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Mitochondrial Diversity and Phylogenetic Structure of *Microtus rossiaemeridionalis* at the Western Range Margin

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Abstract

The mitochondrial genetic diversity and phylogenetic relationships of the sibling vole (*Microtus rossiaemeridionalis*) in Lithuania were investigated by analysing partial sequences of the cytochrome *b* (*cytb*) gene and the mitochondrial control region. A total of 38 individuals from four locations were analysed and compared with previously published sequences from Eurasia. Two haplotypes were identified at each mitochondrial locus, indicating low genetic diversity. In the 727-base pair (bp) *cytb* dataset, mitochondrial diversity was found to be low (Hd = 0.053 ± 0.049; π = 0.00007 ± 0.00007). The Lithuanian haplotypes clustered within the European lineage of *M. rossiaemeridionalis* and were closely related to sequences from Ukraine, Romania, Finland, Norway, and Western Siberia. There was pronounced genetic differentiation among several Eurasian samples, whereas the degree of geographic structuring within Lithuania could not be reliably assessed due to uneven sampling. These findings provide the first comparative assessment of mitochondrial diversity in Lithuanian populations, contributing to our understanding of the phylogeographic relationships of the species across its Eurasian range.

Keywords: sibling vole; *cytb*; control region; phylogeny; genetic diversity; phylogeography; Lithuania

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1. Introduction

The common vole complex (*Microtus arvalis* group) comprises closely related species whose identification is complicated by morphological similarity and chromosomal differentiation. One such species is the sibling vole (*Microtus rossiaemeridionalis* Ognev, 1924), which differs from *M. arvalis* (Pallas, 1779) in chromosome number ($2n$ = 54 versus $2n$ = 46). However, their external morphology is similar, which makes identification difficult in sympatric areas [1–3]. Consequently, *M. rossiaemeridionalis* has historically been underrecognised [3,4]. Some populations otherwise designated as *M. rossiaemeridionalis* are also referred to as *M. levis* in taxonomic literature [5,6]; here, the latter name is used consistently, except when referring to specific taxonomic treatments.

In Europe, *M. rossiaemeridionalis* is found in the Balkan Peninsula, Turkey, eastern Europe, Lithuania, Latvia, Estonia, and southern Finland [7]. The absence of reported

Polish records suggests that Lithuania may represent a western range margin area. A revision of 2266 skulls previously assigned to *M. arvalis* sensu lato in Lithuania identified *M. rossiaemeridionalis* in 40 localities across 21 districts. Literature records extend its occurrence to 23 districts, and its characteristic karyotype ($2n = 54$, NF = 56) has been confirmed [3]. Subsequent molecular studies corroborated its occurrence, which had previously been obscured by identification difficulties. More broadly, the distributions of these sibling species in eastern Europe include sympatric, allopatric, and contact areas [5,8].

Mitochondrial DNA (mtDNA) provides information on maternal lineages and historical population relationships [9]. Previous studies have identified distinct European and Turkish mitochondrial components in *M. rossiaemeridionalis*, while broader studies have demonstrated phylogeographic differentiation among *Microtus* taxa [10,11]. However, mitochondrial patterns do not necessarily reflect genome-wide evolutionary relationships, as mitochondrial, nuclear, and chromosomal markers may yield contrasting results [12,13]. Therefore, while mtDNA is particularly informative for maternal phylogeography, conclusions about overall species history and genome-wide differentiation require caution.

There is limited genetic information on Lithuanian *M. rossiaemeridionalis*. An early allozyme study of 16 individuals from two administrative districts detected polymorphism at eight loci, but the geographical coverage was insufficient for assessing population structure [14]. Regional mitochondrial data are also limited. Mashkov and Gajduchenko [15] analysed a 385 bp *cytb* fragment in 27 specimens from seven countries, including two from Belarus, while Sheremetyeva et al. [16] examined the control region in two introduced Russian Far Eastern populations. Although these studies provide regional insights, they do not allow a detailed assessment of genetic variation within individual European countries. As the control region and *cytb* provide complementary information, their combined analysis can improve the assessment of mitochondrial diversity.

