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NEW DATA ON PHYLOGEOGRAPHY OF THE BOREAL OWL, *AEGOLIUS FUNEREUS* (STRIGIFORMES, STRIGIDAE), IN EURASIA

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New Data on Phylogeography of the Boreal Owl, *Aegolius funereus* (Strigiformes, Strigidae), in Eurasia. **Homel, K. V., Nikiforov, M. E., Kheidorova, E. E., Valnisty, A. A. —** In the article the research's results of phylogeography, genetic diversity, genetic structure and demographic characteristics of the Boreal Owl population in Eurasia are given. The first domain of control region of mtDNA is used as a genetic marker. The sample size was 59 specimens. The population of Boreal Owl is characteristic of high genetic diversity and it has signs of rapid expansion in the past as revealed by analysis of CR1 mtDNA polymorphism. Genetic differentiation between birds from the west and the east part of the species range is shown. The level of found population genetic differentiation isn't high that can be explained by gene flow in the past and possible at the present time. We didn't reveal any signs of genetic differentiation for Boreal Owl population according to subspecies (between *A. f. funereus* and *A. f. pallens*) which are distinguished for the studying area.
Key words: boreal owl, *Aegolius funereus*, phylogeography, genetic diversity, genetic structure, Eurasia.

Introduction

Highly reliable data on the biological diversity of a species provides increased efficiency of directed conservation efforts. Defining a species' phylogeographic structure through the genetic differentiation of its populations allows for selecting more appropriate and adequate conservation approaches, compared with those based on intraspecific taxonomic structure as it is defined by morphological criteria for a given species. A solid understanding of intraspecific genetic status of a species is vital for determining vulnerable genetic lines, as well as determining fitting localities for efficient efforts towards their conservation (Allendorf et al., 2013).

Phylogeographic studies are also vital for unearthing the history of developing intraspecific structure and the influences of historical geoclimatic processes on a species and its adaptability (Avise & Walker, 1998; Hewitt, 2001). Establishing the role of climatic and biogeographical processes in determining the genetic makeup of populations serves to make the impact of future environmental transformations on such populations more predictable.

Serious contradictions can frequently be seen between classic intraspecific taxonomic units and molecular genetic marker data. Examples of such contradictions for mitochondrial markers include Willow Ptarmigan, *Lagopus lagopus* (Linnaeus, 1758) (Höglund et al., 2013), Northern Goshawk, *Accipiter gentilis* (Linnaeus, 1758) (Kunz et al., 2019), Golden Eagle, *Aquila chrysaetos* (Linnaeus, 1758) (Nebel et al., 2015), Eurasian Three-toed Woodpecker, *Picoides tridactylus* (Linnaeus, 1758) (Zink et al., 2002 b), Great Spotted Woodpecker, *Dendrocopos major* (Linnaeus, 1758) (Zink et al., 2002 a), Western Capercaillie, *Tetrao urogallus* Linnaeus, 1758 (Duriez et al., 2007), Black Grouse, *Lyrurus tetrix* (Linnaeus, 1758) (Corrales et al., 2014) and White Wagtail, *Motacilla alba* Linnaeus, 1758 (Li et al., 2016). These situations of ambiguous intraspecific genetic division showcase the complexity and variety of historic phylogeographic structure among birds. Investigating the roots of phylogeographic patters in other species facilitates better understanding of avian microevolution processes and climatic adaptability. What in turn supports the efforts for biodiversity conservation.

In this study we analyze the phylogeographic structure of a polytypic species — the Boreal Owl, *Aegolius funereus* (Linnaeus, 1758), in the Eurasian part of its range.

The Boreal Owl inhabits the Holarctic ecozone. In Eurasia this species has a continuous range stretching from boreal forests of Northern and Central Europe across Urals and to Kamchatka and Kuril isles. The dispersed populations across Europe stretch south to Pyrenees, Alps and Caucasus mountains, and in Asia along Tarbagatai, Tien Shan and Zervshan mountain ranges (Hayward & Hayward, 2020). The species' habitat includes boreal, subalpine and mixed temperate forests (Hayward & Hayward, 2020).

The Boreal Owl is characteristic of settled behavior. Males are more site tenacious than females, but both sexes prefer to hold to their home range all year round, undertaking distant migrations only as the numbers of their prey dwindle (Hayward & Hayward, 2020).

The species is traditionally divided into six subspecies of different localization (Hayward & Hayward, 2020), five subspecies are traditionally counted across its Eurasian part of the range and one subspecies — across its North American part of the range.

Present subspecies and wide distribution across Eurasia could suggest significant genetic distancing between subspecies due to separation of subpopulations, as well as intraspecific competition. However, the current Boreal Owl subspecies were defined according to morphological characteristics, primarily differences in body size and plumage coloration. Variability of those traits can have clinal nature. This uncertainty could be resolved by employing molecular genetic analysis for determining the intraspecific relations between the subspecies on the Eurasian part of the species' range, which could supply an insight into the species' development through its phylogeographic history during the Pleistocene glacial periods as well as postglacial events.

At the moment there is a series of researches on genetic differentiation and diversity of the species existed. The preliminary research on the phylogeography of Boreal Owl in Eurasia was already conducted by us in 2013. A 648 bp fragment of mitochondrial COI gene was used as the genetic marker. The results of that study did not suggest any noticeable genetic divergence between the Boreal Owl subspecies, or significant genetic distancing between various subspecies specimens from geographically distant subpopulations (Belarus, Russia (Sakhalin, Kirov oblast, Magadan oblast), Norway, Sweden), according to the chosen molecular marker. The procured genetic diversity data suggested a recent expansion of the Boreal Owl across Norther Europe and Russia, marking the subspecies divergence as being currently in its early stages.

