S-192707	
14		EthS1	–	(7b) Eastern Plateau, Bale Mountains, Sodota (N 7.000; E 39.683; 3500 m ASL)
15	*C. harenna*	Char 2295	S-192705	(7c) Eastern Plateau, Bale Mountains, Katcha (N 6.700; E 39.773; 2190 m ASL)
16		Char 2316	S-192706	(7d) Eastern Plateau, Bale Mountains (N 6.731; E 39.718; 2502 m ASL)
17	*C. baileyi*	1089	S-172690	(2) Mount Guna near Yitba (N 11.717; E 38.250; 3800 m ASL)
18	*C. bottegi*	Cbot 1924	S-189291	(6) Western Plateau, Debre-Sina (N 9.826; E 39.735; 3233 m ASL)
19	*C. yaldeni*	CspB30	S-165342	(8) Western Plateau, Beletta Forest (N 7.547; E 36.564; 1900 m ASL)
20		CspB35	S-165343	
21		Csp19	S-165340	
22	*C. glassi*	Cgl 378	S-164856	(7e) Eastern Plateau, Bale Mountains, Dinsho area (N 7.100; E 39.768; 3170 m ASL)
23		Cgl 2103	S-190445	(9a) Eastern Plateau, Arsi Mountains, Shirka Area (N 7.540; E 39.345; 3294 m ASL)
24		Cgl 2258	S-192690	(7f) Eastern Plateau, Bale Mountains, Rira area (N 6.743; E 39.717; 2610 m ASL)
25		Cgl 2324	S-192708	(7 g) Eastern Plateau, Bale Mountains, Sanetti Plateau (N 6.788; E 39.766; 3730 m ASL)
26		Cgl 2383	S-192710	(7a) Eastern Plateau, Bale Mountains, Sanetti Plateau (N 6.851; E 39.883; 4110 m ASL)
27		Cgl 2850	S-197815	(9b) Eastern Plateau, Arsi Mountains, Shirka area (N 7.533; E 39.333; 3294 m ASL)
28		Cgl 2859	S-197816	(9c) Eastern Plateau, Arsi Mountains, Badda area (N 7.825; E 39.414; 3791 m ASL)
29		Cgl 2860	S-197817	
30		Cgl 2865	S-197818	
31		Cgl 2866	–	
32		Cgl 2886	S-197819	(9d) Eastern Plateau, Arsi Mountains, eastern slope of the Galama ridge (N 7.813; E 39.463; 3100 m ASL)
33		Cgl 2888	S-197820	
34		Cgl 03	pers.coll	(9e) Eastern Plateau, Arsi Mountains, western slope of the Chilalo Mt. (N 7.928; E 39.218; 3700 m ASL)
35		Cgl 15	pers.coll	(9f) Eastern Plateau, Arsi Mountains, western slope of the Chilalo Mt. (N 7.917; E 39.200; 3400 m ASL)
36		Cgl 16	pers.coll	(9e) Eastern Plateau, Arsi Mountains, western slope of the Chilalo Mt. (N 7.928; E 39.218; 3700 m ASL)

Table 1 (continued)

No.	Species	Field/tissue and Figs. 2, 3, S1 code	Museum collection code	Collecting site
37	*C. thalia*	Cth 468	S-165165	(7 h) Eastern Plateau, Bale Mountains, Shawei River, Harenna Forest (N 6.633; E 39.733;1935 m ASL)
38		Cth 473	S-165166	
39		Cth 06	pers.coll	(9 g) Eastern Plateau, Arsi Mountains, western slope of the Galama ridge (N 7.913; E 39.321; 3400 m ASL)
40		Cth 12	pers.coll	(9 h) Eastern Plateau, Arsi Mountains, western slope of the Galama ridge (N 7.907; E 39.370; 3885 m ASL)
41		Cth 13	pers.coll	(9i) Eastern Plateau, Arsi Mountains, western slope of the Chilalo Mt. (N 7.949; E 39.203; 3200 m ASL)
42	*C. russula*	Spain2	S-197238	Spain, Andalusia, Sierra Nevada
43		Spain1	S-197237	
44	*C. leucodon*	Crimea12-19	S-196948	Crimea, Feodosia
45		907A	ZMMU	Russia, Dagestan Republic
46	*C. suaveolens*	priaz12-1	ZMMU	Russia, Krasnodar region
47	*C. lasiura*	NED171/2004	pers.coll	Russia, Khabarovsk reg., Lazovsky district
48	*Diplomesodon pulchellum*	01,122,015	ZMMU	Astrakhan region, Aktyubinsk, Dosang

munissii Stanley, Hutterer, Giarla and Esselstyn 2015, *C. mdumai* Stanley, Hutterer, Giarla and Esselstyn 2015, *C. fumosa* Thomas, 1904, and *C. luna* Dollman, 1910.

In addition to our original material, a further 118 sequences of *cytb* and 151 sequences of the eight nuclear genes belonging to different African and Asian species of *Crocidura* and four species of *Suncus* with special attention to the Tanzanian sample, were downloaded from GenBank (Table S1) for inclusion in the final dataset.

DNA extraction, PCR amplification, and sequencing

Genomic DNA from ethanol-preserved tissues was extracted using a standard protocol consisting of proteinase K digestion, phenol–chloroform deproteinization, and isopropanol precipitation (Sambrook et al. 1989). We sequenced the mitochondrial cytochrome *b* gene (*cytb*) and portions of eight independent nuclear loci: the autosomal exon, Apolipoprotein B (*ApoB*); recombination-activating gene 1 (*RAG1*); Willebrand factor exon 28 (*vWF*); exon 11 of the breast cancer type 1 susceptibility protein (*BRCA1*); exon 10 of the growth hormone receptor (*GHR*); brain-derived neurotrophic factor (*BDNF*); prostaglandin E receptor 4 (*PTGER4*) gene; and the autosomal Mast Cell Growth Factor Introns 5–6 (*MCGF*).

Cytb was amplified by PCR using the primer combination and conditions for amplification described in our previous studies (Lavrenchenko et al. 2009; Bannikova et al. 2011). Primers and the polymerase chain reaction protocols for all nuclear loci, except *BRCA1*, were developed and described by Esselstyn et al. (2009, 2013). The primers for exon 11 of *BRCA1* (F60: 5′–GCCTAGGAAGAAGCCAACAGAACA GATG–3′ and R843: 5′–GCACTTTGTTTTTCTGATGAT GCTGTTGAG–3′) were designed by our group. The PCR products were visualised on 1.5% agarose gel and purified using Diatom DNA Clean-Up kit (Isogen). Approximately 10–30 ng of the purified PCR product was used for sequencing with each primer on the autosequencing system, ABI 3100-Avant, using the ABI PRISM®BigDyeTM Terminator v. 3.1 (Applied Biosystems, Foster City, CA, USA). The sequences obtained in this study can be accessed via GenBank (Accession Nos. MW286411–MW410134, Table S2).

