

Supplementary Material

Footprints of adaptive evolution in mitochondrial protein-coding genes of endemic Ethiopian brush-furred rats (*Lophuromys*) living at high altitude

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Mammalian Biology

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Fig. S1 Distribution of Ethiopian *Lophuromys* species along the elevational gradient, exemplified by the Bale Mountains (Harar Plateau).

Table S1 Characteristics of the Ethiopian *Lophuromys flavopunctatus* s.l. samples analyzed in this study

[illegible]

868	<i>L.menageshae</i>	menageshae O	Menagesha forest	8.95	38.55	2600	MZ353592.1	ZMMU_S-165969
873	<i>L.menageshae</i>	menageshae O	Menagesha forest	8.95	38.55	2600	X	ZMMU_S-165971
<i>Lophuromys chrysopus</i> [LF]								
938	<i>L.chrysopus</i>	chrysopus	Sheko forest	7.067	35.5	1930	X	ZMMU_S-167315
1058	<i>L.chrysopus</i>	chrysopus	Godare forest	7.35	35.217	1200	X	ZMMU_S-168913
2341	<i>L.chrysopus</i>	chrysopus	Bale NP	6.645	39.733	1900	MZ353590.1	ZMMU_S-192730
2470	<i>L.chrysopus</i>	chrysopus	Dhati Welel	9.226	34.877	1427	X	ZMMU_S-192915
3598	<i>L.chrysopus</i>	chrysopus	Chebera Churchura NP	06.951704	36.663703	1636	X	-
<i>Lophuromys pseudosikapusi</i> [LF]								
951	<i>L.pseudosikapusi</i>	pseudosikapusi	Sheko forest	7.067	35.5	1930	X	ZMMU_S-179563
952	<i>L.pseudosikapusi</i>	pseudosikapusi	Sheko forest	7.067	35.5	1930	X	ZMMU_S-179564
<i>Lophuromys chercherensis</i> [LF]								
1006	<i>L.chercherensis</i>	chercherensis	Chercher Mts	9.333	41.267	2700	X	ZMMU_S-168902

1011	<i>L.chercherensis</i>	chercherensis	Chercher Mts	9.333	41.267	2700	X	ZMMU_S-168906
3460	<i>L.chercherensis</i>	chercherensis	Haremaya University	9.418	42.031	2023	X	ZMMU_S-203250
<i>Lophuromys simensis</i> [GEN]								
1111	<i>L.simensis</i>	simensis II	Guna Mt	11.717	38.25	3800	X	ZMMU_S-171633
1119	<i>L.simensis</i>	simensis I	Guna Mt	11.717	38.25	3800	X	ZMMU_S-171634
1148	<i>L.simensis</i>	simensis I	Guna Mt (Vanzay)	11.783	37.667	1800	X	ZMMU_S-171637
1188	<i>L.simensis</i>	simensis II	Guna Mt (Vanzay)	11.917	37.95	2250	X	ZMMU_S-175386
1345	<i>L.simensis</i>	simensis II	Simien NP	13.233	38.05	3250	X	ZMMU_S-178491
1398	<i>L.simensis</i>	simensis II	Simien NP	13.25	38.217	3800	X	ZMMU_S-178504
3247	<i>L.simensis</i>	simensis I	Choke Mt	10.706	37.843	3965	MZ353589.1	ZMMU_S-202836
3306	<i>L.simensis</i>	simensis I	Debre-Marcos	10.421	37.563	2342	X	ZMMU_S-202893
ETH1260	<i>L.simensis</i>	menageshae N	Borena Saynt NP	10.85	38.792	3273	X	USB_ETH1260
ETH1261	<i>L.simensis</i>	menageshae N	Borena Saynt NP	10.85	38.792	3273	X	USB_ETH1261

Lophuromys brevicaudus [MH]

2152	<i>L.brevicaudus</i>	brevicaudus	Arsi	7.54	39.345	3300	X	ZMMU_S-190475
2257	<i>L.brevicaudus</i>	brevicaudus	Bale NP	6.743	39.717	2610	X	ZMMU_S-192714
2772	<i>L.brevicaudus</i>	brevicaudus	Arsi	7.918	39.187	3119	X	ZMMU_S-197601

Lophuromys melanonyx [HA]

2392	<i>L.melanonyx</i>	melanonyx II	Bale NP	6.851	39.883	4110	X	ZMMU_S-192746
2395	<i>L.melanonyx</i>	melanonyx II	Bale NP	6.851	39.883	4110	X	ZMMU_S-192747
2643	<i>L.melanonyx</i>	melanonyx II	Arsi	7.825	39.414	3791	X	ZMMU_S-197610
2763	<i>L.melanonyx</i>	melanonyx II	Arsi	7.825	39.414	3791	X	ZMMU_S-197645
2666	<i>L.melanonyx</i>	melanonyx I	Arsi	7.825	39.414	3791	X	ZMMU_S-197615
2672	<i>L.melanonyx</i>	melanonyx I	Arsi	7.825	39.414	3791	X	ZMMU_S-197617
2673	<i>L.melanonyx</i>	melanonyx I	Arsi	7.825	39.414	3791	MZ353591.1	ZMMU_S-197618

Out group

	<i>Deomys ferrugineus</i>	outgroup	-	-	-	-	MZ159975.1	-
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Note Designation of "Mitochondrial lineage" correspond to clades of mtDNA identified in Komarova et al. (2021). New sequences obtained in this study are highlighted in green. Species-level habitat–altitude categories used in comparative analyses were defined as follows: low-elevation forest-associated species [LF] — *L. chrysopus*, *L. pseudosikapusi*, *L. brunneus*, *L. menageshae*, *L. chercherensis*; mid-elevation heathland-associated species [MH] — *L. brevicaudus* and *L. flavopunctatus*; high-elevation Afro-alpine species [HA] — *L. melanonyx*; and generalist species with a wide elevational range [GEN] — *L. simensis*. These broad categories were assigned according to typical habitat preferences and elevational ranges rather than the exact sampling elevation of each individual

Table S2 Primer sequences used for long-range PCR amplification of mitochondrial genome fragments

Nº	Primer Name	Primer Sequence (5'-3')	Data Source
1	L1091	AAACTGGGATTAGATACCCCACTAT	Kocher et al. 1989
	H6913_Loph	ATAGAGGTTATGRKGTGGCTTGAAAC	Designed for this study
2	L5310_Loph	CTTGCTCAGCCATHTTACCTATG	Designed for this study
	H11719_Loph	CTTTTATTTGGAGTTGCACCAAG	Designed for this study
3	L11910_Loph	RVAGTAATCCRTTGGTCTTAGGAAC	Designed for this study
	H1478_Loph	TAATTTGAGGAGGGTGACGGGCGGTGTGT	Designed for this study

Note. Sequences selected specifically for this study are indicated in bold and green

Table S3 Long-range PCR protocol.

mix (25 ul)	1 reaction (ul)	final concentration
H2O	14.45	
5X Taq-Buffer	5	1X
10 mM dNTPs	0.75	300 µM
Mg	0.8	
10 µM Primers (F+R)	1	0.4 µM (0.05–1 µM)
Amp Pol	1	5 units/50 µl PCR
DNA	2	0.5-250 ng
Total volume	25	

The PCR conditions were:

D- 94°C/ 30 sec	30X cycl	50sec/kb
94°C /20 sec		
58°C/ 30 seconds		
65°C /8min		
Fin ex- 65°C/10 min		
Hold 4–10°C		