A publication by Koopman et al. from 2005 describes a study of the Boreal Owl subspecies structure using 7 microsatellites as genetic markers. The sample size was 275 specimens from North America, 36 from Norway, and 5 from eastern Russia. Microsatellite analysis data did not indicate any significant genetic divergence between Norwegian and Russian Boreal Owl populations, but a certain degree of divergence between Eurasian and North American populations. Authors tied close genetic admixture of populations within the same continent to extensive dispersal ranges of the species, facilitating stable migration rate between subpopulations. In the light of these results, the authors declared the necessity of further genetic research into Boreal Owl populations in order to better determine intraspecific structure. They also highlight that North American populations don't display any significant genetic divergence — Alaskan, Canadian, Idaho, Montana, Wyoming and Colorado were characterized by very low genetic differentiation ($\theta = 0{,}004$) according to chosen microsatellite panel data (Koopman et al., 2005).

Another publication reported the study of genetic differentiation between the westernmost Boreal Owl population of Pyrenees and the Fennoscandian population (Broggi et al., 2013). A part of mitochondrial DNA's control region (369 base pairs in length) was utilized as the genetic marker. The sample consisted of 19 individual birds from the region of Andorra and Catalonia, and 17 individuals from Finland. A total of 26 marker sequence haplotypes was noted. The genetic diversity coefficients were similar for both studied populations. The hierarchical molecular variance analysis indicated a lack of clear genetic differentiation between the studied populations with the $\Phi_{ST} = 0.0194$ ($P = 0.1711$). A presence of genetic flow between the sampled groups in the range between 11 and 43 individuals per generation was determined, further highlighting the absence of a clear division between populations.

This study aims to continue the investigation of boreal owl's phylogeography and intraspecific genetic structure through analysis of mtDNA control region sequences including the easternmost samples.

Material and methods

The study utilized original sequences of boreal owl's first control region domain of the mtDNA from Belarus (n = 2) and Russia (n = 12), as well as confirmed and approved publicly available sequences of the same marker obtained from the NCBI database, the latter being sequences from individuals sampled in the Pyrenees (n = 18), Fennoscandia (n = 16), Norway (n = 9), as well as 2 sequences identical to Pyrenees and Fennoscandia (Appendix, table 1).

The study included a total of 59 mtDNA CR1 sequences of the Boreal Owl (fig. 1).

The sampled Boreal Owl sequences source locations correspond to the ranges of two Eurasian subspecies of Boreal Owl out of five, namely *A. f. funereus* and *A. f. pallens* (Hayward & Hayward, 2020).

DNA for further amplification and sequencing of original samples was extracted from muscle tissues deposited in the genetic collection of SSPA "Scientific and Practical Center of the National Academy of Sciences of Belarus on Bioresources" and provided by MSU Zoological Museum. Extraction was carried out using a commercial "Blood, Animal, Plant DNA Preparation Kit" (Jena Bioscience, Germany) utilizing silica membrane spin-columns. The concentrations of extracted DNA samples were measured via IMPLEN spectrophotometer P330 (IMPLEN, Germany). Every utilized original DNA sample was deposited into the Wildlife genetic bank of the Scientific and Practical Center of the National Academy of Sciences of Belarus on Bioresources for further reference.

The amplification of the Boreal Owl mtDNA CR1 was conducted using primers AftRNAglu (5'-GGCCTGAAAAACCACCGTTAA-3') and AfH535 (5'-AGATTATTTGGTTATGGTGGG-3') (Broggi et al., 2013).

PCR-amplification was performed in 25мl volume reaction mixes, containing 2.5 мl of 10X Taq buffer with $(NH_4)_2SO_4$, 2.5 мl of 10X dNTPs mix (2mM of each dNTP), 3 мl of

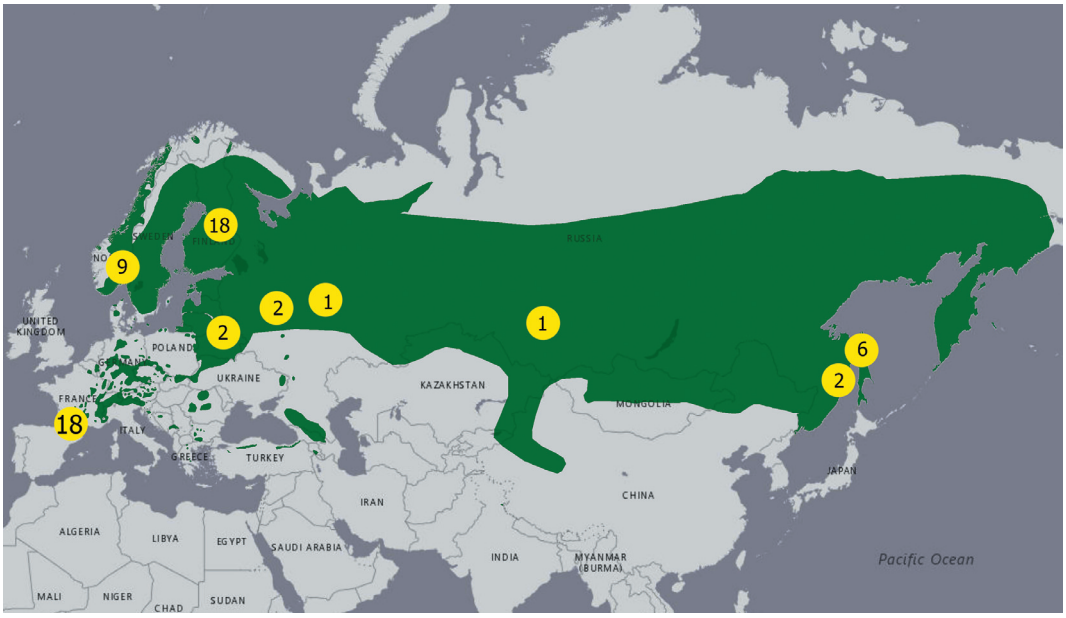

Fig. 1. The source regions of Boreal Owl mtDNA CR1 sequences used in the present study. Numbered yellow circles indicate sampling regions for utilized Boreal Owl mtDNA CR1 sequences, as well as the number of sequences per each region. Green coloration indicates boreal owl's range.

