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Genetic Structure and Diversity of Eurasian Otter (*Lutra lutra*) in Northern Eurasia and Caucasus: Are There Any Differences Between the Two Subspecies?

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Abstract: The Eurasian otter (*Lutra lutra*) is a widespread semiaquatic carnivorous mammal in Eurasia. The nominate subspecies (*L. l. lutra*) occupies vast areas between Western Europe and the Russian Far East, but its phylogeography and genetic diversity are still unclear across Northern Eurasia. Another subspecies, *L. l. meridionalis*, located in the Caucasus mountains, is morphologically almost identical to *L. l. lutra* but needs genetic revision. We compared the genetic diversity of Eurasian otters from Russia and Armenia using a mtDNA fragment (820 bp) and 20 autosomal microsatellite loci (N = 117). A total of 32 haplotypes were observed with 17 novel haplotypes. The MtDNA median-joining network was mostly star-shaped with a branch of haplotypes from Far Eastern Russian otters. Both mtDNA analysis and Bayesian clustering of microsatellite data indicated that Far Eastern otters are more genetically differentiated than European and Siberian otters (Φst = 0.565 and 0.467; Rst = 0.306 and 0.256), as well as Caucasian otters (*L. l. meridionalis*) from Russia and Armenia (Φst = 0.515, Rst = 0.253). Haplotype and nucleotide diversities of Far Eastern otters are also the highest between sample groups (H = 0.882, π = 0.003) and, of Caucasian otters, the lowest (H = 0.464, π = 0.001). Our results suggest Caucasian otters are more similar to the otters from European Russia than to the other groups (but with lower genetic diversity) and lack the genetic variability typical to different subspecies. On the contrary, otters from the Russian Far East are more genetically differentiated, have higher genetic diversity than otters from Europe, and likely belong to another genetic lineage.

Keywords: genetic diversity; *Lutra lutra*; microsatellites; mtDNA; phylogeography

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1. Introduction

The Eurasian otter (*Lutra lutra* L., 1778) is a specialized semiaquatic species. Its range spans areas between Western Europe and North Africa to the Russian Far East and South and Southeast Asia [1]. There are 12 subspecies of *L. lutra* [2], but this differentiation is contentious [3–5]. Two subspecies live in Russia: *L. l. lutra* is widely distributed across the whole country and *L. l. meridionalis* [6], which occupies Northern Caucasus. The Caucasian subspecies of the Eurasian otter (*L. l. meridionalis)* is listed as Near Threatened by the IUCN and is critically endangered and highly protected in Russia and Transcaucasian countries (Georgia, Armenia, and Azerbaijan) [1]. The contact zone between these two subspecies

is supposed to be along the North Caucasian River basins (such as Terek and Kuban basins) [6], but it is unclear if they can possibly cooccur there.

Otters have gone through several drastic population declines in Europe, especially during the 20th century [7,8]. After reintroduction efforts in the 1990s and 2000s, the Eurasian otter population in Europe started to grow and is currently stable [9]. The Eurasian otter is also an indicator of freshwater ecosystem health [10,11]. In some regions of Northwestern Russia and Siberia, there were insufficient declines in the middle of the 20th century due to anthropogenic pressure (poaching and habitat loss from deforestation and oil extraction), but otters were always abundant [12–14]. Despite their wide distribution in Russia and fur commercial value in the recent past, the genetic diversity of the Eurasian otter remains unclear.

Genetic diversity plays an important role in population and individual fitness helping to adapt to environmental changes. Hence, assessing genetic diversity and connections between populations is relevant for conservation policy and management. The genetic structure of Eurasian otters is well known for European populations, including genetic changes after reintroduction efforts [15–19]. In general, European otters have low genetic diversity (except for Ireland) as a result both of the post-Pleistocene bottlenecks and recent anthropogenic population declines due to habitat destruction, poaching, and organochlorine pesticides [16]. In Asia, mostly South Korea and China [20–23], genetic diversity is greater than in European populations. For Caucasian otters, there is only a little evidence that they also lack morphological variability as compared to nominate subspecies [24], and there were no samples of this subspecies included in previous genetic studies. Du Plessis et al. [25] derived their measures of genetic diversity in Russia from just two samples that span an enormous geographic area. Limited availability of samples from Russia, Caucasus, and Central/Eastern Asia in general has resulted in an incomplete understanding of the phylogeography of Eurasian otters. Therefore, our study aimed to evaluate the genetic variability and structure of Eurasian otters in the Northern Palearctic and Caucasus. These results can help to explain whether the current population structure is caused only by anthropogenic factors or if other factors are at play.

2. Materials and Methods

We used 148 Eurasian otter samples (tissues, bones, fur, and feces) from different parts of the study area (Figure 1).

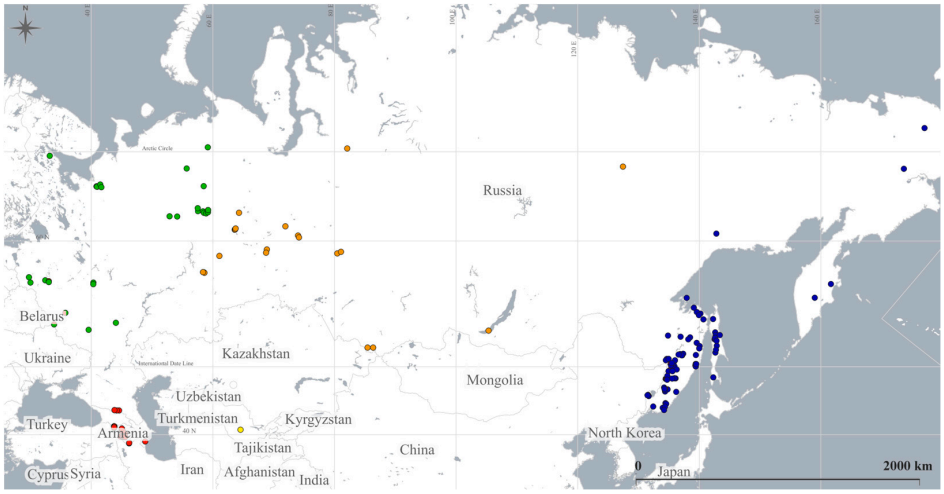

Figure 1. Eurasian otter (*L. lutra*) sample locations. Group colors: red—Caucasus, green—European Russia, orange—Siberia, blue—Russian Far East, and yellow—Uzbekistan. Provided via QGIS 3.28.1 software with Natural Earth map layers (https://www.naturalearthdata.com/ (accessed on 1 September 2024)).

Tissue samples were collected from the Joint Usage Center "Instrumental Methods in Ecology" at the Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, and stored in 95% ethanol at −25 °C. Bone and fur samples were drilled and collected in the Zoological Museum of Moscow State University (ZMMU), Craniological Laboratory of Central Forest Nature Reserve, the Museum of the Institute of Plant and Animal Ecology of Ural Branch of Russian Academy of Sciences (IPAE UB RAS), collection of Zoological Museum of Syktyvkar State University and Museum of Institute of Biology of Komi Science Centre UB RAS, collection of National Academy of Science of the Republic of Armenia, and Tashkent Zoo (Uzbekistan). Fecal samples were collected in Nature Reserves (Kaluzhskie Zaseki, Bryanskiy Les, Central Forest, Caucasus, and Botchinsky) and Nature Park (Kondinskie Ozyora) and Armenia, then stored in 95% ethanol at room temperature. Further information about the samples is shown in Table S1.

Molecular genetic analysis was provided by the Joint Usage Center "Instrumental Methods in Ecology" at the Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow. Degraded DNA from feces, bones, and fur was processed in rooms with no exposure to PCR products with negative controls. DNA was extracted in a laminar flow hood with UV light from tissues with a QIAamp Blood & Tissue kit (Qiagen, Hilden, Germany), bone and fur samples with a QIAamp Investigator kit (Qiagen, Germantown, MA, USA), and COrDIS extract decalcine (Gordiz, Moscow, Russia) and excrements with a QIAamp DNA Stool Mini kit (Qiagen, USA), according to the manufacturer's protocols. Extracted DNA was stored at −25 °C.

For the mtDNA analysis, we amplified and sequenced two fragments. The first was from the 5′ end of the control region (255 bp) to compare with known haplotypes of otters in the NCBI database (Table S2). The second fragment (820 bp) contained the 3′ end of the cytb gene, tRNA-Thr, tRNA-Pro, and the 5′ end of the control region (including 15 previously described haplotypes for European Russia (NCBI Accession Numbers: OQ059035-OQ059049 [26]) (Table S3).