Table S4 Partitions and substitution models used for the phylogenetic analysis

Best Model	Partitions
GTR+I+G	12SrRNA, 16SrRNA, tRNA (Thr)
TRN+G	ND6b, ATP6a
K81UF	ATP6b, CYTBb
GTR+I+G	ND5c, ATP6c
TRN+I+G	ATP8a, tRNA_Pro
F81+G	ATP8b
TRN+G	ATP8c
SYM+I+G	COX1a, tRNA (Leu), COX3a, tRNA (Tyr)
HKY+I	COX1b
GTR+I+G	ND4Lc, COX1c, COX2c
TRN+I+G	COX2a, ND1a, CYTBa
HKY+I	COX3b, COX2b
TIM+I+G	COX3c, ND1c, ND6a, ND3c, ND4c
TRN+I+G	ND2c, CYTBc
TRN+I+G	ND1b
GTR+I+G	ND2a, ND4a, tRNA (Val)
TVM+I+G	ND2b, ND4Lb, ND3b, ND5b, ND4b
GTR+G	tRNA (Met), ND3a, ND4La
TVM+I+G	ND5a, tRNA (Gln), tRNA (Cys), tRNA (Ser), tRNA (Trp), tRNA (Gly), tRNA (phe)
TRN+I	ND6c
HKY+I+G	tRNA (Lys), tRNA (Glu), tRNA (Ile), tRNA (Ala), tRNA (Arg)
HKY	tRNA (Asn), tRNA (Ser)
HKY+I	tRNA (Asp), tRNA (His)
HKY+I	tRNA (Leu)

Note. For the phylogenetic analysis 24 partitions were designated according to their models of evolution. For PCGs, the first codon position is indicated with the letter “a”, second position with “b”, and third position with “c”

Table S5 Results of codon-based tests for positive selection across 12 protein-coding genes

Gene	M0–M3 (LRT/p/ω)	M1a–M2a (LRT/p/ω)	M7–M8 (LRT/p/ω)	M8a – M8 (LRT/p)	BEB sites (PP>0.90)	MEME sites ω>1 (p<0.05)	FUBAR sites ω>1 (PP≥0.95)	FEL sites ω>1 (p≤0.05)	Consistency	Codons
ND1	50.93/<0.001 M3: ω ₂ =14.53 	2.00/1.000 M2a: ω ₂ =50.86 	25.60/<0.001 M8: ω=1.28 	0.37/0.541 	-	-	252 (0.95) *	-	✗	319
ND2	40.52/<0.001 M3: ω ₂ =2.62 	0/1.000 M2a: ω ₂ =1.00 	3.16/0.206 M8: ω=1.00 	0/0.993 	-	91 (0.04) *	-	-	✗	345
ND3	7.78/0.100 M3: ω ₂ =1.44 	0/1.000 M2a: ω ₂ =18,35 	0.001/0.999 M8: ω=1.00 	2.00/0.999 	-	-	-	-	✗	115
ND4L	0.98/0.913 M3: ω ₂ =153.71 	0.001/0.999 M2a: ω ₂ =3,14 	0.001/0.999 M8: ω=1.00 	0/1.000 	-	-	-	-	✗	98
ND4	85.53/<0.001 M3: ω ₂ =0.81 	0/1.000 M2a: ω ₂ =1.00 	24.70/<0.001 M8: ω=1.00 	6.00/0.998 	144 (0.93) *	343 (0.02) *	-	-	✗	467
ND5	113.6/<0.001 M3: ω ₂ =40.41 	0/1.000 M2a: ω ₂ =1.00 	30.14/<0.001 M8: ω=1.00 	0/1.000 	- - 578 (0.92) *	447 (0.01) * 578 (0.01) *	533 (0.93) * 603 (0.92) * 578 (0.97) *	-	✓ (578)	604
CYTB	56.26/<0.001 M3: ω ₂ =19.50 	0/1.000 M2a: ω ₂ =1.00 	25.88/<0.001 M8: ω=1.00 	0/1.000 	-	-	-	-	✗	380

COX1	0/1.000 M3: $\omega_2=80.31$ 	5.22/0.999 M2a: $\omega_2=31,71$ 	0.12/0.942 M8: $\omega=1.00$ 	9.40/0.976 	-	-	-	-	X	514
COX2	37.26/< 0.001 M3: $\omega_2=4.80$ 	0/1.000 M2a: $\omega_2=10,89$ 	20.15/< 0.001 M8: $\omega=1.00$ 	2.12/0.988 	-	-	-	-	X	227
COX3	15.12/ 0.005 M3: $\omega_2=34.10$ 	0/1.000 M2a: $\omega_2=24,97$ 	7.19/ 0.027 M8: $\omega=1.00$ 	2.00/0.999 	-	-	-	-	X	261
ATP6	13.88/ 0.008 M3: $\omega_2=115.5$ 	1.88/0.999 M2a: $\omega_2=28,36$ 	3.15/0.207 M8: $\omega=1.00$ 	2.00/0.999 	-	-	-	-	X	226
ATP8	34.58/< 0.001 M3: $\omega_2=5.81$ 	7.93/ 0.019 M2a: $\omega_2=3.68$ 	16.24/< 0.001 M8: $\omega=3.74$ 	0/ 0.006 	15 (0.99) *	15 (0.05) *	15 (0.99) *	15 (0.037) *	✓ (15)	67

Note. Selection was tested using CODEML site models and DataMonkey methods (MEME, FUBAR, FEL). For CODEML, LRT statistics and p-values are reported for comparisons of nested models (M0–M3, M1a–M2a, M7–M8, M8a–M8). Positively selected sites were identified under M2a and M8 using Bayes Empirical Bayes (BEB) with posterior probability (PP) > 0.90 following significant LRT results ($p < 0.05$). DataMonkey results include MEME ($p \leq 0.05$), FUBAR (PP ≥ 0.95), and FEL ($p \leq 0.05$). Bold font indicates statistically significant p-values ($p < 0.05$) for model comparisons where evidence of positive selection was detected. The "Consistency" column summarizes the agreement between approaches: ✓ — full match of positively selected codons by at least two methods (e.g., codon 578 identified by both SM and Datamonkey), △ — partial match (neighboring or overlapping sites), X — disagreement (codons detected in one method but not in others). Color circles indicate selection strength:  Strong positive ($\omega > 1.3$ and $p < 0.01$),  Weak positive ($1.0 < \omega \leq 1.3$ and $p < 0.05$) and  Neutral/Borderline ($p < 0.05$ and $\omega \approx 1$)  No significant signal ($p \geq 0.05$) (Yang and Nielsen. 2002). Sites under positive selection are marked by asterisks indicating support level: * High (PP > 0.95 in BEB/FUBAR or $p \leq 0.01$ in MEME/FEL), * Medium ($0.90 \leq \text{PP} \leq 0.95$ in BEB/FUBAR or $0.01 < p \leq 0.05$ in MEME/FEL), * Low (PP < 0.90 in BEB/FUBAR or $p > 0.05$ in MEME/FEL)

Table S6 Results of branch-site tests for positive selection in protein-coding genes

