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GENETIC DIVERSITY AND PLACE IN THE GENERAL PHYLOGEOGRAPHIC STRUCTURE OF CAPERCAILLIE, *TETRAO UROGALLUS* (GALLIFORMES, PHASIANIDAE), FROM BELARUS

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Genetic Diversity and Place in the General Phylogeographic Structure of Capercaillie, *Tetrao urogallus* (Galliformes, Phasianidae), from Belarus. Homel, K. V., Pavlushchick, T. E., Nikiforov, M. E., Kheidorova, E. E., Dmitrenok, M. G., Pakul, P. A. — We report on the assessment of the level of genetic diversity of the capercaillie in Belarus. This species of birds is a valuable biological resource, and also acts as a natural indicator for the state of large forests and the degree of their disturbance by human activities. Two subspecies of the capercaillie —*Tetrao urogallus major* (C. L. Brehm, 1831) and *Tetrao urogallus pleskei* (Stegmann, 1926) have been described for Belarus. The first domain of the mitochondrial DNA control region was used as the molecular marker for the study. An additional assessment of the level of genetic diversity of the capercaillie was carried out by calculating the effective size of its population (*Ne*) in Belarus. We found that the absence of subspecific structure is characteristic for all samples of the capercaillie from Belarus. The data on the intraspecific structure and genetic diversity of the capercaillie from Belarus allows us to considerits population in the central and eastern parts of the country as stable and sustainable. This can be explained by its inclusion in the general phylogeographic structure of birds of the boreal lineage. The low values of the effective population size for the partially isolated capercaillie population from the western part of the country indicate the need for increased attention and further monitoring of that population.
Key words: capercaillie, genetic diversity, subspecies, phylogeography, Belarus.

Introduction

The capercaillie (*Tetrao urogallus* Linnaeus, 1758) is considered an "umbrella" species in the ecosystems of boreal and mountain forests (Suter et al., 2002; Pakkala et al., 2003, *quoted* from Rutkowski et al., 2017 a). Thus, the species is an indicator of the "health" of important reservoirs of biological diversity. Also, the capercaillie is a valuable biological resource. The latter entails a number of adverse effects on the species, and the most significant one is hunting. Destruction and change of the capercaillie natural habitats is, as for many other species, the most pernicious (BirdLife International, 2016). This is especially relevant in the light of anthropogenic

transformation of territories and climatic changes (Moss, 2001; Moss et al., 2001), which can affect their habitation ranges, causing their disjunction and fragmentation. In isolated fragments of populations, the population risks related to genetic factors increase substantially, leading, among other things, to decrease in their viability.

In order to assess the level of viability and sustainability of the capercaillie populations under conditions of disturbance and extinction of their habitats, it is important to have an idea of the condition of genetic diversity within its populations. This stands as the reasoning behind the focus on the molecular methods in the effort to study of the aforementioned state of the capercaillie.

Significant results were obtained from studying the genetic structure of the capercaillie within the European part of the range (Segelbacher, Piertney, 2007). That study employing mitochondrial DNA markers has allowed its authors to show that the greatest degree of separation can be observed between haplotypes from the Pyrenees and other European haplotypes. The authors of that paper argue to consider the Pyrenean capercaillie population as a separate evolutionarily significant unit. These results were later confirmed in another work (Duriez et al., 2007).

An equally important conclusion was the indication of the absence of a subspecific genetic structure in the birds studied outside the Pyrenees. Other authors have reached a similar conclusion (Duriez et al., 2007; Bajc et al., 2011; Strzała et al., 2015; Rutkowski et al., 2017 a). The importance of local populations in maintaining overall genetic diversity is highlighted in the study of the genetic mtDNA polymorphism of birds from the south-eastern part of the European range of the species (from the Rhodope Mountains and Rila in Bulgaria, through the Dinaric Mountains to the Slovenian Alps) (Bajc et al., 2011). The confirmation of *rudolfi* subspecies presence for the Rhodope and the Rila Mountains was a tangible result of this study (Bajc et al., 2011).

The issue of the importance of connection of the capercaillie local populations has been studied in more detail using microsatellite markers in the study of the metapopulation structure of the capercaillie in the Alps (Segelbacher, Storch, 2002). Similar studies were conducted on the capercaillie in the Bavarian Alps (Segelbacher et al., 2003 a).

The consequences of population fragmentation for capercaillie population were studied using microsatellite markers in the European part of the range at various levels along the gradient of spatial structuring (Segelbacher et al., 2003 b). As expected, the genetic separation was least pronounced within the continuous range of boreal forests. Based on the obtained data on the genetic variability of microsatellite markers in the studied region, the authors concluded that anthropogenic habitat disturbance and fragmentation not only have significant genetic and evolutionary consequences for the survival of populations, but also can lead to decrease in range and extinction (Segelbacher et al., 2003 b).

The importance of the capercaillie protection was shown in a Polish a research paper (Strzała et al., 2015). A reintroduction program was developed to save the local capercaillie population in Western Carpathians. To control the success of this program, the authors studied the genetic diversity of birds, both raised in captivity and reintroduced (Strzała et al., 2015). In order to implement a rational program to preserve the genetic diversity of the capercaillie population in Poland, independent management units were identified and polymorphism of microsatellite markers was estimated. It was shown that isolation of populations and a recent decrease in numbers have led to a decrease in genetic diversity (Rutkowski et al., 2017 b).

Additional work in the same direction was carried out using the mitochondrial genetic marker (Rutkowski et al., 2017 a). Regarding independent management/conservation units for different capercaillie populations in Poland, similar results were obtained using microsatellite markers (Rutkowski et al., 2017 b).

In Belarus, no study of the capercaillie population genetic structure and diversity was carried out before. There is only fragmentary information on this issue. This concerns the work done on the issue of the phylogeography of a species within its range (Bajc et al., 2011), as well as research on the reintroduction of a species to Poland (Strzała et al., 2015). In both studies only samples used were from the south of Belarus, the Polesye region. There was no information about capercaillie genetic diversity for other regions of Belarus.