Alignment and partitioning

The sequences for each specimen and loci were assembled and edited using Bioedit version 7.0.9.0 (Hall 1999). Sequences of the nuclear genes were used as unphased genotypes in the analysis of concatenated matrix. Heterozygous positions (where two peaks of approximately equal intensity were observed) were coded using the IUB ambiguity codes. Tests for base composition homogeneity in the 3rd codon positions and estimation of the K2P distances were conducted with MEGA6 (Tamura et al. 2007).

Phylogenetic reconstructions were performed with (1) the sequences of *cytb* mtDNA gene and (2) all of the nuclear genes combined. The program, PartitionFinder (Lanfear et al. 2012), was used to determine the best partitioning strategy for nuclear concatenation among the following five a priori candidate schemes: (1) partitioning by gene; (2) partitioning by codon position; (3) partitioning by gene and codon position (three subsets per gene); (4) as in variant 3, but with the 1st and 2nd codon positions combined (2

subsets per gene); and (5) no partitioning. The *cytb* dataset was always partitioned into three codon positions.

Phylogenetic tree reconstruction

Phylogenetic analysis was performed using Maximum Likelihood (ML) and Bayesian criteria. Maximum likelihood reconstructions were conducted in IQTREE version 1.6 (Nguen et al. 2015) using the partitioning scheme and optimum models, as inferred in PartitionFinder (Kalyaanamoorthy et al. 2017). Clade stability was tested using Ultrafast Bootstrap (Minh et al. 2013), with 10,000 replicates. Bayesian tree reconstructions were performed in MrBayes 3.2 (Ronquist et al. 2012). Models with either two or six rate matrix parameters were defined for each subset in accordance with the results obtained from PartitionFinder. The analysis included two independent runs of four chains using the default heating scheme. The chain length was set at 5 million generations, with sampling of every 2,000 generation. Tracer 1.6 software (Rambaut and Drummond 2005) was used to scan for convergence and to determine the necessary burn-in fraction, which was 10% of the chain length. The effective sample size exceeded 200 for all estimated parameters. Estimations of the genetic *p*-distances were conducted in MEGA6 (Tamura et al. 2007).

Species tree and molecular dating

To estimate the ages of divergence of the main lineages, we reconstructed the species tree using a Bayesian coalescent framework, as implemented in *BEAST ver. 1.84 (Heled and Drummond 2010). All nuclear genes were phased using Phase module (Stephens et al. 2001; Stephens and Donnelly 2003) implemented in the software, DnaSP (ver.5; Librado and Rozas 2009). The partitioning scheme was set to match that in the ML analysis. Following the results of hierarchical Likelihood Ratio tests performed in PAML v. 4.9 (Yang 2007), separate strict clock models were employed for each gene. The divergence time at each node was estimated simultaneously. The analysis was performed with the assumption of a Yule prior for the tree shape and optimum models (as in the ML analysis) for each partition. Two runs of 200 million generations were conducted. The node ages were calibrated using the estimate of a *BRCA1* substitution rate equal to 4.27E−03 substitutions per site per Mya (standard deviation = 0.61E−03). This estimate was obtained via an additional analysis of the extended *BRCA1* alignment containing 41 sequences of *Crocidura* and *Suncus* retrieved from GenBank (one sequence per species). To calibrate this analysis, we employed the age of the oldest fossils of *Crocidura* and *Suncus* from the latest Miocene [i.e., 6 Mya as the hard lower bound for the time of divergence between the two genera as per Butler (2010)]. Stratigraphic bracketing was used to define the soft (95%) upper bound, which was placed at the MN11/MN12 boundary (7.75 Mya). Exponential distribution was used as the prior density.

Results

Sequence characteristics and partitions

The final alignment of *cytb* sequences contained 1,140 bp for 111 specimens, including the three outgroups. The combined analyses of the eight nuclear genes were performed using the data for 40 specimens, including three specimens of *Suncus murinus*, *S. montanus*, and *S. etruscus*, which were used as outgroups. The final alignment contained 4366 nucleotide positions: 600 bp of *ApoB*, 713 bp of *BRCA1*, 273 bp of *RAG1*, 695 bp of *vWF*, 563 bp of *GHR*, 408 bp of *BDNF*, 472 bp of *PTGER4*, and 642 bp of *MCGF*. The optimum partitioning scheme for the nuclear genes identified by Partition Finder under the BIC criterion corresponded to scheme 4 (two subsets per gene with the 1st and 2nd codon positions combined and the 3rd position separate). The best-fitting substitution models employed for each gene and subset are listed in Table S3. The reconstruction of species tree was based on data for 55 specimens, including nine specimens of *Suncus murinus*, *S. montanus*, and *S. etruscus* used as outgroups.

Mitochondrial phylogeny of Crocidura including the Eastern Afromontane species

The mitochondrial phylogeny of *Crocidura* including the Eastern Afromontane species and multiple other lineages as inferred from the *cytb* sequences is presented in the Fig. 2 and Fig. S1. In both the BI and ML analyses, species endemic to Ethiopia and Tanzania as well as *C. fumosa*, *C. luna*, and *C.* aff *monax* form a single monophyletic group (designated as the Eastern Afromontane clade) (Fig. 2). However, the reconstructed phylogenetic tree indicates that neither the Ethiopian nor Tanzanian groups of endemic species are monophyletic.

In the *cytb* tree, the endemic Ethiopian species (including sub-endemic *C. bottegi*) fall into seven branches with a genetic (*p*-) distance ranging from 6.4 to 11.8% between them (Supporting Information Table S4). These branches correspond to species *C. bottegi*, *C. macmillani*, *C. baileyi*, *C. harenna*, *C. lucina*, *C. yaldeni*, and *C. thalia/C. glassi* species group. *C. bottegi* is inferred to be the sister branch to the remainder of the Eastern Afromontane clade (*p*-distance of approximately 12%).The highly supported monophyletic group of *C. baileyi* + *C. lucina* is placed as the sister clade to the *C. thalia/C. glassi* species group. Within the latter, haplotypes of *C. thalia* and *C. glassi* are highly similar