Gene	Branch code	ω 1 (class 2a)/ LRT p-value	InL (Model A/Anull)	BEB Sites (PP > 0.90)	MEME Sites (p ≤ 0.05)	aBSREL Sites (p ≤ 0.05)	Consistency
ND1	A	ω 1=1.000/1.000 	-3338.259 / -3338.259	—	—		—
ND1	B	ω 1=1.000/1.000 	-3338.871 / -3338.871	—	—		—
ND1	C	ω 1=1.036/1.000 	-3338.904 / -3338.904	—	—		—
ND1	E	ω 1=1.000/0.980 	-3338.904 / -3338.904	—	—		—
ND1	F	ω 1=1.000/1.000 	-3337.627 / -3337.627	—	—		—
ND1	H	ω 1=1.000/1.000 	-3338.904 / -3338.904	—	—		—
ND1	I	ω 1=52.684/0.109 	-3337.442 / -3338.726	—	—		—
ND1	J	ω 1=1.000/1.000 	-3338.904 / -3338.904	—	—		—
ND1	K	ω 1=1.000/0.997 	-3338.482 / -3338.482	—	—		—
ND1	L	ω 1=1.000/1.000 	-3338.904 / -3338.904	—	—		—
ND1	N	ω 1=1.000/0.998 	-3334.496 / -3334.496	—	—		—
ND1	O	ω 1=1.000/0.256 	-3338.259 / -3338.904	—	—		—
ND2	A	ω 1=3.528/0.321 	-3683.230 / -3683.722	—	—		—
ND2	B	ω 1=1.000/1.000 	-3685.799 / -3685.799	—	—		—
ND2	C	ω 1=1.000/1.000 	-3686.837 / -3686.837	—	—		—
ND2	E	ω 1=1.490/0.988 	-3686.837 / -3686.837	—	—		—
ND2	F	ω 1=36.295/ 0.01 	-3681.618 / -3684.951	91 (0.987) *	91 (0.04) *		✓ (91)
ND2	H	ω 1=1.000/1.000 	-3686.837 / -3686.837	—	—		—
ND2	I	ω 1=1.000/1.000 	-3686.837 / -3686.837	—	—		—
ND2	J	ω 1=1.000/1.000 	-3686.739 / -3686.739	—	—		—
ND2	K	ω 1=1.000/1.000 	-3686.837 / -3686.837	—	—		—
ND2	L	ω 1=1.000/0.637 	-3686.768 / -3686.879	—	—		—
ND2	N	ω 1=1.000/1.000 	-3686.474 / -3686.474	—	—		—

ND2	O	$\omega_1=1.000/1.000$ 	-3686.837 / -3686.837	—	—		—
ND4	A	$\omega_1=1.000/1.000$ 	-4841.058 / -4841.058	—	—		—
ND4	B	$\omega_1=1.000/1.000$ 	-4841.143 / -4841.143	—	343 (0.02) *		X
ND4	C	$\omega_1=8.790/1.000$ 	-4841.143 / -4841.143	—	343 (0.02) *		X
ND4	E	$\omega_1=1.000/1.000$ 	-4841.143 / -4841.143	—	—		—
ND4	F	$\omega_1=60.669/0.132$ 	-4840.010 / -4841.143	—	—		—
ND4	H	$\omega_1=1.000/1.000$ 	-4840.768 / -4840.768	—	—		—
ND4	I	$\omega_1=1.000/1.000$ 	-4841.143 / -4841.143	—	—		—
ND4	J	$\omega_1=1.000/1.000$ 	-4841.143 / -4841.143	—	—		—
ND4	K	$\omega_1=24.662/0.787$ 	-4840.496 / -4840.533	—	—		—
ND4	L	$\omega_1=1.000/1.000$ 	-4841.143 / -4841.143	—	—		—
ND4	N	$\omega_1=1.000/1.000$ 	-4841.143 / -4841.143	—	—		—
ND4	O	$\omega_1=1.000/1.000$ 	-4841.143 / -4841.143	—	—		—
ND5	A	$\omega_1=1.000/1.000$ 	-6141.372 / -6141.372	—	578 (0.01) *		X
ND5	B	$\omega_1=1.000/0.999$ 	-6143.191 / -6143.191	—	578 (0.01) *		X
ND5	C	$\omega_1=602.508/<0.001$ 	-6134.956 / -6141.836	447 (0.996) *	447 (0.01) *	447 (0.035) *	✓(447)
ND5	E	$\omega_1=1.000/1.000$ 	-6143.191 / -6143.191	—	—		—
ND5	F	$\omega_1=1.000/0.994$ 	-6143.191 / -6143.191	—	—		—
ND5	H	$\omega_1=1.000/0.982$ 	-6143.191 / -6143.191	—	—		—
ND5	I	$\omega_1=1.000/0.998$ 	-6143.191 / -6143.191	—	—		—
ND5	J	$\omega_1=1.000/1.000$ 	-6143.191 / -6143.191	—	—		—
ND5	K	$\omega_1=1.000/0.999$ 	-6143.191 / -6143.191	—	—		—
ND5	L	$\omega_1=1.000/1.000$ 	-6143.007 / -6143.007	—	—		—
ND5	N	$\omega_1=1.000/0.999$ 	-6143.191 / -6143.191	—	—		—
ND5	O	$\omega_1=89.950/0.758$ 	-6143.076 / -6143.124	—	—		—
CYTB	A	$\omega_1=1.000/1.000$ 	-3815.030 / -3815.030	—	—		—
CYTB	B	$\omega_1=1.000/1.000$ 	-3815.030 / -3815.030	—	—		—

CYTB	C	$\omega_1=1.000/1.000$		-3815.030 / -3815.030	—	—	—
CYTB	E	$\omega_1=1.000/1.000$		-3815.030 / -3815.030	—	—	—
CYTB	F	$\omega_1=1.000/0.999$		-3815.031 / -3815.030	—	—	—
CYTB	H	$\omega_1=1.000/1.000$		-3814.797 / -3814.797	—	—	—
CYTB	I	$\omega_1=1.000/1.000$		-3815.030 / -3815.030	—	—	—
CYTB	J	$\omega_1=22.176/0.388$		-3813.636 / -3814.009	—	—	—
CYTB	K	$\omega_1=1.000/0.999$		-3815.030 / -3815.031	—	—	—
CYTB	L	$\omega_1=1.000/1.000$		-3815.030 / -3815.030	—	—	—
CYTB	N	$\omega_1=1.000/1.000$		-3812.617 / -3812.617	—	—	—
CYTB	O	$\omega_1=1.000/0.999$		-3815.030 / -3815.031	—	—	—
COX2	A	$\omega_1=1.000/1.000$		-2137.937 / -2137.937	—	—	—
COX2	B	$\omega_1=1.000/0.999$		-2137.688 / -2137.688	—	—	—
COX2	C	$\omega_1=20.987/1.000$		-2137.937 / -2137.937	—	—	—
COX2	E	$\omega_1=1.000/1.000$		-2137.937 / -2137.937	—	—	—
COX2	F	$\omega_1=1.000/0.994$		-2137.937 / -2137.937	—	—	—
COX2	H	$\omega_1=1.000/1.000$		-2137.937 / -2137.937	—	—	—
COX2	I	$\omega_1=1.000/1.000$		-2137.937 / -2137.937	—	—	—
COX2	J	$\omega_1=1.000/1.000$		-2137.937 / -2137.937	—	—	—
COX2	K	$\omega_1=1.000/1.000$		-2137.937 / -2137.937	—	—	—
COX2	L	$\omega_1=1.000/1.000$		-2137.937 / -2137.937	—	—	—
COX2	N	$\omega_1=1.000/1.000$		-2137.937 / -2137.937	—	—	—
COX2	O	$\omega_1=1.000/1.000$		-2137.937 / -2137.937	—	—	—
COX3	A	$\omega_1=2.613/1.000$		-2385.777/-2385.777	—	—	—
COX3	B	$\omega_1=9.507/0.665$		-2384.924/-2385.018	—	—	—
COX3	C	$\omega_1=13.319/1.000$		-2385.777/-2385.777	—	—	—
COX3	E	$\omega_1=1.000/1.000$		-2385.777/-2385.777	—	—	—
COX3	F	$\omega_1=1.000/1.000$		-2385.777/-2385.777	—	—	—
COX3	H	$\omega_1=1.000/1.000$		-2385.777/-2385.777	—	—	—