$MgCl_2$ solution (25 mM), 2мl of forward and reverse primer solution (5 pmol/мl), 1 unit of Taq-polymerase, 2 мl of DNA matrix solution and 10.9 мl of ddH_2O.

Amplification of the Boreal Owl mtDNA CR1 was done according to the following protocol: initial denaturation at 95 °C for 2 minutes; 35 cycles of — denaturation at 95 °C for 30 seconds, primer annealing at 50 °C for 30 seconds, elongation at 72 °C for 90 seconds; final elongation at 72 °C for 5 minutes. The reaction was carried out with a C1000 Touch thermal cycler (Bio-Rad Laboratories, Inc. USA).

The Boreal Owl mtDNA CR1 amplicons were Sanger-sequenced using a GenomeLab GeXP Genetic Analysis System (Beckman Coulter, Germany) with Dye Terminator Cycle Sequencing Quick Start Kits (Beckman Coulter, Germany) according to manufacturer's protocols, utilizing the abovementioned mtDNA CR1 primers.

Obtained sequence data was manually checked using MEGA 6 software and aligned using Muscle algorithm with the same software (Tamura et al., 2013). Polymorphism sites were detected using MEGA 6 and DnaSP v. 6.10.04 (Rozas et al., 2017). Nucleotide diversity (π), number of haplotypes, average number of nucleotide differences (k), haplotype diversity (Hd), number of segregation sites (S) and θ per site from S for the studied sequence set were calculated in DnaSP. Haplotype network of the studied sequences was built using POPART ("PopART," 2020) with the Median Joining Network algorithm.

Demographic indexes were calculated with DnaSP — specifically, Fu's Fs, Tajima's D, raggedness index (r) and Ramos-Onsin's and Roza's R_2. Low values of R_2 and negative values of Fs and D indicate population expansion in the past (Ramos-Onsins & Rozas, 2002). P-values for the abovementioned indexes were determined via coalescent simulation in DnaSP utilizing theta and segregating sites number both. Additionally, a mismatch distribution graph (the distribution of the number of site differences between pairs of sequences) was constructed using DnaSP. The last test indicates population expansion in the case if the distribution is a unimodal, raggedness index (quantitative assessment of the smoothness of the mismatch distribution for the demographic scenarios of population expansion and stability in the past) and the sum of squared deviations (SSD) from the sudden expansion model also have low values in this situation (Rogers & Harpending, 1992; Maltagliati et al., 2010). The sum of squared deviations (SSD) was calculated in Arlequin 3.5.1.2. (Excoffier et al., 2007).

Divergence rate values of 4 % and 14 % per Myr for mtDNA CR were used to calculate the time of beginning population expansion (Marthinsen et al., 2009). It was calculated as $t = \tau \div 2\mu$ (Shephard et al., 2013; Klinga et al., 2015), τ was taken from mismatch distribution calculation in DnaSP, and μ being [divergence rate/2/10^6 * sequence length in base pairs (210 bp)* generation time in years] (Marthinsen et al., 2009). Boreal Owl generation was taken as 2 years (Hayward & Hayward, 2020). The resulting μ equaled $8{,}4*10^{-6}$ for divergence rate value of 4 % and $2{,}94*10^{-5}$ for 14 % rate.

Population differentiation within the Boreal Owl population across the Eurasian range was determined using Arlequin. The population was divided into three nominal groups: Pyrenees (n = 19), Fennoscandia/Eastern Europe (n = 31) and Russian Far East (n = 9). Genetic divergence of populations was determined through pairwise Fst (Tamura & Nei genetic distance with 10 000 permutations) and an exact test of sample differentiation based on haplotype frequencies (default settings).

Results and discussion

Aligning original mtDNA CR1 sequences and GenBank sequences (with a total of 59) produced a 261 bp alignment. A 210 bp continuous segment of this alignment was fit for analysis (after excluding sites with gaps/missing data). Pyrimidine transitions were more prevalent across the alignment; 31 variable sites were detected, with 22 of them being parsimony-informative ones. Thirty-two distinct haplotypes were identified among 59 aligned sequences (Appendix, table 2).

Table 1. Molecular diversity metrics for the Eurasian population of Boreal Owl according to mtDNA CR1 polymorphism

Metric	Value
N	59
h	32
S	22
Hd ± SD	0.953 ± 0.017
π ± SD	0.01337 ± 0.00102
k	2.808
Theta per site (from S) (Theta-W)	0.02255
Fu's Fs	–31.39***
Tajima's D	–1.28 NS
R_2	0.0602 NS
SSD	0.00286 NS
Raggedness index (r)	0.0438 NS

Note. N — sample size, SD — standard deviation, Fu's Fs, Tajima's D — mutation neutrality indexes, SSD — sum of squared deviations for the sudden expansion model (Rogers & Harpending, 1992 (Maltagliati et al., 2010), raggedness index (r) (Harpending's (1994) (Maltagliati et al., 2010), NS — statistically not significant, *** $p < 0.001$.

One of the identified haplotypes (Afuner_2) included 11 individuals of the sample; two haplotypes (Afuner_8, Afuner_12) including 4 individuals each; two haplotypes (Afuner_10, Afuner_16) including 3 individuals each and seven haplotypes (Afuner_5, Afuner_11, Afuner_13, Afuner_21, Afuner_27, Afuner_30, Afuner_40) including 2 individuals each. The remaining 20 haplotypes all included unique individual sequences.