These two fragments were amplified with two pairs of primers: LlucybL996/H16498 [16] and Lut4F/Lut4R [26]. The 20 µL PCR mix included 4 µL 5× Mas^{DD}TaqMIX-2025 (Dialat Ltd., Moscow, Russia), 1.5 µL each of forward and reverse primers at a concentration of 5 picomoles, 1 unit of Hot Start Taq DNA polymerase (SibEnzyme, Novosibirsk, Russia), and 13 µL of DNA and H_2O. The cycling program for PCR pair LlucybL996/H16498 was taken from Mucci et al. [16] and, for PCR pair Lut4F/Lut4R, it was 94 °C 2 min (94 °C 40 s, 57 °C 40 s, and 72 °C 40 s), 40 cycles, then 72 °C 10 min.

Amplification was carried out using a MiniAmp Thermal Cycler (Applied Biosystems, Foster City, CA, USA). The quality of the PCR product was controlled by electrophoresis in a 1.5% agarose gel. PCR products were purified by precipitation in ethanol or with a Cleanup Mini kit (Evrogen, Moscow, Russia). Sequencing was performed on an ABI 3130 genetic analyzer (Applied Biosystems, USA) with the BigDye Terminator kit v 3.1/1.1 (Applied Biosystems, USA).

For microsatellite analysis, we used 20 autosomal microsatellite loci and SRY sex-linked loci, previously developed for Eurasian otter and pine marten (*Martes martes* L., 1758), which we combined in 6 multiplexes (Table S4). The 15 µL PCR mix included 3 µL 5× Mas^{DD}TaqMIX-2025 (Dialat, Russia) from 0.5 to 3 µL of forward and reverse primers at a concentration of 5 picomoles and 1 or 2 µL of DNA and H_2O. The cycling program for each multiplex was 94 °C 3 min (95 °C 10 s, 57 °C 30 s, and 68 °C 1 min) for 28–42 cycles, then 68 °C 25 min. Molecular size standard SD-450 (Syntol, Moscow, Russia) was added to 1 µL of PCR product and 20 µL formamide and analyzed with an ABI 3130 genetic analyzer (Applied Biosystems, USA). For microsatellites derived from bones and feces, we repeated PCRs three times to minimize the chances for false allele and allelic dropout. All the observed alleles were scored using Genemapper v.4.0 (Applied Biosystems, USA).

Some populations of Eurasian otters may have genetic differences across river basins [16], so we divided all the samples of the Eurasian otter into four sample groups according to their geographical distribution and river basins: European Russia, Caucasus, Siberia, and

the Russian Far East. The number of samples from Caucasus and Siberia is relatively small compared to the other groups, so there could be a possibility of some sampling error. For more information on the samples, see Table S1.

The received sequences for 111 samples were aligned by eyes in Bioedit 7.05 software [27] with mtDNA haplotypes from GenBank (Lut1-19, RU1-7, ARM1-2, UZ1, and 820-1–820-15) (see NCBI accession numbers in Table S2) using BioEdit 7.05 software [27]. The sample from Uzbekistan was used only as a reference in median-joining networks and was excluded from further analysis. Haplotype diversity (H), nucleotide diversity (π), number of haplotypes (N_H), nucleotide substitutions (N_S), Tajima's index (D), Fu's index (Fs), Φst, mismatch distribution (Tau), Mantel test, and Harperding's raggedness (R) [28] were computed using Arlequin 3.5 software [29] with Kimura 2-parameter [30] substitution model (equal rates for all sites), picked with MrModeltest 2.3 [31]. Median-joining networks were constructed with PopART 1.7 software [32]. The network calculations were carried out with the default value for the epsilon parameter ($\varepsilon = 0$).

For microsatellite analysis of 117 received Eurasian otter samples, we computed the number of alleles (Na), the effective number of alleles (Ne), expected observed heterozygosity (He, Ho), fixation index (Fis), Rst, and Mantel test in Arlequin 3.5 software [33]. Principal coordinate analysis (PCoA) and deviations from Hardy–Weinberg equilibrium (HWE) were provided in GenAlEx 6.5 [33]. The presence of null alleles was tested in Microchecker v 2.2.3 [34]. Allelic richness (A_R) was tested in Fstat 2.9.4 [35]. Bayesian clustering was performed with STRUCTURE 2.3 [36]. Analyses were carried out by running 500,000 Markov chain Monte Carlo (MCMC) iterations with a burn-in period of 50,000 with and without prior location information (LOCPRIOR). All simulations were replicated five times for K from 1 to 8. The best number of clusters was determined by ΔK [37] in Structure Harvester [38]. The output was summarized and visualized by CLUMPAK 1.1 [39].

3. Results

3.1. Mitochondrial DNA Diversity

The Eurasian otters had mtDNA haplotype diversity H ranging from 0.464 in Caucasian otters to 0.882 in otters from the Russian Far East. The nucleotide diversity π was 0.001 in the Caucasian and European groups and 0.003 in the Russian Far East group. Tajima's D was negative for the Caucasian, European Russian, and Siberian groups with multiple low-frequency polymorphisms and positive for the Far East Russian group, showing a low level of high- and low-frequency polymorphisms. Fu's Fs was positive for Caucasian otters, indicating the deficiency of alleles and probable recent population bottleneck, and negative for each other group, showing the recent population expansion. Mismatch distributions of pairwise differences showed a unimodal peak in the European and Russian Far East groups and a bimodal peak in the Caucasian and Siberian groups (Figure S1).

Harperding's raggedness indexes were positive in each group, showing population equilibrium. The average mean number of pairwise differences was 3.227 ± 1.68, and 27 polymorphic sites were observed (Table 1).

A total of 13 haplotypes of Eurasian otter were observed in this study for the short CR fragment (255 bp), including eight haplotypes reported earlier (Table S2). First of all, we divided the total sequences according to their subspecies rank. In Figure 2, with a 255 bp fragment of mtDNA control region, 5 haplotypes are newly sequenced, in addition to the 30 previously sequenced in NCBI haplotypes. Two haplotypes of *L. l. meridionalis* samples from Armenia differ from common central haplotype Lut1 on one and two substitutions, respectively. A similar situation occurred with only the sample of *L. l. seistanica* from Uzbekistan. It differs from Lut1 by two nucleotide substitutions and from Lut3 by one nucleotide substitution.

Table 1. Genetic diversity parameters among the selected groups of Eurasian otter (*Lutra lutra*) according to the 820 bp mtDNA fragment.

Diversity Estimates	CAU	EUR	SIB	RFE	TOTAL
N_S	8	52	10	40	110
N_H	3	14	4	15	33
H	0.464 ± 0.2	0.836 ± 0.04	0.644 ± 0.152	0.882 ± 0.033	0.901 ± 0.018
π	0.001 ± 0.001	0.001 ± 0.001	0.002 ± 0.001	0.003 ± 0.002	0.004 ± 0.002
Pairwise difference	0.751 ± 0.61	1.220 ± 0.79	1.381 ± 0.93	2.808 ± 1.51	3.227 ± 1.68
Tajima's D	−1.45	**−1.58**	−0.01	0.555	−0.93
Fu's Fs	0.20	**−7.31**	−0.21	**−5.11**	**−17.49**
Raggedness index	0.22832	**0.06068**	0.13926	**0.09953**	0.12672

N_S number of samples, N_H number of haplotypes, H haplotype diversity, π nucleotide diversity. EUR European Russia, CAU Caucasus, SIB Siberia, and RFE Russian Far East. Significant *p*-values (>0.05) in bold.

