

Phylogenetic relationships of water voles (*Arvicola*, Rodentia) of Azerbaijan according to the cytochrome *b* gene

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ABSTRACT. For the first time, phylogenetic relationships within the genus *Arvicola* (Lacepede, 1799) have been analyzed for Azerbaijani water voles. Complete *cytb* gene sequences have been obtained for individuals from two localities of the Kura-Araks Lowland trapped in 1965 and stored in the Institute of Zoology of the Ministry of Science and Education of Azerbaijan. Phylogenetic reconstructions have shown that water voles from the territory of Azerbaijan are significantly differentiated from *A. amphibius* and are clustered together with *A. persicus* from Iran as two distinct genetic lineages. Earlier, morphological analysis of the dental characteristics of water voles, including individuals from this work, showed a high degree of differentiation of the Azerbaijani water vole from the Eurasian water vole and proximity to *A. persicus*. The genetic differences between individuals from the Kura-Araks Lowland and the Persian water vole from Iran amount to 6.99%, which corresponds to the level of interspecific distances established for the genus *Arvicola* according to *cytb* variability data. The obtained results are consistent with the assumption that *A. persicus* may represent a group of cryptic species that are morphologically similar but genetically differentiated. To resolve issue concerning taxonomic status of the water voles from Azerbaijan, bearing a distinct mitochondrial lineage, additional material from Western Asia and the analysis of other markers of the mitochondrial and nuclear genomes are needed.

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Филогенетические связи водяных полевок (*Arvicola*, Rodentia) Азербайджана по данным гена цитохром *b*

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РЕЗЮМЕ. Впервые для водяных полевок Азербайджана проведен анализ филогенетических связей в пределах рода *Arvicola*. Для особей из двух локалитетов Кура-Араксинской низменности из сборов 1965 года, хранящихся в фондах Института зоологии Министерства науки и образования Азербайджана, получены полные последовательности гена *cytb*. Филогенетические реконструкции показали, что водяные полёвки с территории Азербайджана значительно дифференцированы от *A. amphibius* и образуют вместе с *A. persicus* из Ирана общую кладу, в пределах которой обособляются в отдельную генетическую линию. Ранее морфологический анализ дентальных характеристик водяных полевок, включая особей из данной работы, показал высокую степень дифференциации водяной полевки Азербайджана от евразийской водяной полевки и близость к *A. persicus*. Генетические различия между особями из Кура-Араксинской низменности и персидской водяной полевкой из Ирана составляют 6.99%, что соответствует уровню

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межвидовых дистанций, установленных для рода *Arvicola* по данным изменчивости *cytb*. Полученные результаты согласуются с предположением о том, что *A. persicus* может представлять собой группу криптических видов, морфологически сходных, но дифференцированных генетически. Для решения вопроса о таксономическом статусе водяных полевок из Азербайджана, обособленных в отдельную генетическую линию, необходимы дополнительный материал из Передней Азии и данные анализа других маркеров митохондриального и ядерного геномов.

КЛЮЧЕВЫЕ СЛОВА: *Arvicola*, филогения, Кура-Араксинская низменность, митохондриальная ДНК, генетическая дифференциация.

Introduction

Representatives of the *Arvicola* genus are widely distributed across Palaearctic, occupying a vast range from the Atlantic coast of Europe to northwestern China (west to east) and from the shores of the Arctic Ocean to the Mediterranean and Caspian coasts (north to south). Despite its widespread use as a model group in ecological, evolutionary, and paleontological studies, the systematics of *Arvicola* remains debated.

Currently, the genus *Arvicola* is considered to comprise between two, three (Abramson & Lisovsky, 2012) and four species (Kryštufek & Shenbrot, 2022). The taxonomic status of two species — the Eurasian (*A. amphibius* Linnaeus, 1758) and Iberian (*A. sapidus* Miller, 1908) water voles — has been established based on a combination of genetic and morphological traits. However, the classification of the Persian (*A. persicus* De Filippi, 1865) and Italian (*A. italicus* Savi, 1838) water voles remains under discussion.

Genetic studies based on *cytb* sequences of water voles from Iran have demonstrated their distinctiveness as a separate species, *A. persicus*, with a restricted distribution to the Alborz Mountain region (Mahmoudi *et al.*, 2020, 2022). An analysis of morphological variability in the cheek teeth of West Asian water voles revealed differences from the Eurasian water vole, suggesting that these regions may also fall within the range of *A. persicus* (Maul *et al.*, 2021). Furthermore, the latest taxonomic review of rodents in the subfamily Arvicolinae proposed expanding the distribution range of the Persian water vole to include parts of Western Asia, particularly Azerbaijan (Kryštufek & Shenbrot, 2022).

A study of dental characteristics of water voles from Azerbaijan, preserved in the collections of the Institute of Zoology of the Ministry of Science and Education of Azerbaijan, demonstrated their distinction from *A. amphibius* and their affinity with *A. persicus* (Markova *et al.*, 2025). Consequently, they were tentatively identified as *Arvicola* cf. *persicus*. The qualifier *conformis* (cf.) indicates that morphological diagnostic criteria suggest their classification as Persian water voles, but further studies incorporating genetic data are required for confirmation.

This study presents the results of a phylogenetic analysis of Azerbaijani water voles based on *cytb* gene sequences.

Materials and methods

The study utilized skins and bone tissue of water voles from Azerbaijan stored in the collections of the Institute of Zoology of the Ministry of Science and Education of Azerbaijan (Appendix 1). The samples for DNA extraction were taken from water vole specimens collected in 1926–1965 and included in a previous study of dental characteristics (Markova *et al.*, 2025).