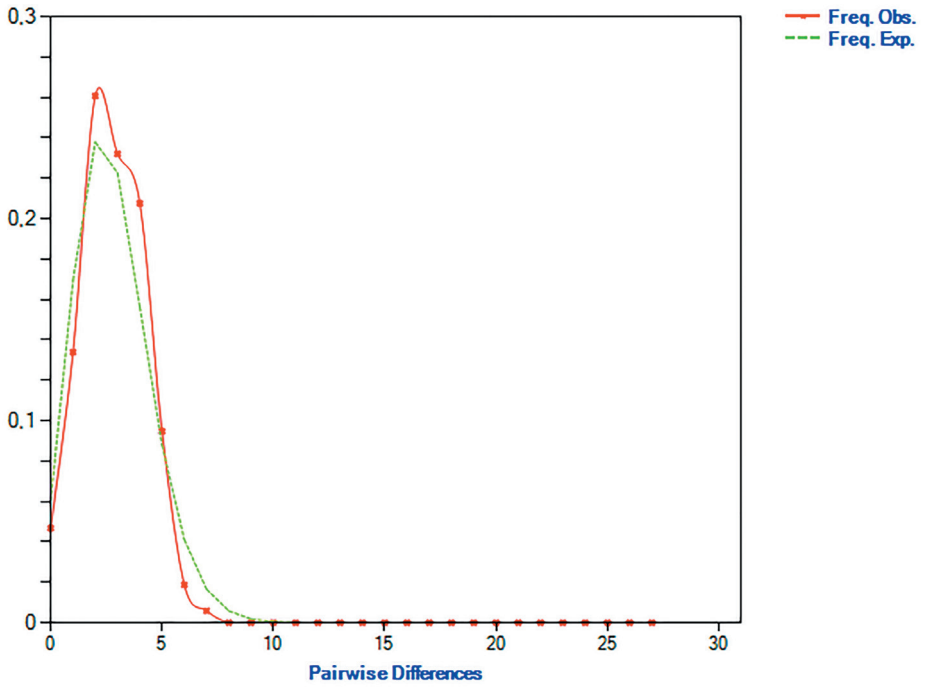

Fig. 2. Mismatch distribution graph for the pairwise comparison of mtDNA CR1 sequences of Eurasian Boreal Owl population. X axis reflects pairwise difference, Y axis reflects frequency of the difference across sequences; Freq. Obs. is the studied sample's observed mismatch frequency graph, Freq. Exp. is the expected frequency for the sudden expansion model.

The genetic diversity metrics for the studied Boreal Owl sample are listed in table 1.

The mtDNA CR1 polymorphism of the studied sample clearly indicates a high genetic diversity level for boreal owl's Eurasian population, despite modest resolution of the marker. Still, it has proven sufficient to determine intraspecific genetic variety for the studied population.

Analysis of demographic metrics of the Eurasian population of the Boreal Owl shows a very likely sudden and rapid population expansion in the species' distant past, as indicated by statistically significant negative Fu's Fs value, low (although not statistically significant) R_2 value, low and statistically not significant values of SSD for sudden expansion model and raggedness index r (table 1). The mismatch distribution graph for the studied sequences, with expected values and the ones obtained from the studied sample's mtDNA CR1 data is presented in fig. 2. The mismatch distribution graph appears to be unimodal, indicating population expansion in the past. The average pairwise distance equaled 2,808, with most differences being between 2 and 4 mismatches. The mtDNA CR1 divergence data, for $\mu = 8{,}4^{*}\ 10^{-6}$ or $2{,}94\ ^{*}10^{-5}$ and $\tau = 2{,}808$ indicates the population expansion 167 142,9 or 47 755,10 years ago respectively, this timeframe value being an order of magnitude lesser than the one reported previously (Broggi et al., 2013). This shows that Boreal Owl expansion happened before the beginning of the last glacial maximum (about 20 000 years ago (Hewitt, 2001)). The high genetic diversity is likely to be tied with the absence of any known major bottleneck events for this species in the late Pleistocene, this possibly being supported by the obtained negative and not significant Tajima's D value.

The genetic diversity and demographic history of the Boreal Owl described above is also reflected in the haplotype network for the studied sample (fig. 3).