Hybridisation between *M. rossiaemeridionalis* and *M. arvalis* has also been documented. Gileva et al. [17] reported a wild hybrid from the Urals, while experimental studies have demonstrated meiotic abnormalities and reduced fertility in hybrids [18,19]. However, these findings do not indicate substantial ongoing introgression or mitochondrial gene flow in natural populations.

Given Lithuania's position at the western edge of the European range of *M. rossiaemeridionalis*, the aim of this study was to characterise the mitochondrial diversity and phylogenetic relationships of this species in Lithuania and to place the findings in a broader Eurasian context. Specifically, we quantified haplotype and nucleotide diversity, assessed the phylogenetic position of Lithuanian sequences relative to published data, and evaluated the geographical structuring of mitochondrial lineages. The results primarily reflect maternal phylogeographic patterns rather than genome-wide diversity or contemporary gene flow.

2. Materials and Methods

2.1. Sample Collection

A total of 38 *M. rossiaemeridionalis* individuals were collected from four Lithuanian localities between 2023 and 2024: Mieliūnai (56.007° N, 24.722° E), Deikiškiai (56.021° N, 24.739° E), the Utena district (55.444° N, 25.464° E), and Sudervė (54.822° N, 25.104° E) (Figure S1). The voles were trapped in anthropogenic habitats using 25 snap traps, spaced approximately 5 metres apart, and baited with sunflower-oil-soaked bread. The sample comprised 23 individuals from Mieliūnai, 13 from Sudervė, and one each from Deikiškiai and Utena. During the same sampling period, 85 *M. arvalis* individuals were captured: 43

in Mieliūnai, 16 in Deikiškiai, 12 in Utena, and 14 in Sudervė. The snap-trapping was justified since the material collected was intended for extensive parasitological and ecological research on small mammals, including those representing *M. rossiaemeridionalis*.

For exploratory genetic comparisons, the sampling sites were grouped into northern Lithuania (Mieliūnai) and eastern Lithuania (Deikiškiai, Utena, and Sudervė). However, given the uneven sample sizes and limited locality representation, these groups should not be considered formal geographic populations.

After the capture, the animals were frozen at −20 °C and subsequently transported to the Laboratory of Mammalian Ecology, State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania, for dissection and further examination. The heart muscles from voles were excised and preserved in 96% ethanol in 1.5 mL microtubes until further processing.

2.2. DNA Extraction, PCR, and Sanger Sequencing

Molecular examination of *M. rossiaemeridionalis* material was performed in the Laboratory of Molecular Ecology, State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania. Genetic characterisation of *M. rossiaemeridionalis* was carried out in two mtDNA loci: the *cytb* gene and the control region.

Genomic DNA was extracted from ethanol-preserved heart tissue using the universal salt-extraction method described [20] and diluted to a concentration of 50 ng/μL. Conventional PCR with Micr-2L (5′-CAACAACAGCATTCTCATCA-3′) and Micr-2R (5′-TGCTCGTTGTTTTGAAGTGT-3′) primers were used for the amplification of partial *cytb*, while Pro+ (5′-CACCATCAGCACCCAAAGCTG-3′) and MicrF (5′-ATTTAAGGGGAACGTATGGACG-3′) primers were utilised to multiply the partial control region [21]. The resulting PCR products yielded sequences of 767 bp for *cytb* and 463 bp for the control region before trimming. PCRs were carried out in a final volume of 25 μL containing 12.5 μL of Taq PCR Master Mix (Vazyme Biotech Co., Nanjing, China), 0.5 μM of each primer, 4 μL of DNA template, and 7.5 μL of nuclease-free water. Thermal cycling conditions, enzymatic purification of PCR products, and bidirectional Sanger sequencing were performed as previously described [21].