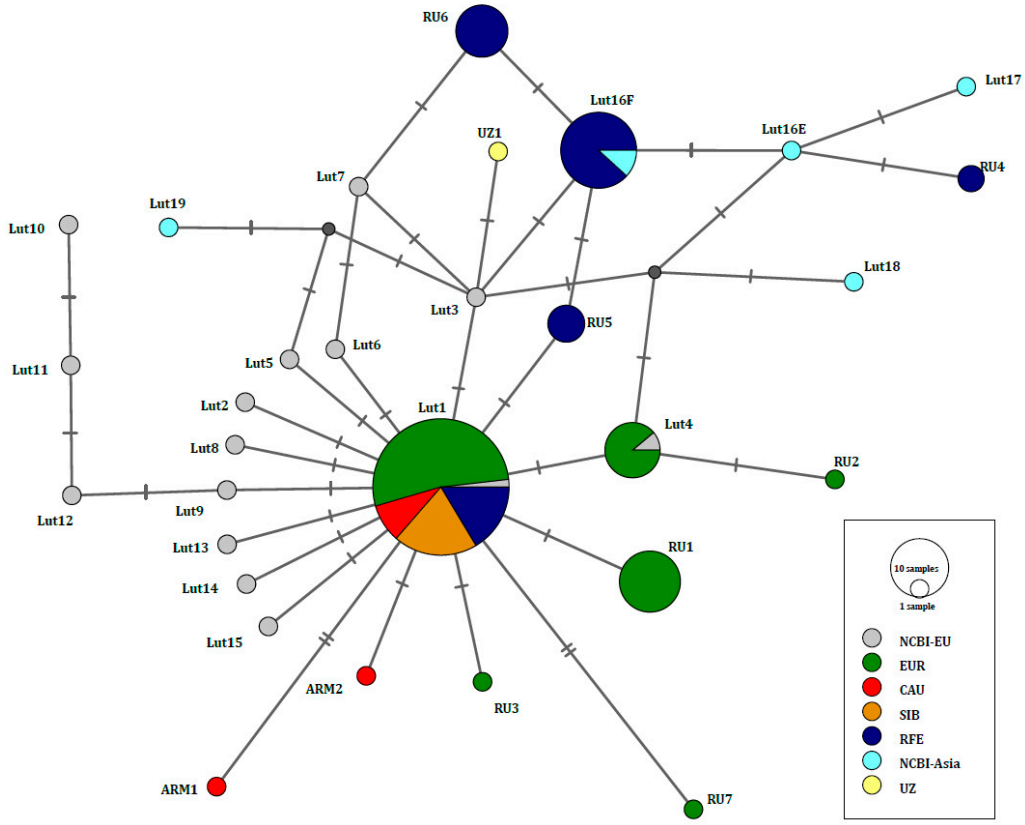

Figure 2. Median-joining network of the 255 bp fragment of the mtDNA control region of Eurasian otters (*L. lutra*). NCBI-EU European haplotypes from NCBI, EUR European Russia, CAU Caucasus (*L. l. meridionalis*), SIB Siberia, RFE Russian Far East, NCBI-Asia otter haplotypes from South Korea, China and Japan from NCBI, and UZ Uzbekistan (*L. l. seistanica*). The number of mutation positions is indicated as hatch marks on the branches, and the nod diameter is proportional to the number of samples.

After dividing the sample into four groups according to their regional origin, the central haplotype Lut1 of the star-shaped pattern occurred in almost every group except the East Asian one (Figure 2)—from Western Europe to Eastern Siberia. We concluded that this haplotype is common at least in North Eurasia. The second major haplotype, Lut16F, was previously described for otters from South Korea, but is also very common in the Russian Far East (Primorsky Krai, Khabarovsky Krai, and Sakhalin Oblast). Haplotypes Lut16E and Lut17 were also from South Korea, haplotype Lut18 was from China, and haplotype Lut19 was from the extinct Japanese otter [3,20]. Lut4 was another shared haplotype between the

two sample groups. This haplotype was described for East Britain [17] and occurred in Bryansk and Kaluga Oblasts (west of European Russia, Volga basin). Haplotype RU1 was found in samples from Pskov, Tver, and Penza Oblasts (west and southeast of European Russia, Volga, and Don basins), and RU2 was found in Kaluga Oblast. RU3 and RU7 were from Sverdlovsk Oblast and Komi Republic, respectively (North and Central Urals). RU4 was from Primorsky Krai (Russian Far East), while both RU5 and RU6 were from Khabarovsky Krai (Russian Far East). Total haplotype frequencies in sample groups EUR, CAU, SIB, and RFE are represented in Table S5.

A more divided median-joining network but with an analogous star-shaped pattern was observed in the longer fragment of mtDNA (820 bp), where only our samples were shown (Figure 3). A total of 32 haplotypes for the longer mtDNA fragments (820 bp) were observed with 15 haplotypes matched previously published haplotypes (Table S3). The Caucasian otter samples 820-12 and 820-13 differed from central 820-4 by three and one nucleotide substitutions, respectively. The only sample of *L. l. seistanica* (820-15) differed from 820-4 by four nucleotide substitutions.

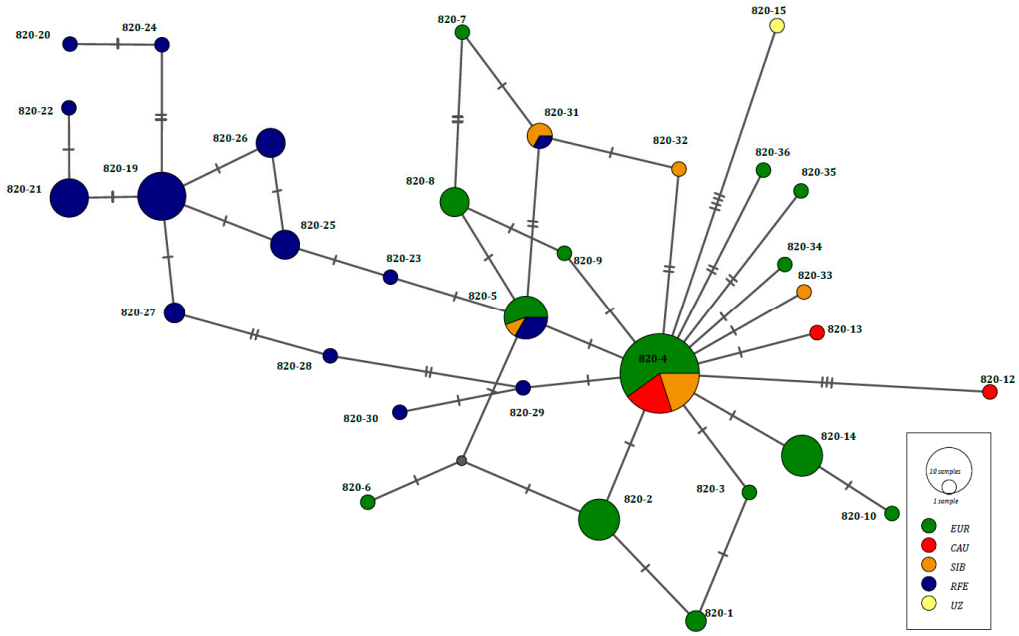

Figure 3. Median-joining network of 820 bp fragment of partial mtDNA of Eurasian otter (*L. lutra*). EUR: European Russia, CAU: Caucasus (*L. l. meridionalis*), SIB: Siberia, RFE: Russian Far East, and UZ: Uzbekistan (*L. l. seistanica*). The number of mutation positions is indicated as hatch marks on the branches, and the nod diameter is proportional to the samples number.

A median-joining network with longer fragments of mtDNA showed that the central haplotype broke on two major haplotypes separated by one substitution (Figure 3). The 820-4 haplotype combined European Russia, Caucasus, Urals, and Siberia, and 820-5 was found in Northern Eurasia, including European Russia, Urals, Siberia, and Russian Far East. Haplotype 820-31 was also shared between Siberia and the Russian Far East (Kamchatka Krai). Other haplotypes were unique for each location group and differed from each other by one to four nucleotide substitutions. Total haplotype frequencies in sample groups EUR, CAU, SIB, and RFE are represented in Table S6.

The spatial distribution of mtDNA haplotypes among the sampled groups is shown in Figure 4.

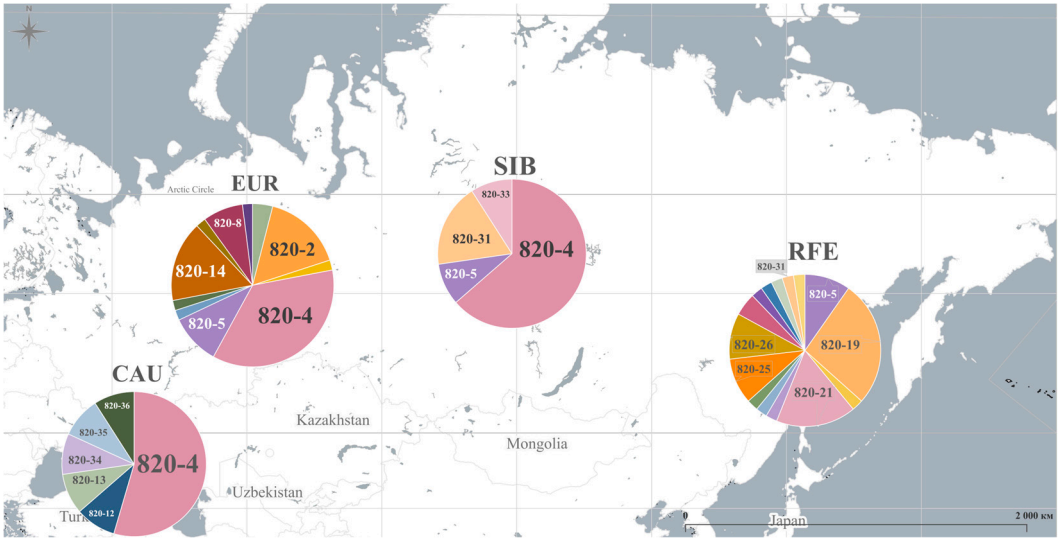

Figure 4. Distribution of mtDNA haplotypes of Eurasian otter (*L. lutra*). EUR: European Russia, CAU: Caucasus (*L. l. meridionalis*), SIB: Siberia, and RFE: Russian Far East. Different haplotypes are in colours.