Total DNA was extracted from museum specimens using the commercial “DNA-Extran-2” kit (Syntol) following the manufacturer's protocol. Although spectrophotometric analysis confirmed DNA presence after extraction, initial PCR attempts using universal primers L7, H2, L8, and H6 (Tougard *et al.*, 2008), designed to amplify *cytb* fragments of approximately 700–800 bp, were unsuccessful. Consequently, we employed primer L7 and nine newly designed primers for PCR and sequencing. This approach allowed us to obtain short fragments (about 250–350 bp), with overlapping regions enabling full-gene sequence assembly (Table 1, Table 2).

Sequencing was performed using the “BrilliantDye Terminator Cycle Sequencing Kit” v. 3.1 (Applied Biosystems, USA) on a “NanoPhore-05” genetic analyzer (Syntol, Russia) at the Shared Facilities Center of the Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Sciences (Yekaterinburg). Raw nucleotide sequences were processed in BioEdit v. 7.2.0 (Hall, 1999) and MEGA v.11 (Tamura *et al.*, 2021).

Due to high DNA degradation, complete *cytb* sequences (1143 bp) were obtained for only three out of 18 museum specimens, all from two localities in the Kura-Araks Lowland (Fig. 1): two individuals from near Sary-Su Lake (Az1, Az2 (field numbers 19 and 12)) and one from near Bahramtepe village (Az3, field number 13) collected in 1965 (Appendix 1).

Phylogenetic analysis was performed using a 941 bp fragment of the *cytb* gene (excluding a 61 bp 5'-end segment and a 138 bp 3'-end segment) to maximize compatibility with *Arvicola* data available in GenBank. The dataset included 143 *cytb* haplotypes (Appendix 2). The following outgroup taxa were used: *Clethrionomys glareolus* Schreber, 1780 (AM392368; Galewski *et al.*, 2006), *Eothenomys melanogaster* Milne-Edwards, 1871 (AM392374; Galewski *et al.*, 2006), *Ellobius tancrei* Blasius, 1884 (MK544900; Bakloushinskaya *et al.*, 2019), and *Microtus arvalis* Pallas, 1778 (AM991045; Tougard *et al.*, 2008).

Phylogenetic reconstructions were performed using Bayesian Inference (BI) in MrBayes v. 3.2.2 (Ronquist *et al.*, 2012), with codon-position-specific models selected via MrModeltest 2.3 (Nylander, 2004). The GTR+G+I model was optimal for all positions. Maximum Likelihood (ML) and Neighbor-Joining

(NJ) analyses were conducted in MEGA v.11, using the GTR+I+G (ML) and Tamura 3-parameter (T3P) (Tamura, 1992) (NJ) models, respectively. A median-joining network was constructed in Network 4.5.1.6 (Bandelt *et al.*, 1999). Genetic distances were calculated in MEGA v.11 using the Kimura 2-parameter

Table 1. Characteristics of primers developed for the genus *Arvicola* for amplification of short fragments of the *cytb* gene.

Name		5'–3' sequence	Primer length, bp	Melting t°	GC%	Amplicon length, bp
L7	1 pair	TGACCAATGACATGAAAAATCATCG	25	56.27	32	310
AT1_R		GCTCCGTTGGCGTGGG	16	56.57	75	
AT2_F	2 pair	CATCAGTCGCCCATATCTGCCG	22	60.89	59	285
AT2_R		GTTGTGCCGATGTAGGGGATGG	22	60.71	59	
AT3_F	3 pair	CCATGAGGACAGATATCCTTTTGAGG	26	59.18	46	394
AT3_R		GGAGTATTGAGTGGATTTCAGGAG	25	59.48	48	
AT4_F	4 pair	TCCCAGATATTCTCGGAGACCCT	23	59.38	52	276
AT4_R		ATTTCATGTAAGGATTAATAGGTCGGATAC	29	57.74	34	
AT5_F	5 pair	CCTCAAAACAACGAGGATTAACATTCC	27	59.45	41	268
AT5_R		TTTTCATTCTGTTTACAAGACC	24	56.04	33	

Table 2. Composition and temperature-time profile of TouchDown PCR for primers developed for amplification of the *cytb* gene from museum samples for the genus *Arvicola*.

Components of the reaction mixture	Volume, ul	Temperature protocol		
H ₂ O	7.75	95°C: 3 min	Initial denaturation	X1
dNTP's 2.5 mM each	2.50			
AS Buffer 10x	2.50	95°C: 15 sec	Denaturation	X44
MgCl2 50mM	1.25			
Primer + 2pM	3.75	60–56°C: 20 sec	Primer annealing	
Primer – 2pM	3.75			
Taq 5U/ul	1.00	72°C: 50 sec	Polymerization	
DNA 50 ng/ul	2.50	72°C: 10 min	Final polymerization	X1