COX3	I	$\omega_1=1.000/1.000$		-2385.777/-2385.777	—	—	—
COX3	J	$\omega_1=1.000/1.000$		-2385.777/-2385.777	—	—	—
COX3	K	$\omega_1=1.000/1.000$		-2385.777/-2385.777	—	—	—
COX3	L	$\omega_1=1.000/1.000$		-2385.777/-2385.777	—	—	—
COX3	N	$\omega_1=1.000/1.000$		-2385.777/-2385.777	—	—	—
COX3	O	$\omega_1=1.000/1.000$		-2385.777/-2385.777	—	—	—
ATP6	A	$\omega_1=1.000/1.000$		-2090.911/-2090.911	—	—	—
ATP6	B	$\omega_1=1.000/1.000$		-2090.911/-2090.911	—	—	—
ATP6	C	$\omega_1=8.475/1.000$		-2090.911/-2090.911	—	—	X
ATP6	E	$\omega_1=1.000/1.000$		-2090.911/-2090.911	—	—	—
ATP6	F	$\omega_1=1.000/1.000$		-2090.911/-2090.911	—	—	—
ATP6	H	$\omega_1=1.000/1.000$		-2088.486/-2088.486	—	—	X
ATP6	I	$\omega_1=1.000/1.000$		-2090.911/-2090.911	—	—	—
ATP6	J	$\omega_1=1.000/1.000$		-2090.911/-2090.911	—	—	—
ATP6	K	$\omega_1=1.000/1.000$		-2090.911/-2090.911	—	—	—
ATP6	L	$\omega_1=1.000/1.000$		-2090.911/-2090.911	—	—	—
ATP6	N	$\omega_1=1.000/1.000$		-2090.911/-2090.911	—	—	—
ATP6	O	$\omega_1=1.000/1.000$		-2090.911/-2090.911	—	—	—
ATP8	A	$\omega_1=1.000/1.000$		-553.3615/-553.3615	—	15 (0.05) *	X
ATP8	B	$\omega_1=1.000/0.993$		-553.4506/-553.4507	—	15 (0.05) *	X
ATP8	C	$\omega_1=1.000/1.000$		-553.4506/-553.4506	—	—	—
ATP8	E	$\omega_1=1.000/1.000$		-553.4506/-553.4506	—	—	—
ATP8	F	$\omega_1=1.000/1.000$		-553.4506/-553.4506	—	—	—
ATP8	H	$\omega_1=1.000/1.000$		-553.4506/-553.4506	—	—	—
ATP8	I	$\omega_1=10.212/0.774$		-553.0346/-553.0760	—	—	—
ATP8	J	$\omega_1=1.000/0.997$		-553.4506/-553.4506	—	—	—
ATP8	K	$\omega_1=1.000/1.000$		-553.4506/-553.4506	—	—	—
ATP8	L	$\omega_1=1.000/1.000$		-553.3615/-553.3615	—	—	—

ATP8	N	$\omega_1=1.000/1.000$ 	-553.4506/-553.4506	—	15 (0.05) *		X
ATP8	O	$\omega_1=1.000/1.000$ 	-553.4506/-553.4506	—	—		—

Note. Results combine CODEML branch-site models (BSM) and DataMonkey methods (MEME, aBSREL). For BSM, ω_1 (dN/dS for class 2a sites) ratios and LRT p-values (vs. Model A null) are reported for foreground lineages. For Model A null, ω_1 was fixed to 1 (neutral evolution) as per standard branch-site test implementation in CODEML. Positively selected sites were inferred under Model A using Bayes Empirical Bayes (BEB; PP > 0.90) only when the LRT was significant ($p < 0.05$) compared to Model A null (where ω is fixed to 1 for foreground branches). MEME results (LRT $p \leq 0.05$) are included for episodic selection detection. Bold font indicates statistically significant p-values ($p < 0.05$) for model comparisons where evidence of positive selection was detected. The "Consistency" column summarizes the agreement between approaches: ✓ — full match of positively selected codons by at least two methods (e.g., codon 578 identified by both SM and Datamonkey), X — disagreement (codons detected in one method but not in others). Color circles indicate selection strength:  Strong positive ($\omega > 1.3$ and $p < 0.01$),  Weak positive ($1.0 < \omega \leq 1.3$ and $p < 0.05$) and  Neutral/Borderline ($p < 0.05$ and $\omega \approx 1$)  No significant signal ($p \geq 0.05$) (Yang and Nielsen. 2002). Sites under positive selection are marked by asterisks indicating support level: * High (PP > 0.95 in BEB or $p \leq 0.01$ in MEME), * Medium ($0.90 \leq \text{PP} \leq 0.95$ in BEB or $0.01 < p \leq 0.05$ in MEME), * Low (PP < 0.90 in BEB or $p > 0.05$ in MEME). Following Yang and Nielsen (2002) and empirical studies (e.g., Zhang et al. 2005), we classify $\omega > 1.3$ as strong positive selection, with BEB PP > 0.90 along the foreground branch. Lineage codes (A–O) match Fig. 2 in the main text

Table S7 Results of branch-specific selection analysis using CODEML Branch Models (BM).

Gene	Branch code	Model	Ln L	Estimates of parameters	LRT P-value	ω for foreground branches
ND1	A	M2	-3364.191	$\omega_0=0.027$, $\omega_1=0.036$	0.686	—
		M0	-3364.273	$\omega=0.028$ 		
ND1	B	M2	-3364.111	$\omega_0=0.027$, $\omega_1=0.038$	0.569	—
		M0	-3364.273	$\omega=0.028$ 		
ND1	C	M2	-3359.802	$\omega_0=0.032$, $\omega_1=0.003$	0.003	$\omega_1=0.003$ 
		M0	-3364.273	$\omega=0.028$		
ND1	E	M2	-3363.873	$\omega_0=0.027$, $\omega_1=0.087$	0.371	—
		M0	-3364.273	$\omega=0.028$ 		
ND1	F	M2	-3363.294	$\omega_0=0.026$, $\omega_1=0.059$	0.162	—
		M0	-3364.273	$\omega=0.028$ 		
ND1	H	M2	-3361.568	$\omega_0=0.030$, $\omega_1 < 0.001$	0.020	$\omega_1 < 0.001$ 
		M0	-3364.273	$\omega=0.028$		
ND1	I	M2	-3364.272	$\omega_0=0.028$, $\omega_1=0.029$	0.966	—
		M0	-3364.273	$\omega=0.028$ 		
ND1	J	M2	-3363.298	$\omega_0=0.028$, $\omega_1 < 0.001$	0.163	—
		M0	-3364.273	$\omega=0.028$ 		
ND1	K	M2	-3363.639	$\omega_0=0.027$, $\omega_1=0.092$	0.260	—
		M0	-3364.273	$\omega=0.028$ 		
ND1	L	M2	-3363.714	$\omega_0=0.028$, $\omega_1 < 0.001$	0.290	—
		M0	-3364.273	$\omega=0.028$ 		
ND1	N	M2	-3360.145	$\omega_0=0.026$, $\omega_1=0.943$	0.004	$\omega_1=0.943$ 
		M0	-3364.273	$\omega=0.028$		
ND1	O	M2	-3363.004	$\omega_0=0.027$, $\omega_1=0.337$	0.111	—
		M0	-3364.273	$\omega=0.028$ 		
ND2	A	M2	-3699.832	$\omega_0=0.054$, $\omega_1=0.121$	0.038	$\omega_1=0.121$ 
		M0	-3701.989	$\omega=0.059$		
ND2	B	M2	-3701.802	$\omega_0=0.058$, $\omega_1=0.080$	0.541	—