Within the territory of Belarus, two subspecies of the capercaillie *T. urogallus* have been recorded: *Tetrao urogallus major* C. L. Brehm, 1831 (western regions) and *Tetrao urogallus pleskei* Stegmann, 1926 (northern, central and eastern regions) (Danilov, 1965; Domaniewski, Rydzewski, 1937; Potapov, 1985; Fedushin, 1928). These subspecies differ in feather coloration, the type of song and some morphological parameters. The populations of both subspecies have been declining since the early 1960s due to fragmentation of pine forests caused by large-scale clearcutting, transformation of ground vegetation and uncontrolled poaching. Another reason of the capercaillie population decline was the transformation of pine forests vegetation in raised bogs due to drainage of mires and surrounding forest areas. Subspecies *T. u. major*, distinguished by a darker color of the plumage, a "warm" hue of a chestnut brown mantle, the absence of a streaky pattern on the feathers of the thoracic plastron, a slight development of white spots on the abdominal side of the body and the presence of an additional element in the song — a cork-pop note — inhabits the western part of Belarus to Volozhin, Slonim and Luninets (Domaniewski, Rydzewski, 1937; Tukallo, 1927 a, b). Further to the east, the subspecies *pleskei* (Fedyushin, 1928) was distributed with a lighter plumage color and oriental type of song without a cork-pop note.

T. u. major is characterized by large body mass and larger size than *T. u. pleskei*. The diagnostically significant features for differentiation of these two subspecies by morphological indicators were measurements of the length of the beak (length from the tip of the beak to the nostrils and to the border of the rhamphotheca). The

ratio of these two measurements for *T. u. major* was 0.64 ± 0.02, and for *T. u. pleskei* — 0.85 ± 0.02 (the differences were statistically significant, td = 5.22, p > 0.99) (Pavlushchick, Cherkas, 1999).

It should be noted that while a positive trend of population dynamics was observed over the past 20 years for *T. u. pleskei* in Belarus, on the other hand for *T. u. major* an approximately 10-fold reduction in numbers occurred during the second half of the twentieth century (Nikiforov et al., 1996; Nikiforov et al., 1997; Pavlushchick et al., 1999). Over the past 15 years, the number of *T. u. major* decreased by 66.4 % and has now reached the minimum size of a viable population of the capercaillie (500 individuals), determined by genetic and population studies (Grimm, Storch, 2000). Complete extinction within the next decade is a real risk to consider, unless timely measures to protect *T. u. major* within western part of Belarus.

Additionally, it is should be noted that the fact that there are two subspecies of the capercaillie in Belarus requires more detailed research both in the light of existing works on the phylogeography of the species (Duriez et al., 2007; Bajc et al., 2011), and for the possibility of further work on identifying independent units of control over the country.

Material and methods

For the study of the intraspecific genetic structure of the capercaillie from Belarus and its position within the general phylogeographic structure of the species, 7 samples were taken. All samples, except for one of the country's west, belong to the population that is considered to belong to the eastern subspecies *T. u. pleskei* (fig. 1).

For a comparative analysis, 64 sequences of the capercaillie region from different parts of the range (Balkan Peninsula, Western Europe, Central Europe, Eastern Europe, Northern Europe, Western Russia, North-Western Russia) were taken from the GenBank database, covering 10 subspecies (*rudolfi*, *major*, *pleskei*, *urogallus*, *uralensis*, *obsoletus*, *volgensis*, *karelicus*, *aquitanicus*, *cantabricus*) out of 13 known (de Juana, Kirwan, 2018) (Appendix, table 1).

For the isolation of DNA from the tissues of the capercaillie, the "Genomic DNA Purification Kit" (Fermentas) commercial kit was used.

To study the subspecies status and genetic diversity of the capercaillie on the territory of Belarus, the first domain of the mtDNA control region was used. For this, the following primers pair was chosen: PHDL (5'-AGGACTACGGCTTGAAAAGC-3') and PH-H521 (5'-TTATGTGCTTGACCGAGGAACCAG-3') (Rodrıguez-Munoz et al., 2007). The PCR protocol for amplification of the control region of the mtDNA capercaillie consisted of 3 min initial denaturation at 95 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 57 °C and 1 min at 72 °C and a final incubation of 3 min at 72 °C.

DNA sequencing was performed using the GenomeLab GeXP (Beckman Coulter) genetic analysis system. Commercial reagents (Dye Terminator Cycle Sequencing with Quick Start Kit) and original protocol were used for this.

The alignment of the sequences of the mtDNA control region of the capercaillie, as well as the construction of phylogenetic trees was carried out in the MEGA 6 (Tamura, 2013). The haplotype networks were built using the POPART (PopArt, 2018) using the Median Joining Network algorithm. Calculation of indicators of genetic diversity: nucleotide diversity (π, Nei 1987), number of haplotypes (h), average number of nucleotide differences (k), haplotypic diversity (Hd, Nei 1987), and θ_s (theta per site, Watterson 1975, Nei 1987) is a measure of θ based on polymorphic sites (S) was performed in the program DnaSP (Rozas, Rozas 1999). The

Fig. 1. Distribution of samples of the capercaillie. Black circles are samples obtained independently, black squares are mtDNA sequences (control region) downloaded from the GenBank database (see Appendix, table 1).

estimation of the differentiation between Belarusian capercaillie population and the populations of the boreal and southern groups by calculating the index of the population differentiation *Fst* (based on haplotypes) and by calculating the exact test of sample differentiation based on genotype frequencies was carried out in the Arlequin 3.5.2.2. (Excoffier et al., 2005).

