



Genetic Diversity and Differentiation in the Forest Dormouse *Dryomys nitedula* (Pallas, 1778) (Rodentia: Gliridae) Population in Latvia

Dainis Ruņģis^{1,*}, *Inese Gavarāne*², *Linda Bankovska*², *Valdis Pilāts*³, *Zanda Segliņa*³ & *Digna Pilāte*¹

¹Latvian State Forest Research Institute “Silava”, 111 Rīgas Street, LV2169 Salaspils, Latvia

²Institute of Life sciences and Technology, Daugavpils University, 1A Parādes Street, LV5401 Daugavpils, Latvia

³The Nature Conservation Agency, 7 Baznīcas Street, LV2150 Sigulda, Latvia

Abstract: The distribution range of the forest dormouse *Dryomys nitedula* (Pallas, 1778) extends from central Europe to central Asia; many isolated subpopulations occur on the edge of its range. Only one isolated forest dormouse population is found in Latvia; it is located in the south-eastern region, bordering Belarus. The forest dormouse is a protected species in the European Union. In Latvia, two sets of monitoring data are collected: (1) range and distribution and (2) population trends. This study extends the monitoring program with an initial assessment of the genetic diversity and structure of the forest dormouse population in Latvia. Its aim is to provide an understanding of population structure and patterns of genetic divergence, which can be further used for the development of species protection and management plans and strategies. One hundred and twenty individual forest dormice were genotyped and no significant genetic differentiation between age and sex groups was found. Bayesian clustering identified three clusters within the Latvian forest dormouse population. The history of this population is unknown but the clustering might be explained by two parameters, i.e. limited mobility of forest dormice and habitat suitability.

Key words: genetic diversity, population structure, RAPD markers, population fragmentation

Introduction

The distribution of the forest dormouse *Dryomys nitedula* (Pallas, 1778) covers a relatively large area: from Switzerland, through eastern and southern Europe, Asia Minor and the Caucasus, to central Russia and central Asia, reaching as far as 90°E. Many isolated subpopulations occur on the edge of its range, including in Latvia, Lithuania and Belarus (BATSAIKHAN et al. 2016). Only one forest dormouse population is found in Latvia, located in the south-eastern region, bordering Belarus (PILĀTS et al. 2012). This population is isolated, and the closest known forest dormouse populations are in Belarus about 110 km

away (<https://www.gbif.org>; D. SHAMOVICH, personal communication, January 5, 2023) and in Lithuania (JUŠKAITIS 2015) about 170 km away.

Genetic diversity within a species is a fundamental aspect of biodiversity and a decrease of genetic variation within populations reduces population viability and sustainability and increases the risk of population extirpation. A key factor affecting genetic diversity levels is population size (MONTGOMERY et al. 2000). Additionally, populations with low genetic diversity have lower levels of resistance to parasites, diseases and fungal pathogens (KING & LIVELY 2012). Genetic diversity plays a crucial role in the fitness, adaptability, survival and plasticity of

*Corresponding author: dainis.rungis@silava.lv

populations in changing environmental conditions (PLUESS & STÖCKLIN 2004). Isolated populations have limited gene flow and have an increased possibility of experiencing genetic drift (CABALLERO 1994). Moreover, in small and closed populations there is a more rapid loss of heterozygosity than in large populations. Therefore, they are particularly vulnerable to decrease of population viability and sustainability (FRANKHAM 2005). Extinction of a small local population of the forest dormouse is documented in Lithuania (JUŠKAITIS 2021). Unfortunately, there is no data available on the genetic diversity of the closest populations to the Latvian forest dormouse population.

Several studies have reported habitat fragmentation and population isolation in various dormouse species (e.g., GRIGORYEVA et al. 2015, HARDAGEN et al. 2016, MOSKA et al. 2016). Limited data are available concerning genetic diversity within the forest dormouse populations. Additionally, understanding of the patterns of population structure and patterns of genetic divergence is important to establish effective conservation programmes. The present study is an initial assessment of the genetic diversity and structure of the forest dormouse population in Latvia using RAPD markers.

The forest dormouse is a protected species listed in *Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora*, Appendix 4. The results obtained in this study provide information for the development of species protection and management strategies and plans.

Materials and Methods

Study area

The study area covers all of the approximately 12 km² of forest inhabited by the forest dormouse in Latvia (Fig. 1). In this area, the species lives in boreo-nemoral forests dominated by premature and mature Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*) and birch (*Betula pendula*) growing on dry, sandy soils. Hazel (*Corylus avellana*) at various densities is an important forest element in the shrub layer (PILĀTS et al. 2012).