2.3. DNA Sequence Analysis

DNA sequences were manually edited using Chromas 2.6.5 (https://technelysium.com.au/wp/chromas/, accessed on 7 September 2026) and assembled into consensus sequences using CLC Sequence Viewer v. 8.0 (QIAGEN, Aarhus, Denmark). After removing primer sequences, the final 727 bp *cytb* and 422 bp control region sequences were deposited in GenBank under accession numbers QB135402–QB135439 and QB135440–QB135477, respectively.

The sequences obtained in the current study were compared with those of the same and most closely related species using the Nucleotide BLAST algorithm [22] to search for the sequences which can be applied for phylogenetic and population structure analysis of *M. rossiaemeridionalis*. The sequences obtained in our present work and retrieved from NCBI GenBank were aligned using the ClustalW algorithm implemented in MEGA12, version 12.0.14. [23]. As *cytb* sequences available in GenBank significantly varied in the length and positions within loci, two *cytb* datasets of 727 bp and 438 bp were further investigated while 370 bp control region sequences were further analysed. Haplotypes of mtDNA loci were established with the help of FaBox v. 1.5 [24].

Parameters of genetic diversity, number of haplotypes (*h*), number of polymorphic/segregating sites (*S*), average number of nucleotide differences (*k*), haplotype diversity (*Hd*), nucleotide diversity (π), and standard deviation (SD) for *Hd* and π were assessed

with a DnaSP v. 6.12.03 [25]. Additionally, values of Tajima's D neutrality test [26] were calculated with the same software.

Phylogenetic inference was assessed using the maximum likelihood (ML) [27] approach. The evolutionary model best fitting a separate alignment was chosen with MEGA 12.0.14. [23]. Accordingly, Tamura-Nei + G + I, Tamura 3-parameter + G, and HKY + I models were set for 727 bp *cytb*, 438 bp *cytb*, and 370 bp control region datasets, respectively. Sequences of Günther's vole, *M. guentheri* Danford and Alston, 1880 were used as the outgroup for the 727 bp *cytb* and control region datasets. The 438 bp *cytb* dataset included fewer taxa than the 727 bp dataset; therefore, *M. arvalis* was selected as the outgroup for this analysis. The credibility of resulted phylogenetic branching was evaluated using the bootstrap method with 1000 replications. The haplotype network analysis was conducted using the median-joining method [28] implemented in NETWORK 10.2.0.0 ((https://www.fluxus-engineering.com/sharenet.htm), accessed on 7 September 2026). Sequence KX581057 of Caspian gray vole, *M. mystacinus* De Filippi, 1863, and sequence OL588495 of *M. arvalis* were used as outgroup for *cytb* and control region haplotype networks, respectively.

The inter-population genetic analyses of *M. rossiaemeridionalis* were performed evaluating genetic differentiation via a calculation of par-wise Φ_{ST} and by conducting principal coordinates analysis (PCoA) of studied vole species samples. The Φ_{ST} values were determined with the help of Arlequin v. 3.5.2.2 [29] using the Tamura 3 parameter model and 0.06, 0.05, and 0.08 gamma values, for 727 bp *cytb*, 438 bp *cytb*, and 370 bp control region datasets, respectively. The statistical significance of Φ_{ST} was considered by 10,000 permutations at a 95% confidence level. The PCoA using Nei's genetic distance [30] was undertaken using GenAlEx v. 6.502 [31].

3. Results

3.1. Mitochondrial Sequence Variation in Microtus rossiaemeridionalis

All animals were successfully genotyped at *cytb* control and region, revealing two haplotypes per locus, differing by a single segregating site. To maximize geographic representation, 727 bp and 438 bp *cytb* datasets and a 370 bp control region dataset were used for subsequent population genetic analyses.