The calculation of the effective population size of the capercaillie, as a measure to estimate the rate of loss of genetic variation due to genetic drift and inbreeding, was made according to the formulas given in Braude, 2010 (Harmon, Braude, 2010).

The effective population size was calculated taking into account inbreeding (*inbreeding effective size*, N_{ef}) (1) — this is the size of an ideal population that would allow the same accumulation of pedigree inbreeding as the actual population of interest; this effective population size indicates the likely loss of heterozygosity across all alleles in population of interest; calculated as a harmonic average population size over time from the founding generation to the penultimate generation:

$$N_{ef} = \frac{t}{\frac{1}{N(0)} + \frac{1}{N(1)} + \ldots + \frac{1}{N(t-1)}} \quad (1),$$

where t is the number of generations for which we have population size data, $N(0)$ is the size of the founding population, $N(1)$ is the size of the population after one generation and so on, and $N(t–1)$ is the size of the penultimate population.

Also *the variance effective population size* (N_{ev}) was calculated (2).The variance effective population size is the size of an ideal population that would accumulate the same amount of variance in allele frequencies as the population of interest; this effective population size indicates how rapidly allele frequencies are likely to change:

$$N_{ev} = \frac{t}{\frac{1}{N(1)} + \frac{1}{N(2)} + \ldots + \frac{1}{N(t)}} \quad (2),$$

where $N(1)$ is the size of the population after one generation, etc., $N(t)$ is the size of the current population for which we have data.

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Results and discussion

After alignment of all sequences of the mtDNA control region of the capercaillie with the sequences of the *Tetrao parvirostris* (von Middendorff, 1853) from GenBank (AF532464.1, AF532463.1), the region of 270 bp was obtained. The resulting region belongs to the first most variable domain of the mtDNA control region. In the analysis of genetic diversity and structure of the capercaillie from Belarus were also included 4 sequences (HQ852196.1 (Prypiat), HQ852184.1 (Prypiat), KT223510.1 (Lielcycy Forestry), KT223509.1 (Lielcycy Forestry)) from the South of the country, which were obtained in other laboratories (see references in Appendix, table 1).

Table 1. Estimation of the level of genetic diversity of the capercaillie according to the mtDNA control region

Parameter	Belarus	Boreal lineage*	Boreal lineage	Southern lineage
N	11	42	53	18
h	6	33	36	9
Hd	0.86	0.98	0.97	0.84
π	0.005	0.01	0.0095	0.007
k	1.38	2.81	2.57	1.89
theta per site (from S) (Theta-W)	0.006	0.03	0.03	0.0086

Note. N is a sample, h is the number of haplotypes, Hd is the level of haplotypic diversity, π is the nucleotide diversity (average number of differences per site between two sequences), k is the average number of nucleotide differences, theta per site (from S) (Theta-W) — assessment of nucleotide diversity based on the number of segregation sites, * — excluding Belarusian samples.