Collection of samples

Samples from 120 forest dormouse individuals were collected. The animals were caught in nest boxes set-up for dormouse monitoring. For dormouse monitoring in Latvia two sets of data are collected: (1) range and distribution and (2) population trends. Small temporary study plots, with 5–15 nest boxes in

each, were established in suitable habitats to detect species occurrence. In addition, 50 nest boxes were arranged in a grid at 50 m distance from each other in permanent study sites (Fig. 1). Samples from dormice were collected at both types of monitoring site. Sampling was done in 2015–2017 from May until September (the active season for dormice). Three tissue samples (hair, the tip of the ear and saliva by buccal swabs) were collected and stored at -20 °C until further analysis. Information on each animal's sex, age class (0 – juvenile, 1 – this year's animal, 2 – adult) was also collected as well the nest box location where the individual was caught. After completion of sampling, the animals were immediately returned to their nest boxes.

DNA extraction and RAPD-PCR analysis

Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany) with incubation time set at 10 min. Genotyping was done with 32 RAPD primers (Table 1). Thermal cycler Verity 96-Well Thermal Cycler (Thermo Scientific, Finland) was used for PCR amplification with a total volume of 15 µL containing 5 µL DNA template, 0.1 µL primer (0.6 µM), 7.5 µL Taq DNA Polymerase (Qiagen, Germany), 2.25 µL Milli-Q water and 0.15 µL DMSO. PCR profile comprised of an initial denaturation for 1 min at 95 °C, followed by 45 cycles of denaturation for 1 min at 94 °C, annealing for 2 min at 36 °C, and extension for 2 min at 72 °C with final extension for 15 min at 72 °C and then stored at 4 °C. RAPD-PCR fragments were visualized using QIAxcel electrophoresis system and ScreenGel Software (Qiagen, Germany). Fragment amplification was recognized as valid and reliable if there were no PCR amplification fragments in negative controls, all fragments in samples were longer than 50 bp and fragments were within the range of the size standard markers. Fragment sizes were determined by comparing them with the DNA size marker. RAPD fragments were scored as present/absent in a binary matrix. Polymorphism Information Content (PIC) values were calculated using the formula $PIC = 1 - (p_i^2 + (1 - p_i)^2)$, where p is the frequency of the i th RAPD locus. GenAIEX 6.501 (PEAKALL & SMOUSE 2012) was used to calculate pairwise Nei genetic distances, as well as to perform analysis of molecular variance (AMOVA) and principal coordinates analysis (PCoA). Pairwise population differentiation (Phipt – an analogue of Fst) was also calculated (using 999 permutations). STRUCTURE 2.3.4 was used to determine clustering of the populations using a 150000 burnin period, followed by 300000 MCMC steps, K was determined for 1 to 10