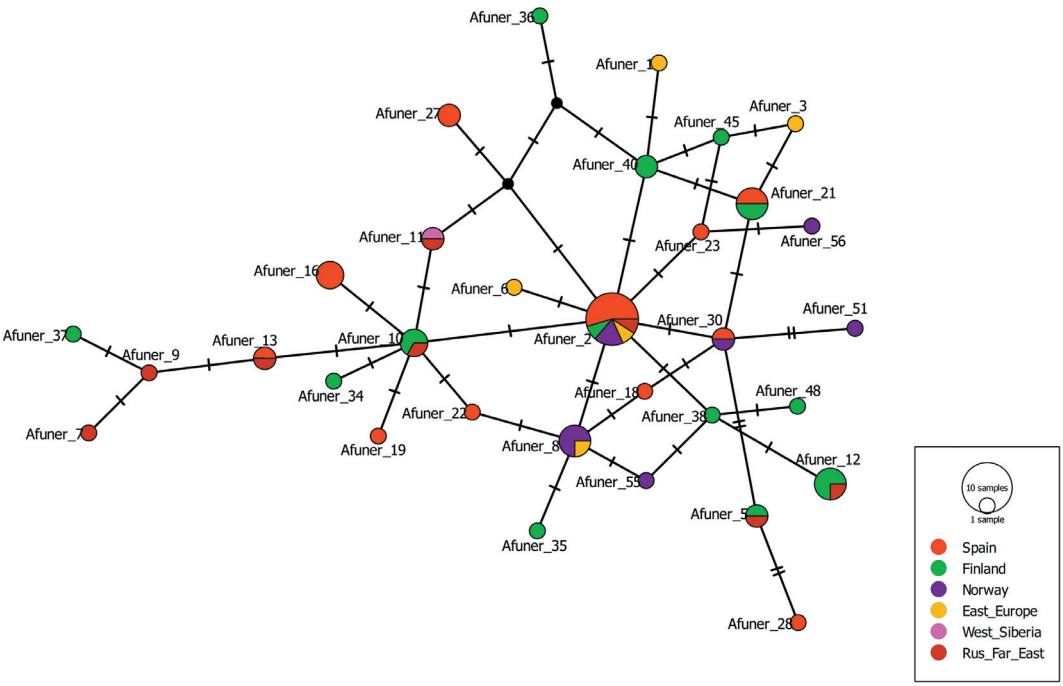

Fig. 3. Median joining network of mtDNA CR1 haplotypes for the studied Boreal Owl sample. Each circle reflects a mtDNA CR1 haplotype. Circle sizes reflect the number of studied individuals possessing the haplotype; circle colors reflect geographic origin of individuals possessing the haplotype. Bars connect related haplotypes, with notches on bars reflecting the number of nucleotide differences between them. Black dots indicate implied haplotypes not present in the sample.

The haplotype network shows a distinct central haplotype Afuner_2, uniting sequences of individuals sampled in Russian Far East (Khabarovsk krai), European Russia (Kirov oblast), Western Europe (Pyrenees) and Northern Europe (Fennoscandia, Norway), marking it as a possible ancestral haplotype. The full haplotype network shows a clear picture of species' expansion in the past with a distinct, interconnected star-like structure with a likely ancestral haplotype in the center, as well as significant genetic diversity though high number of haplotypes including distant and unique ones. Geographic distribution of the studied haplotypes across the Boreal Owl range is shown in figure 4.

Geographic distribution of Boreal Owl mtDNA CR1 haplotypes shows the presence of common haplotypes between extremely distant regions, such as Fennoscandia and Russian Far East sharing 4 common haplotypes (Afuner_2, Afuner_5, Afuner_10, Afuner_12), the Pyrenees and Russian Far East sharing 2 common haplotypes (Afuner_2, Afuner_13), and Scandinavia and Pyrenees sharing 3 (Afuner_2, Afuner_21, Afuner_30). Unique haplotype distribution possibly shows more active microevolution processes in Pyrenees and Scandinavia (fig. 4), although this is more likely to be an indication of local genetic diversity reflected through the local sample size.

The Fst pairwise comparison unsurprisingly shows the most significant genetic difference between Fennoscandia/East Europe group and the Russian Far East group (Fst = 0.099, p = 0.006), with the one between Pyrenees group and Russian Far East group being a close second (Fst = 0.089, p = 0.019), the difference between the Pyrenees group and the Fennoscandia/East Europe group one being much smaller and statistically not significant (Fst = 0.024, p = 0.077). Exact test of sample differentiation based on haplotype frequencies has failed to provide robust differences ($p > 0.05$), aside from a reliable but very minor difference between Pyrenees and Fennoscandia/East Europe groups (p = 0.03). These results lead to the following conclusions: there is a degree of genetic differentiation

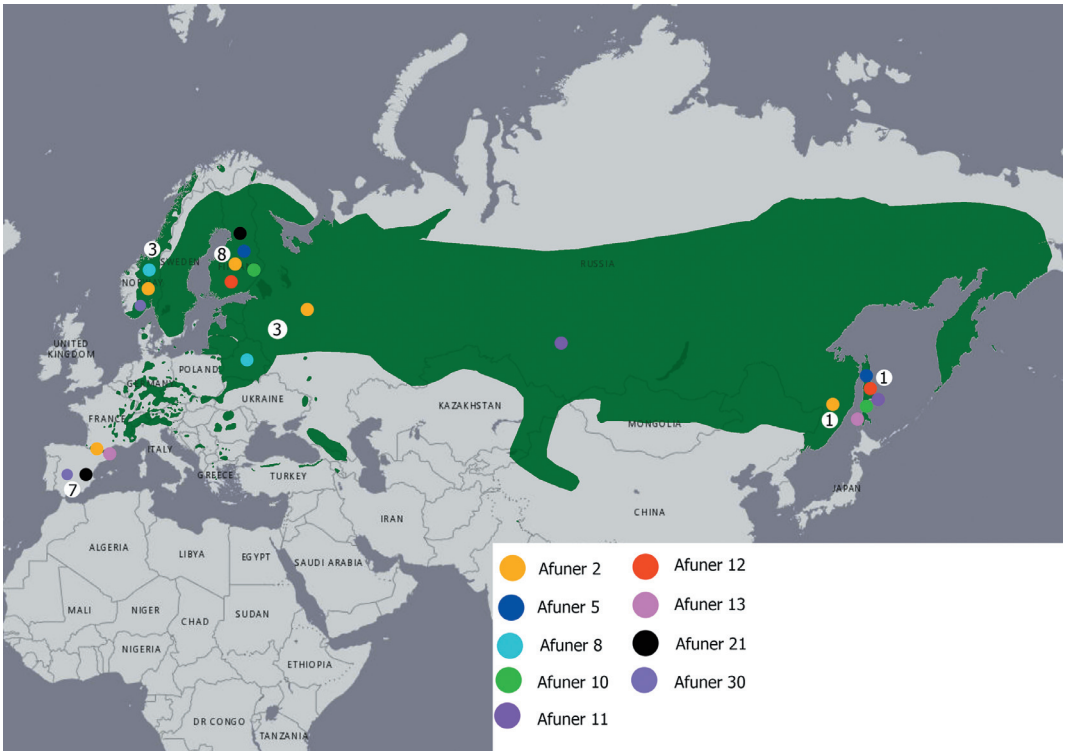

Fig. 4. Boreal Owl mtDNA CR1 haplotype distribution across it's Eurasian range. Colored dots indicate approximate regions of sampling for individuals possessing the corresponding haplotype. White dots with numbers reflect the total number of unique haplotypes for this region.

between the individuals present in the easternmost and the westernmost parts of the range — which is natural, given the extreme distances. On the other hand, the haplotype frequencies distribution is extremely unlikely to be caused entirely by homoplasy shows a lack of any definite line of genetic division of any nature between parts of the range, introducing a degree of gene flow across them in the past and, most likely, present. The observed minor differentiation between the Pyrenees and Fennoscandia/East Europe groups can be attributed to the effect of individual unique haplotypes under the limited sample size.