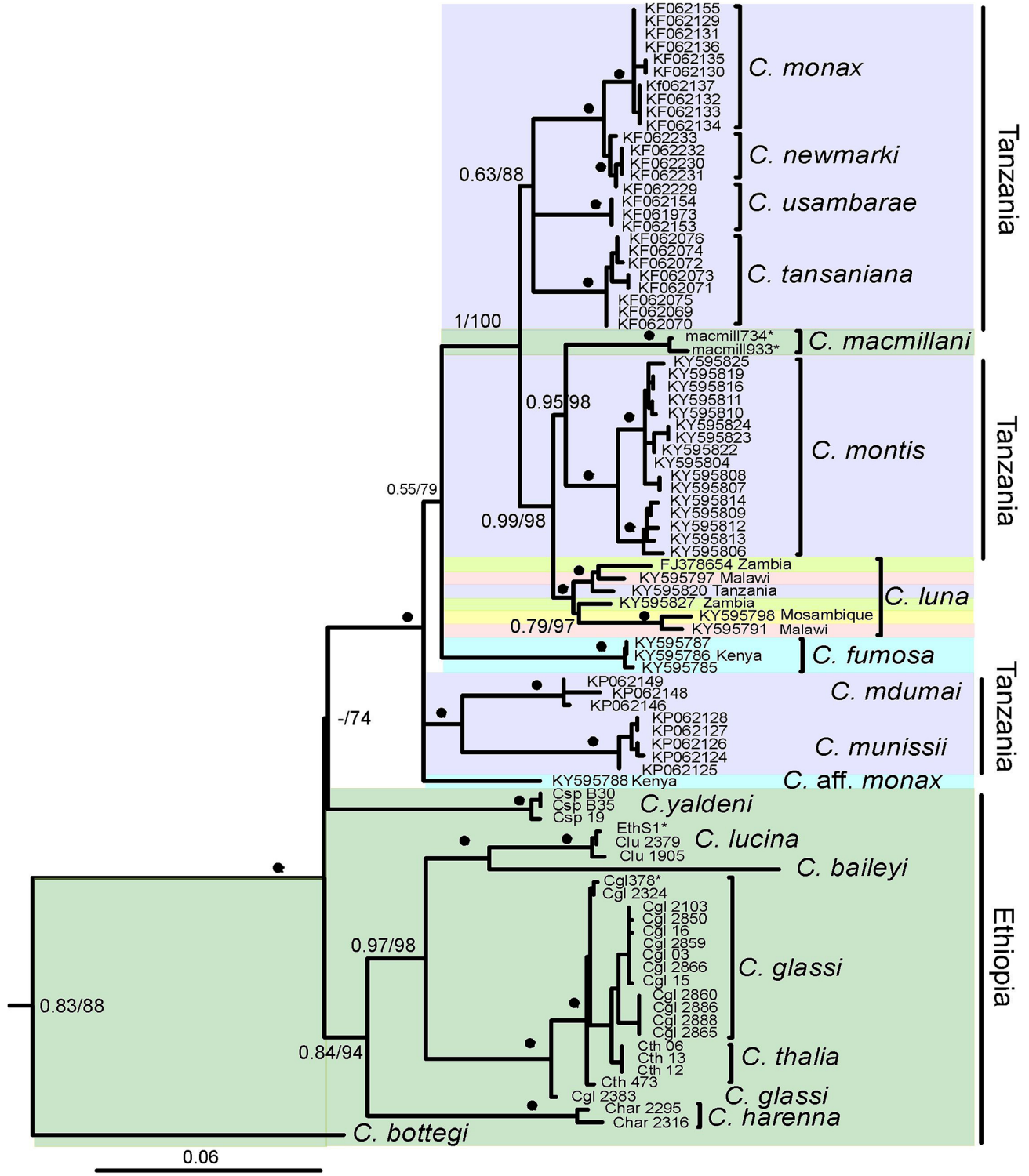

Fig. 2 The ML phylogeny of the Eastern Afromontane endemic species of *Crocidura* based on the *cytb* gene sequences. Values above the branches correspond to bootstrap support (1000 pseudoreplicates) and posterior probabilities in the ML and BI analyses, respectively. Asterisks mark the sequences we obtained in the previous studies. Filled circles in tree nodes indicate absolute support in all analyses. Colours indicate geographic origin of specimens. Relationships among outgroup lineages are shown in the Fig. S1

(*p*-distance of 0.7–1%) and do not form species-specific clusters. *C. harenna* appears as the sister group of the (*C. baileyi* + *C. lucina*)/(*C. thalia* + *C. glassi*) clade with moderate support. The position of *C. yaldeni* remains unresolved. Another endemic Ethiopian species, *C. macmillani,* appears deep within the highly supported clade comprising mostly Tanzanian/Kenyan species.

The species of Tanzania are divided into three branches. The first branch includes *C. usambarae*, *C. tanzaniana*, and *C. monax* + *C. newmarki*. The second branch corresponds to endemic *C. munissii* and *C. mdumai*. The third branch includes *C. montis* that forms a clade with *C. macmillani* from Ethiopia (*p*-distance of 4.1%). This latter grouping stands as sister to *C. luna* (from different localities) with high support. The ((*C. montis* + *C. macmillani*) + *C. luna*) clade is joined by the first endemic group (*C. usambarae*/*C. tanzaniana*/(*C. monax* + *C. newmarki*)). The genetic (*p*-) distance among endemic Tanzanian species ranges from 1.2 to 7.5% (Table S5), which is noticeably lower than that of the Ethiopian species. The position of the Kenyan species *C. fumosa* and *Crocidura* aff. *monax* within theTanzanian/Kenyan clade remains unresolved.

Concatenated nuclear tree

No contradicting nodes with high support were found among the gene genealogies inferred from separate nuclear genes. The ILD test did not reject the H0 of partition homogeneity ($P = 0.105$).

The topology of the combined nuclear ML tree (Fig. 3) shows the paraphyly of the Ethiopian endemic group relative to the Tanzanian endemic clade. The united Eastern Afromontane clade has a high bootstrap support (1.0/100), with *C. bottegi* standing as the sister branch to the rest of the clade. The next branching clade contains *C. yaldeni* and *C. thalia/C. glassi* and is followed by the *C. lucina*/*C. baileyi* clade. *C. harenna* is the sister taxon to the entire Tanzanian clade. Similar to the mitochondrial tree, *C. macmillani* occupies the position within the Tanzanian clade; however, the sister-group relationship among *C. macmillani* and *C. tanzaniana* (C. *usumbarae/C. monax/C. newmarki*) has low support (0.91/70). The monophyly of the *C. tanzaniana* (C. *usumbarae/C. monax/C. newmarki*) grouping is highly supported (0.99/98).

Species tree

The *BEAST species tree (Fig. 4) strongly supports the paraphyly of the Ethiopian group of species, with reference to the species from Tanzania. In addition, this species tree is similar to the ML tree based on the concatenated matrix; however, the PP support for the joint clade of the Ethiopian and Tanzanian species is low. Compared to the nuclear genes concatenated tree, the position of *C. macmillani* is different. Nonetheless, it is supported as the sister group to all Tanzanian species (PP = 0.9). The relationship among the three groups of Ethiopian–Tanzanian species (i.e., *C. lucina* + *C. baileyi*, *C yaldeni* + *C. thalia/C. glassi* and *C. harenna* + *C. macmillani* + Tanzanian clade) remains unresolved.