For the longer fragment of mtDNA (820 bp), the European Russian and Siberian groups had a minimum Φst of 0.053; European and Far Eastern otters had a maximum Φst of 0.565 (Figure 5, Table S7). Caucasian otters did not differ from European Russian and West Siberian otters Φst at 0.054 and 0.085, respectively). Far Eastern otters differed from other groups dramatically: Φst = 0.467 between Siberian and Far Eastern otters; Φst = 0.515 between Caucasian and Far Eastern otters. The Mantel test did not show any correlation between genetic and geographic distance matrices among the sampled groups r = 0.685 (p = 0.09).

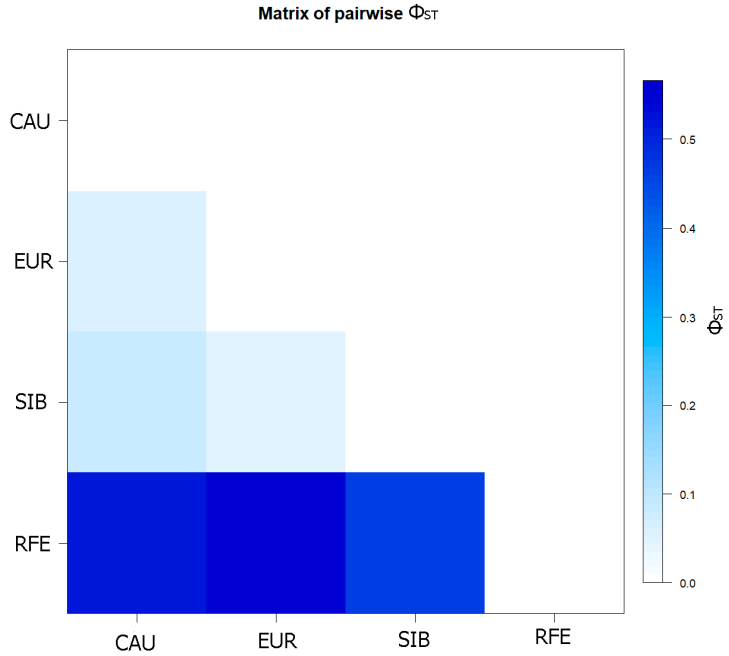

Figure 5. Genetic differentiation Φst between Eurasian otter populations based on mtDNA fragment (820 bp). EUR: European Russia, CAU: Caucasus, SIB: Siberia, and RFE: Russian Far East.

3.2. Microsatellite Diversity

The genetic diversity of 20 microsatellite loci is shown in Table S8. Two loci (OT19 and Mar08) were discarded from the following analyses because they showed high levels of null alleles. Thus, the estimated genetic diversity of 18 microsatellites in 117 otter samples, divided by sample groups, is shown in Table 2.

Table 2. Estimated genetic diversity in sampling groups by 18 microsatellite loci of Eurasian otters (*Lutra lutra*). All Fis *p*-values are significant (<0.05).

Geographical Area	N	Na	Ne	Ho	He	Fis
EUR	44	9.5 ± 0.51	4.9 ± 0.33	0.68 ± 0.03	0.78 ± 0.02	0.14 ± 0.03
CAU	8	4.7 ± 0.36	3.3 ± 0.25	0.46 ± 0.09	0.65 ± 0.04	0.30 ± 0.07
SIB	5	5.2 ± 0.37	4.0 ± 0.36	0.64 ± 0.09	0.71 ± 0.03	0.08 ± 0.08
RFE	60	12.0 ± 0.97	5.8 ± 0.44	0.62 ± 0.07	0.81 ± 0.01	0.23 ± 0.02
TOTAL	117	7.8 ± 0.47	4.5 ± 0.20	0.59 ± 0.05	0.74 ± 0.02	0.19 ± 0.03

EUR: European Russia, CAU: Caucasus, SIB: Siberia, and RFE: Russian Far East. N: number of samples, Na means number of alleles per locus, Ne: effective alleles per locus, Ho: observed heterozygosity, He: expected heterozygosity, and Fis: inbreeding coefficient.

All loci were polymorphic in all sampled groups. Allele number per locus (Na) ranged from Na = 4.7 (Caucasian otter) to Na = 12.0 (Russian Far East), with an average number of Na = 7.8. The average effective allele number was Ne = 4.5 and ranged from the lowest in the Caucasian group (Ne = 3.3) to the highest in the Russian Far East (Ne = 5.8).

The observed heterozygosity was moderate (Ho = 0.59), from Ho = 0.46 in Caucasian otters to Ho = 0.68 in otters from European Russia. Otherwise, the expected heterozygosity was rather high (He = 0.74) and higher than Ho in each sampled group. The lowest He was in the Caucasian group (He = 0.65), and the highest He was in the Far Eastern samples (He = 0.81). the allelic richness (A_R) of different loci was 3.306–4.981. The fixation index (Fis) was significantly greater than zero in every group (from 0.08 to 0.30).

The PCoA plot of multi-locus genotypes showed the lack of geographical differentiation among the sampled groups (Figure 6a–c).

Although no distinct clusters were observed, individuals tended to aggregate according to their population groups, with otters from the Russian Far East notably segregated from the other groups along PC1, which accounted for 6.80% of the population variance (Figure 6a,b). There were no significant clusters along PC2 (4.50% of the population variance, Figure 6a), as well as along PC3 (3.68% of the population variance, Figure 6b). Thus, our results demonstrated several trends of segregation (Figure 6a,b): Far Eastern otters (blue) tended to be distant to the other sample groups, while the other samples were intermixed. The PCoA for population variation (Figure 6c) also showed that Far Eastern and Caucasian otters were distant from each other, which is geographically confirmed. PC1 described 48.85% of the total variation and 32.36% for PC2. Rst values were the largest between otters from the European part of Russia and Far East Russian otters (Rst = 0.306) (Figure 7, Table S9). The lowest Rst, confirmed by a significant *p*-value, was identified between European Russian and Caucasian otters (Rst = 0.11). An analysis of the molecular variance (AMOVA) showed that 22% of the genetic variability was distributed among the groups ($p < 0.001$), and 78% within the groups. The Mantel test show correlation between genetic and geographic distance matrices among the sampled groups r = 0.829 ($p = 0.04$).

STRUCTURE analysis, performed without any information about sample locations (admixture model and no LOCPRIOR function) showed that the optimal number of clusters was K = 2 (Figure 8). Caucasian and European otters were shown as a monomorphic cluster, as well as the Far Eastern cluster. Otherwise, Siberian otters tended to be intermediate (half-blue half-red samples in the center of the plot). Further clusterization (i.e., at K = 5) showed that Caucasian otters were not separated from European otters.