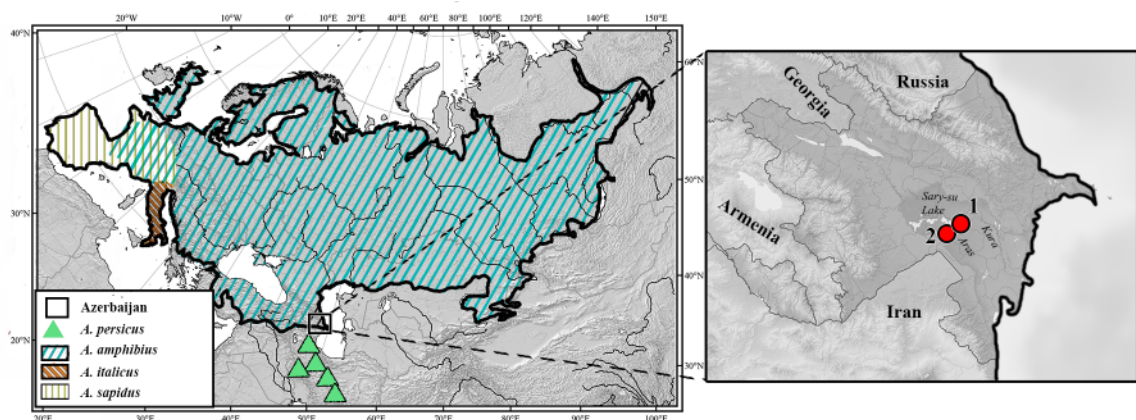


Fig. 1. Distribution ranges of *Arvicola* species (Kryštufek *et al.*, 2015; Mahmoudi *et al.*, 2020) and sampling locations of specimens used for phylogenetic analysis of Azerbaijani water voles: 1 — vicinity of Sary-Su Lake, Saatly District; 2 — Bahramtepe settlement, Imishli District.

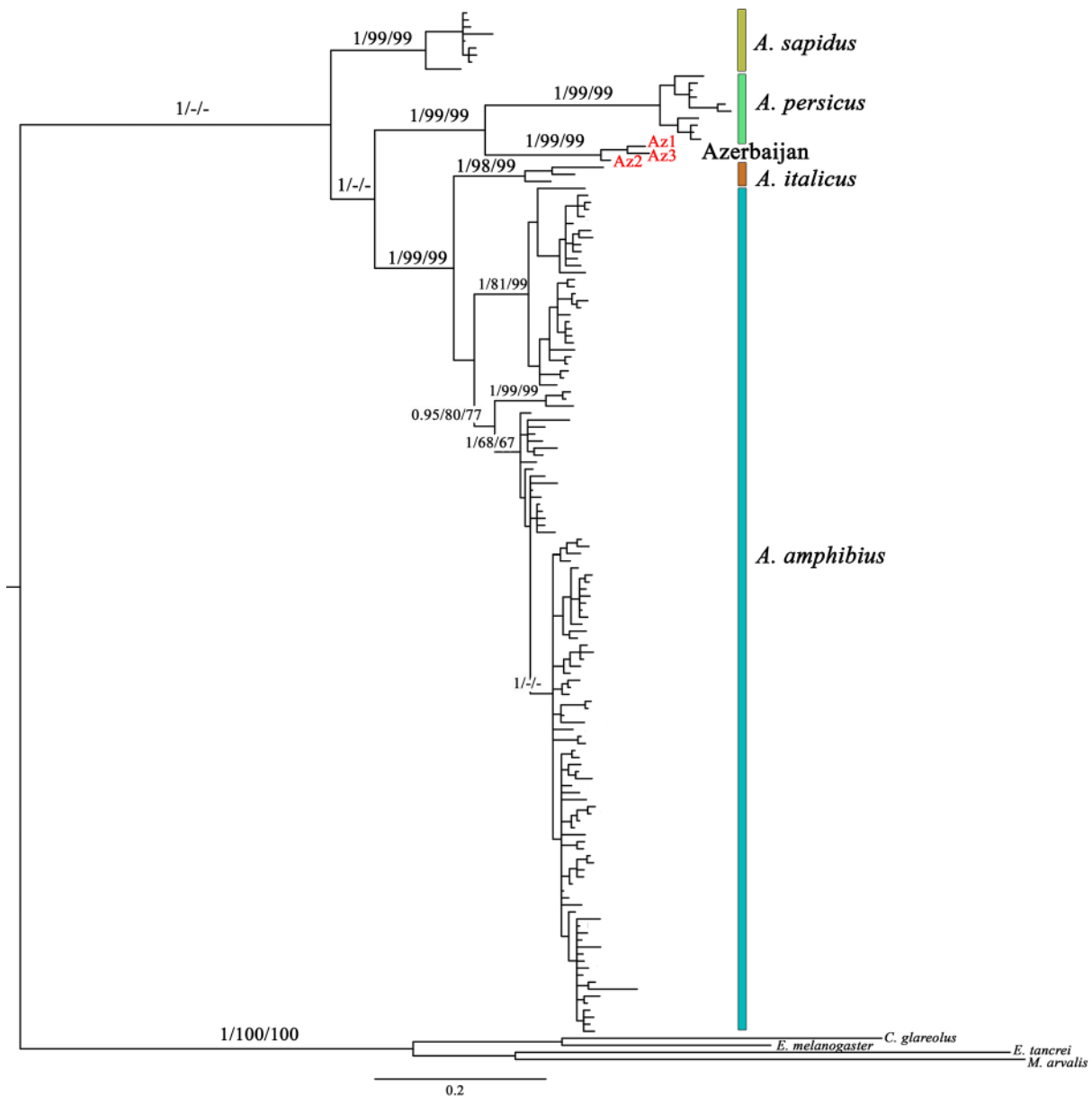


Fig. 2. Phylogenetic tree (BI) of 143 *cytb* haplotypes (941 bp). The values above the branches are a posteriori probabilities and bootstrap support (BI>0.90/NJ>60/ML>60). The dashes indicate absence of this node in particular analysis.

model (Kimura, 1980). The Automatic Barcode Gap Discovery procedure (ABGD) that sorts the sequences into hypothetical species (Puillandre *et al.*, 2012) was also used to partition dataset.