Molecular time estimates

The node age estimates produced by species tree analysis are presented in Table 2 and Fig. 4. The age of the basal node within *Crocidura* is estimated to be 4.1 Mya, thereby corresponding with Early Pliocene. The results of molecular dating suggest that the clade comprising of the Ethiopian and Tanzanian species diverged at ca. 3.4 Mya (2.45–4.7 Mya) while the time of the basal radiation within this clade corresponds to the first half of the Early Pleistocene (ca. 2 Mya, 95%CI: 1.37–2.96 Mya). Thus, Ethiopian endemics represent a relatively young group within the ancient taxon.

Discussion

Phylogenetic relationships between the Eastern Afromontane clade and other African species

Our data confirm the existence of the Eastern Afromontane clade distributed in the the EABH including EP and mountain chains in Tanzania and Kenya. However, to date, the phylogenetic relationships between the Eastern Afromontane clade and other African species are not clear. A single species of this clade, *C. luna*, was included in the phylogenetic study by Dubey et al. (2008), in which it was recovered as a member of the Old World clade, where it stands as the sister taxon to *C. zimmermanni.* According to Lavrenchenko et al. (2009), endemic Ethiopian species form a subclade of the Mediterranean major clade including *C. zimmermanni*, *C. sicula, C. canariensi,* and *C. tarfaiensis*. However, neither current mitochondrial results nor previous studies provide robust support for the Mediterranean clade, the validity of which should be tested based on multi-locus data.

Phylogenetic relationship within the Eastern Afromontane clade

Our analyses of the mitochondrial *cytb* data, concatenated alignment of eight nuclear genes, and the species tree reconstruction produced generally similar results, including (1) the close relation between the Ethiopian group of species and the Tanzanian/Kenyan endemic species, with evident paraphyly of the first group relative to the second; and (2) highly supported sister relations between *C. bottegi* and the rest of the Eastern Afromontane clade. Molecular dating

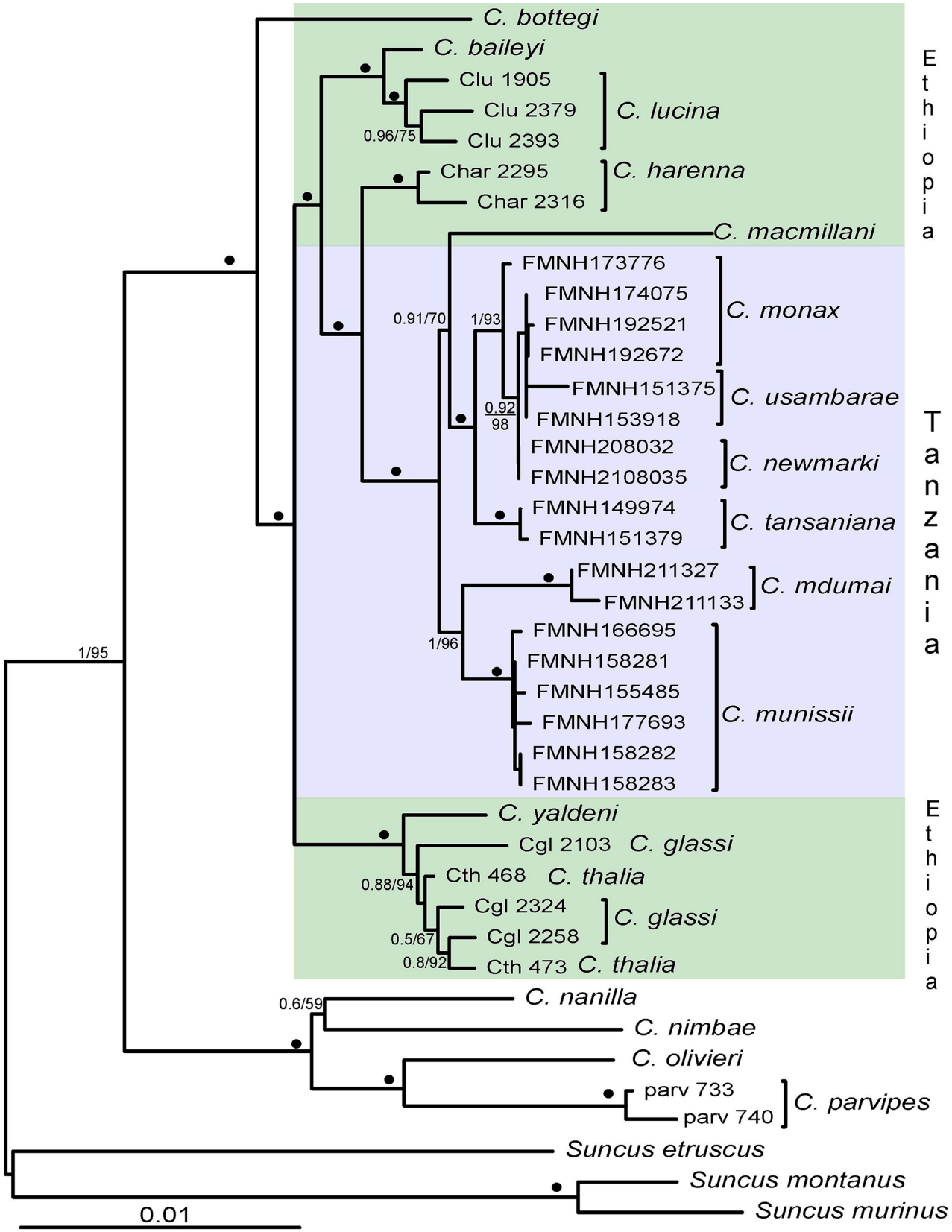

Fig. 3 The ML phylogeny of the Eastern Afromontane species of *Crocidura* based on the concatenated alignment of eight nuclear genes. The designations are as in Fig. 2