Based on both mtDNA loci, the Lithuanian sample of *M. rossiaemeridionalis* had limited genetic variability. Similar values of haplotype diversity and average number of nucleotide differences were estimated in both regions (k = 0.47795, 0.05263; Hd = 0.478 ± 0.045, 0.053 ± 0.049), whereas higher nucleotide diversity was established in the control region (π = 0.00129 ± 0.00012) than in *cytb* (π = 0.00007 ± 0.00007) (Table S1). By *cytb*, moderate genetic diversity was assessed for Ukraine, Iran, Greece, and Armenia samples (Hd = 0.617 ± 0.135–0.929 ± 0.084; π = 0.00320 ± 0.00120–0.00731 ± 0.00415), while high genetic diversity was determined for the Turkish sample (k = 5.84784, Hd = 0.953 ± 0.013, π = 0.01335 ± 0.00068). Based on the control region, highest genetic variation was established for the Greece sample (k = 3.36316, Hd = 0.937 ± 0.043, π = 0.00909 ± 0.00091), followed by seemingly different diversity in two geographically close samples from the Russian Far East. Finally, no variability in either loci was observed when comparing eight animals collected from Krasnodar, the Russian Federation.

3.2. Phylogenetic Relationships of Microtus rossiaemeridionalis

Based on 727 bp *cytb* sequences *M. rossiaemeridionalis* formed a monophyletic well-supported cluster which showed the closest relationship with Kerman vole, *M. kermanensis* Roguin, 1988, and *M. mystacinus* (Figure 1a). Notably, *M. mystacinus* was paraphyletic species. Altogether, these above-mentioned species clustered with sequences of *M. arvalis*

and Altai vole, *M. obscurus* Eversmann, 1841. Within the *M. rossiaemeridionalis* group, a single sequence from Kazakhstan formed a separate branch; sequences originating from Turkey and Iran were grouped in two separate clades, while sequences generated in the current work were placed with those from European locations, including Ukraine, North Macedonia, Romania, Finland, Norway, and one sequence from Western Siberia (Novosibirsk). In the phylogenetic tree constructed using 438 bp *cytb* sequences, lower bootstrap values were given. Nevertheless, relatively high support was obtained for the grouping of sequences from Lithuania with those from Ukraine, North Macedonia, Romania, Finland, Norway, and some of those from Turkey (Figure 1b). Finally, in the phylogram based on the 370 bp control region, sequences from *M. rossiaemeridionalis* grouped together with maximum support (Figure 1c). At the control region, *M. rossiaemeridionalis* clustered with *M. arvalis* and the *M. obscurus* group, while *M. kermanensis* and *M. mystacinus* sequences were not yet available. Phylogenetic relationships among *M. rossiaemeridionalis* control region haplotypes were not revealed using the ML method.