		M0	-3701.989	$\omega=0.059$ 		
ND2	C	M2	-3701.037	$\omega_0=0.063, \omega_1=0.033$	0.168	—
		M0	-3701.989	$\omega=0.059$ 		
ND2	E	M2	-3701.772	$\omega_0=0.059, \omega_1=0.104$	0.511	—
		M0	-3701.989	$\omega=0.059$ 		
ND2	F	M2	-3700.874	$\omega_0=0.057, \omega_1=0.134$	0.135	—
		M0	-3701.989	$\omega=0.059$ 		
ND2	H	M2	-3700.746	$\omega_0=0.062, \omega_1=0.016$	0.115	—
		M0	-3701.989	$\omega=0.059$ 		
ND2	I	M2	-3699.168	$\omega_0=0.062, \omega_1 < 0.001$	0.018	$\omega_1 < 0.001$ 
		M0	-3701.989	$\omega=0.059$		
ND2	J	M2	-3701.232	$\omega_0=0.058, \omega_1=0.131$	0.219	—
		M0	-3701.989	$\omega=0.059$ 		
ND2	K	M2	-3701.802	$\omega_0=0.058, \omega_1=0.080$	0.541	—
		M0	-3701.989	$\omega=0.059$ 		
ND2	L	M2	-3701.981	$\omega_0=0.060, \omega_1=0.054$	0.903	—
		M0	-3701.989	$\omega=0.059$ 		
ND2	N	M2	-3701.869	$\omega_0=0.059, \omega_1=0.108$	0.625	—
		M0	-3701.989	$\omega=0.059$ 		
ND2	O	M2	-3701.802	$\omega_0=0.058, \omega_1=0.080$	0.541	—
		M0	-3701.989	$\omega=0.059$ 		
ND3	A	M2	-1223.992	$\omega_0=0.028, \omega_1 < 0.001$	0.105	—
		M0	-1225.307	$\omega=0.025$ 		
ND3	B	M2	-1224.544	$\omega_0=0.027, \omega_1 < 0.001$	0.217	—
		M0	-1225.307	$\omega=0.025$ 		
ND3	C	M2	-1223.598	$\omega_0=0.029, \omega_1 < 0.001$	0.064	—
		M0	-1225.307	$\omega=0.025$ 		
ND3	E	M2	-1225.242	$\omega_0=0.026, \omega_1 < 0.001$	0.718	—
		M0	-1225.307	$\omega=0.025$ 		
ND3	F	M2	-1225.064	$\omega_0=0.027, \omega_1=0.014$	0.486	—

		M0	-1225.307	$\omega=0.025$ 		
ND3	H	M2	-1224.992	$\omega_0=0.026, \omega_1 < 0.001$	0.428	—
		M0	-1225.307	$\omega=0.025$ 		
ND3	I	M2	-1224.812	$\omega_0=0.027, \omega_1 < 0.001$	0.320	—
		M0	-1225.307	$\omega=0.025$ 		
ND3	J	M2	-1224.978	$\omega_0=0.026, \omega_1 < 0.001$	0.418	—
		M0	-1225.307	$\omega=0.025$ 		
ND3	K	M2	-1225.218	$\omega_0=0.026, \omega_1 < 0.001$	0.674	—
		M0	-1225.307	$\omega=0.025$ 		
ND3	L	M2	-1224.748	$\omega_0=0.027, \omega_1 < 0.001$	0.290	—
		M0	-1225.307	$\omega=0.025$ 		
ND3	N	M2	-1225.246	$\omega_0=0.026, \omega_1 < 0.001$	0.728	—
		M0	-1225.307	$\omega=0.025$ 		
ND3	O	M2	-1225.178	$\omega_0=0.026, \omega_1 < 0.001$	0.612	—
		M0	-1225.307	$\omega=0.025$ 		
ND4	A	M2	-4878.721	$\omega_0=0.040, \omega_1=0.049$	0.616	—
		M0	-4878.847	$\omega=0.040$ 		
ND4	B	M2	-4878.794	$\omega_0=0.040, \omega_1=0.033$	0.744	—
		M0	-4878.847	$\omega=0.040$ 		
ND4	C	M2	-4876.093	$\omega_0=0.045, \omega_1=0.014$	0.019	$\omega_1=0.014$ 
		M0	-4878.847	$\omega=0.040$		
ND4	E	M2	-4878.687	$\omega_0=0.040, \omega_1 < 0.001$	0.571	—
		M0	-4878.847	$\omega=0.040$ 		
ND4	F	M2	-4878.826	$\omega_0=0.040, \omega_1=0.036$	0.835	—
		M0	-4878.847	$\omega=0.040$ 		
ND4	H	M2	-4878.738	$\omega_0=0.039, \omega_1=0.054$	0.640	—
		M0	-4878.847	$\omega=0.040$ 		
ND4	I	M2	-4878.846	$\omega_0=0.040, \omega_1=0.038$	0.959	—
		M0	-4878.847	$\omega=0.040$ 		
ND4	J	M2	-4878.846	$\omega_0=0.040, \omega_1=0.038$	0.959	—

		M0	-4878.847	$\omega=0.040$ 		
ND4	K	M2	-4878.763	$\omega_0=0.040, \omega_1=0.064$	0.682	—
		M0	-4878.847	$\omega=0.040$ 		
ND4	L	M2	-4877.937	$\omega_0=0.039, \omega_1=0.138$	0.177	—
		M0	-4878.847	$\omega=0.040$ 		
ND4	N	M2	-4878.379	$\omega_0=0.040, \omega_1 < 0.001$	0.333	—
		M0	-4878.847	$\omega=0.040$ 		
ND4	O	M2	-4878.404	$\omega_0=0.040, \omega_1=0.135$	0.346	—
		M0	-4878.847	$\omega=0.040$ 		
ND4L	A	M2	-1070.370	$\omega_0=0.020, \omega_1=0.172$	0.054	—
		M0	-1072.221	$\omega=0.024$ 		
ND4L	B	M2	-1071.597	$\omega_0=0.026, \omega_1 < 0.001$	0.264	—
		M0	-1072.221	$\omega=0.024$ 		
ND4L	C	M2	-1072.129	$\omega_0=0.026, \omega_1=0.018$	0.669	—
		M0	-1072.221	$\omega=0.024$ 		
ND4L	E	M2	-1072.168	$\omega_0=0.024, \omega_1 < 0.001$	0.745	—
		M0	-1072.221	$\omega=0.024$ 		
ND4L	F	M2	-1072.034	$\omega_0=0.023, \omega_1=0.049$	0.541	—
		M0	-1072.221	$\omega=0.024$ 		
ND4L	H	M2	-1071.785	$\omega_0=0.025, \omega_1 < 0.001$	0.351	—
		M0	-1072.221	$\omega=0.024$ 		
ND4L	I	M2	-1071.759	$\omega_0=0.022, \omega_1=0.079$	0.336	—
		M0	-1072.221	$\omega=0.024$ 		
ND4L	J	M2	-1071.980	$\omega_0=0.025, \omega_1 < 0.001$	0.488	—
		M0	-1072.221	$\omega=0.024$ 		
ND4L	K	M2	-1071.974	$\omega_0=0.025, \omega_1 < 0.001$	0.482	—
		M0	-1072.221	$\omega=0.024$ 		
ND4L	L	M2	-1072.051	$\omega_0=0.025, \omega_1 < 0.001$	0.560	—
		M0	-1072.221	$\omega=0.024$ 		
ND4L	N	M2	-1072.169	$\omega_0=0.024, \omega_1 < 0.001$	0.747	—