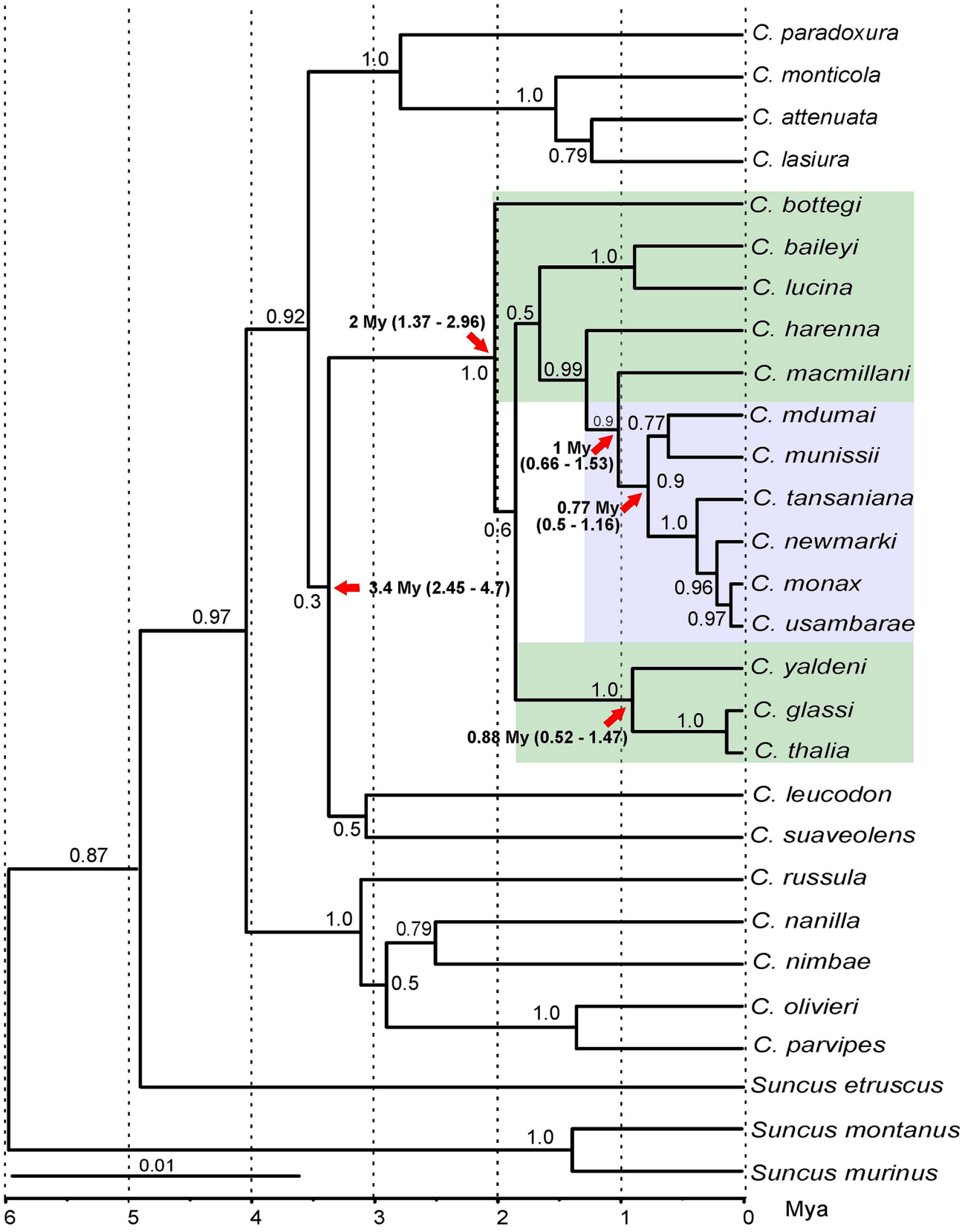

Fig. 4 Species tree of the genus *Crocidura* based on eight nuclear genes and produced by the *BEAST algorithm using the Bayesian multispecies coalescent approach. Values above the branches correspond to Bayesian posterior probabilities

Table 2 Molecular times for the major divergence events of the Ethiopian and Tanzanian species of *Crocidura*

Clades of species tree and major divergence events	Absolute time (My)	95%HPD
Crocidura/*Suncus* of Asia	6.01	4.24–8.51
Basal divergence of *Crocidura*	4.07	2.94–5.63
Ethiopia + Tanzania/sister group	3.39	2.45–4.70
Crocidura of Ethiopia	2.02	1.37–2.96
C. macmillani/Tanzania	1.01	0.66–1.53
Basal divergence of Tanzanian clade	0.77	0.50–1.16
C. thalia/*C.glassi*	0.09	0.01–0.69
C. baileyi/*C. lucina*	0.86	0.52–1.44
C. yaldeni/(*C. thalia* + *C. glassi*)	0.88	0.52–1.47
(*C. yaldeni* + *C. thalia* + *C. glassi*)/(*C. baileyi* + *C. lucina* + Tanzania)	1.85	1.30–2.64
(*C. baileyi* + *C. lucina*)/Tanzania	1.65	1.15–2.37
C. harenna/(*C. macmillani* + Tanzania)	1.27	0.86–1.86

suggests that the latter clade diverged around the Early/Late Pliocene boundary. Further, the radiation among Ethiopian species should be attributed to Early Pleistocene.

According to our estimates, the age of the basal node in *Crocidura* dates back to Early Pliocene (4.1 Mya). Notably, this genus is sometimes believed to be significantly older and its origin is attributed to the Middle/Late Miocene boundary (Butler 1998) or Late Miocene (~6.2 My; Dubey et al. 2008). The discrepancy between molecular dates in the latter study and our time estimates can be explained by two factors: (1) the calibration method used [soft upper bound in our analysis versus high hard bound for the tree root (= Soricinae/Crocidurinae) in Dubey et al. (2008)]; (2) the sample of loci analysed [eight nuclear loci in our study versus a concatenation of two nuclear and two mitochondrial genes in Dubey et al. (2008)]. It is important to note that the mtDNA-based date estimates usually suffer from rate decay, which results in upwards bias for younger nodes and downward bias for more ancient nodes (Molak and Ho 2015).

For the Ethiopian–Tanzanian clade, Ethiopia is the primary centre of diversity owing to the tree structure, higher genetic variation among endemic Ethiopian lineages relative to Tanzanian lineages, and their relatively old age (~2 Mya). Tanzanian species can be viewed as a later derivative of Ethiopian radiation. In fact, the Tanzanian species, *C. monax*, *C. usambara*, and *C. newmarki*, appear to be very similar genetically, thereby aligning with a recent speciation pattern. The low level of divergence among these species is comparable to that between *C. thalia*/*C. glassi* from Ethiopia (see below). However, in contrast to the latter, *C. monax*, *C. usambara*, and *C. newmarki* are allopatric and reciprocally monophyletic in the mitochondrial DNA (mtDNA).

One of the intriguing results is the phylogenetic position of the Ethiopian endemic species *C. macmillani*. Ecologically, *C. macmillani* differs from other lineages as it is found at low altitudes, thereby inhabiting plains rather than mountains. Earlier cytogenetic analysis revealed that unlike all other karyologically studied Ethiopian endemics (*C. bottegoides*, *C. glassi*, *C. thalia*, *C. harenna*, *C. lucina*, and *C. yaldeni*), with structurally similar karyotype (2n = 36, NF = 52–56), *C. macmillani* possesses a karyotype that consists entirely of biarmed elements (2*n* = 28, NFa = 52) and might be a derivative of the 36-chromosome karyotype of the remaining Ethiopian endemics (Lavrenchenko et al. 1997). A very similar karyotype with 28 biarmed chromosomes was found in *C. luna* from Zambia (Castiglia et al. 2009), despite the description of an earlier karyotype of *C. luna*, with 2*n* = 36, NF = 56, from Burundi. Based on these data and the position of *C. macmillani* and *C. luna* in our *cytb* tree, we suggest that the 2*n* = 28 karyotype evolved from the 2*n* = 36 karyotype, and is thus a synapomorphy of the *C. macmillani*/*C. luna* clade. To test this hypothesis, karyotype variation across *C. montis* sensu lato should be assessed.