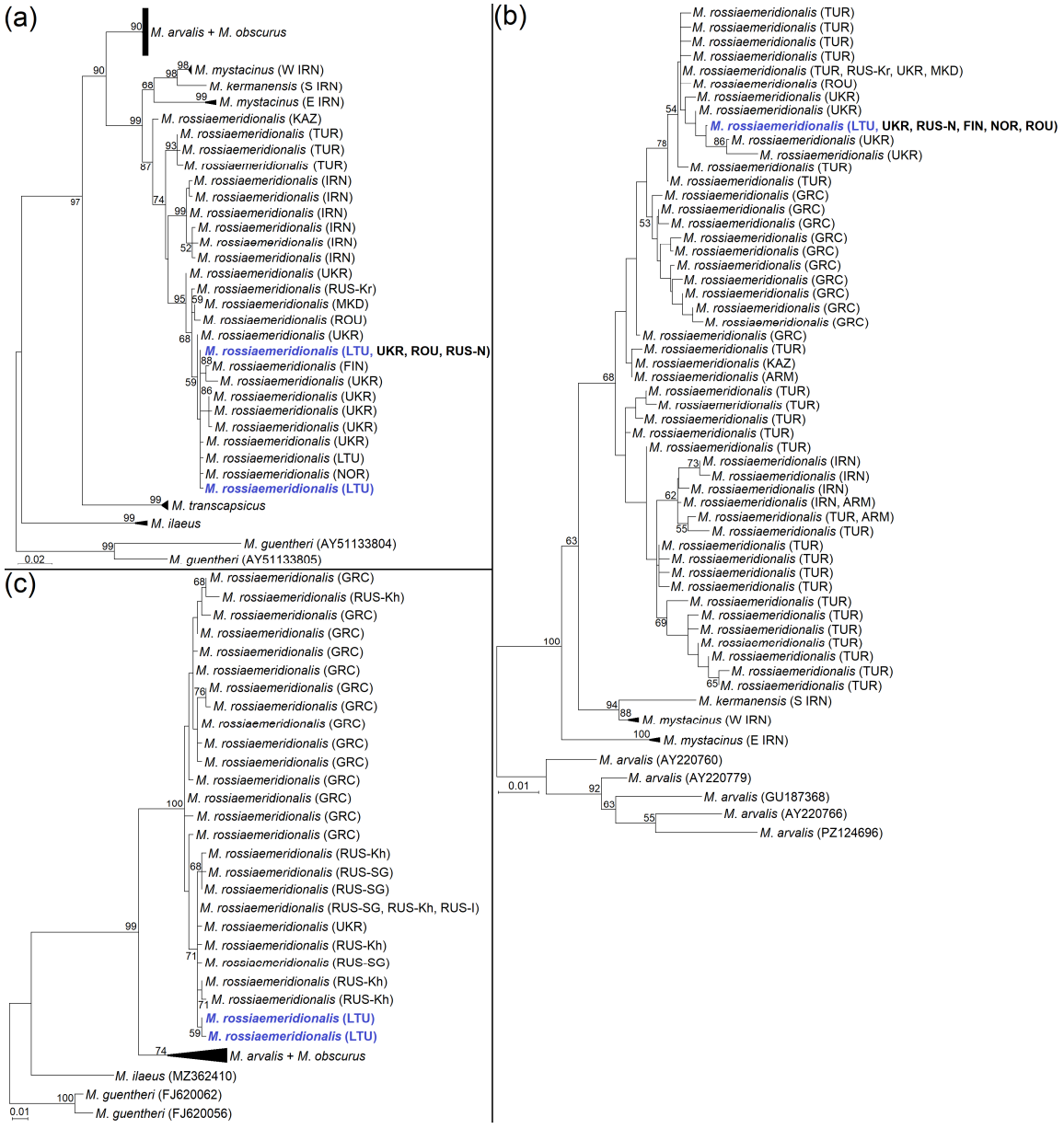

Figure 1. Phylogenetic analysis showing relationships of *M. rossiaemeridionalis* with closely related *Microtus* species and the placement of mitochondrial haplotypes of *M. rossiaemeridionalis* identified in Lithuania. Trees were constructed based on 727 bp *cytb* (**a**), 438 bp *cytb* (**b**), and 370 bp control

region (**c**) sequences. Bootstrap support values higher than 50 are indicated next to branches. Sequences determined in the present study are displayed in indigo. Geographical locations or GenBank accession numbers are given in brackets. Armenia (ARM), Finland (FIN), Greece (GRE), Iran (IRN), Kazakhstan (KAZ), Lithuania (LTU), North Macedonia (MKD), Norway (NOR), Romania (ROU), Russia (RUS), Turkey (TUR), Ukraine (UKR), Irkutsk (RUS-I), Krasnodar (RUS-Kr), Khabarovsk (RUS-Kh), Novosibirsk (RUS-N), Sovetskaya Gavan (RUS-SG).

3.3. Relationships of M. rossiaemeridionalis Mitochondrial Haplotypes Using Network Analysed

The haplotype networks did not reveal a clear star-like structure or clearly exhibited dominant haplotypes shared among multiple geographic locations (Figure 2). On the contrary, haplotypes were distributed across the networks in a relatively complex pattern, with most connections involving one-two mutational steps. However, some more common haplotypes were shared among several locations, which were geographically relatively close to one another.

Specifically, the MJ haplotype network constructed using 727-bp *cytb* sequences showed two haplotypes identified in *M. rossiaemeridionalis* from Lithuania, the dominant of which was detected in 37 animals and the other differing by single mutational step and found only in one vole (Figure 2a). Haplotypes from Lithuania shared or were most closely related with those established in voles from Ukraine, Romania, Finland, Norway, and Western Siberia (Novosibirsk). Notably, two clades of haplotypes originating from Iran and Turkey were distinguished.