Results

The phylogenetic tree of *cytb* haplotypes (941 bp) constructed using Bayesian analysis (Fig. 2) revealed four well-supported clades, posterior probability (PP) = 1, generally corresponding to currently recognized species within the genus *Arvicola*. These include: *A. sapidus* (PP = 1)

— an endemic species of southwestern Europe; *A. italicus* (PP = 1) with clear geographical restriction to the Apennine Peninsula; and *A. amphibius* (PP = 1), represented by haplotypes ranging from Central Europe to Krasnoyarsk region. The haplotypes of Azerbaijani water voles formed a fourth clade (PP = 1) together with *A. persicus*, within which they differentiated into a distinct subgroup (PP = 1). Phylogenetic reconstructions using ML and NJ methods showed similar topologies (differing only in the branching order of *A. sapidus* and *A. persicus*).

The median-joining haplotype network also revealed clades corresponding to *A. sapidus*, *A. persi-*

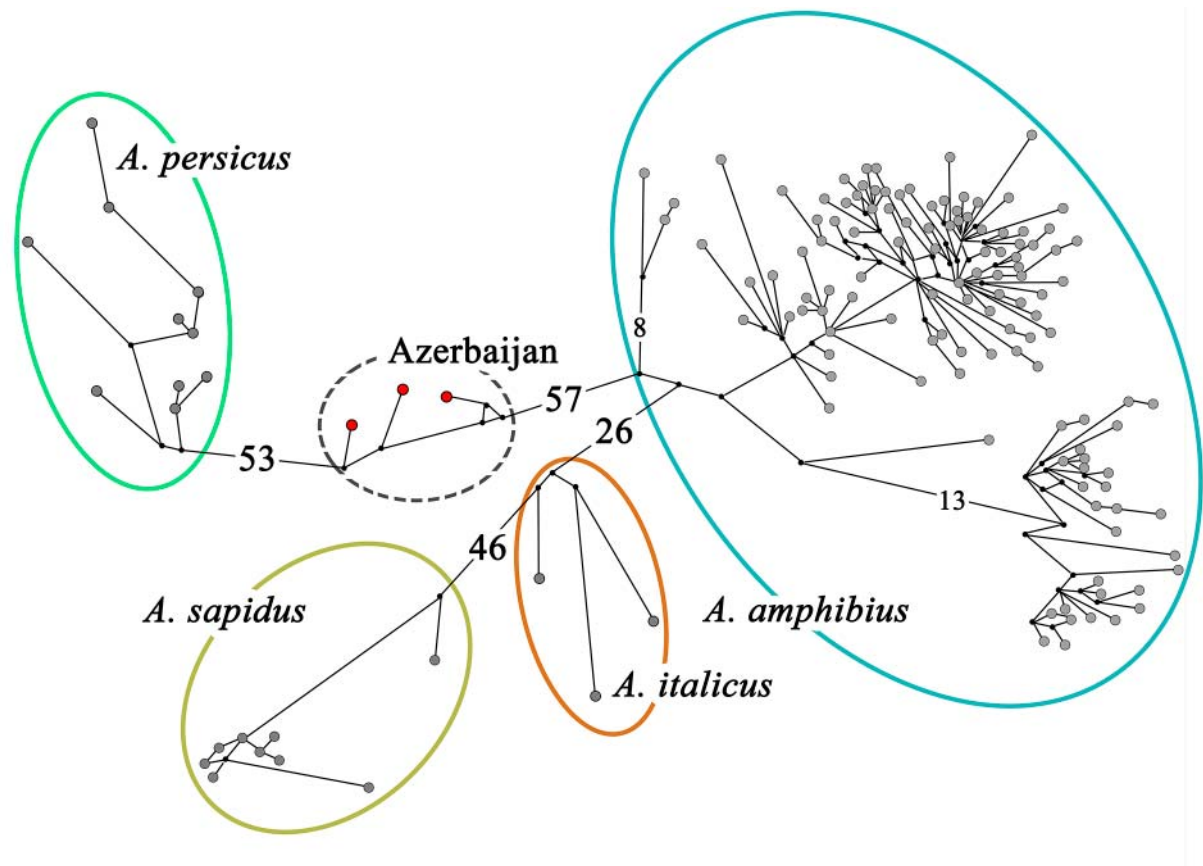


Fig.3. Median-joining network of 143 *cytb* haplotypes, numbers on branches correspond to the number of mutational steps, in the other cases the branch length is equivalent to the number of substitutions between haplotypes (the minimum branch length is one substitution).

Table 3. Mean intragroup and intergroup genetic distances in percent among phylogenetic lineages of the genus *Arvicola*.