		M0	-1072.221	$\omega=0.024$ 		
ND4L	O	M2	-1072.221	$\omega_0=0.024, \omega_1=2.319$	0.996	—
		M0	-1072.221	$\omega=0.024$ 		
ND5	A	M2	-6196.265	$\omega_0=0.054, \omega_1=0.096$	0.069	—
		M0	-6197.921	$\omega=0.057$ 		
ND5	B	M2	-6196.560	$\omega_0=0.059, \omega_1=0.026$	0.099	—
		M0	-6197.921	$\omega=0.057$ 		
ND5	C	M2	-6197.762	$\omega_0=0.058, \omega_1=0.049$	0.573	—
		M0	-6197.921	$\omega=0.057$ 		
ND5	E	M2	-6196.128	$\omega_0=0.057, \omega_1=999.000$	0.058	—
		M0	-6197.921	$\omega=0.057$ 		
ND5	F	M2	-6195.924	$\omega_0=0.060, \omega_1=0.025$	0.046	$\omega_1=0.025$ 
		M0	-6197.921	$\omega=0.057$		
ND5	H	M2	-6196.366	$\omega_0=0.059, \omega_1=0.021$	0.078	—
		M0	-6197.921	$\omega=0.057$ 		
ND5	I	M2	-6197.800	$\omega_0=0.058, \omega_1=0.046$	0.623	—
		M0	-6197.921	$\omega=0.057$ 		
ND5	J	M2	-6196.874	$\omega_0=0.059, \omega_1=0.023$	0.148	—
		M0	-6197.921	$\omega=0.057$ 		
ND5	K	M2	-6197.671	$\omega_0=0.057, \omega_1=0.106$	0.479	—
		M0	-6197.921	$\omega=0.057$ 		
ND5	L	M2	-6197.513	$\omega_0=0.056, \omega_1=0.098$	0.367	—
		M0	-6197.921	$\omega=0.057$ 		
ND5	N	M2	-6197.461	$\omega_0=0.057, \omega_1 < 0.001$	0.338	—
		M0	-6197.921	$\omega=0.057$ 		
ND5	O	M2	-6197.600	$\omega_0=0.057, \omega_1=0.165$	0.423	—
		M0	-6197.921	$\omega=0.057$ 		
CYTB	A	M2	-3840.715	$\omega_0=0.017, \omega_1=0.007$	0.330	—
		M0	-3841.088	$\omega=0.017$ 		
CYTB	B	M2	-3840.715	$\omega_0=0.017, \omega_1=0.008$	0.388	—

		M0	-3841.088	$\omega=0.017$ 		
CYTB	C	M2	-3837.878	$\omega_0=0.020, \omega_1=0.003$	0.011	$\omega_1=0.003$ 
		M0	-3841.088	$\omega=0.017$		
CYTB	E	M2	-3840.884	$\omega_0=0.017, \omega_1 < 0.001$	0.524	—
		M0	-3841.088	$\omega=0.017$ 		
CYTB	F	M2	-3840.250	$\omega_0=0.018, \omega_1=0.006$	0.196	—
		M0	-3841.088	$\omega=0.017$ 		
CYTB	H	M2	-3841.083	$\omega_0=0.017, \omega_1=0.015$	0.923	—
		M0	-3841.088	$\omega=0.017$ 		
CYTB	I	M2	-3840.777	$\omega_0=0.016, \omega_1=0.028$	0.430	—
		M0	-3841.088	$\omega=0.017$ 		
CYTB	J	M2	-3840.411	$\omega_0=0.016, \omega_1=0.036$	0.245	—
		M0	-3841.088	$\omega=0.017$ 		
CYTB	K	M2	-3841.048	$\omega_0=0.017, \omega_1=0.034$	0.778	—
		M0	-3841.088	$\omega=0.017$ 		
CYTB	L	M2	-3841.038	$\omega_0=0.017, \omega_1=0.027$	0.753	—
		M0	-3841.088	$\omega=0.017$ 		
CYTB	N	M2	-3839.500	$\omega_0=0.016, \omega_1=0.289$	0.075	—
		M0	-3841.088	$\omega=0.017$ 		
CYTB	O	M2	-3841.042	$\omega_0=0.017, \omega_1 < 0.001$	0.762	—
		M0	-3841.088	$\omega=0.017$ 		
COX1	A	M2	-4867.823	$\omega_0=0.004, \omega_1=0.006$	0.691	—
		M0	-4867.902	$\omega=0.004$ 		
COX1	B	M2	-4867.484	$\omega_0=0.004, \omega_1 < 0.001$	0.361	—
		M0	-4867.902	$\omega=0.004$ 		
COX1	C	M2	-4866.542	$\omega_0=0.005, \omega_1 < 0.001$	0.099	—
		M0	-4867.902	$\omega=0.004$ 		
COX1	E	M2	-4867.866	$\omega_0=0.004, \omega_1 < 0.001$	0.788	—
		M0	-4867.902	$\omega=0.004$ 		
COX1	F	M2	-2391.947	$\omega_0=0.016, \omega_1 < 0.001$	0.201	—

		M0	-2392.765	$\omega=0.015$ 		
COX1	H	M2	-4867.484	$\omega_0=0.004, \omega_1 < 0.001$	0.361	—
		M0	-4867.902	$\omega=0.004$ 		
COX1	I	M2	-4867.316	$\omega_0=0.004, \omega_1=0.015$	0.279	—
		M0	-4867.902	$\omega=0.004$ 		
COX1	J	M2	-4867.673	$\omega_0=0.004, \omega_1 < 0.001$	0.498	—
		M0	-4867.902	$\omega=0.004$ 		
COX1	K	M2	-4867.842	$\omega_0=0.004, \omega_1 < 0.001$	0.728	—
		M0	-4867.902	$\omega=0.004$ 		
COX1	L	M2	-4866.901	$\omega_0=0.004, \omega_1=0.025$	0.157	—
		M0	-4867.902	$\omega=0.004$ 		
COX1	N	M2	-4867.807	$\omega_0=0.004, \omega_1 < 0.001$	0.662	—
		M0	-4867.902	$\omega=0.004$ 		
COX1	O	M2	-4867.866	$\omega_0=0.004, \omega_1 < 0.001$	0.787	—
		M0	-4867.902	$\omega=0.004$ 		
COX2	A	M2	-2156.351	$\omega_0=0.023, \omega_1=0.019$	0.811	—
		M0	-2156.380	$\omega=0.022$ 		
COX2	B	M2	-2156.052	$\omega_0=0.021, \omega_1=0.042$	0.418	—
		M0	-2156.380	$\omega=0.022$ 		
COX2	C	M2	-2153.125	$\omega_0=0.026, \omega_1 < 0.001$	0.011	$\omega_1 < 0.001$ 
		M0	-2156.380	$\omega=0.022$		
COX2	E	M2	-2156.263	$\omega_0=0.022, \omega_1 < 0.001$	0.628	—
		M0	-2156.380	$\omega=0.022$ 		
COX2	F	M2	-2391.947	$\omega_0=0.016, \omega_1 < 0.001$	0.201	—
		M0	-2392.765	$\omega=0.015$ 		
COX2	H	M2	-2155.904	$\omega_0=0.023, \omega_1 < 0.001$	0.329	—
		M0	-2156.380	$\omega=0.022$ 		
COX2	I	M2	-2155.838	$\omega_0=0.023, \omega_1 < 0.001$	0.298	—
		M0	-2156.380	$\omega=0.022$ 		
COX2	J	M2	-2155.838	$\omega_0=0.023, \omega_1 < 0.001$	0.298	—

		M0	-2156.380	$\omega=0.022$ 		
COX2	K	M2	-2156.123	$\omega_0=0.023, \omega_1 < 0.001$	0.473	—
		M0	-2156.380	$\omega=0.022$ 		
COX2	L	M2	-2156.121	$\omega_0=0.023, \omega_1 < 0.001$	0.472	—
		M0	-2156.380	$\omega=0.022$ 		
COX2	N	M2	-2156.190	$\omega_0=0.022, \omega_1 < 0.001$	0.537	—
		M0	-2156.380	$\omega=0.022$ 		
COX2	O	M2	-2156.284	$\omega_0=0.022, \omega_1 < 0.001$	0.660	—
		M0	-2156.380	$\omega=0.022$ 		
COX3	A	M2	-2392.747	$\omega_0=0.014, \omega_1=0.017$	0.849	—
		M0	-2392.765	$\omega=0.015$ 		
COX3	B	M2	-2392.483	$\omega_0=0.014, \omega_1=0.026$	0.453	—
		M0	-2392.765	$\omega=0.015$ 		
COX3	C	M2	-2392.026	$\omega_0=0.017, \omega_1=0.005$	0.224	—
		M0	-2392.765	$\omega=0.015$ 		
COX3	E	M2	-2392.720	$\omega_0=0.015, \omega_1 < 0.001$	0.763	—
		M0	-2392.765	$\omega=0.015$ 		
COX3	F	M2	-2391.947	$\omega_0=0.016, \omega_1 < 0.001$	0.201	—
		M0	-2392.765	$\omega=0.015$ 		
COX3	H	M2	-2392.615	$\omega_0=0.014, \omega_1=0.027$	0.584	—
		M0	-2392.765	$\omega=0.015$ 		
COX3	I	M2	-2391.802	$\omega_0=0.016, \omega_1 < 0.001$	0.165	—
		M0	-2392.765	$\omega=0.015$ 		
COX3	J	M2	-2392.601	$\omega_0=0.014, \omega_1=0.028$	0.567	—
		M0	-2392.765	$\omega=0.015$ 		
COX3	K	M2	-2392.673	$\omega_0=0.015, \omega_1 < 0.001$	0.667	—
		M0	-2392.765	$\omega=0.015$ 		
COX3	L	M2	-2392.516	$\omega_0=0.015, \omega_1 < 0.001$	0.480	—
		M0	-2392.765	$\omega=0.015$ 		
COX3	N	M2	-2392.765	$\omega_0=0.015, \omega_1=2.080$	0.991	—