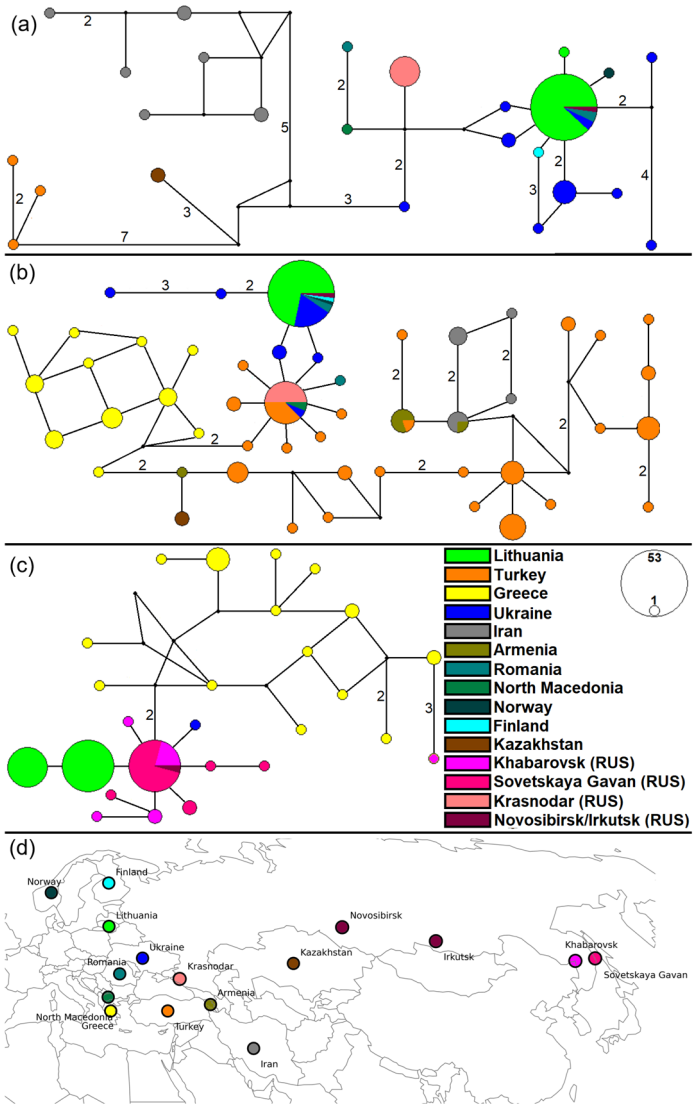

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Figure 2. Haplotype networks of *M. rossiaemeridionalis* generated using median-joining method. Networks were formed based on 727 bp *cytb* (**a**), 438 bp *cytb* (**b**), and 370 bp (**c**) control region sequences. Figures represent mutational steps, with only values above 1 shown. Circles are scaled according to frequencies of haplotypes. Hypothetical intermediate haplotypes are presented as black circles. The geographic distribution of the sampling locations represented is shown on the Eurasian map in Robinson projection (**d**).

The shorter 438 bp *cytb* MJ network exhibited more complex arrangements of haplotypes (Figure 2b). Eleven haplotypes exclusively determined in voles from Greece were placed together in network, while those from Turkey were widely distributed across the network, and some of these haplotypes were also identified in Russia, Ukraine, North Macedonia, and Armenia. At the shorter *cytb* fragment, only one haplotype was identified in voles from Lithuania, being genetically closely related to those from Ukraine, North Macedonia, Romania, Finland, Norway, Turkey, Southern Russia (Krasnodar), and Western Siberia (Novosibirsk).