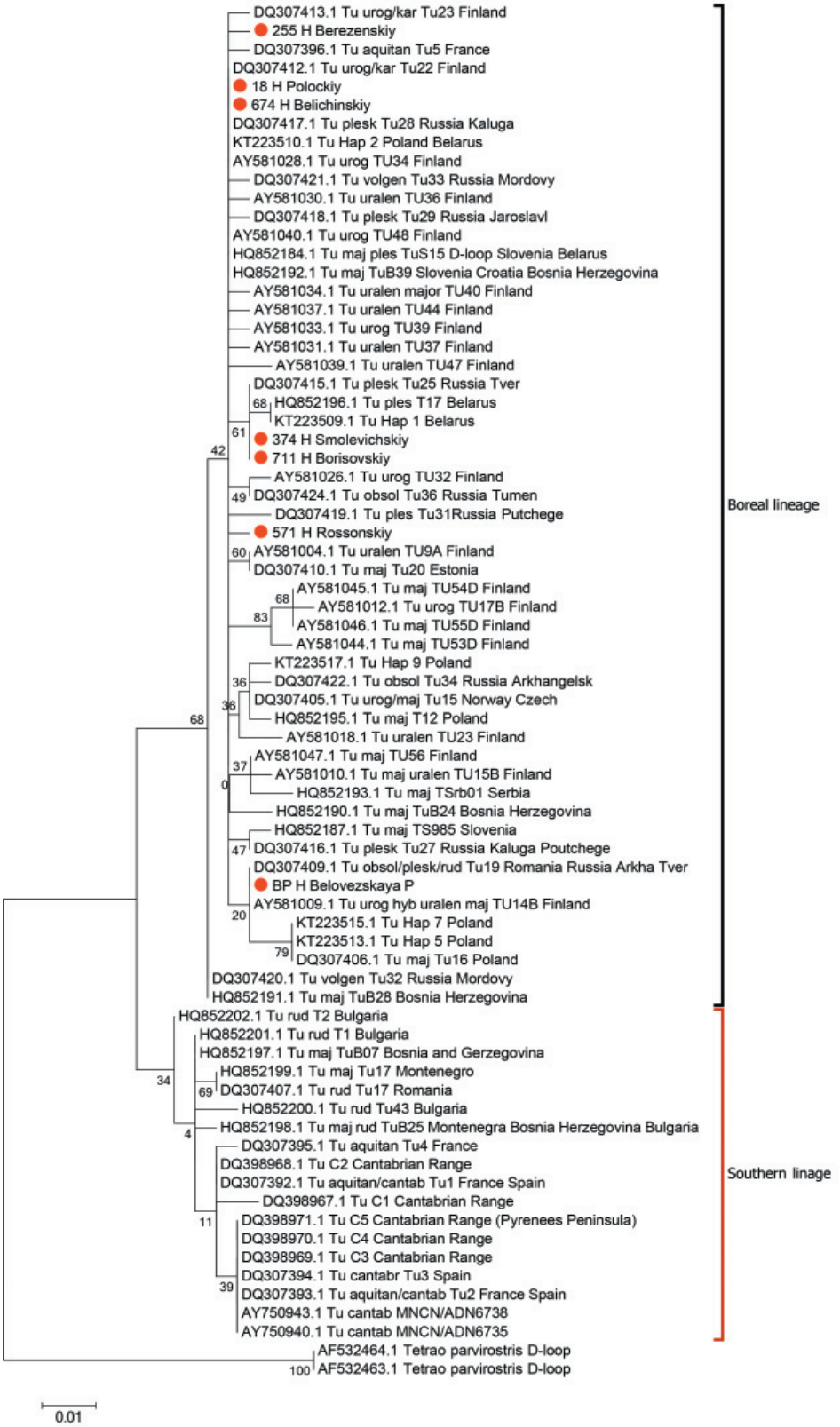

Fig. 2. Reconstruction of the phylogeny of the capercaillie according to the polymorphism of the control region of mtDNA. Red dots — sequences from Belarus (this study).

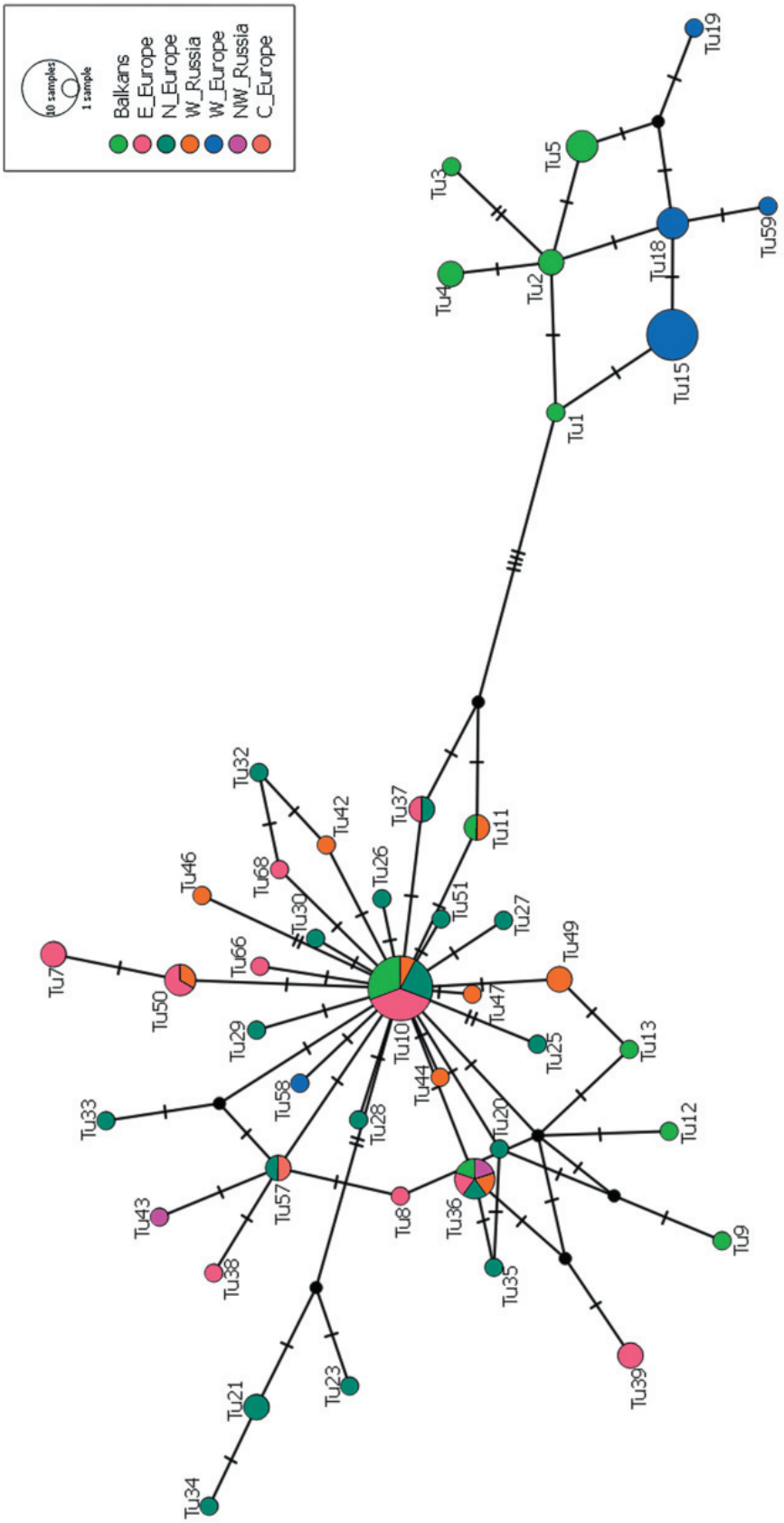

Fig. 3. Network of capercaillie haplotypes according to the mtDNA control region. Balkans — the Balkan Peninsula, E_Europe — Eastern Europe, N_Europe — Northern Europe, W_Russia — Western Russia (up to Ural Mountains), W_Europe — Western Europe, NW_Russia — Northwest Russia, C_Europe — Central Europe.

The nucleotide composition of 11 sequences from Belarus had the following ratio: T — 27.4 %, C — 30 %, A — 29.1 %, G — 13.5 %. The ratio of transitions to transversion R = 6.64. The number of monomorphic (without mutations, insertions / deletions) sites are 265. The number of polymorphic sites, respectively, are 5. Of the latter sites, 2 sites are parsimony-informative and 3 are singleton sites (fig. 2).

K2 + G (Kimura 2-parameter + Gamma distribution) was chosen as the best model for estimating the substitution model for building a phylogenetic tree. For calculating the reliability of tree nodes, the bootstrap method with 500 iterations was used.