The Eurasian population of Boreal Owl is characterized by high level of genetic diversity, lack of intraspecific differentiation and absence of signs of drastic population decline events (bottlenecks) in the observable past.

The obtained genetic diversity characteristics and absence of intraspecific structure in the Eurasian population of Boreal Owl are in agreement with similar earlier publications on the subject (Koopman et al., 2005; Broggi et al., 2013). Broggi et al. (2013) have reported a similar level of differentiation between boreal owls from the Pyrenees and Fennoscandia ($\Phi_{ST} = 0.0194$, $p = 0.1711$), as well as similar conclusion on demographic dynamics concerning absence of probable bottleneck events in the species' past (Broggi et al., 2013). The most significant difference of the present study's outcomes from the results presented by Broggi et al. (2013) is in the haplotype network structure and the time of population divergence. The previous haplotype network lacked any structural differentiation between the Pyrenees and Fennoscandian groups. This difference is most likely tied to the sampling limitations of both studies, rather than any objective effect or other differences in methodology. The significant difference in the predicted time of population divergence (between 600 000 and 2 million years ago according to (Broggi et al., 2013)) also most likely stem from different samples, as well as a higher resolution marker used by Broggi et al. (2013) (369 bp sequence against a 210 bp one in the present study), and other minor differences in methodology.

The absence of any clear intraspecific structure within the Eurasian Boreal Owl population is supported by earlier studies utilizing microsatellite markers (Koopman et al., 2005). That study compared *A. f. funereus* and *A. f. sibiricus* (currently designated as *A. f. pallens* (Hayward & Hayward, 2020)) populations, and determined absence of any significant genetic differentiation between the subspecies. The same conclusions are made for other subspecies of boreal owl, exclusing *A. f. caucasicus*.

The basic reason for the lack of intraspecific differentiation in the Eurasian Boreal Owl population can be the species' tendency for long-distance migrations, characteristic of the species under the conditions of diminishing prey numbers (Hayward & Hayward, 2020), as well as the species' exclusion from the historic phenomenon of prolonged isolation in the Mediterranean refugia characteristic of species from temperate and more southern regions (Hewitt, 1996, 2000 quoted in (Broggi et al., 2013)). For example, a related species of tawny owl (*Strix aluco* Linnaeus, 1758) was determined to possess the genetic differentiation within Western Europe populations according to both mitochondrial and nuclear DNA markers data (Brito, 2007). This was tied to reason that tawny owl is not prone for making long-range migrations and such behavior prevents constant admixture between partially isolated populations. It is suggested that extended isolation of tawny owl in Mediterranean refugia during the Pleistocene glaciation, which would form a base for the current genetic differentiation (Brito, 2005). A fitting example for a clear role of historic refugia isolation playing a role in the current genetic structure of a species would be the western capercaillie. Most of its subspecies, inhabiting boreal forests of Europe, lack any intraspecific structure, while the populations historically tied to the glacial refugia of Balkans and Iberia present clearly defined genetic lineages (Bajc et al., 2011; Rodríguez-Muñoz et al., 2007). The northern goshawk can serve as an example of another wide-ranging species without any clear intraspecific structure akin to the situation of the Boreal Owl (Kunz et al., 2019). The cause for this can be tied both to frequent long-range migrations (Squires et al., 2020) and historic expansion from a singular refugium (Kunz et al., 2020).

Conclusion

The obtained results on genetic differentiation within the Eurasian population of the Boreal Owl allow us to conclude that this species belongs to the phylogeographic model type with lacking any significant genetic differentiation within its continental range, forming a singular expansive population, with any significant genetic differences being possible mainly within the global range including the North American population (Koopman et al., 2005).

The authors would like to thanks to our colleagues from the Zoological Museum of Moscow University (Russia) and Laboratory of ornithology of SSPA "Scientific and Practical Center of the National Academy of Sciences of Belarus on Bioresources" for providing and transferring samples of the Boreal Owl from the Russian part of the species range. We also thank the anonymous referees for important comments.

Appendix. Table 1. A list of Boreal Owl mtDNA CR1 sequences used in the present study

N	Sequence ID	Geographic region of origination
	Sequences obtained from GenBank	
1	KC495114.1	
2	KC495113.1	
3	KC495112.1	
4	KC495111.1	
5	KC495110.1	
6	KC495109.1	
7	KC495107.1	
8	KC495106.1	
9	KC495105.1	
10	KC495104.1	Pyrenees (Spain)
11	KC495103.1	
12	KC495102.1	
13	KC495101.1	
14	KC495100.1	
15	KC495099.1	
16	KC495098.1	
17	KC495097.1	
18	KC495096.1	
19	KC495095.1	
20	KC495094.1	
21	KC495093.1	
22	KC495092.1	
23	KC495091.1	
24	KC495090.1	
25	KC495089.1	
26	KC495088.1	
27	KC495087.1	Fennoscandia
28	KC495086.1	
29	KC495085.1	
30	KC495084.1	
31	KC495082.1	
32	KC495081.1	
33	KC495080.1	
34	KC495079.1	