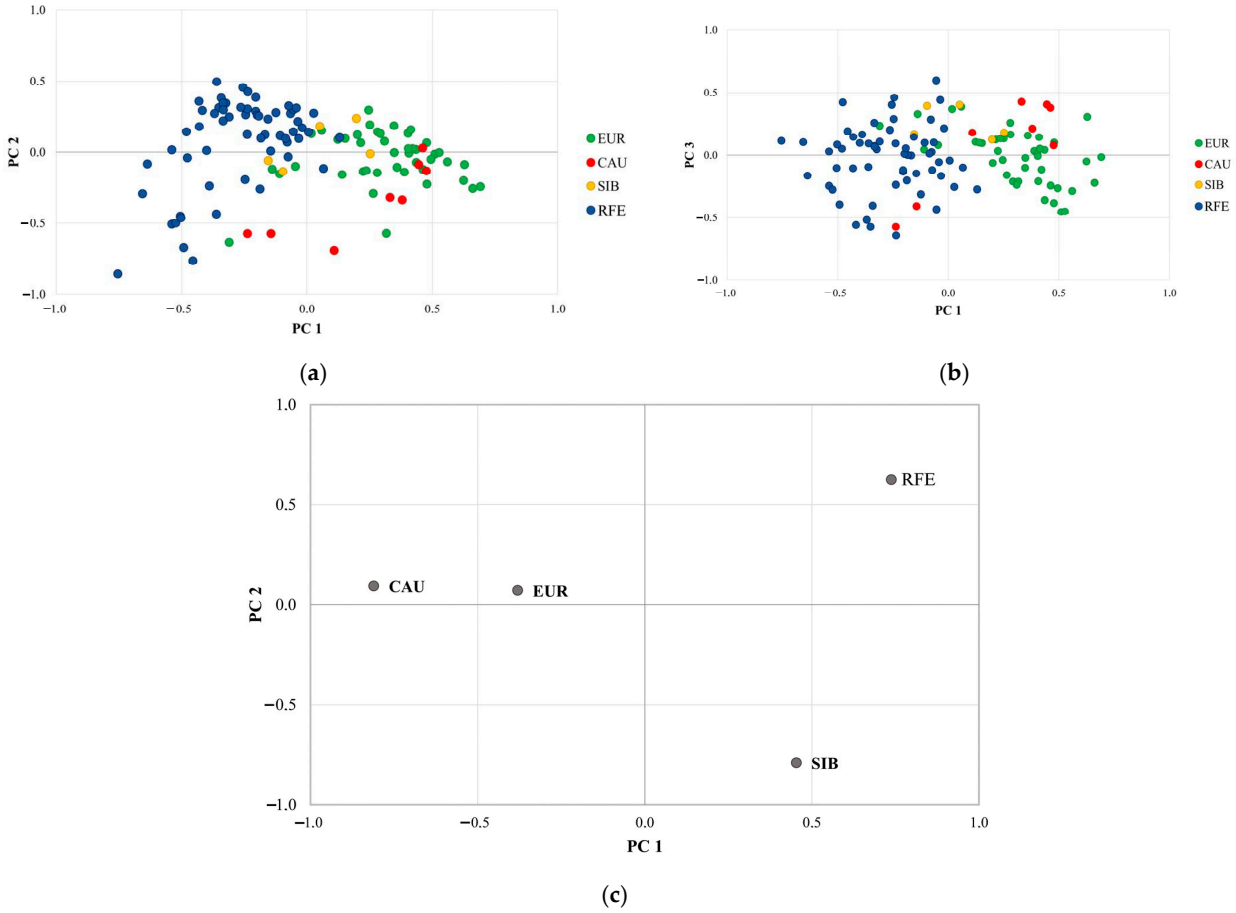

Figure 6. Principal coordinate analysis (PCoA) of Eurasian otter (*L. lutra*) individual (**a**,**b**) and population (**c**) multi-locus variations. EUR: European Russia, CAU: Caucasus, SIB: Siberia, and RFE: Russian Far East.

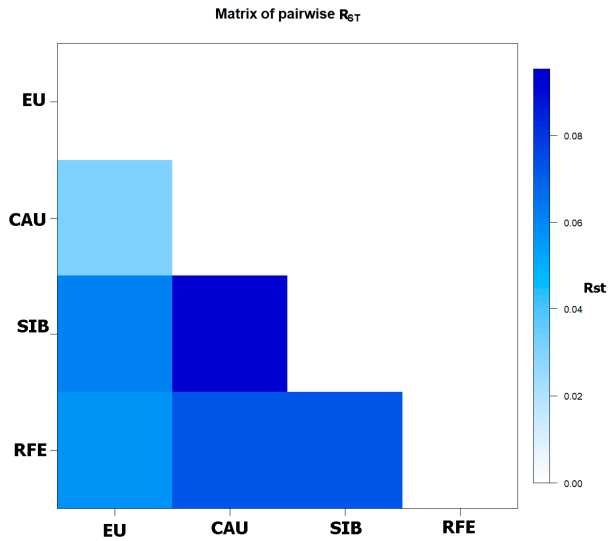

Figure 7. Genetic differentiation Rst between Eurasian otter populations based on mtDNA fragment (820 bp). EUR European Russia, CAU Caucasus, SIB Siberia, RFE Russian Far East.

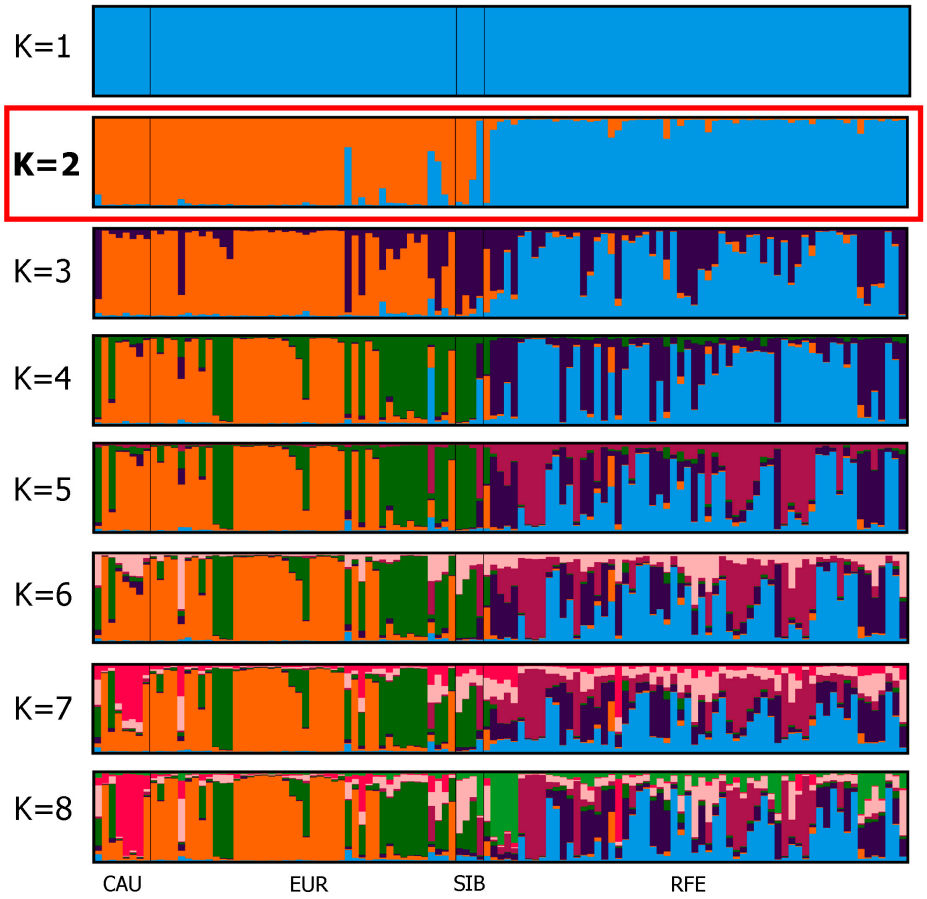

Figure 8. The genetic clustering of 117 Eurasian otter samples based on STRUCTURE analysis. The colors indicate the percentage of assignment of an individual to each cluster. The best K value was K = 2 (in bold and red frame). EUR European Russia, CAU Caucasus, SIB Siberia, RFE Russian Far East.

4. Discussion

The Eurasian otter has unusually low genetic diversity [15,16,20,40–43], even for carnivorous mammals (especially in Western Eurasia). Compared to other carnivore species with similarly wide distributions, for example, brown bear [44], grey wolf [45], or red fox [46], the levels of genetic variability for Eurasian otters are significantly lower. The phylogeographic structure of these species has some common patterns, such as the presence of major common haplotypes with several well-distinct genetic lineages or even clades. While these carnivorous mammals have greater distribution variability and mobility, the Eurasian otter is a habitat-specialized species, which perhaps explains the lower diversity and slower gene flow [47].