The phylogenetic tree showed the division of the entire capercaillie sample into two main clades: IB = 68 and IB = 34. The first clade (IB = 68) included all sequences of the capercaillie, which relate mainly to the eastern and northern parts of the species range. The first clade includes all sequences from Belarus. The exception was one sequence from the western part of the range (DQ307396.1) —*T. u. aquitanicus* (Ingram, 1915) from the Pyrenees (France). The second clade includes sequences of the capercaillie from the western (the Pyrenees and the Cantabrian Mountains) and southern (Balkan Peninsula) parts of the species range.

In the studies on the phylogeography of the capercaillie (Duriez et al., 2007; Bajc et al., 2011), these two clades refer to the boreal (or line A) and southern (or line B) lineages. As in the above mentioned works, it can be noted that in the boreal lineage (first clade) no structure is observed in accordance with the subspecific division based on the morphological criterion. Whereas in the southern lineage, a separate cluster from the group of sequences belonging to the subspecies *cantabricus*/*aquitanicus* can be distinguished. The latter may indicate their division into a separate evolutionary lineage (Duriez et al., 2007; Rodrıguez-Munoz et al., 2007).

The phylogeographic structure of the capercaillie is more clearly represented using haplotypes' network (mtDNA control region; fig 3).

The haplotype network clearly distinguishes two haplogroups: the first group includes haplotypes from Eastern Europe, Northern Europe, Western Russia, Northwest Russia and Central Europe, while the second group includes haplotypes from the Balkan Peninsula and Western Europe. The distribution of sequences by haplotype is presented in Appendix, table 2.

Considering the distribution of sequences of the capercaillie from Belarus it can be noted the absence, also true for the entire boreal lineage, of subspecific structuring. Thus, the sequence from the south-west of Belarus (BP_H_Belovezskaya_P, Ivatsevichi District) is included in the Tu36 (Bel) haplotype, which includes sequences of the capercaillie from Finland (subspecies *urogallus, uralensis, major*), from Romania (subspecies *rudolfi*) and Russia (subspecies *obsoletus, pleskei*). Sequences from the south of Belarus (Prypiat: HQ852184.1, Lielcycy Forestry: KT223510.1), together with sequences from the north (Polotsk District, 18_H_Polockiy) and the east (Belynichy District, 674_H_Belinichskiy) are included in the dominant haplotype of the boreal lineage Tu10 (Bel).The latter haplotype includes sequences of the capercaillie, which were assigned to the subspecies *major*, *pleskei*, *urogallus* and *karelicus*. Two sequences from the center of Belarus (Smolevichi Region: 374_H_Smolevichskiy, Borisov District: 711_H_Borisovskiy) are included in the Tu50 (Bel) haplotype, which also includes a sequence of the capercaillie from Russia (Tver), assigned to the *pleskei* subspecies. Two sequences of the capercaillie from Belarus, drawn from GenBank, belong to the south of

Table 2. The number of the capercaillie in Belarus

Year	Number
1990	6000
1995	6710
2000	8630
2005	9667
2010	8927
2015	8800

the country (HQ852196.1 (Prypiat), KT223509.1 (Lielcycy Forestry) and form a separate haplotype Tu7 (Bel). The sequences HQ852196.1 belongs to the capercaillie, which was classified as subspecies *pleskei*. Two more sequences from the center (Berezinsky District: 255_H_Berezenskiy) and the north of Belarus (Rossony District: 571_H_Rossonskiy) are allocated in two separate haplotypes — Tu66(Bel) and Tu68 (Bel) respectively.

Despite the presence of individual sequences, grouped into clusters according to a subspecific trait, there is no general structure that would be built on the basis of subspecific status.

To assess the level of genetic diversity of the capercaillie from Belarus, the main indicators were calculated (table 1).

Comparing the level of genetic diversity of the capercaillie from Belarus with the level of genetic diversity of the capercaillie of boreal and southern lineages, it is clear that the indicators of the diversity of Belarusian birds are close to those of birds from the southern lineage. The level of genetic diversity of birds from the boreal lineage, excluding Belarusian samples, is higher than both Belarusian samples and samples from the southern lineage. Regarding Belarusian birds, this can be explained by the small sample, whereas genetic diversity of birds from southern lineage has been decreased as consequences of their isolated position.

An assessment of the difference between Belarusian birds and birds of boreal (excluding Belarusian birds) and southern lineages according to the Fst index (for the haplotype data) showed a relatively small differentiation (Fst = 0.04, $p < 0.05$) between Belarusian birds and birds of boreal lineage in comparison with the separation between the latter and the birds of the southern lineage (0.70, $p < 0.05$). In confirmation of a close connection of the Belarusian birds to the rest of the birds of the boreal lineage, an additional test was conducted to determine the exact differentiation of populations (similar to the Fisher's exact test). According to the latter test, the birds from Belarus are part of the general population of the capercaillie from the boreal lineage ($p > 0.88$) While the birds from Belarus and the rest of birds of the boreal lineage are reliably differentiated from the birds of the southern lineage ($p < 0.05$).

Based on the data obtained on the structure and genetic diversity of the Belarusian capercaillie, it can be concluded that its population is stable due to its inclusion in the population of the boreal lineage (there are no obvious differentiation between them).The latter implies the existence of a gene flow and the maintenance of total genetic diversity, which, despite a small sample, is characterized by a moderate level corresponding to similar populations from this region (Bajc et al., 2011; Duriez et al., 2007; Klinga et al., 2015; Rutkowski et al., 2017 a). Evidence of a favorable level of the genetic diversity is given by data on the effective population size (N_e) of the capercaillie in Belarus on the basis of long-term information on its population (table 2).

Taking into account data on the number of the capercaillie for the 25 year period (from 1990 to 2015; 5 generations), two indicators of the effective population size for this species were calculated. The value of the variance effective population size (N_{ev}) was 8417 individuals, and the value of the effective population size, taking into account inbreeding, was 7727 individuals.