35	EU411019.1	Norway (Vest-Agder)
36	EU411018.1	
37	EU411017.1	
38	EU411016.1	
39	EU411015.1	
40	EU411014.1	
41	EU411013.1	
42	EU411012.1	
43	EU411011.1	
44	KC495108.1	Pyrenees/Fennoscandia
45	KC495083.1	
	Original sequences from individuals sampled in Russia	
46	AV02202 (RYA 2831)	Sakhalin island
47	AV02203 (RYA 2839)	
48	AV02204 (RYA 2845)	
49	AV02205 (RYA 2846)	
50	AV02209 (CBH 3372)	
51	AV02210 (CBH 3371)	
52	AV02206 (CBH 2153)	Khabarovsk krai
53	AV02207 (CBH 2154)	
54	AV02201 (NIA 458)	Tomsk oblast, Seversk city
55	AV02208 (CBH 869)	Kirovsk oblast
56	AV01751 (RYA 3246)	Moscow
57	AV01752 (TMA 410)	
	Original sequences from individuals sampled in Belarus	
58	AV00133	Brest oblast., Lyakhovichi district, Tukhovichi village
59	AV02760	State environmental institution «Berezinsky biosphere reserve»

Appendix. Table 2. Boreal Owl mtDNA CR1 haplotypes identified in the aligned sequences

Haplotype	Number of individuals	Individuals with the haplotype
Afuner_1	1	1751_Russia_Moscow
Afuner_2	11	2207_Russia_Khabarovsk, 2208_Russia_Kirov_region, KC495114.1_AF92_Spain, KC495112.1_AF90_Spain, KC495105.1_AF82_Spain, KC495104.1_AF81_Spain, KC495103.1_AF80_Spain, KC495096.1_AF60_Spain, KC495085.1_AF15_Finland, EU411018.1_perle9_Norway, EU411012.1_perle2_Norway
Afuner_3	1	1752_Russia_Moscow
Afuner_5	2	2210_Russia_Sakhalin, KC495090.1_AF33_Finland
Afuner_6	1	2760_Belarus_Berezinsky_Reserve
Afuner_7	1	2203_Russia_Sakhalin
Afuner_8	4	133_Belarus_Lyakhovichi_district, EU411017.1_perle7_Norway, EU411016.1_perle6_Norway, EU411011.1_perle1_Norway
Afuner_9	1	2206_Russia_Khabarovsk
Afuner_10	3	2209_Russia_Sakhalin, KC495080.1_AF4_Finland, KC495079.1_AF1_Finland
Afuner_11	2	2201_Russia_Tomsk_region, 2205_Russia_Sakhalin
Afuner_12	4	2202_Russia_Sakhalin, KC495087.1_AF24_Finland, KC495086.1_AF21_Finland, KC495082.1_AF12_Finland

Afuner_13	2	2204_Russia_Sakhalin, KC495098.1_AF62_Spain
Afuner_16	3	KC495113.1_AF91_Spain, KC495109.1_AF86_Spain, KC495097.1_AF61_Spain
Afuner_18	1	KC495111.1_AF89_Spain
Afuner_19	1	KC495110.1_AF88_Spain
Afuner_21	2	KC495108.1_AF85_Spain_Finland, KC495083.1_AF13_Spain_Finland
Afuner_22	1	KC495107.1_AF84_Spain
Afuner_23	1	KC495106.1_AF83_Spain
Afuner_27	2	KC495102.1_AF66_Spain, KC495100.1_AF64_Spain
Afuner_28	1	KC495101.1_AF65_Spain
Afuner_30	2	KC495099.1_AF63_Spain, EU411013.1_perle3_Norway
Afuner_34	1	KC495095.1_AF53_Finland
Afuner_35	1	KC495094.1_AF50_Finland
Afuner_36	1	KC495093.1_AF49_Finland
Afuner_37	1	KC495092.1_AF39_Finland
Afuner_38	1	KC495091.1_AF34_Finland
Afuner_40	2	KC495089.1_AF32_Finland, KC495088.1_AF30_Finland
Afuner_45	1	KC495084.1_AF14_Finland
Afuner_48	1	KC495081.1_AF11_Finland
Afuner_51	1	EU411019.1_perle10_Norway
Afuner_55	1	EU411015.1_perle5_Norway
Afuner_56	1	EU411014.1_perle4_Norway

References

Allendorf, F. W., Luikart, G., Aitken, S. N. 2013. *Conservation and the genetics of populations*, 2nd ed. John Wiley & Sons, Hoboken, 1–602.

Avise, J. C., Walker, D. E. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **265** (1395), 457–463. https://doi.org/10.1098/rspb.1998.0317

Bajc, M., Čas, M., Ballian, D., Kunovac, S., Zubić, G., Grubešić, M., Zhelev, P., Paule, L., Grebenc, T., Kraigher, H. 2011. Genetic differentiation of the western capercaillie highlights the importance of South-Eastern Europe for understanding the species phylogeography. *PLoS ONE*, **6** (8), e23602. https://doi.org/10.1371/journal.pone.0023602

Brito, P. H. 2005. The influence of Pleistocene glacial refugia on tawny owl genetic diversity and phylogeography in western Europe. *Molecular Ecology*, **14** (10), 3077–3094. https://doi.org/10.1111/j.1365-294X.2005.02663.x

Brito, P. H. 2007. Contrasting patterns of mitochondrial and microsatellite genetic structure among Western European populations of tawny owls (*Strix aluco*). *Molecular Ecology*, **16** (16), 3423–3437. https://doi.org/10.1111/j.1365-294X.2007.03401.x

Broggi, J., Copete, J. L., Kvist, L., Mariné, R. 2013. Is there genetic differentiation in the pyrenean population of tengmalm's owl *Aegolius funereus*? *Ardeola*, **60** (1), 123–132. https://doi.org/10.13157/arla.60.1.2012.123

Corrales, C., Pavlovska, M., Höglund, J. 2014. Phylogeography and subspecies status of Black Grouse. *Journal of Ornithology*, **155** (1), 13–25. https://doi.org/10.1007/s10336-013-0982-z

Duriez, O., Sachet, J.-M., Ménoni, E., Pidancier, N., Miquel, C., Taberlet, P. 2007. Phylogeography of the capercaillie in Eurasia: what is the conservation status in the Pyrenees and Cantabrian Mounts? *Conservation Genetics*, **8** (3), 513–526. https://doi.org/10.1007/s10592-006-9165-2