Eurasian otter fossils are rarely found in paleontological deposits. One of the earliest paleontological records of this species is from the late Middle Pleistocene in Grotta Romanelli, Italy [48]. Several other species survived the last glacial maximum (LGM) in European refugia like Iberia, Apennines, and the Balkans, and it was hypothesized that otters have also benefited from the same refugia [16]. Also, Siberia and Northeast Eurasia were not completely covered by LGM ice shields [49], leaving opportunities for otters to survive. During the Holocene, the Eurasian otter was a typical game species in North Eurasia, but its abundance in archaeological sites was always low due to otters' semiaquatic specialization [50,51]. Morphological studies of Eurasian otters provided both craniological [24,52] and odonatological data [53,54], suggesting that Eurasian otters are significantly different in the western and eastern parts of its range, with a border in East Siberia. This is assuming that the geographic patterns in morphology and genetics are

congruent. Furthermore, it was suggested that, in the Late Pleistocene, the otter range was divided into western and eastern parts, with further connection during the Holocene [53]. Eurasian otters share one common haplotype of mtDNA control region throughout the whole of Northern Eurasia, but other clades or other distinctive lineages can hardly be distinguished. This pattern is well known from previous studies [16,55–57], and our data also confirmed this. Moreover, we compared three Eurasian otter subspecies on the median network. The Caucasian subspecies (*L. l. meridionalis*) hardly differs from the nominate subspecies (*L. l. lutra*); however, we found two unique haplotypes for Caucasian otters, but the distances between other haplotypes from the center of a star-shaped phylogeny were comparable with the nominate subspecies (Figure 3). The only sample from Uzbekistan (*L. l. seistanica*) also diverged from the central haplotype but again only by four nucleotide substitutions. Φst values confirmed that Caucasian otters were not genetically distant from otters from European Russia and thus from Western and Eastern Europe [26]. Eurasian otters from the southeastern part of the Russian Far East (Khabarovsky Krai, Primorsky Krai) were more different and unique. Based on our median-joining networks, Far Eastern otters tended to form their star-shaped clade with another center (haplotypes Lut16 and 820-19) with unique haplotypes (except for 820-5 and 820-31), which suggested the existence of another refugium. Φst values also showed that Far Eastern otters were genetically more distant from the other groups. The Russian Far East has a well-known unique fauna with different clades (i.e., brown bears [44], wolves [45], and sables (*M. zibellina*, L., 1758) [58] or even different subspecies (i.e., wapiti (*Cervus canadensis*, Erxleben 1777 [59,60]) and musk deer (*Moschus moschiferus*, L. 1758 [61]) inhabiting this zone. The Stanovoy Ridge and other mountain ridges surround this area and serve as geographical barriers for species distribution, leading to limited gene flow and geographical isolation of animal populations. However, for Eurasian otters, the distribution of ice shields probably formed a meridional barrier in Eastern Siberia.

Recent studies of the whole mitogenome and cytochrome b of Eurasian otters from Great Britain to South Korea [5,25] showed that otters, as expected, are more diverse genetically than was previously believed, but the whole pattern of genetic differentiation is still the same. According to these studies, otters have five mitogenomic lineages [5], two of which live in Russia: lineage 3 is found in otters of Western Europe, Middle Asia, and Siberia, and lineage 2 in otters from South Korea and Sakhalin Island. The Caucasian otter is supposed to be included in lineage 3, and our results also confirmed this. Additionally, we confirmed the segregation of at least two lineages with a shorter fragment of the control region: lineage 3, which is widely distributed across the European part of Russia, Western Siberia, and the Caucasus, and lineage 2, which is similar to lineage 1 from South China and inhabits Sakhalin Island and Northeastern Russia. Also, we found the contact zone in Siberia (Yakutia for the 820-5 haplotype and Tomsk and Altai Oblast for the 820-31 haplotype). The quite distant otter subspecies from Uzbekistan, according to our data, could belong to yet another undiscovered lineage. Previous studies of Eurasian otters from the European population showed moderate microsatellite diversity. There were several explanations suggested for this feature: population fragmentation [42,62], expansion from a refugial population [16], and recent severe population decline [63]. In addition, differentiation of local populations was also observed, which can be a consequence of post-glacial population changes and relatively recent isolation [16,43]. On the contrary, otters from Kinmen Island, China [23], have moderate microsatellite (Ho = 0.60; He = 0.61 on average) and allelic diversity (Na = 4.25). Moreover, there was no evidence of a bottleneck. Our results for every sampled group showed that both Na and Ne are higher than previously observed values from both Europe and East Asia (Na values from 4.7 in Caucasian otters to 12.2 in otters from Far East Russia), but Ho (0.59) and He (0.74) are similarly rather moderate. Principal coordinate analysis and Rst values also showed moderate differentiation of Far Eastern otters from the other groups and a close relationship between Caucasian and European otters. The STRUCTURE analysis showed Far Eastern otters as a distinct cluster and the absence of any clusterization of Caucasian otters. Also,

the clinal pattern of clusterization from West to East is possible. These results could be a consequence of a large geographic distribution of samples and could reflect the presence of minor subpopulations in the area.

As a possible phylogeographic scenario, we propose a scenario similar to that for tigers [64] or the recent scenario for otters [5]. Eurasian otter's center of origin is Southeast Asia, and most of the otter genetic diversity is also observed in Southeast Asia. On the one hand, it is likely that the nominate subspecies *L. l. lutra* spread rapidly northwestward along the Himalayas and Western Asia and then quickly populated throughout Northern Eurasia (lineage 3). As a result, the main central haplotype is still present in vast areas. However, rather low levels of genetic variability could be a result of population decline and habitat destruction in Western Europe in the 20th century. On the other hand, there could be an otter population that went to Northeastern Russia (lineage 2) along the Pacific shore and then joined the North Eurasian branch (lineage 3) in Central Siberia after the last glacial minimum (LGM), which explains the minor star-shaped pattern in the Russian Far East otter population and further microsatellite clusterization.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d16120764/s1, Table S1. Samples of Eurasian otter (*L. lutra*) used in the study; Table S2. The list of all Eurasian otter (*L. lutra*) control region haplotypes (255 bp) from NCBI GenBank and newly described haplotypes; Table S3. The list of Eurasian otter (*L. lutra*) control region haplotypes (820 bp) from NCBI GenBank and newly described haplotypes; Table S4. Developed multiplexes of STR and sex loci of Eurasian otter (*L. lutra*), used in the study; Table S5. Number of haplotypes in of Eurasian otter (*L. lutra*) population for 255 bp mtDNA; Table S6. Number of haplotypes in of Eurasian otter (*L. lutra*) population for 820 bp mtDNA; Table S7. Genetic diffeentiation Φst of Eurasian otter (*L. lutra*) groups; Table S8. Estimated genetic diversity of 117 Eurasian otter (*L. lutra*) samples of 20 microsatellite loci; Table S9. Genetic differentiation Rst of Eurasian otter (*L. lutra*) groups. Figure S1. Mismatch distributions of pairwise differences of Eurasian otter (*L. lutra*) for 820 bp mtDNA fragment.

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References

1. Loy, A.; Kranz, A.; Oleynikov, A.; Roos, A.; Savage, M.; Duplaix, N. Lutra lutra (Amended Version of 2021 Assessment). *IUCN Red List. Threat. Species* **2022**. [CrossRef]
2. Hung, N.; Law, C.J. *Lutra lutra* (Carnivora: Mustelidae). *Mamm. Species* **2016**, *48*, 109–122. [CrossRef]
3. Waku, D.; Segawa, T.; Yonezawa, T.; Akiyoshi, A.; Ishige, T.; Ueda, M.; Ogawa, H.; Sasaki, H.; Ando, M.; Kohno, N.; et al. Evaluating the Phylogenetic Status of the Extinct Japanese Otter on the Basis of Mitochondrial Genome Analysis. *PLoS ONE* **2016**, *11*, e0149341. [CrossRef] [PubMed]
4. Park, H.C.; Kurihara, N.; Kim, K.S.; Min, M.S.; Han, S.; Lee, H.; Kimura, J. What Is the Taxonomic Status of East Asian Otter Species Based on Molecular Evidence?: Focus on the Position of the Japanese Otter Holotype Specimen from Museum. *Anim. Cells Syst.* **2019**, *23*, 228–234. [CrossRef] [PubMed]