Both indices obtained have high values and show a very low level of inbreeding and gene drift in the populations of the capercaillie in the country. According to Lande (1995), populations will be at high risk of genetic drift if their N_{ev} is less than 5000 individuals (Harmon, Braude, 2010). In other words, the genetic diversity in the population of the capercaillie has high values and, consequently, the population has a good adaptive capacity.

The main factors affecting the number of the capercaillie include the reduction of suitable habitat biotopes due to the cutting of mature and maturing pine forests (Dolbik, 1980; Dolbik, 1974; Ivanyutenko, Semashko, 1989), the growing recreational load and the press of predatory mammals. However, based on the data of the total population abundance, the

Table 3. Spring numbers (individuals) of *T. u. pleskei* and *T. u. major* in Belarus

Year	*T. u. pleskei*	*T. u. major*
1990	5000	1000
1995	5720	990
2000	7440	1190
2005	8728	939
2010	8206	721
2015	8305	495

values of the effective population size and the data on the genetic structure and diversity we can conclude that the current capercaillie population does not experience a critical negative impact from these adverse changes in its main biotopes.

A slightly different picture is observed for local populations of the capercaillie living in the west of the country and belonging to the subspecies *T. u. major*. As it was shown above, the sequence of the capercaillie from the south-west of Belarus (BP_H_Belovezskaya_P, Ivatsevichi District) is not included in a separate cluster with other sequences that were assigned to the *major* subspecies, but on the contrary included in the haplotype, combining several different subspecies from different countries. The latter may indicate the presence of connection of the capercaillie from the south-west of Belarus with populations from other countries. On the other hand, this sequence, together with the sequences from the center (Berezinsky District: 255_H_Berezenskiy) and the north of Belarus (Rossonsky District: 571_H_Rossonskiy) are not grouped into haplotypes with other Belarusian of the capercaillie sequences studied in this paper. This can be characterized as an indicator of the haplotypic diversity of the species in the country, as the presence of isolated subpopulations of the species or as an insufficiently large size of the studied region of the mtDNA control region.

In contrast to the rest of the population, for the capercaillie from the west of Belarus an unfavorable situation in population abundance is noted (Nikiforov et al., 1996; Pavluschik et al., 1999; Pavlushchick, 2018). According to the abundance data (table 3) for two subspecies of the capercaillie — *T. u. major* and *T. u. pleskei* values of effective population size were calculated.

The effective population size has the following values: *T. u. pleskei* — N_{ev} = 7507, N_{ef} = 6705; *T. u. major* N_{ev} = 930, N_{ef} = 1022. Thus, it can be seen that the overall state of the genetic diversity of the *T. u. pleskei* population in Belarus is sufficient, whereas for the population *T. u. major* we can expect a decrease in genetic diversity due to the processes of genetic drift and inbreeding. The latter is most likely associated with more pronounced changes in key biotopes in the zone of subspecies habitation — in the Grodno, Brest and partially Minsk Regions (Pavlushchick, 2018).

Conclusion

The obtained data on the intraspecific structure and genetic diversity of the Belarusian capercaillie is indicating sufficient stability and sustainability of its population in the central and eastern part of the country, which is associated with its inclusion in the general phylogeographic structure of the birds of the boreal lineage. There is no notable distinction between them. The latter involves the presence of gene flow that supports overall genetic diversity. Data on the effective population size (N_e) of the capercaillie in Belarus also confirm favorable levels of genetic diversity. However, despite the general favorable situation for the species, the partially isolated capercaillie population, belonging

to the *major* subspecies in the west of the country, requires special attention and further monitoring. This subpopulation has low values of effective population size, which in the future may lead to negative consequences in the form of loss of adaptability due to the reduction of genetic diversity.

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Author contributions

K. V. Homel: contributed to conception, design, analysis and interpretation of data. T. E. Pavlushchick, M. E. Nikiforov: contributed to conception, design; drafted and revised the article. E. E. Kheidorova: revised the article. M. G. Dmitrenok, P. A. Pakul: collection of material.

Appendix. Table 1. Capercaillie samples from the GenBank

GenBank	Haplotype	Locality	Subspecies	Reference
		Balkan Peninsula		
HQ852202.1	T2	Bulgaria	*rudolfi*	(Bajc et al., 2011)
HQ852201.1	T1	Bulgaria	*rudolfi*	(Bajc et al., 2011)
HQ852200.1	Tu43	Bulgaria	*rudolfi*	(Bajc et al., 2011)
HQ852199.1	Tu17	Montenegro	*major*	(Bajc et al., 2011)
HQ852198.1	TuB25	Montenegro, Bosnia and Herzegovina, Bulgaria	*rudolfi, major*	(Bajc et al., 2011)
HQ852197.1	TuB07	Bosnia and Herzegovina	*major*	(Bajc et al., 2011)
HQ852193.1	TSrb01	Serbia	*major*	(Bajc et al., 2011)
HQ852192.1	TuB39	Slovenia, Croatia, Bosnia and Herzegovina	*major*	(Bajc et al., 2011)
HQ852191.1	TuB28	Bosnia and Herzegovina	*major*	(Bajc et al., 2011)
HQ852190.1	TuB24	Bosnia and Herzegovina	*major*	(Bajc et al., 2011)
HQ852187.1	TS985	Slovenia	*major*	(Bajc et al., 2011)
DQ307407.1	Tu17	Romania	*rudolfi*	(Duriez et al., 2007)
		Western Europe		
DQ398971.1	C5	Cantabrian Mountains	–	(Rodrıguez-Munoz et al., 2007)
DQ398970.1	C4	Cantabrian Mountains	–	(Rodrıguez-Munoz et al., 2007)
DQ398969.1	C3	Cantabrian Mountains	–	(Rodrıguez-Munoz et al., 2007)
DQ398968.1	C2	Cantabrian Mountains	–	(Rodrıguez-Munoz et al., 2007)
DQ398967.1	C1	Cantabrian Mountains	--	(Rodrıguez-Munoz et al., 2007)
DQ307396.1	Tu5	France (Pyrenees)	*aquitanicus*	(Duriez et al., 2007)
DQ307395.1	Tu4	France (Pyrenees)	*aquitanicus*	(Duriez et al., 2007)
DQ307394.1	Tu3	Spain (Cantabrian Mountains)	*cantabricus*	(Duriez et al., 2007)
DQ307393.1	Tu2	France (Pyrenees)/Spain (Cantabrian Mountains)	*aquitanicus, cantabricus*	(Duriez et al., 2007)
DQ307392.1	Tu1	France (Pyrenees)/Spain (Cantabrian Mountains)	*aquitanicus, cantabricus*	(Duriez et al., 2007)
AY750943.1	–	Spain	*cantabricus*	–
AY750940.1	–	Spain	*cantabricus*	–
		Northern Europe		
AY581047.1	TU56	Finland	*major*	(Liukkonen-Anttila et al., 2004)
AY581046.1	TU55D	Finland	*major*	(Liukkonen-Anttila et al., 2004)
AY581045.1	TU54D	Finland	*major*	(Liukkonen-Anttila et al., 2004)
AY581044.1	TU53D	Finland	*major*	(Liukkonen-Anttila et al., 2004)
AY581040.1	TU48	Finland	*urogallus*	(Liukkonen-Anttila et al., 2004)
AY581039.1	TU47	Finland	*uralensis*	(Liukkonen-Anttila et al., 2004)