Excoffier, L., Laval, G., Schneider, S. 2007. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Hayward, G. D., Hayward, P. H. 2020. Boreal Owl (*Aegolius funereus*). *In*: *Birds of the World*. https://doi.org/10.2173/bow.borowl.01

Hewitt, G. M. 2001. Speciation, hybrid zones and phylogeography - or seeing genes in space and time. *Molecular Ecology*, **10** (3), 537–549. https://doi.org/10.1046/j.1365-294x.2001.01202.x

Höglund, J., Wang, B., Axelsson, T., Quintela, M. 2013. Phylogeography of willow grouse (*Lagopus lagopus*) in the Arctic: taxonomic discordance as inferred from molecular data. *Biological Journal of the Linnean Society*, **110** (1), 77–90. https://doi.org/10.1111/bij.12109

Klinga, P., Mikoláš, M., Zhelev, P., Höglund, J., Paule, L. 2015. Genetic differentiation of western capercaillie in the Carpathian Mountains: the importance of post glacial expansions and habitat connectivity. *Biological Journal of the Linnean Society*, **116** (4), 873–889. https://doi.org/10.1111/bij.12643

Koopman, M. E., McDonald, D. B., Hayward, G. D., Eldegard, K., Sonerud, G. A., Sermach, S. G. 2005. Genetic similarity among Eurasian subspecies of boreal owls *Aegolius funereus*. *Journal of Avian Biology*, **36** (3), 179–183. https://doi.org/10.1111/j.0908-8857.2005.03509.x

Kunz, F., Gamauf, A., Zachos, F. E., Haring, E. 2019. Mitochondrial phylogenetics of the goshawk *Accipiter* [*gentilis*] superspecies. *Journal of Zoological Systematics and Evolutionary Research*, **57** (4), 942–958. https://doi.org/10.1111/jzs.12285

Li, X., Dong, F., Lei, F., Alström, P., Zhang, R., Ödeen, A., Fjeldså, J., Ericson, P. G. P., Zou, F., Yang, X. 2016. Shaped by uneven Pleistocene climate: mitochondrial phylogeographic pattern and population history of white wagtail *Motacilla alba* (Aves: Passeriformes). *Journal of Avian Biology*, **47** (2), 263–274. https://doi.org/10.1111/jav.00826

Maltagliati, F., Di Giuseppe, G., Barbieri, M., Castelli, A., Dini, F. 2010. Phylogeography and genetic structure of the edible sea urchin *Paracentrotus lividus* (Echinodermata: Echinoidea) inferred from the mitochondrial cytochrome b gene. *Biological Journal of the Linnean Society*, **100** (4), 910–923. https://doi.org/10.1111/j.1095-8312.2010.01482.x

Marthinsen, G., Wennerberg, L., Solheim, R., Lifjeld, J. T. 2009. No phylogeographic structure in the circumpolar snowy owl (*Bubo scandiacus*). *Conservation Genetics*, **10** (4), 923–933. https://doi.org/10.1007/s10592-008-9581-6

Nebel, C., Gamauf, A., Haring, E., Segelbacher, G., Villers, A., Zachos, F. E. 2015. Mitochondrial DNA analysis reveals Holarctic homogeneity and a distinct Mediterranean lineage in the Golden eagle (*Aquila chrysaetos*). *Biological Journal of the Linnean Society*, **116** (2), 328–340. https://doi.org/10.1111/bij.12583

PopART (Population Analysis with Reticulate Trees) [WWW Document] 2020. URL http://popart.otago.ac.nz/index.shtml (accessed 9.7.20).

Ramos-Onsins, S. E., Rozas, J. 2002. Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution*, **19** (12), 2092–2100. https://doi.org/10.1093/oxfordjournals.molbev.a004034

Rodríguez-Muñoz, R., Mirol, P. M., Segelbacher, G., Fernández, A., Tregenza, T. 2007. Genetic differentiation of an endangered capercaillie (*Tetrao urogallus*) population at the Southern edge of the species range. *Conservation Genetics*, **8** (3), 659–670. https://doi.org/10.1007/s10592-006-9212-z

Rogers, A. R., Harpending, H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9** (3), 552–569. https://doi.org/10.1093/oxfordjournals.molbev.a040727

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., Sánchez-Gracia, A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution*, **34** (12), 3299–3302. https://doi.org/10.1093/molbev/msx248

Shephard, J. M., Ogden, R., Tryjanowski, P., Olsson, O., Galbusera, P. 2013. Is population structure in the European white stork determined by flyway permeability rather than translocation history? *Ecology and Evolution*, **3** (15), 4881–4895. https://doi.org/10.1002/ece3.845

Squires, J. R., Reynolds, R. T., Orta, J., Marks, J. S. 2020. Northern Goshawk (*Accipiter gentilis*). *In*: Billerman, S. M., Keeney, B. K., Rodewald, P. G., Schulenberg, T. S., eds. *Birds of the World*. Cornell Lab of Ornithology. https://doi.org/10.2173/bow.norgos.01

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, **30** (12), 2725–2729. https://doi.org/10.1093/molbev/mst197

Zink, R. M., Drovetski, S. V., Rohwer, S. 2002 a. Phylogeographic patterns in the great spotted woodpecker *Dendrocopos major* across Eurasia. *Journal of Avian Biology*, **33** (2), 175–178. https://doi.org/10.1034/j.1600-048X.2002.330208.x

Zink, R. M., Rohwer, S., Drovetski, S., Blackwell-Rago, R. C., Farrell, S. L. 2002 b. Holarctic Phylogeography and Species Limits of Three-Toed Woodpeckers. *The Condor*, **104** (1), 167–170. https://doi.org/10.1093/condor/104.1.167

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