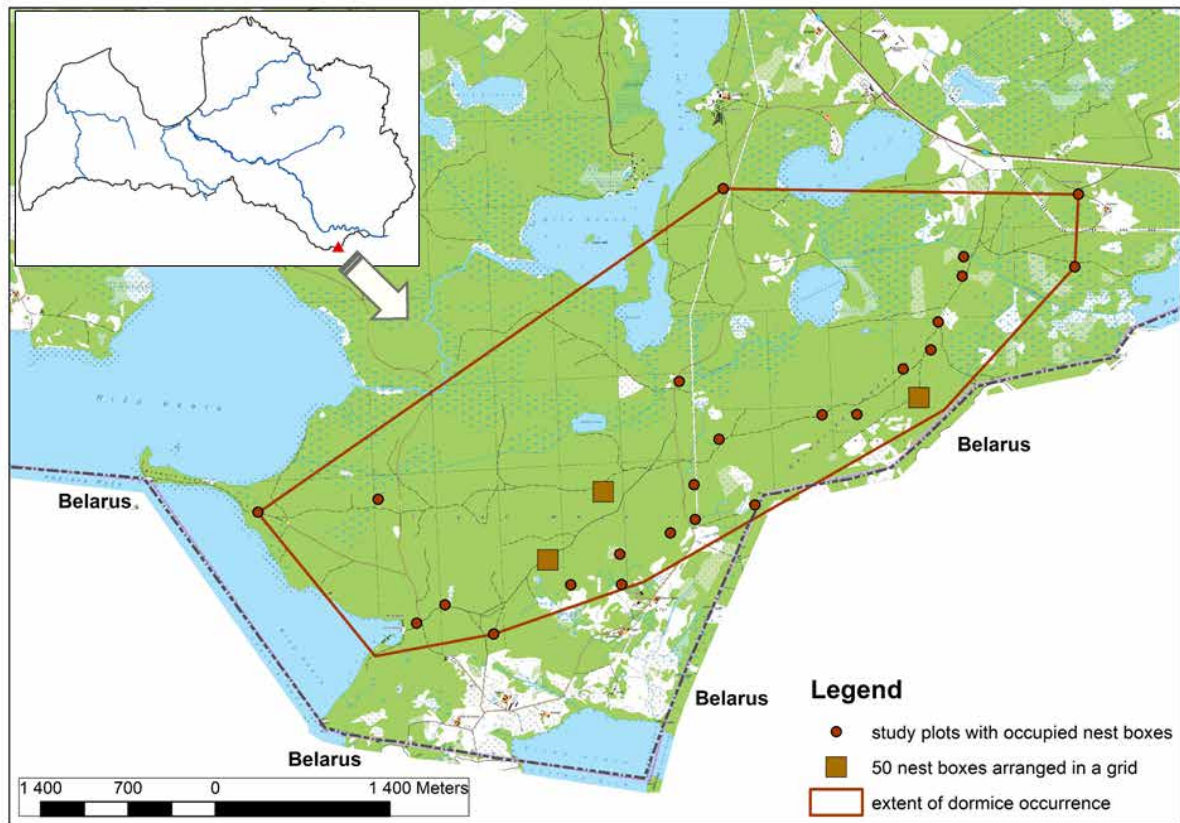


Fig. 1. Location of nest box sites: temporary plots (with small groups of nest boxes) and permanent study sites (grids) in the area inhabited by forest dormice in Latvia. These were used for monitoring dormice and for sampling materials needed for research. The extent of dormice occurrence (area with occupied nest boxes during 2015-2018) was recorded according to IUCN guidelines (<http://www.iucnredlist.org/>).

Table 1. RAPD primers assessed for genotyping of the forest dormouse population in Latvia

Primer ID	Sequence	Primer ID	Sequence
OPA-01	CAGGCCCTC*	OPA-11	CAATCGCCGT#
OPA-02	TGCCGAGCTG*	OPA-12	TCGGCGATAG#
OPA-03	AGTCAGCCAC*	OPA-15	TTCCGAACCC#
OPA-04	AATCGGGCTG*	OPA-16	AGCCAGCGAA#
OPA-08	GTGACGTAGG*	OPM-2	ACAACGCCTC#
OPB-01	GTTTCGCTCC*	OPM-4	GGCGTTGTC#
OPB-02	TGATCCCTGG*	OPM-5	GGGAACGTGT#
OPB-03	CATCCCCTG*	OPM-6	CTGGGCAACT#
OPB-04	GGA CTGGAGT*	OPM-12	GGGACGTTGG#
OPB-06	TGCTCTGCCC*	OPP-5	CCCCGGTAAC#
OPD-02	GGACCCAACC*	OPP-6	GTGGCTGAC#
OPD-04	TCTGGTGAGG*	OPP-9	GTGGTCCGCA#
OPA-6	GGTCCCTGAC#	OPP-11	AACGCGTCGG#
OPA-7	GAAACGGGTG#	OPP-12	AAGGGCGAGT#
OPA-9	GGGTAACGCC#	OPP-13	GGAGTGCCTC#
OPA-10	GTGATCGCAG#	OPP-16	CCAAGTGCC#

* – SELÇUK et al. (2012), # – MISHARA et al. (2002)

with 25 runs for each value of K. The LOCPRIOR model was used, using the sampling location as default information to assist clustering, which is recommended for use with datasets with weak structure signal (PRITCHARD et al. 2000). The optimal value of K was determined using the ΔK method (EVANNO et al. 2005) using STRUCTURE HARVESTER (EARL 2012) and the probability by K using median values of Ln(Pr Data) implemented via the CLUMPAK server (KOPELMAN et al. 2015). Coefficients of membership to the most likely K cluster number were visualized using Distruct via the CLUMPAK server.

Results

DNA was extracted from 120 forest dormouse individuals. Reliable genotype data was obtained with 16 of the 32 tested RAPD markers: OPA-01, OPA-02, OPA-03, OPA-08, OPB-01, OPB-02, OPB-04, OPD-02, OPA-10, OPM-2, OPM-4, OPM-5, OPM-6, OPM-12, OPP-13, OPP-16. Totally, 65 RAPD loci were obtained from genotyping, of which 89% were polymorphic, average expected heterozygosity over all loci was 0.321, average polymorphism information content (PIC) was 0.246. There were no significant differences in average PIC values between individuals of different age classes (0, 1, 2), or male and female individuals. Similarly, there was no significant genetic differentiation between age and sex groups, overall F_{st} (a modification of F_{st}) values were 0.010 ($p=0.101$), and 0 ($p=0.872$), respectively.