AY581037.1	TU44	Finland	*uralensis*	(Liukkonen-Anttila et al., 2004)
AY581034.1	TU40	Finland	*uralensis, major*	(Liukkonen-Anttila et al., 2004)
AY581033.1	TU39	Finland	*urogallus*	(Liukkonen-Anttila et al., 2004)
AY581031.1	TU37	Finland	*uralensis*	(Liukkonen-Anttila et al., 2004)
AY581030.1	TU36	Finland	*uralensis*	(Liukkonen-Anttila et al., 2004)
AY581028.1	TU34	Finland	*urogallus*	(Liukkonen-Anttila et al., 2004)
AY581026.1	TU32	Finland	*urogallus*	(Liukkonen-Anttila et al., 2004)
AY581018.1	TU23	Finland	*uralensis*	(Liukkonen-Anttila et al., 2004)
AY581012.1	TU17B	Finland	*urogallus*	(Liukkonen-Anttila et al., 2004)
AY581010.1	TU15B	Finland	*uralensis, major*	(Liukkonen-Anttila et al., 2004)
AY581009.1	TU14B	Finland	*urogallus, uralensis, major, hybrid*	(Liukkonen-Anttila et al., 2004)
AY581004.1	TU9A	Finland	*uralensis*	(Liukkonen-Anttila et al., 2004)
DQ307413.1	Tu23	Finland	*urogallus, karelicus*	(Duriez et al., 2007)
DQ307412.1	Tu22	Finland	*urogallus, karelicus*	(Duriez et al., 2007)
		Eastern Europe		
HQ852196.1	T17	Belarus (Prypiat)	*pleskei*	(Bajc et al., 2011)
HQ852195.1	T12	Poland	*major*	(Bajc et al., 2011)
KT223517.1	Hap_9	Poland	–	(Strzała et al., 2015)
KT223515.1	Hap_7	Poland	–	(Strzała et al., 2015)
KT223510.1	Hap_2	Poland/Belarus (Lielcycy Forestry)	–	(Strzała et al., 2015)
KT223509.1	Hap_1	Belarus (Lielcycy Forestry)	–	(Strzała et al., 2015)
DQ307410.1	Tu20	Estonia	*major*	(Duriez et al., 2007)
DQ307406.1	Tu16	Poland	*major*	(Duriez et al., 2007)
		Western Russia		
DQ307424.1	Tu36	Tumen	*obsoletus*	(Duriez et al., 2007)
DQ307421.1	Tu33	Mordovy Republic	*volgensis*	(Duriez et al., 2007)
DQ307420.1	Tu32	Mordovy Republic	*volgensis*	(Duriez et al., 2007)
DQ307419.1	Tu31	Ivanovo-Poutchege	*pleskei*	(Duriez et al., 2007)
DQ307418.1	Tu29	Jaroslav'l	*pleskei*	(Duriez et al., 2007)
DQ307417.1	Tu28	Kaluga	*pleskei*	(Duriez et al., 2007)
DQ307416.1	Tu27	Kaluga/Ivanovo-Poutchege	*pleskei*	(Duriez et al., 2007)
DQ307415.1	Tu25	Tver	*pleskei*	(Duriez et al., 2007)
		Northwest Russia		
DQ307422.1	Tu34	Arkhangelsk	*obsoletus*	(Duriez et al., 2007)
		Balkan Peninsula/Eastern Europe		
HQ852184.1	TuS15	Slovenia, Belarus (Prypiat)	*major, pleskei*	(Bajc et al., 2011)
		Balkan Peninsula/Western, Northwest Russia		
DQ307409.1	Tu19	Romania, Russia (Tver, Arkhangelsk)	*obsoletus, pleskei, rudolfi*	(Duriez et al., 2007)
		Central, Northern Europe		
DQ307405.1	Tu15	Norway, Czech Republic	*urogallus, major*	(Duriez et al., 2007)

Appendix. Table 2. Distribution of the studied sequences of the capercaillie mtDNA control region