		M0	-2392.765	$\omega=0.015$ 		
COX3	O	M2	-2392.720	$\omega_0=0.015, \omega_1 < 0.001$	0.763	—
		M0	-2392.765	$\omega=0.015$ 		
ATP6	A	M2	-2094.705	$\omega_0=0.023, \omega_1 < 0.001$	0.135	—
		M0	-2095.823	$\omega=0.021$ 		
ATP6	B	M2	-2095.807	$\omega_0=0.020, \omega_1=0.025$	0.857	—
		M0	-2095.823	$\omega=0.021$ 		
ATP6	C	M2	-2093.526	$\omega_0=0.024, \omega_1 < 0.001$	0.032	$\omega_1 < 0.001$ 
		M0	-2095.823	$\omega=0.021$		
ATP6	E	M2	-2095.736	$\omega_0=0.021, \omega_1 < 0.001$	0.677	—
		M0	-2095.823	$\omega=0.021$ 		
ATP6	F	M2	-2094.830	$\omega_0=0.022, \omega_1 < 0.001$	0.159	—
		M0	-2095.823	$\omega=0.021$ 		
ATP6	H	M2	-2095.221	$\omega_0=0.022, \omega_1 < 0.001$	0.273	—
		M0	-2095.823	$\omega=0.021$ 		
ATP6	I	M2	-2094.748	$\omega_0=0.022, \omega_1 < 0.001$	0.143	—
		M0	-2095.823	$\omega=0.021$ 		
ATP6	J	M2	-2095.209	$\omega_0=0.022, \omega_1 < 0.001$	0.268	—
		M0	-2095.823	$\omega=0.021$ 		
ATP6	K	M2	-2095.741	$\omega_0=0.021, \omega_1 < 0.001$	0.686	—
		M0	-2095.823	$\omega=0.021$ 		
ATP6	L	M2	-2095.727	$\omega_0=0.021, \omega_1 < 0.001$	0.661	—
		M0	-2095.823	$\omega=0.021$ 		
ATP6	N	M2	-2094.091	$\omega_0=0.019, \omega_1=0.418$	0.063	—
		M0	-2095.823	$\omega=0.021$ 		
ATP6	O	M2	-2095.774	$\omega_0=0.021, \omega_1 < 0.001$	0.755	—
		M0	-2095.823	$\omega=0.021$ 		
ATP8	A	M2	-566.577	$\omega_0=0.142, \omega_1=0.285$	0.695	—
		M0	-566.654	$\omega=0.152$ 		
ATP8	B	M2	-566.353	$\omega_0=0.164, \omega_1=0.071$	0.437	—

		M0	-566.654	$\omega=0.152$ 		
ATP8	C	M2	-565.274	$\omega_0=0.184, \omega_1=0.037$	0.097	—
		M0	-566.654	$\omega=0.152$ 		
ATP8	E	M2	-566.654	$\omega_0=0.152, \omega_1=107.267$	0.993	—
		M0	-566.654	$\omega=0.152$ 		
ATP8	F	M2	-566.324	$\omega_0=0.156, \omega_1 < 0.001$	0.416	—
		M0	-566.654	$\omega=0.152$ 		
ATP8	H	M2	-566.541	$\omega_0=0.159, \omega_1 < 0.001$	0.634	—
		M0	-566.654	$\omega=0.152$ 		
ATP8	I	M2	-566.883	$\omega_0=0.157, \omega_1=0.127$	0.499	—
		M0	-566.654	$\omega=0.152$ 		
ATP8	J	M2	-566.218	$\omega_0=0.163, \omega_1 < 0.001$	0.350	—
		M0	-566.654	$\omega=0.152$ 		
ATP8	K	M2	-566.332	$\omega_0=0.156, \omega_1 < 0.001$	0.422	—
		M0	-566.654	$\omega=0.152$ 		
ATP8	L	M2	-566.654	$\omega_0=0.152, \omega_1=283.914$	0.994	—
		M0	-566.654	$\omega=0.152$ 		
ATP8	N	M2	-566.682	$\omega_0=0.149, \omega_1=0.396$	0.815	—
		M0	-566.654	$\omega=0.152$ 		
ATP8	O	M2	-566.899	$\omega_0=0.155, \omega_1=395.517$	0.484	—
		M0	-566.654	$\omega=0.152$ 		

Notes. Reported are ω (dN/dS) ratios for foreground lineages (ω_1) and background branches (ω_0) under the two-ratio model (M2), compared to the one-ratio model (M0). Likelihood ratio test (LRT) p-values evaluate whether foreground ω_1 differs significantly from background ω_0 . Bold values indicate statistically significant LRT results ($p < 0.05$) comparing M2 model versus model M0. When the test is non-significant ($p \geq 0.05$), the foreground ω_1 cannot be distinguished from background evolution and the genome-wide ω value from M0 (one-ratio) is shown instead; non-significant foreground ω_1 values are marked with "—". Selection strength is color-coded as follows:  Strong purifying selection ($\omega < 0.3$),  Moderate purifying selection ($0.3 \leq \omega < 1$),  Neutral evolution ($\omega = 1$),  Strong positive selection ($\omega > 1.3$),  Weak positive selection ($1.0 < \omega \leq 1.3$). Color highlighting applies to uniform ω from M0 when $p \geq 0.05$ or ω_1 (foreground) when $p < 0.05$. Following Yang and Nielsen (2002) and

empirical studies (e.g., Zhang et al. 2005), we classify $\omega > 1.3$ as strong positive selection, with BEB PP > 0.90 along the foreground branch. Lineage codes (A–O) correspond to Fig. 2 in the main text

Table S8 Branch-specific evolutionary rate estimates for 12 PCGs across *Lophuromys* lineages, inferred using aBSREL

Branch code	ND1	ND2	ND3	ND4	ND4 L	ND5	CYTB	COX1	COX2	COX3	ATP6	ATP8	Selection (branch-level, $p < 0.05$)
C	0.004	0.035	0	0.014	0.020	0.054	0.004	0	0	0.008	0	0.048	Yes (ND5-447)
F	0.062	0.112*	0.016	0.040	0.034	0.027	0.008	0	0	0	0	0	No ($p = 1$)
A	0.037	0.121*	0	0.038	0.148	0.112	0.009	0.007	0.024	0.022	0	0.330	No ($p = 1$)
B	0.042	0.080	0	0.039	0	0.030	0.009	0	0.049	0.036	0.022	0.080	No ($p = 1$)
E	0.073	0	0	0.044	0	0.095	0	0.042	0	0	0.055	N/A	No ($p = 1$)
H	0	0.016	0	0.065	0	0.019	0.019*	0	0	0.034	0	0	No ($p = 1$)
I	0.029	0	0	0.043	0.065	0.050	0.035	0.017	0	0	0	0.140	No ($p = 1$)
J	0	0.129	0	0.036	0	0.025	0.044	0	0	0.035	0	0	No ($p = 1$)
K	0.102	0.043	0	0.059	0	0.117	0.080	0	0	0	0	0	No ($p = 1$)
N	0.663	0.111	0	0	0	0	0.350	0	0	N/A	0.360	N/A	No ($p = 1$)
O	0.331	0	0	0.123	N/A	0.179	0	0	0	0	0	N/A	No ($p = 1$)
L	0	0.056	0	0.127	0	0.104	0	0.030	0	0	N/A	0	No ($p = 1$)