	<i>A. persicus</i> (+Az)	<i>A. sapidus</i>	<i>A. italicus</i>	<i>A. amphibius</i>	<i>A. persicus</i>	Az
<i>A. persicus</i> (+Az)	3.21±0.36	0.96	0.92	0.9		
<i>A. sapidus</i>	9.16	0.72±0.15	0.82	0.77	1.01	0.95
<i>A. italicus</i>	8.81	6.83	1.34±0.28	0.53	0.92	0.94
<i>A. amphibius</i>	8.99	6.86	4.25	2.12±0.26	0.94	0.9
<i>A. persicus</i>		9.36	8.82	9.17	1.25±0.24	0.87
Az		8.36	8.75	8.27	6.99	1.07±0.29

Note: “Az” denotes water voles from Azerbaijan. The lower triangular matrix shows intergroup genetic distances. The upper triangular matrix (italicized) contains standard deviations (SD). Bold diagonal entries represent intragroup variation (mean ± SD). Gray-shaded cells denote ineligible comparisons between pooled (*A. persicus* + Az) and non-pooled datasets (*A. persicus* and Az separately).

cus, *A. italicus* and *A. amphibius* (Fig. 3). The Azerbaijani water voles clustered on the same branch with Persian water voles, but were nearly equidistant from both *A. amphibius* and *A. persicus* in terms of mutational steps.

The latest review of Arvicolinae subfamily (Kryštufek & Shenbrot, 2022) suggests that Azerbaijani water voles belong to *A. persicus*. However, our phy-

logenetic reconstructions and median network analysis revealed certain differences between haplotypes from the Kura-Araks Lowland and Iranian *A. persicus* haplotypes. Considering these findings, we calculated genetic distances using two approaches. First, we analyzed *A. persicus* and Azerbaijani voles as a single group (*A. persicus* + Az), while in the second approach we treated them as two separate phylogenetic lineages.

In the first approach, the smallest genetic distance (4.25%) was observed between *A. amphibius* and *A. italicus*, while the largest distance (9.16%) occurred between *A. persicus* and *A. sapidus* (Table 3). Genetic distances between the *A. persicus* + Az group and other groups ranged from 8.81% to 9.16%. When considering Azerbaijani water voles as a separate phylogenetic lineage (second approach), their intergroup genetic distances fell within the range observed for *A. amphibius*, *A. persicus*, *A. sapidus* and *A. italicus*, with the smallest value occurring in comparison with Persian voles (Table 3). In both calculation approaches, intragroup genetic distances were several times smaller than intergroup distances, with the exception of the combined *A. persicus* + Az group (first approach), where intragroup distances approached the intergroup values observed between *A. amphibius* and *A. italicus*.

The ABGD procedure splits the dataset into four groups: *A. sapidus*, *A. persicus*, Azerbaijani water voles, and *A. amphibius* + *A. italicus*. With respect to differentiation between *A. persicus* and Azerbaijani water voles, the results of the ABGD method support the second approach used here to identify phylogenetic lineages and suggest that the water voles from Azerbaijan might represent a hypothetical species.

Discussion

Our phylogenetic reconstructions generally do not contradict previously published data on the taxonomic structure of the genus *Arvicola*. The four identified clades (Fig. 2) generally correspond to the currently proposed species *A. sapidus*, *A. amphibius*, *A. italicus* and *A. persicus* (Kryštufek *et al.*, 2015; Chevret *et al.*, 2020; Mahmoudi *et al.*, 2020; Solano *et al.*, 2024). Water voles from the territory of Azerbaijan (Kura-Araks Lowland) belong to the same clade as Persian voles and, according to genetic distances, are closer to them than to the Eurasian water vole (Table 3). These results are consistent with the data of the analysis of dental characteristics of individuals, in particular the SDQ dental enamel differentiation quotient (Markova *et al.*, 2025), which serves as a criterion for identifying chronospecies of the genus *Arvicola* in the fossil record (Heinrich, 1978), and was recently proposed for the diagnosis of modern Eurasian and Persian water voles (Maul *et al.*, 2021; Kryštufek & Shenbrot, 2022). However, on the Bayesian tree within the clade, the haplotypes of Azerbaijani water voles form a separate group, significantly differentiated from *A. persicus* occurring in Iran (Fig. 2), and on the median network they are significantly distant from both Eurasian water voles and Persian water voles (Fig. 3). In rodents, *cytb* distance exceeding 5% is considered as evidence of possible interspecific divergence (Baker & Bradley, 2006). For the genus *Arvicola*, threshold values for species delimitation based on *cytb* have been proposed at 4.9%, and values below 4.1% as corresponding to intraspecific variability (Kryštufek *et al.*, 2015). According to genetic distances revealed in our study, dif-

ferences between the Azerbaijani water voles and other phylogenetic lineages considered as species, including *A. persicus* from Iran, correspond to the interspecific level (Table 3).

The discovery of a differentiated mitochondrial lineage of *Arvicola* cf. *persicus* in Azerbaijan is consistent with the previously proposed hypothesis that *A. persicus* may represent a group of cryptic species that are morphologically similar but genetically differentiated (Maul *et al.*, 2021). According to the results of morphological analysis of dental characteristics (Markova *et al.*, 2025), water voles occurring in the Kura-Araks Lowland exhibit a plesiomorphic pattern of dental enamel differentiation in cheek teeth bringing it closer to *A. persicus*. Genetically dated specimens Az1–Az3 also show the plesiomorphic variant of dental enamel differentiation, in which the enamel on the leading cutting edges of the tooth crown prisms is thinner than on the trailing edges. The enamel differentiation quotient (SDQ sensu Heinrich, 1978), expressed as a percentage and calculated for the first lower tooth, m1 (m1_SDQ index) for specimens Az1–Az3 is 119.6, 145.9 and 137.2, respectively (see Appendix 3 for some morphological characteristics of the individuals Az1–Az3). Such SDQ values are not characteristic of *A. amphibius* (in which the SDQ values typically do not exceed 100) and fall within the variability range of the Persian water vole. Thus, the genetic and morphological data are consistent with each other and confirm a high degree of differentiation of the studied water vole individuals Az1–Az3 from the Eurasian water vole. Sampling localities are situated in the Kura-Araks Lowland at a distance of about 60 km from each other and might represent a single population. The mountain systems surrounding the lowland from the north and west — the Greater Caucasus and the Lesser Caucasus also host the water voles with the plesiomorphic dental features (Markova *et al.*, 2025) but molecular genetic data for *Arvicola* are yet to be obtained. Questions about the degree of geographic differentiation of the water vole within the lowland and mountainous regions of Azerbaijan and the degree of genetic difference of the mitochondrial lineage occurring in the Kura-Araks Lowland from Persian water voles occurring in Iran remain open and require further research.