The phylogenetic relations of *C. macmillani* are not completely clear. In fact, in the nuclear concatenated tree, its position within the Tanzanian groups remains poorly supported while in the species tree, *C. macmillani* is recovered, with moderate support, as the sister group to all Tanzanian species. The mitochondrial data robustly support the former arrangement and suggest that *C. macmillani* branches off deep within a clade that is mainly comprised of Kenyan and Tanzanian species. According to the mtDNA results reported by Sabuni et al. (2018), *C. macmillani* is the sister group of the Kenyan *C. montis* sublineage. If the mitochondrial hypothesis, which is consistent with the nuclear concatenated tree, and the chromosomal data are accepted, *C. macmillani* can be regarded as the only Ethiopian endemic species that colonized its contemporary range through a northward dispersal from a more southerly centre of origin (possibly in Kenya).

Whether the Tanzanian (and Kenyan) highlands were colonised via a single or several dispersal waves originating in Ethiopia is a related but unanswered question. Considering the high level of support for the Tanzanian/Kenyan clade and the non-Ethiopian origin that is suggested for *C. macmillani*, one may hypothesize that a single colonisation event occurred between 0.66 and 1.27 Mya according to our date estimates. This interval is similar to those estimated for the colonization of large areas southward of Ethiopia by other groups of small mammals (*Lophuromys flavopuctatus* species complex, genus *Tachyoryctes*) that might use the EP as their cradle (Lavrenchenko 2008; Šumbera et al. 2018). Molecular dating estimates suggest that this relatively recent "out-of-Ethiopia" dispersal event for different small mammal groups might have occurred in the time synchrony. The period between 1.1 and 0.9 Mya was characterized by both

humid conditions (Trauth et al. 2005, 2007) and substantial climate variability (Potts 2013) in East Africa. We can expect that during this period, some "corridors" of humid habitats connected the EP and the highlands of Kenya and Tanzania. Accordingly, representatives of the three groups (*Crocidura*, *Lophuromys flavopuctatus* s.l. and *Tachyoryctes*) could colonize the highlands southward of Ethiopia. A further study involving an extended species set (including the *Crocidura* taxa from the Kenyan highlands) is necessary to support and describe the proposed scenario for the Eastern Afromontane *Crocidura* species.

Is the gradient model of speciation practical?

The ecological (gradient) model of diversification suggests that (1) divergent selection across strong environmental gradients causes adaptive divergence and speciation, and (2) reproductive isolation is caused by the pleiotropic and hitchhiking genetic effects that results in adaptation to different habitats (Smith et al. 1997; Orr and Smith 1998; Moritz et al. 2000; Couvreur et al. 2020). Although divergence, with the gene flow model underlying the gradient hypothesis, is supported by two experiments with laboratory populations of *Drosophila* (see Rice and Hostert, 1993 for review) and mathematical models (Doebeli and Dieckmann 2003; Mizera and Meszéna 2003; Geritz et al. 2004), selection across ecological gradients is yet to be unequivocally demonstrated as a major cause of speciation. The gradient model differs fundamentally from the allopatric (refugia) model that postulates that spatial isolation is a necessary prerequisite for divergence and speciation. However, by examining patterns of molecular diversity, these two alternative speciation models can be assessed (Patton and Smith 1992; Orr and Smith 1998; Moritz et al. 2000; Lara et al. 2005; Linck et al. 2020a, b). The gradient model predicts that sister taxa should occupy habitats that are geographically adjacent but distinct. Our findings support a previous suggestion (Lavrenchenko 2011) that the diversification of two species, *C. glassi* and *C. thalia*, can be explained by the gradient model of speciation. This pair of recently diverged *Crocidura* sister taxa is represented by species replacing each other in the adjacent altitudinal belts of the Bale Massif (*C. glassi* in the afroalpine zone and ericaceous belt and *C. thalia* in the tropical forest). By analysing the mitochondrial and nuclear genes, exceptional similarity was found between the two *Crocidura* species, with no marker depicting reciprocal monophyly. Whether this pattern can be explained according to ongoing or historical introgression has yet to be established using genomic data. However, *C. glassi* and *C. thalia* display markedly pronounced morphological distinctions (Dippenar 1980). Moreover, the validity of these species has never been doubted. The specific distribution patterns of both species match the predictions of the gradient model. In fact, they are parapatric and inhabit different altitudinal belts over their entire areas. In addition, they are phenetically divergent but genetically very similar, thereby aligning with the predictions of the gradient model (Moritz et al. 2000). Notably, the highland *C. glassi* is significantly smaller in size relative to the forest *C. thalia*; they also belong to different size groups of Ethiopian shrews (i.e., "medium-sized" and "large-sized", respectively) (Lavrenchenko et al. 2016). However, this fact contradicts Bergman's rule and can be explained by the considerable differences in physiology between the two species, which are associated with their adaptations to markedly different habitats.

As summarized by Lavrenchenko (2011), previous studies rejected the hypothesis that for terrestrial vertebrates, ecological (gradient) speciation occurs along an altitudinal gradient in tropical mountains. However, most of these studies were mainly based on mtDNA only. Further analysis of nuclear markers supported the gradient model of speciation for two species pairs of narrow-headed rats (*Stenocephalemys*) distributed along the remarkable altitudinal gradient of the ecological conditions located at the north-western (*S. zimai/S. albipes*) and south-eastern (*S. griseicauda–S. albocaudata*) massifs of the EP (Bryja et al. 2018; Mizerovská et al. 2020). By analysing nuclear markers, this modus of speciation was found to be used by another pair of species (*C. glassi/C. thalia*) endemic to Ethiopia. Therefore, ecological speciation may be an uncommon, but possible, natural evolutionary phenomenon, at least for small mammals that inhabit the EP.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42991-021-00120-7.

Acknowledgements This study was supported by the Russian Foundation for Basic Research (project numbers 20-04-00081, 18-04-00563, and 19-54-26003). All fieldwork complied with the legal regulations of Ethiopia, and sampling was carried out with the permission of the Ethiopian Wildlife Conservation Authority and the Oromia Forest and Wildlife Enterprise. We would like to express our gratitude to Dr. Andrei Darkov (Joint Ethio-Russian Biological Expedition, Fourth Phase—JERBE IV) for managing the expedition. We also thank D.S. Kostin, A.R. Gromov, D.Y. Alexandrov, A.A. Warshavsky, Yu. F. Ivlev, and M. Kasso for their assistance with field sampling.

Declarations

Conflict of interest The authors declare that they have no competing interests.