Compared to *cytb*, considerably less data is available for *M. rossiaemeridionalis* from the control region. Consequently, the haplotype network included multiple individuals from only four locations: Lithuania, Greece, and two locations from the Russian Far East (Sovetskaya Gavan and Khabarovsk). No common haplotypes for different countries of sample origin were detected, and seemingly higher haplotype diversity was observed in Greece than in Russia and Lithuania (Figure 2c). Overall, two fairly common haplotypes found in Lithuania were most closely connected to those from Russia and Ukraine.

3.4. The Genetic Relatedness of M. rossiaemeridionalis Samples

Particularly high and significant genetic differentiation was detected among samples of *M. rossiaemeridionalis* within *cytb* (Table S2). Pairwise Φ_{ST} values in most cases exceeded 0.3. Based on the 438 bp *cytb* fragment, the lowest differentiation was observed between Lithuanian and Ukrainian samples (Φ_{ST} = 0.157). In contrast, the Lithuanian sample had 0.600–1.000 Φ_{ST} values as compared to other samples. Somewhat smaller genetic differentiation was also identified between Iranian and Armenian samples (Φ_{ST} = 0.255) as well as between Armenian and Turkish samples (Φ_{ST} = 0.210). Based on the control region, voles from Lithuania showed highly significant 0.501–0.723 Φ_{ST} values as compared with samples from Russia and Greece. Geographically close samples from Khabarovsk and Sovetskaya Gavan (Russian Far East), however, exhibited average genetic differentiation (Φ_{ST} = 0.061). The principal coordinates analysis (PCoA) demonstrated highest relatedness of voles from Lithuania with those from Ukraine and Russia (Figure S2).

3.5. The Inter-Population Genetic Comparison of M. rossiaemeridionalis from Lithuania

A restricted mitochondrial genetic variation was found in *M. rossiaemeridionalis* from Lithuania. Having combined *cytb* and control region (CR) data, we identified three haplotype combinations: *cytb*-H1/CR-H1, *cytb*-H1/CR-H2, and *cytb*-H2/CR-H2 (H1 and H2 indicate different haplotypes in certain locus) (Table 1). Three haplotype combinations were detected in North Lithuania, while a single combination (*cytb*-H1/CR-H1) was observed in East Lithuania. So, no mitochondrial variability was identified in East Lithuania, and low genetic variation was assessed in *M. rossiaemeridionalis* from North Lithuania (k = 0.58498, Hd = 0.549 ± 0.060, π = 0.00051 ± 0.00008).

Table 1. Mitochondrial haplotype combinations of *M. rossiaemeridionalis* defined in two Lithuanian samples (East and North) based on the sequences of *cytb* and control region.

Haplotype Combination	Frequency	Samples		Nucleotide Position	
		East	North	515	902
cytb-H1/CR-H1	24	15	9	T	A
cytb-H1/CR-H2	13		13	T	G
cytb-H2/CR-H2	1		1	C	G

H1 and H2—haplotypes, *cytb*—cytochrome *b*, CR—control region. 1–721 nucleotide positions encompass *cytb*, while 722–1149 nucleotide positions cover control region.

4. Discussion

4.1. Lithuanian and Eurasian Comparisons of Mitochondrial Diversity

Previous studies have demonstrated divergence between karyotypically distinct sibling vole species [32] and incongruence between chromosomal and mitochondrial relationships within the *M. arvalis* group [13]. Thus, the low diversity observed here characterises the maternal lineages sampled rather than genome-wide variation. Compared with other vole species sampled in Lithuania, *M. rossiaemeridionalis* exhibited significantly lower *cytb* diversity than *M. arvalis*, root vole (*Alexandromys oeconomus* Pallas, 1776), and, in particular, *M. agrestis* (Table S3) [21,33,34].

Regional comparisons are limited by small sample sizes and heterogeneous datasets. The Belarusian material included only two *M. rossiaemeridionalis* individuals, which prevented reliable population-level diversity estimates [15,35]. Similarly, the relatively high diversity reported by Mashkov and Gajduchenko was based on 21 pooled European sequences [15]. Data from two Russian Far Eastern populations likewise cannot represent the native Eurasian range [16].