Notes. The values represent mean baseline ω ratios (MG94xREV model), reflecting the background (average) dN/dS (ω) ratios across individual branches. Lineages with significant evidence of episodic diversifying selection ($p < 0.05$ after Holm-Bonferroni correction) are highlighted in green. Non-significant branches (LRT $p = 1$) uniformly exhibited pervasive purifying selection across 100% of sites ($\omega \ll 1$), as confirmed by Rate distributions analysis. For branches with statistically supported positive selection (LRT $p < 0.05$), additional parameters from Rate distributions were reported in the Results section: (1) specific ω values for each rate category, and (2) the proportion of sites under positive and purifying selection. Extreme ω values (>100), typically occurring in short branches with fewer than 5 substitutions, were excluded from analysis (marked as N/A and highlighted in light gray) due to model estimation artifacts. Asterisks (*) mark branches with non-

significant rate heterogeneity (LRT $p=1$) showing two evolutionary modes: branch F (ND2) with $\omega=0.022$ (98.4% sites) and $\omega=18.857$ (1.6%); branch A (ND2) with $\omega=0$ (94.1% sites) and $\omega=3.716$ (5.9%); and branch H (CYTB) with $\omega=0$ (99.7% sites) and $\omega\approx 116$ (0.3%). These ω values likely represent computational artifacts due to limited substitutions in short branches. While slightly inflating baseline ω estimates, they don't affect our conclusion of dominant purifying selection across lineages since they (1) affect insignificant site proportions (<6%) and (2) preserve baseline $\omega<1$ in all cases. Branches with ω values >2 are marked as NA due to model estimation artifacts, primarily occurring in lineages with few substitutions and being biologically implausible in most proteins. Lineage codes (A–O) correspond to Fig. 2 in the main text

Table S9 Proportion of sites under purifying selection ($\omega < 1$) in mitochondrial PCGs using FEL

Gene	N sites ($\omega < 1$)	% purifying selection	Gene length (codons)
Complex I	639	32,80287474	1948
ND1	118	37.00	319
ND2	102	29.60	345
ND3	46	40.00	115
ND4L	37	37.80	98
ND4	148	31.70	467
ND5	188	31.10	604
Complex III			
CYTB	165	43.40	380
Complex IV	351	35,02994012	1002
COX1	187	36.40	514
COX2	69	30.40	227
COX3	95	36.40	261
Complex V	78	26,62116041	293
ATP6	68	30.10	226
ATP8	10	14.90	67
Total	1233	34.00	3623

Table S10 Localization of positively selected radical amino acid changes among the individual mitochondrial lineages in Ethiopian *Lophuromys*, as detected by TreeSAAP

[illegible]

[illegible]

CYTB (39)	–	–	ATC→G TC	ATC→GT C	–	–	ATC→GT C	–	–	–	–	–
Ile→Val												
CYTB (43)	–	–	–	–	–	–	–	CTT→AT C	–	–	–	–
Leu→Ile												
CYTB (232)	–	–	–	–	–	–	ATT→GT T	–	–	–	–	–
Ile→Val												
ATP8 (15)	–	–	GCC→A CC	GCC→AC C	–	–	–	–	–	GCC→AC C	–	–
Ala→Thr												

Notes. For each amino acid replacement, the program evaluates changes across 31 physicochemical properties and assigns a magnitude category from 1 to 8, ranking the extent to which a given substitution alters the intrinsic property scale of the encoded amino acid (McClellan and Ellison 2010). Categories 1–2 reflect minimal (conservative) changes, 3–5 moderate, and 6–8 the most radical shifts in property values. TreeSAAP then compares the observed frequency of such property-altering amino acid replacements to the expectations under a neutral model by calculating a z-score for each property. A significantly positive z-score ($z > 1.645$; $p < 0.05$) indicates an excess of replacements in a given category compared to neutral expectations, suggesting that positive selection is acting on that property. This approach enabled us to identify which physicochemical properties were evolving under directional (positive) selection and to map the respective signals across protein regions.

Amino acid abbreviations: Ala, Alanine; Thr, Threonine; Ile, Isoleucine; Val, Valine; Tyr, Tyrosine; His, Histidine; Ser, Serine; Phe, Phenylalanine; Met, Methionine; Leu, Leucine

Table S11 Standardized number of adaptive amino acid changes across mitochondrial PCGs in Ethiopian *Lophuromys*

Gene/Complex	Complex	Protein length (aa)	Radical changes	Weight factor	Standardized number of adaptive amino acid changes
Complex I	I	1948	17	6.65	2.56
ND1	I	319	2	1.09	1.84
ND2	I	345	3	1.18	2.55
ND3	I	115	0	0.39	0.0
ND4	I	467	0	1.59	0.0
ND4L	I	98	0	0.33	0.0
ND5	I	604	12	2.06	5.82
Complex III					
CYTB	III	380	3	1.3	2.31
Complex IV	IV	1002	2	3.42	0.58
COX1	IV	514	0	1.75	0.0
COX2	IV	227	2	0.77	2.58
COX3	IV	261	0	0.89	0.0
Complex V	V	293	1	1.0	1.0
ATP6	V	226	0	0.77	0.0
ATP8	V	67	1	0.23	4.37

Notes. Instead of counting the total number of radical physicochemical property changes per site, we treated each positively selected amino acid site with radical effects as a single adaptive event—representing one structural impact—regardless of how many properties were

affected. This approach assumes that all changes at a given residue act on the same structural unit of the protein and thus more accurately reflects adaptive pressure as the number of discrete selection 'hits' on protein structure. We then quantified such adaptive sites— defined as codons under strong positive selection associated with radical physicochemical property changes—in each gene and complex. These counts were normalized relative to the amino acid length of Complex V (ATP synthase), the shortest of the mitochondrial OXPHOS complexes. First, we calculated a weigh factor for each complex as the ratio of its total length to that of Complex V. We then divided the observed number of adaptive sites by this factor, effectively standardizing adaptive signal magnitude across complexes of different sizes

Table S12 Standardized counts of radical amino acid changes among mitochondrial lineages of Ethiopian *Lophuromys*

Lineage	N rad	Branch length (subs/site)	R branch
C	8	0.069	3.13
I	2	0.035	1.47
J	3	0.024	3.22
E	2	0.005	10.29
O	0	0.003	0.00
H	0	0.031	0.00
F	3	0.050	1.54
A	11	0.070	4.04
L	1	0.013	1.98
B	5	0.045	2.86
K	1	0.008	3.22
N	1	0.005	5.15

Notes. Nrad is the number of TreeSAAP-inferred radical amino acid substitutions in magnitude categories 6–8 ($z > 3.72$, $p < 0.0001$). Branch length is given in substitutions per nucleotide site and was estimated from the mitochondrial phylogeny. The standardized value (R branch) was calculated by normalizing Nrad by the cumulative length of the 12 analyzed mitochondrial protein-coding genes (ND6 excluded) and by branch length, and then scaling the value to the length of complex V (293 codons). Because branch lengths were estimated per nucleotide site, they were multiplied by 3 to approximate substitutions per codon. This metric allows comparison of radical amino acid changes among lineages while accounting for differences in branch length and analyzed sequence length. Lineage codes (A–O) correspond to Fig. 2 in the main text