References

Bannikova AA, Abramov AV, Borisenko AV, Rozhnov LVS, VV (2011) Mitochondrial diversity of the white-toothed shrews (Mammalia, Eulipotyphla, *Crocidura*) in Vietnam. Zootaxa 2812:1–20

Bannikova AA, Lavrenchenko LA, Kramerov DA (2005) Phylogenetic relationships between Afrotropical and Palaearctic *Crocidura* species inferred from Inter-SINE-PCR. Biochem Syst Ecol 33:45–59. https://doi.org/10.1016/j.bse.2004.05.014

Bannikova AA, Lavrenchenko LA, Lomov AA, Mednikov BM (2001) Molecular diversity of some *Crocidura* species (Insectivora, Soricidae) from Ethiopia. In: Denis C, Granjon L, Poulet A (eds), African Small Mammals. IRD Editions, Paris, pp 55–64. http://horizon.documentation.ird.fr/exl-doc/pleins_textes/divers09-03/010028448.pdf

Bryja J, Kostin D, Meheretu Y, Šumbera R, Bryjová A, Kasso M, Mikula O, Lavrenchenko LA (2018) Reticulate Pleistocene evolution of Ethiopian rodent genus along remarkable altitudinal gradient. Mol Phyl Evol 118:75–87. https://doi.org/10.1016/j.ympev.2017.09.020

Bryja J, Meheretu Y, Šumbera R, Lavrenchenko LA (2019a) Annotated checklist, taxonomy and distribution of rodents in Ethiopia. Folia Zool 68:117–213. https://doi.org/10.2525/fozo.030.2019

Bryja J, Colangelo P, Lavrenchenko LA, Meheretu Y, Šumbera R, Bryjová A, Castiglia R (2019b) Diversity and evolution of African Grass Rats (Muridae: Arvicanthis)—from radiation in East Africa to repeated colonization of north-western and south-eastern savannas. J Zool Syst Evol Res 57:970–988

Butler PM (2010) Neogene insectivora. In: Werdelin L, Sanders WJ (eds) Cenozoic mammals of Africa. University of California Press, Berkeley, pp 579–586

Butler PM (1998) Fossil history of shrews in Africa. In: Wójcik JM, Wolsan M (eds) Evolution of shrews. Mammal Research Institute, Polish Academy of Science, Bialowieza, pp 121–132

Castiglia R, Annesi F, Sichilima AM, Hutterer R (2009) A molecular and chromosomal study of the moonshine shrew, *Crocidura luna* Dollman, 1910 from Zambia with a description of a new remarkable karyotype. Mammalia 73:56–59. https://doi.org/10.1515/MAMM.2009.005

Couvreur TLP, Dauby G, Blach-Overgaard A, Deblauwe V, Dessein S, Droissart V, Hardy OJ, Harris DJ, Janssens SB, Ley AC, Mackinder BA, Sonké B, Sosef MSM, Stévart T, Svenning J-C et al (2020) Tectonics, climate and the diversification of the tropical African terrestrial flora and fauna: diversification of tropical African biodiversity. Biol Rev. https://doi.org/10.1111/brv.12644

Dippenaar NJ (1980) New species of *Crocidura* from Ethiopia and Northern Tanzania (Mammalia: Soricidae). Ann Transvaal Mus 32:125–154

Doebeli M, Dieckmann U (2003) Speciation along environmental gradients. Nature 421:259–264

Dubey S, Salamin N, Ruedi M, Barriere P, Colyn M, Vogel P (2008) Biogeographic origin and radiation of the Old World crocidurine shrews (Mammalia: Soricidae) inferred from mitochondrial and nuclear genes. Mol Phylogenet Evol 48:953–963. https://doi.org/10.1016/j.ympev.2008.07.002

Esselstyn JA, Achmadi MAS, Siler CD, Evans BJ (2013) Evans Carving out turf in a biodiversity hotspot: multiple, previously unrecognized shrew species co-occur on Java Island, Indonesia. Mol Ecol 22:4972–4987. https://doi.org/10.1111/mec.12450

Esselstyn JA, Timm RM, Brown RM (2009) Do geological or climatic processes drive speciation in dynamic archipelagos? The tempo and mode of diversification in Southeast Asian shrews. Evolution 63:2595–2610. https://doi.org/10.1111/j.1558-5646.2009.00743.x

Geritz SAH, Kisdi E, Meszena G, Metz JAJ (2004) Adaptive dynamics of speciation: ecological Underpinnings. In: Dieckmann U, Doebeli M, Metz JAJ, Tautz D (eds) Adaptive speciation. Cambridge University Press, Cambridge, pp 54–75

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Heled J, Drummond AJ (2010) Bayesian inference of species trees from multilocus data. Mol Biol Evol 27:570–580

Hutterer R, Yalden DW (1990) Two new species of shrews from a relic forest in the Bale Mountains, Ethiopia. In: Peters G, Hutterer R (eds) Vertebrates in tropics. Museum Alexander Koenig, Bonn, pp 63–72

Kalyaanamoorthy S, Minh BQ, Wong TKF et al (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods 14:587–589

Konečný A, Hutterer R, Meheretu Y, Bryja J (2020) Two new species of *Crocidura* (Mammalia: Soricidae) from Ethiopia and updates on Ethiopian shrew fauna. J Vertebr Biol 69:20064

Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol Biol Evol 29:1695–1701

Lara MC, Geise L, Schneider CJ (2005) Diversification of small mammals in the Atlantic Forest of Brazil: testing the alternatives, in Mammalian Diversification: From Chromosomes to Phylogeography (a Celebration of the Career of JL Patton). Univ Calif Publ Zool 133:311–333

Lavrenchenko LA (2008) The Ethiopian mammals: a model for research in evolutionary biology. Ecological and faunistic studies in Ethiopia. KMK, Moscow, pp 114–139

Lavrenchenko LA (2011) Testing of alternative hypotheses for speciation: a case study on ground vertebrates in tropical mountains. Biol Bull 38:551–557

Lavrenchenko LA, Bekele A (2017) Diversity and conservation of Ethiopian mammals: what have we learned in 30 years? Ethiop J Biol Sci 16:1–20. http://213.55.95.79/index.php/EJBS/article/view/1058

Lavrenchenko LA, Verheyen E (2005) An assessment of the systematics of the genus *Desmomys* Thomas, 1910 (Rodentia: Muridae) using mitochondrial DNA sequences. In: Huber BA, Sinclair BJ, Lampe K-H (eds) African biodiversity: molecules, organisms, ecosystems. Springer, New York, pp 363–369

Lavrenchenko LA, Milishnikov AN, Aniskin VM et al (1997) The genetic diversity of small mammals of the Bale Mountains Ethiopia. SINET: Ethiop J Sci 20:213–233

Lavrenchenko LA, Verheyen E, Potapov SG et al (2004) Divergent and reticulate processes in evolution of Ethiopian *Lophuromys flavopunctatus* species complex: evidence from mitochondrial and nuclear DNA differentiation patterns. Biol J Linn Soc 83:301–316. https://doi.org/10.1111/j.1095-8312.2004.00390.x