4.2. Phylogeographic Position and Population Differentiation of Lithuanian Lineages

Consistent with previous studies [10,15], phylogenetic trees, haplotype networks, and PCoA placed Lithuanian samples within the broader European mitochondrial assemblage of *M. rossiaemeridionalis*. Population differentiation analyses further revealed the least *cytb* differentiation between Lithuania and Ukraine ($\Phi_{ST} = 0.157$; $p < 0.0001$), which is consistent with the results of the haplotype network and PCoA. However, Φ_{ST} reflects differences in mitochondrial haplotype frequencies and does not independently demonstrate contemporary gene flow or population connectivity. Similarly, phylogenetic proximity may reflect shared ancestry rather than recent dispersal. Furthermore, differences in geographic coverage and marker selection limit detailed comparisons [1,11].

4.3. Demographic History, Species Boundaries, and Limitations

The low mitochondrial diversity observed in Lithuania may be indicative of a small or fluctuating population, founder effects, or other demographic processes. However, the available data cannot distinguish between these scenarios. Although hybridisation between *M. rossiaemeridionalis* and *M. arvalis* has been documented in the Urals, including wild F1 hybrids [17], reproductive abnormalities were observed in experimental crosses [18,19]. Therefore, such findings do not indicate ongoing introgression in Lithuania, which cannot be assessed using mitochondrial data alone.

4.4. Mitochondrial Diversity at the Western Range Margin

Peripheral populations may exhibit reduced genetic diversity and increased differentiation due to smaller effective population sizes, founder effects, genetic drift, and limited connectivity [36]. However, contrasting patterns can be produced by demographic

history, postglacial colonisation, refugia, and secondary contact [37–42]. Comparative studies of European small mammals, including *Microtus*, demonstrate that the relationship between geographic position and genetic diversity is species specific [40,41,43]. Furthermore, mitochondrial patterns may reflect historical divergence and local adaptation [44], whereas genome-wide studies of *M. arvalis* have identified balancing and positive selection as contributing to geographic genetic structure [45].

The low mitochondrial diversity observed in Lithuanian *M. rossiaemeridionalis* is consistent with a range-margin effect; however, the present data do not allow us to establish its causes. These findings provide an initial characterisation of the maternal lineages sampled within the broader European mitochondrial assemblage.

5. Conclusions

The Lithuanian populations of *Microtus rossiaemeridionalis* exhibited low mitochondrial diversity, with only two haplotypes identified at each analysed locus. These haplotypes belonged to the European lineage and were found to be similar to sequences from several other parts of Eurasia. Further sampling is required to clarify the geographical structure and diversity of the species in Lithuania.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/life16122040/s1, Figure S1: Sampling sites and their habitat structure: (1) Mieliūnai, (2) Deikiškiai, (3) Utena, (4) Sudervė; Figure S2: Principal coordinate analysis (PCoA) of genetic distances between *M. rossiaemeridionalis* samples. Analyses were conducted using Nei's genetic distances of 438 bp *cytb* (a) and 370 bp (b) control region sequences; Table S1: Parameters of intra-population genetic diversity and neutrality test of *cytb* and control region sequences in *M. rossiaemeridionalis*. Samples are ordered by π values; Table S2: Genetic differentiation of *M. rossiaemeridionalis* samples. Pairwise Φ_{ST} values obtained based on *cytb* (438 bp and 727 bp fragments) and control region sequences are displayed below and above the diagonal, respectively. Statistical significance is presented with asterisks; Table S3: Comparison of mitochondrial genetic diversity among vole species sampled in Lithuania.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The *cytb* and control region sequences of *Microtus rossiaemeridionalis* generated in the present study were submitted to the GenBank database under accession numbers QB135402–QB135477.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

mtDNA	Mitochondrial DNA
cytb	Cytochrome *b*
PCR	Polymerase chain reaction
Hd	Haplotype diversity
π	Nucleotide diversity
Φ_{ST}	Fixation index
PCoA	Principal coordinates analysis
BLAST	Basic Local Alignment Search Tool

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