Conclusion

For the first time, phylogenetic relationships of Azerbaijani water voles within the genus *Arvicola* were analyzed using *cytb* gene sequences. The three studied specimens from the Kura-Araks Lowland showed closer genetic affinity to Iranian *A. persicus* than to *A. amphibius*. However, the significant genetic differences observed, reaching species-level divergence, currently prevent definitive classification of these Azerbaijani water voles as Persian water voles (*A. persicus*). To accurately determine the taxonomic status and distribution range of this phylogenetic lineage, further studies should incorporate more representative sampling from

this region and other parts of Western Asia, as well as include additional genetic markers, particularly from the nuclear genome.

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References

- Abramson N.I. & Lisovsky A.A. 2012. [Subfamily Arvicolinae] // [Mammals of Russia: systematic and geographical reference book]. Moscow: KMK Scientific Press. P.127–141 [in Russian].
- Abramson N.I., Bodrov S.Y., Bondareva O.V., Genelt-Yanovskiy E.A. & Petrova T.V. 2021. A mitochondrial genome phylogeny of voles and lemmings (Rodentia: Arvicolinae): Evolutionary and taxonomic implications // PLoS One. Vol.16. No.11. P.e0248198. DOI: 10.1371/journal.pone.0248198
- Baker R.J. & Bradley R.D. 2006. Speciation in mammals and the genetic species concept // Journal of Mammalogy. Vol.87. No.4. P.643–662. DOI: 10.1644/06-MAMM-F-038R2.1
- Bakloushinskaya I., Lyapunova E. A., Saidov A. S., Romanenko S. A., O'Brien P. C., & Serdyukova N. A. 2019. Rapid chromosomal evolution in enigmatic mammal with XX in both sexes, the Alay mole vole *Ellobius alaicus* Vorontsov et al., 1969 (Mammalia, Rodentia) // Comparative Cytogenetics. Vol.13. No.2. P.147–160. DOI: 10.3897/CompCytogen.v13i2.34673
- Bandelt H.-J., Forster P. & Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies // Molecular Biology and Evolution. Vol.16. No.1. P.37–48. DOI: 10.1093/oxfordjournals.molbev.a026036
- Barbosa S., Pauperio J., Searle J.B. & Alves P.C. 2013. Genetic identification of Iberian rodent species using both mitochondrial and nuclear loci: application to noninvasive sampling // Molecular Ecology Resources. Vol.13. No.1. P.43–56. DOI: 10.1111/1755-0998.12024
- Castiglia R., Annesi F., Aloise G. & Amori G. 2016. The Italian peninsula hosts a divergent mtDNA lineage of the water vole, *Arvicola amphibius* s.l., including fossorial and aquatic ecotypes // Hystrix. Vol.27. No.2. P.1–5. DOI: 10.4404/hystrix-27.2-11748
- Centeno-Cuadros A. & Godoy J.A. 2010. Structure, organization and nucleotide diversity of the mitochondrial control region and cytochrome *b* of southern water vole (*Arvicola sapidus*) // Mitochondrial DNA. Vol.21. P.48–53. DOI: 10.3109/19401736.2010.490582
- Centeno-Cuadros A., Delibes M. & Godoy J.A. 2009. Phylogeography of Southern Water Vole (*Arvicola sapidus*): evidence for refugia within the Iberian glacial refugium? // Molecular Ecology. Vol.18. No.17. P.3652–3667. DOI: 10.1111/j.1365-294X.2009.04297.x
- Chevret P., Renaud S., Helvacı Z., Ulrich R.G., Quéré J.P., & Michaux J.R. 2020. Genetic structure, ecological versatility, and skull shape differentiation in *Arvicola* water voles (Rodentia, Cricetidae) // Journal of Zoological Systematics and Evolutionary Research. Vol.58. No.4. P.1323–1334. DOI: 10.1111/jzs.12384
- Conroy C.J. & Cook J.A. 1999. MtDNA evidence for repeated pulses of speciation within arvicoline and murid rodents // Journal of Mammalian Evolution. Vol.6. No.3. P.221–245. DOI: 10.1023/A:1020561623890
- D'Elia G. 2003. Phylogenetics of Sigmodontinae (Rodentia, Muroidea, Cricetidae), with special reference to the akodont group, and with additional comments on historical biogeography // Cladistics. Vol.19. No.4. P.307–323. DOI: 10.1111/j.1096-0031.2003.tb00363.x
- Galewski T., Tilak M.K., Sanchez S., Chevret P., Paradis E. & Douzery E.J. 2006. The evolutionary radiation of Arvicolinae rodents (voles and lemmings): relative contribution of nuclear and mitochondrial DNA phylogenies // BMC Evolutionary Biology. Vol.6. P.1–17. DOI: 10.1186/1471-2148-6-80
- [Information system of the fauna of Azerbaijan (vertebrates)]. 2023. Baku: Tereggi MMC. 598 p. [in Azerbaijani].
- Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT // Nucleic Acids Symposium Series. Vol.41. P.95–98.
- Heinrich W.-D. 1978. [Biometric studies on fossil small mammals from a Late Pleistocene roof sequence over the interglacial travertine of Burgtonna, Thuringia] // Quartärpaläontologie. Vol.3. P.255–268 [in German].
- Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences // Journal of Molecular Evolution. Vol.16. No.2. P.111–120. DOI: 10.1007/BF01731581
- Kryštufek B., Koren T., Engelberger S., Horváth G.F., Purger J.J., Arslan A., Chisamera G. & Murariu D. 2015. Fossorial morphotype does not make a species in water voles // Mammalia. Vol.79. No.3. P.293–303. DOI: 10.1515/mammalia-2013-0181
- Kryštufek B. & Shenbrot G.I. 2022. Voles and Lemmings (Arvicolinae) of the Palaearctic Region. Maribor: University of Maribor Press. 449 p.
- Lisovsky A.A., Petrova T.V., Yatsentyuk S.P., Golenishchev F.N., Putincev N.I., Kartavtseva I.V., Sheremetyeva I.N. & Abramson N.I. 2018. Multilocus phylogeny and taxonomy of East Asian voles *Alexandromys* (Rodentia, Arvicolinae) // Zoologica Scripta. Vol.47. No.1. P.9–20. DOI: 10.1111/zsc.12261
- Mahmoudi A., Darvish J., Aliabadian M. & Kryštufek B. 2020. Evolutionary history of water voles revisited: confronting a new phylogenetic model from molecular data with the fossil record // Mammalia. Vol.84. No.2. P.171–184. DOI: 10.1515/mammalia-2018-0169
- Mahmoudi A., Arslan A., Khoshyar M. & Kryštufek B. 2022. C-heterochromatin and NOR distribution in the karyotype of Persian water vole, *Arvicola persicus* (Mammalia; Rodentia) from Iran // Journal of Animal Diversity. Vol.4. No.2. P.110–114. DOI: 10.52547/JAD.2022.4.2.4
- Margaryan A., Noer C.L., Richter S.R., Restrup M.E., Bülow-Hansen J.L., Leerhøi F., Langkjær E.M.R., Gopalakrishnan S., Carøe C. & Gilbert M.T.P. 2021. Mitochondrial genomes of Danish vertebrate species generated for the national DNA reference database, DNAmark // Environmental DNA. Vol.3. No.2. P.472–480. DOI: 10.1002/edn3.137
- Markova E.A., Nadirli L.V. & Zykov S.V. 2025. The dental enamel differentiation quotient of modern water voles of the genus *Arvicola* in Azerbaijan: variation and diagnostic value // Biology Bulletin. Vol.52. P.235.
- Martin Y., Gerlach G., Schlötterer C. & Meyer A. 2000. Molecular phylogeny of European murid rodents based on complete cytochrome *b* sequences // Molecular Phy-