The sequence	Haplotype
HQ852202.1_Tu_rud_T2_Bulgaria	Tu1(Bel)
HQ852201.1_Tu_rud_T1_Bulgaria	Tu2(Bel)
HQ852197.1_Tu_maj_TuB07_Bosnia_and_Gerzegovina	
HQ852200.1_Tu_rud_Tu43_Bulgaria	Tu3(Bel)
HQ852199.1_Tu_maj_Tu17_Montenegro	Tu4(Bel)
DQ307407.1_Tu_rud_Tu17_Romania	
HQ852198.1_Tu_maj_rud_TuB25_Montenegra_Bosnia_Herzegovina_Bulgaria	Tu5(Bel)
HQ852196.1_Tu_ples_T17_Belarus	Tu7(Bel)
KT223509.1_Tu_Hap_1_Belarus	
HQ852195.1_Tu_maj_T12_Poland	Tu8(Bel)
HQ852193.1_Tu_maj_TSrb01_Serbia	Tu9(Bel)
HQ852192.1_Tu_maj_TuB39_Slovenia_Croatia_Bosnia_Herzegovina	Tu10(Bel)
HQ852184.1_Tu_maj_ples_TuS15_D-loop_Slovenia_Belarus	
AY581040.1_Tu_urog_TU48_Finland	
AY581028.1_Tu_urog_TU34_Finland	
KT223510.1_Tu_Hap_2_Poland_Belarus	
DQ307417.1_Tu_plesk_Tu28_Russia_Kaluga	
DQ307412.1_Tu_urog/kar_Tu22_Finland	
MK482512.118_H_Polockiy	
674_H_Belinichskiy	
HQ852191.1_Tu_maj_TuB28_Bosnia_Herzegovina	Tu11(Bel)
DQ307420.1_Tu_volgen_Tu32_Russia_Mordovy	
HQ852190.1_Tu_maj_TuB24_Bosnia_Herzegovina	Tu12(Bel)
HQ852187.1_Tu_maj_TS985_Slovenia	Tu13(Bel)
DQ398971.1_Tu_C5_Cantabrian_Range_(Pyrenees_Peninsula)	Tu15(Bel)
DQ398970.1_Tu_C4_Cantabrian_Range	
DQ398969.1_Tu_C3_Cantabrian_Range	
DQ307394.1_Tu_cantabr_Tu3_Spain	
DQ307393.1_Tu_aquitan/cantab_Tu2_France_Spain	
AY750943.1_Tu_cantab_MNCN/AND6738	
AY750940.1_Tu_cantab_MNCN/AND6735	
DQ398968.1_Tu_C2_Cantabrian_Range	Tu18(Bel)
DQ307392.1_Tu_aquitan/cantab_Tu1_France_Spain	
DQ398967.1_Tu_C1_Cantabrian_Range	Tu19(Bel)
AY581047.1_Tu_maj_TU56_Finland	Tu20(Bel)
AY581046.1_Tu_maj_TU55D_Finland	Tu21(Bel)
AY581045.1_Tu_maj_TU54D_Finland	
AY581044.1_Tu_maj_TU53D_Finland	Tu23(Bel)
AY581039.1_Tu_uralen_TU47_Finland	Tu25(Bel)
AY581037.1_Tu_uralen_TU44_Finland	Tu26(Bel)
AY581034.1_Tu_uralen_major_TU40_Finland	Tu27(Bel)
AY581033.1_Tu_urog_TU39_Finland	Tu28(Bel)
AY581031.1_Tu_uralen_TU37_Finland	Tu29(Bel)
AY581030.1_Tu_uralen_TU36_Finland	Tu30(Bel)
AY581026.1_Tu_urog_TU32_Finland	Tu32(Bel)
AY581018.1_Tu_uralen_TU23_Finland	Tu33(Bel)
AY581012.1_Tu_urog_TU17B_Finland	Tu34(Bel)
AY581010.1_Tu_maj_uralen_TU15B_Finland	Tu35(Bel)

AY581009.1_Tu_urog_hyb_uralen_maj_TU14B_Finland	Tu36(Bel)
DQ307409.1_Tu_obsol/plesk/rud_Tu19_Romania_Russia_Arkha_Tver	
MK482513.1BP_H_Belovezskaya_P	
AY581004.1_Tu_uralen_TU9A_Finland	Tu37(Bel)
DQ307410.1_Tu_maj_Tu20_Estonia	
KT223517.1_Tu_Hap_9_Poland	Tu38(Bel)
KT223515.1_Tu_Hap_7_Poland	Tu39(Bel)
DQ307406.1_Tu_maj_Tu16_Poland	
DQ307424.1_Tu_obsol_Tu36_Russia_Tumen	Tu42(Bel)
DQ307422.1_Tu_obsol_Tu34_Russia_Arkhangelsk	Tu43(Bel)
DQ307421.1_Tu_volgen_Tu33_Russia_Mordovy	Tu44(Bel)
DQ307419.1_Tu_ples_Tu31Russia_Putchege	Tu46(Bel)
DQ307418.1_Tu_plesk_Tu29_Russia_Jaroslavl	Tu47(Bel)
DQ307416.1_Tu_plesk_Tu27_Russia_Kaluga_Poutchege	Tu49(Bel)
DQ307415.1_Tu_plesk_Tu25_Russia_Tver	Tu50(Bel)
374_H_Smolevichskiy	
MK482514.1711_H_Borisovskiy	
DQ307413.1_Tu_urog/kar_Tu23_Finland	Tu51(Bel)
DQ307405.1_Tu_urog/maj_Tu15_Norway_Czech	Tu57(Bel)
DQ307396.1_Tu_aquitan_Tu5_France	Tu58(Bel)
DQ307395.1_Tu_aquitan_Tu4_France	Tu59(Bel)
MK482515.1255_H_Berezenskiy	Tu66(Bel)
MK482516.1571_H_Rossonskiy	Tu68(Bel)

Note. **Bold —** sequences received by the authors of this work, *italics*— the capercaillie sequences from Belarus taken from GenBank.

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