5. du Plessis, S.J.; Hong, S.; Lee, B.; Koepfli, K.P.; Chadwick, E.A.; Hailer, F. Mitochondrial Genome-Based Synthesis and Timeline of Eurasian Otter (*Lutra lutra*) Phylogeography. *Anim. Cells Syst.* **2023**, *27*, 366–377. [CrossRef]
6. Ognev, S.I. *Zveri Vostochnoi Evropy i Severnoi Azii (The Mammals of Eastern Europe and Northern Asia)*; Glavnauka: Moscow, Russia, 1931; Volume 2.
7. MacDonald, S.M.; Mason, C.F.; Council of Europe; Convention on the Conservation of European Wildlife and Natural Habitats. *Status and Conservation Needs of the Otter (Lutra lutra) in the Western Palaearctic*; Council of Europe: Strasbourg, France, 1994; Volume 54.
8. Conroy, J.W.H.; Yoxon, P.; Gutleb, A.C. Proceedings of the First Otter Toxicology Conference. In *Proceedings of the Journal of the International Otter Survival Fund*; International Otter Survival Fund: Broadford, UK, 2000; Volume 1.
9. Loy, A.; Duplaix, N. Decline and Recovery of the Otter in Europe. Lessons Learned and Future Challenges. *J. Mt. Ecol.* **2020**, *13*, 1–8.
10. Van Den Brink, N.W.; Jansman, H.A.H. Applicability of Spraints for Monitoring Organic Contaminants in Free-Ranging Otters (*Lutra lutra*). *Environ. Toxicol. Chem.* **2006**, *25*, 2821–2826. [CrossRef]
11. Baos, R.; Cabezas, S.; González, M.J.; Jiménez, B.; Delibes, M. Eurasian Otter (*Lutra lutra*) as Sentinel Species for the Long-Term Biomonitoring of the Guadiamar River after the Aznalcóllar Mine Spill. *Sci. Total Environ.* **2022**, *802*, 149669. [CrossRef]
12. Vorobyov, V.N. Multiyear Dynamics of Numbers of Hunting and Fishing Animals of the Reserve 'Verkhne-Kondinsky' (Khanty-Mansi Autonomous Okrug—Yugra). *Fauna Ural. Sib.* **2015**, *1*, 107–118. (In Russian)
13. Danilov, P.I.; Panchenko, D.V.; Tirronen, K.F.; Fyodorov, F.V.; Belkin, V.V. Changes in the Fauna of Mammals of the Northern Palaearctic and Dynamics of the Areals of its Component Species. Izvestia RAS. *Biol. Ser.* **2018**, *3*, 301–314. (In Russian) [CrossRef]
14. Chertkova, E.P.; Kalinkin, Y.N. Population Dynamics and Distribution of the River Otter (*Lutra lutra*) in the Altai State Reserve over the Period from 2010–2020. *Monit. State Nat. Complexes Long-Term Stud. Spec. Prot. Nat. Areas Sayano-Shushensky State Nat. Reserve* **2020**, *4*, 81–89. (In Russian)
15. Mucci, N.; Pertoldi, C.; Madsen, A.B.; Loeschcke, V.; Randi, E. Extremely Low Mitochondrial DNA Control-Region Sequence Variation in the Otter *Lutra lutra* Population of Denmark. *Hereditas* **1999**, *130*, 331–336. [CrossRef] [PubMed]
16. Mucci, N.; Arrendal, J.; Ansorge, H.; Bailey, M.; Bodner, M.; Delibes, M.; Ferrando, A.; Fournier, P.; Fournier, C.; Godoy, J.A.; et al. Genetic Diversity and Landscape Genetic Structure of Otter (*Lutra lutra*) Populations in Europe. *Conserv. Genet.* **2010**, *11*, 583–599. [CrossRef]
17. Ferrando, A.; Lecis, R.; Domingo-Roura, X.; Ponsà, M. Genetic Diversity and Individual Identification of Reintroduced Otters (*Lutra lutra*) in North-Eastern Spain by DNA Genotyping of Spraints. *Conserv. Genet.* **2008**, *9*, 129–139. [CrossRef]
18. Honnen, A.C.; Roos, A.; Stjernberg, T.; Zachos, F.E. Genetic Analysis of Eurasian Otters (*Lutra lutra*) Reveals High Admixture in Finland and Pronounced Differentiation in Sweden. *Mamm. Biol.* **2015**, *80*, 47–53. [CrossRef]
19. Geboes, A.L.; Rosoux, R.; Lemarchand, C.; Hansen, E.; Libois, R. Genetic Diversity and Population Structure of the Eurasian Otter (*Lutra lutra*) in France. *Mamm. Res.* **2016**, *61*, 121–129. [CrossRef]
20. Hwang, J.-Y.; Cho, G.-J. Identification of Novel Haplotypes and Interpretation of Gene Flow of Mitochondrial DNA Control Region of Eurasian Otter (*Lutra lutra*) for the Effective Conservation. *J. Vet. Med. Sci.* **2018**, *80*, 1791–1800. [CrossRef]
21. Hung, C.M.; Li, S.H.; Lee, L.L. Faecal DNA Typing to Determine the Abundance and Spatial Organisation of Otters (*Lutra lutra*) along Two Stream Systems in Kinmen. *Anim. Conserv.* **2004**, *7*, 301–311. [CrossRef]
22. Huang, C.C.; Hsu, Y.C.; Lee, L.L.; Li, S.H. Isolation and Characterization of Tetramicrosatellite DNA Markers in the Eurasian Otter (*Lutra lutra*). *Mol. Ecol. Notes* **2005**, *5*, 314–316. [CrossRef]
23. Jang-Liaw, N.H.; Tan, Y.C.; Chang, C.J.; Juan, C.H.; Hou, H.Y.; Chung, L.W.; Cao, H.S.; Waku, D.; Chang, S.W.; Lee, L.L. Genetic Diversity and Structure of Eurasian Otters on Kinmen Island. *Conserv. Genet.* **2023**, *24*, 589–606. [CrossRef]
24. Baryshnikov, G.F. Late Pleistocene Ursidae and Mustelidae remains (Mammalia, Carnivora) from geographical society cave in the Russian Far East. *Proc. Zool. Inst. RAS* **2015**, *319*, 3–22. (In Russian) [CrossRef]
25. du Plessis, S.J.; Blaxter, M.; Koepfli, K.P.; Chadwick, E.A.; Hailer, F. Genomics reveals complex population history and unexpected diversity of Eurasian otters (*Lutra lutra*) in Britain relative to genetic methods. *Mol. Biol. Evol.* **2023**, *40*, msad207. [CrossRef] [PubMed]
26. Sokolova, N.A.; Korablev, N.P.; Korablev, P.N.; Hernandez-Blanco, J.A.; Kaloyan, G.A.; Gyonjyan, A.A.; Malkhasyan, A.H.; Sorokin, P.A. Mitochondrial Genetic Diversity of Eurasian Otter (*Lutra lutra*) from European Russia and Transcaucasian Countries. *Russ. J. Genet.* **2023**, *59*, 804–812. [CrossRef]
27. Hall, T.A. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **1999**, *41*, 95–98.
28. Harperding, H.C. Signature of Ancient Population Growth in a Low-Resolution Mitochondrial DNA Mismatch Distribution. *Hum. Biol.* **1994**, *66*, 591–600.
29. Excoffier, L.; Lischer, H.E.L. Arlequin Suite Ver 3.5: A New Series of Programs to Perform Population Genetics Analyses under Linux and Windows. *Mol. Ecol. Resour.* **2010**, *10*, 564–567. [CrossRef]
30. Kimura, M. A Simple Method for Estimating Evolutionary Rates of Base Substitutions through Comparative Studies of Nucleotide Sequences. *J. Mol. Evol.* **1980**, *16*, 111–120. [CrossRef]
31. Nylander, J.A.A. MrModeltest v2. Program Distributed by the Author. 2004. Available online: https://www.researchgate.net/publication/285805344_MrModeltest_V2_Program_Distributed_by_the_Author (accessed on 17 November 2024).