Lavrenchenko LA, Verheyen WN, Verheyen EK, Hulselmans J, Leirs H (2007) Morphometric and genetic study of Ethiopian *Lophuromys flavopunctatus* Thomas, 1888 species complex with description of three new 70-chromosomal species (Muridae, Rodentia). Bull Inst R Des Sci Nat Belg 77:77–117

Lavrenchenko LA, Bannikova AA, Lebedev VS (2009) Shrews (*Crocidura* spp.) endemic to Ethiopia: recent adaptive radiation of an ancient lineage. Dokl Biol Sci 424:57–60. https://doi.org/10.1134/S0012496609010177 (**Original Russian published in Doklady Akademii Nauk, 2009, 424: 705–708**)

Lavrenchenko LA, Mengistu S, Bulatova NSh, Bekele A, Potapov SG, Nadjafova RS, Belay G (2014) Genetic and cytogenetic variation of African root-rats *Tachyoryctes splendens* (Mammalia: Rodentia) from Ethiopia. Russ J Theriol 13:109–118

Lavrenchenko LA, Voyta LL, Hutterer R (2016) Diversity of shrews in Ethiopia, with the description of two new species of *Crocidura* (Mammalia: Lipotyphla: Soricidae). Zootaxa 4196:38–60

Lavrenchenko LA, Nadjafova RS, Bekele A, Mironova TA, Bryja J (2017) Phylogenetic position of a monotypic Ethiopian endemic rodent genus *Megadendromus* (Nesomyidae). Mammalia 81:71–82. https://doi.org/10.1515/mammalia-2015-0148

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Linck E, Freeman BG, Dumbacher JP (2020a) Speciation and gene flow across an elevational gradient in New Guinea kingfishers. J Evol Biol 33:1643–1652

Linck E, Freeman BG, Dumbacher JP (2020b) Speciation and gene flow across an elevational gradient in New Guinea kingfishers. J Evol Biol. 33:1643–1652. https://doi.org/10.1111/jeb.13698

Minh BQ, Nguyen MAT, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. Mol Biol Evol 30:1188–1195. https://doi.org/10.1093/molbev/mst024

Mittermeier RA, Turner WR, Larsen FW, Brooks TM, Gascon C (2011) Global biodiversity conservation: the critical role of hotspots. In: Zachos F, Habel J (eds), Biodiversity Hotspots. pp 3–22. Springer, Heidelberg, Berlin. https://doi.org/10.1007/978-3-642-20992-5_1

Mizera F, Meszéna G (2003) Spatial niche packing, characteristic displacement and adaptive speciation along an environmental gradient. Evol Ecol Res 5:1–20

Mizerovská D, Mikula O, Meheretu Y, Bartáková V, Bryjová A, Kostin DS, Šumbera R, Lavrenchenko LA, Bryja J (2020) Integrative taxonomic revision of the Ethiopian endemic rodent genus *Stenocephalemys* (Muridae: Murinae: Praomyini) with the description of two new species. J Vertebr Biol 69:20031. https://doi.org/10.25225/jvb.20031

Molak M, Ho SY (2015) Prolonged decay of molecular rate estimates for metazoan mitochondrial DNA. PeerJ 3:e821

Moritz C, Patton JL, Schneider CJ, Smith TB (2000) Diversification of rainforest faunas: an integrated molecular approach. Ann Rev Ecol Syst 31:533–563. https://doi.org/10.1146/annurev.ecolsys.31.1.533

Nguyen L-T, Schmidt HA, von Haeseler et al (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Mol Biol Evol 32:268–274. https://doi.org/10.1093/molbev/msu300

Nosil P (2012) Ecological speciation. Oxford series in ecology and evolution. Oxford University Press, Oxford

Orr MR, Smith TB (1998) Ecology and speciation. Trends Ecol Evol 13:502–506. https://doi.org/10.1016/S0169-5347(98)01511-0

Patton J, Smith MF (1992) MtDNA phylogeny of Andean mice: a test of diversification across ecological gradients. Evolution 46:174–183

Potts R (2013) Hominin evolution in settings of strong environmental variability. Quat Sci Rev 73:1–13. https://doi.org/10.1016/j.quascirev.2013.04.003

Rambaut A, Drummond A (2005) Tracer version 1.5.Computer program distributed by the authors. Department of Zoology, University of Oxford, UK. Available via https://www.evolve.zoo.ox.ac.uk/software.html

Rice WR, Hostert EE (1993) Laboratory experiments on speciation: what have we learned in 40 years? Evolution 47:1637–1653. https://doi.org/10.1111/j.1558-5646.1993.tb01257.x

Ronquist F, Teslenko M, van der Mark P et al (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. https://doi.org/10.1093/sysbio/sys029

Sabuni C, Aghová T, Bryjová A, Šumbera R, Bryja J (2018) Biogeographic implications of small mammals from Northern Highlands in Tanzania with first data from the volcanic Mount Kitumbeine. Mammalia 82:360–372. https://doi.org/10.1515/mammalia-2017-0069

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Lab. Press, New York

Smith TB, Wayne RK, Girman D, Bruford M (1997) A role for ecotones in generating rainforest biodiversity. Science 276:1855–1857. https://doi.org/10.1126/science.276.5320.1855

Stanley WT, Hutterer R, Giarla TC, Esselstyn JA (2015) Phylogeny, phylogeography and geographical variation in the *C. monax* (Soricidae) species complex from the montane islands of Tanzania, with descriptions of three new species. Zool J Linn Soc 174:185–215. https://doi.org/10.1111/zoj.12230

Stephens M, Donnelly P (2003) A Comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 73:1162–1169. https://doi.org/10.1086/379378

Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 68:978–989. https://doi.org/10.1086/319501

Šumbera R, Krásová J, Lavrenchenko LA, Mengistu S, Bekele A, Mikula O, Bryja J (2018) Ethiopian highlands as a cradle of the African fossorial root-rats (genus *Tachyoryctes*), the genetic evidence. Mol Phyl Evol 126:105–115. https://doi.org/10.1016/j.ympev.2018.04.003

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular evolutionary geneticsanalysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599. https://doi.org/10.1093/molbev/msm092

Trauth MH, Maslin MA, Deino AL, Strecker MR (2005) Late Cenozoic moisture history of East Africa. Science 309:2051–2053. https://doi.org/10.1126/science.1112964

Trauth MH, Maslin MA, Deino AL, Strecker MR, Bergner AGN, Dühnforth M (2007) High- and low-latitude forcing of Plio–Pleistocene East African climate and human evolution. J Hum Evol 53:475–486. https://doi.org/10.1016/j.jhevol.2006.12.009

Yang Z (2007) PAML 4: a program package for phylogenetic analysis by maximum likelihood. Mol Biol Evol 24:1586–1591. https://doi.org/10.1093/molbev/msm088