- logenetics and Evolution. Vol.16. No.1. P.37–47. DOI: 10.1006/mpev.1999.0760
- Maul L.C., Rabinovich R. & Biton R. 2021. At the southern fringe: extant and fossil water voles of the genus *Arvicola* (Rodentia, Cricetidae, Arvicolinae) from Israel, with the description of a new species // *Historical Biology*. Vol.33. No.11. P.2773–2793. DOI: 10.1080/08912963.2020.1835889
- Nylander J.A.A. 2004. MrModeltest v2. Program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University.
- Puillandre N., Lambert A., Brouillet S. & Achaz G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation // *Molecular Ecology*. Vol.21. No.8. P.1864–1877.
- Ronquist F., Teslenko M., Van Der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu L., Suchard M.A. & Huelsenbeck J.P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space // *Systematic Biology*. Vol.61. No.3. P.539–542. DOI: 10.1093/sysbio/sys029
- Ruedi M., Manzinalli J., Dietrich A., & Vinciguerra L. 2023. Shortcomings of DNA barcodes: a perspective from the mammal fauna of Switzerland // *Hystrix*. Vol.34. No.1. P.54–61. DOI: 10.4404/hystrix-00642-2023
- Solano E., Castiglia R., Amori G., Gentile G., Bertolino S., Capizzi D., Kranebitter P., Ladurner E., Lapini L. & Colangelo P. 2024. Phylogeography and population genetics of the endemic Italian water voles, *Arvicola italicus* // *Zoologischer Anzeiger*. Vol.313. P.120–129. DOI: 10.1016/j.jcz.2024.01.006
- Tamura K., Stecher G. & Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11 // *Molecular Biology and Evolution*. Vol.38. No.7. P.3022–3027. DOI: 10.1093/molbev/msab120
- Tamura K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases // *Molecular Biology and Evolution*. Vol.9. No.4. P.678–687. DOI: 10.1093/oxfordjournals.molbev.a040752
- Tamura K., Nei M. & Kumar S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method // *Proceedings of the National Academy of Sciences*. Vol.101. No.30. P.11030–11035. DOI: 10.1073/pnas.0404206101
- [Taxonomic spectrum of the fauna of Azerbaijan (vertebrates)] 2020. Baku: Institute of Zoology of ANAS. 141 p. [in Azerbaijani].
- Tougaard C., Renvoisé E., Petitjean A. & Quéré J.-P. 2008. New insight into the colonization processes of common voles: inferences from molecular and fossil evidence // *PLoS One*. Vol.3. No.10. P.e3532. DOI: 10.1371/journal.pone.0003532
- Tougaard C., Delefosse T., Hänni C. & Montgelard C. 2001. Phylogenetic relationships of the five extant rhinoceros species (Rhinocerotidae, Perissodactyla) based on mitochondrial cytochrome *b* and 12S rRNA genes // *Molecular Phylogenetics and Evolution*. Vol.19. No.1. P.34–44. DOI: 10.1006/mpev.2001.0903

Appendix 1. Museum material from Azerbaijan used in this study and number of successful amplifications of short fragments *cytb*.