32. Leigh, J.W.; Bryant, D. POPART: Full-Feature Software for Haplotype Network Construction. *Methods Ecol. Evol.* **2015**, *6*, 1110–1116. [CrossRef]
33. Peakall, R.; Smouse, P.E. GenAlEx 6.5: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research—An Update. *Bioinformatics* **2012**, *28*, 2537–2539. [CrossRef]
34. Van Oosterhout, C.; Weetman, D.; Hutchinson, W.F. Estimation and Adjustment of Microsatellite Null Alleles in Nonequilibrium Populations. *Mol. Ecol. Notes* **2006**, *6*, 255–256. [CrossRef]
35. Goudet, J. Computer Note FSTAT (Version 1.2): A Computer Program to Calculate F-Statistics. *J. Hered.* **1995**, *86*, 485–486. [CrossRef]
36. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of Population Structure Using Multilocus Genotype Data. *Genetics* **2000**, *155*, 945–959. [CrossRef] [PubMed]
37. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the Number of Clusters of Individuals Using the Software Structure: A Simulation Study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [CrossRef] [PubMed]
38. Earl, D.A.; Vonholdt, B.M. STRUCTURE HARVESTER: A Website and Program for Visualizing STRUCTURE Output and Implementing the Evanno Method. *Conserv. Genet. Resour.* **2012**, *4*, 359–361. [CrossRef]
39. Kopelman, N.M.; Mayzel, J.; Jakobsson, M.; Rosenberg, N.A.; Mayrose, I. Clumpak: A Program for Identifying Clustering Modes and Packaging Population Structure Inferences across K. *Mol. Ecol. Resour.* **2015**, *15*, 1179–1191. [CrossRef]
40. Ki, J.S.; Hwang, D.S.; Park, T.J.; Han, S.H.; Lee, J.S. A Comparative Analysis of the Complete Mitochondrial Genome of the Eurasian Otter *Lutra lutra* (Carnivora; Mustelidae). *Mol. Biol. Rep.* **2010**, *37*, 1943–1955. [CrossRef]
41. Jo, Y.S.; Won, C.M.; Jung, J. Testing Microsatellite Loci and Preliminary Genetic Study for Eurasian Otter in South Korea. *J. Species Res.* **2012**, *1*, 240–248. [CrossRef]
42. Cohen, T.M.; Narkiss, T.; Dolev, A.; Ben-Ari, Y.; Kronfeld-Schor, N.; Guter, A.; Saltz, D.; Bar-Gal, G.K. Genetic Diversity of the Eurasian Otter (*Lutra lutra*) Population in Israel. *J. Hered.* **2013**, *104*, 192–201. [CrossRef]
43. Tison, J.L.; Blennow, V.; Palkopoulou, E.; Gustafsson, P.; Roos, A.; Dalén, L. Population Structure and Recent Temporal Changes in Genetic Variation in Eurasian Otters from Sweden. *Conserv. Genet.* **2015**, *16*, 371–384. [CrossRef]
44. Salomashkina, V.V.; Kholodova, M.V.; Tuten'kov, O.Y.; Moskvitina, N.S.; Erokhin, N.G. New Data on the Phylogeography and Genetic Diversity of the Brown Bear *Ursus arctos* Linnaeus, 1758 of Northeastern Eurasia (MtDNA Control Region Polymorphism Analysis). *Biol. Bull.* **2014**, *41*, 38–46. [CrossRef]
45. Ersmark, E.; Klütsch, C.F.C.; Chan, Y.L.; Sinding, M.H.S.; Fain, S.R.; Illarionova, N.A.; Oskarsson, M.; Uhlén, M.; Zhang, Y.P.; Dalén, L.; et al. From the Past to the Present: Wolf Phylogeography and Demographic History Based on the Mitochondrial Control Region. *Front. Ecol. Evol.* **2016**, *4*, 134. [CrossRef]
46. Kutschera, V.E.; Lecomte, N.; Janke, A.; Selva, N.; Sokolov, A.A.; Haun, T.; Steyer, K.; Nowak, C.; Hailer, F. A Range-Wide Synthesis and Timeline for Phylogeographic Events in the Red Fox (*Vulpes vulpes*). *BMC Evol. Biol.* **2013**, *13*, 114. [CrossRef] [PubMed]
47. Pasinelli, G. Genetic Diversity and Spatial Genetic Structure Support the Specialist-Generalist Variation Hypothesis in Two Sympatric Woodpecker Species. *Conserv. Genet.* **2022**, *23*, 821–837. [CrossRef]
48. Mecozzi, B.; Iannucci, A.; Bona, F.; Mazzini, I.; Pieruccini, P.; Sardella, R. Rediscovering Lutra Lutra from Grotta Romanelli (Southern Italy) in the Framework of the Puzzling Evolutionary History of Eurasian Otter. *PalZ* **2022**, *96*, 161–174. [CrossRef]
49. Ehlers, J.; Gibbard, P. *Quaternary Glaciation*; Springer: Berlin/Heidelberg, Germany, 2011; Volume 3.
50. Gimranov, D.O.; Kosintsev, P.A. Otter (*Lutra lutra* L.) and badger (*Meles* sp.) hunting in Holocene in Southern Urals. *Izv. Samar. Nauchnogo Tsentra RAN* **2011**, *13*, 39–42. (In Russian)
51. Bachura, O.P.; Kosintsev, P.A. Larger mammal fauna of the central part of the East European Plain in the Holocene. *Russ. J. Zool.* **2020**, *99*, 569–585. (In Russian) [CrossRef]
52. Baryshnikov, G.F.; Puzachenko, A.Y. Cranimetrical variability of the Eurasian otter (*Lutra lutra*: Carnivora: Mustelidae) from the Northern Eurasia. *Proc. Zool. Inst. RAS* **2012**, *316*, 203–222. (In Russian) [CrossRef]
53. Gimranov, D.O.; Kosintsev, P.A. Geographic Distribution of the Tooth Morphotypes of the River Otter (Carnivora, Mustelidae, *Lutra lutra* L., 1758) in Northern Eurasia. *Dokl. Biol. Sci.* **2012**, *443*, 84–86. [CrossRef]
54. Ranyuk, M.; Ansorge, H. Low Epigenetic Variability of the Eurasian Otter *Lutra lutra* (L.). *Russ. J. Ecol.* **2015**, *46*, 195–201. [CrossRef]
55. Ferrando, A.; Ponsà, M.; Marmi, J.; Domingo-Roura, X. Eurasian Otters, *Lutra lutra*, Have a Dominant MtDNA Haplotype from the Iberian Peninsula to Scandinavia. *J. Hered.* **2004**, *95*, 430–435. [CrossRef]
56. Stanton, D.W.G.; Hobbs, G.I.; Chadwick, E.A.; Slater, F.M.; Bruford, M.W. Mitochondrial Genetic Diversity and Structure of the European Otter *(Lutra lutra*) in Britain. *Conserv. Genet.* **2009**, *10*, 733–737. [CrossRef]
57. Finnegan, L.A.; Néill, L.Ó. Mitochondrial DNA Diversity of the Irish Otter, *Lutra lutra*, Population. *Conserv. Genet.* **2010**, *11*, 1573–1577. [CrossRef]
58. Li, B.; Lu, J.; Monakhov, V.; Kang, H.; Xu, Y.; An, B.; Ghani, M.U.; Li, M.; Peng, W.; Ma, X. Phylogeography of Subspecies of the Sable (*Martes zibellina* L.) Based on Mitochondrial Genomes: Implications for Evolutionary History. *Mamm. Biol.* **2021**, *101*, 105–120. [CrossRef]

59. Doan, K.; Niedziałkowska, M.; Stefaniak, K.; Sykut, M.; Jędrzejewska, B.; Ratajczak-Skrzatek, U.; Piotrowska, N.; Ridush, B.; Zachos, F.E.; Popović, D.; et al. Phylogenetics and Phylogeography of Red Deer MtDNA Lineages during the Last 50 000 Years in Eurasia. *Zool. J. Linn. Soc.* **2022**, *194*, 431–456. [CrossRef]
60. Golosova, O.S.; Kholodova, M.V.; Volodin, I.A.; Seryodkin, I.V.; Okhlopkov, I.M.; Argunov, A.V.; Sipko, T.P. Genetic Diversity of the Eastern Subspecies of Red Deer (*Cervus elaphus*) in Russia Revealed by MtDNA and Microsatellite Polymorphism. *Biol. Bull. Rev.* **2023**, *13*, 482–494. [CrossRef]
61. Kholodova, M.V.; Prikhodko, V.I. Molecular Genetic Diversity of Musk Deer Moschus Moschiferus L., 1758 (Ruminantia, Artiodactyla) from the Northern Subspecies Group. *Russ. J. Genet.* **2006**, *42*, 783–789. [CrossRef]
62. Dallas, J.F.; Marshall, F.; Piertney, S.B.; Bacon, P.J.; Racey, P.A. Spatially Restricted Gene Flow and Reduced Microsatellite Polymorphism in the Eurasian Otter *Lutra lutra* in Britain. *Conserv. Genet.* **2002**, *3*, 15–29. [CrossRef]
63. Macdonald, S.M.; Mason, C.F. Some Factors Influencing the Distribution of Otters (Lutra Lutra). *Mamm. Rev.* **1983**, *13*, 1–10. [CrossRef]
64. Driscoll, C.A.; Yamaguchi, N.; Bar-Gal, G.K.; Roca, A.L.; Luo, S.; Macdonald, D.W.; O'Brien, S.J. Mitochondrial Phylogeography Illuminates the Origin of the Extinct Caspian Tiger and Its Relationship to the Amur Tiger. *PLoS ONE* **2009**, *4*, e4125. [CrossRef]