No.	No. tissue sample	No. museum sample	Locality	Year of capture	Collector	Number of short fragments <i>cytb</i> (5 in total)
1	7	11	Mil steppe, Sary-Su Lake	1965	Kafarov M.K.	2
2	3	3	Khanlar district, village of Gabakgala 1.5 km tributary of the river	1964	Kasumov	2
3	19	19	Saatly district, Sary-Su Lake	1965	Kafarov M.K.	5 (Az1)
4	11	13	Saatly district, Sary-Su Lake	1965	Kafarov M.K.	4
5	2916/1270	2916	Chukhuryurt village	1926	Nazarov	–
6	2854/899	2854	Chukhuryurt village	1926	Nazarov	–
7	8	12	Mughan steppe, Sary-Su Lake	1965	Kafarov M.K.	4
8	12	10	Saatly district, Sary-Su Lake	1965	Kafarov M.K.	5 (Az2)
9	2910/1152	2910	Chukhuryurt village	1926	Nazarov	–
10	13	2	Mil steppe, Bahrantepe	1965	Kafarov M.K.	5 (Az3)
11	10	17	Saatly district, Sary-Su Lake	1965	Kafarov M.K.	3
12	2886/900	2886	Chukhuryurt village	1926	Nazarov	–
13	14	7	Saatly district, Sary-Su Lake	1965	Kafarov M.K.	–
14	18	15	Saatly district, Sary-Su Lake	1965	Kafarov M.K.	–
15	17	8	Saatly district, Sary-Su Lake	1965	Kafarov M.K.	4
16	6	14	Saatly district, Sary-Su Lake	1965	Kafarov M.K.	3
17	15	18	Saatly district, Sary-Su Lake	1965	Kafarov M.K.	4
18	16	16	Saatly district, Sary-Su Lake	1965	Kafarov M.K.	–

Appendix 2. Data used for phylogenetic analysis of Azerbaijani water voles.

Species	Geographical locations	GenBank access. number	Reference
<i>Arvicola</i> cf. <i>persicus</i>	Azerbaijan	PX244611–PX244613	This study
<i>A. amphibius</i>	France	LR746352–354; 357–360; 363; 366; 367; 370; 371; 374; 377; 379; 382–384; 386–392; 398; 399; 403; 406; 407; 457; 458; 460; 471; 473; 477; 478; 484–487; 489–493	direct submission (Michaux, 2020)
	Denmark	LR746482; MF122828; 34	direct submission (Michaux, 2020); Margaryan <i>et al.</i> , 2021
	Germany	LR746427–37; 41; 43–47	direct submission (Michaux, 2020)
	Finland	AF119269	Conroy <i>et al.</i> , 1999
	United Kingdom	LR746408; 09; 12–17; 20; 22–26; 53; 56	direct submission (Michaux, 2020)
	Switzerland	AF159400; AY275106; OQ885451; 52	Martin <i>et al.</i> , 2000; D’Elia, 2003; Ruedi <i>et al.</i> , 2023
	Slovenia	KM005036–43	Krystufek <i>et al.</i> , 2015
	Hungary	KM005011–27	Krystufek <i>et al.</i> , 2015
	Bosnia and Herzegovina	KM005009; 10	Krystufek <i>et al.</i> , 2015
	Austria	KM005000–008; 497–499	Krystufek <i>et al.</i> , 2015
	Serbia	KM005044	Krystufek <i>et al.</i> , 2015
	Romania	KM005028–KM005030	Krystufek <i>et al.</i> , 2015
	Turkey	KM005045– KM005047	Krystufek <i>et al.</i> , 2015
	Russia	KM005031–35; MT381921; MF099519	Krystufek <i>et al.</i> , 2015; Lisovsky <i>et al.</i> , 2018; Abramson <i>et al.</i> , 2021
	Belgium	LR746394–396; 448; 450; 451	direct submission (Michaux, 2020)
	Italy	PQ429244; 50; 51; 54	Solano <i>et al.</i> , 2024
	Spain	JX457750; 51	Barbosa <i>et al.</i> , 2013
<i>A. italicus</i>	Italy	LT546155–58	Castiglia <i>et al.</i> , 2016
	Switzerland	OQ885453	Ruedi <i>et al.</i> , 2023
<i>A. persicus</i>	Iran	MN165740–51	Mahmoudi <i>et al.</i> , 2020
<i>A. sapidus</i>	Spain	FJ539341–45	Centeno-Cuadros <i>et al.</i> , 2010
	Portugal	JX457744–49	Barbosa <i>et al.</i> , 2013

Appendix 3. Photos of individuals Az1–Az3 of *Arvicola cf. persicus* from Azerbaijan: skulls (1–3) and occlusal surface of molars of the adult individual Az1 (4–6). 1 — Az3, collection number 2, subadult male; 2 — Az2, collection number 10, subadult female; 3–6 — Az1, collection number 19, adult, sex unknown.