Analysis of the clustering results obtained from the STRUCTURE software with the deltaK method indicated that the most likely number of clusters within the Latvian forest dormouse population was three (Fig. 2). Individuals were divided into three groups according to the most likely inferred cluster from the STRUCTURE results. The first group contained 47 individuals, the second 32 and the third 41. Assignment of individuals to a particular cluster mostly corresponded with the location where the individual was captured (Fig. 3), however, the inferred ancestry to a particular cluster was above 0.95 for only 22 of the 120 tested individuals.

Analysis of molecular variance (AMOVA) indicated that there was moderate genetic differentiation between the three clusters, with an overall F_{st} value of 0.148 ($p=0.001$). Pairwise F_{st} values between the clusters ranged from 0.118 to 0.180. However, this grouping is based on the STRUCTURE results, and therefore differentiation is higher than it would be if the groups were based on capture location or some other grouping criteria. Pairwise Nei

genetic distances were calculated between all individuals, and principal coordinates analysis indicated the differentiation of the three clusters (Fig. 4). While some individuals from a particular STRUCTURE group are clustered together (e.g. individuals from group 3), there is overlap between individuals from all of the STRUCTURE groups. This is a reflection of the previously mentioned inferred ancestry coefficients, which were above 0.95 for only 22 of the 120 analysed individuals. Average PIC values in cluster 1 were 0.211 (47 individuals), in cluster 2 (32 individuals) 0.263, and in cluster 3 (41 individuals) 0.181. The highest average PIC values were in cluster 2, which consisted of the smallest number of individuals. Average PIC values were significantly different between clusters 2 and 3 (t -value 2.563, $p=0.011$).

Discussion

This study reports an initial assessment of the genetic diversity and population differentiation of the forest dormouse population found in Latvia. This is an isolated population located on the northern edge of the distribution of this species (BATSIAKHAN et al. 2016). Most probably, it is cross-border population. Unfortunately, we do not know its geographical limits within Belarus. Nevertheless, it should be very limited, as no records are known from the Belarus side of the border. In addition, the population size is unknown. Despite the small area occupied by this population, significantly differentiated genetic clusters were identified.

A similar pattern to the differentiation into three clusters shown by principal coordinates analysis can be also seen on a map showing records of sampled dormice (Fig. 3). Although the records of dormice with different clusters overlap and dormice from different clusters were sometimes found in the same nest box, dormice from cluster 2 and especially from cluster 3 tend to dwell in different parts of the occupied area. Moreover, this is despite the fact that sampled animals are living close to each other. This might be explained by two parameters: mobility of forest dormice and habitat suitability. In general, forest dormice have small home ranges and usually move no further than 100–200 m from their nests (Ściński & BOROWSKI 2006, PILĀTS et al. 2012). During the sampling, we recorded two dormice at a distance of 2 km from the previous site of capture. Nevertheless, such long-distance movements are probably rare. Small home ranges might hinder the gene flow between individuals, even within a population occupying a small area, such as

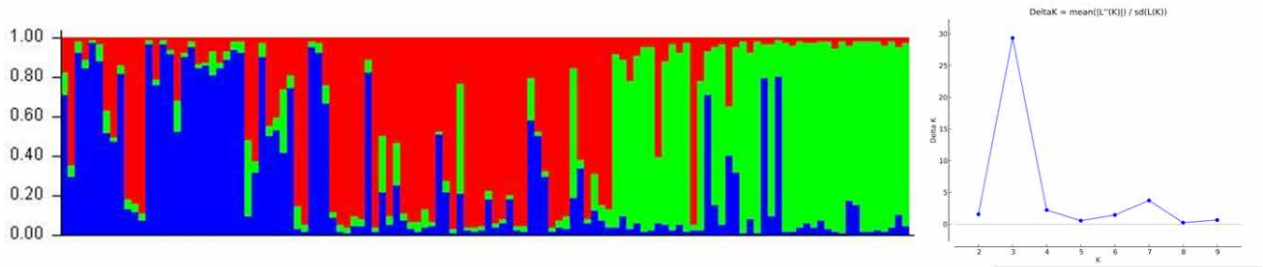


Fig. 2. Clustering results obtained from the STRUCTURE software with the deltaK method indicating that the most likely number of clusters was $K=3$.

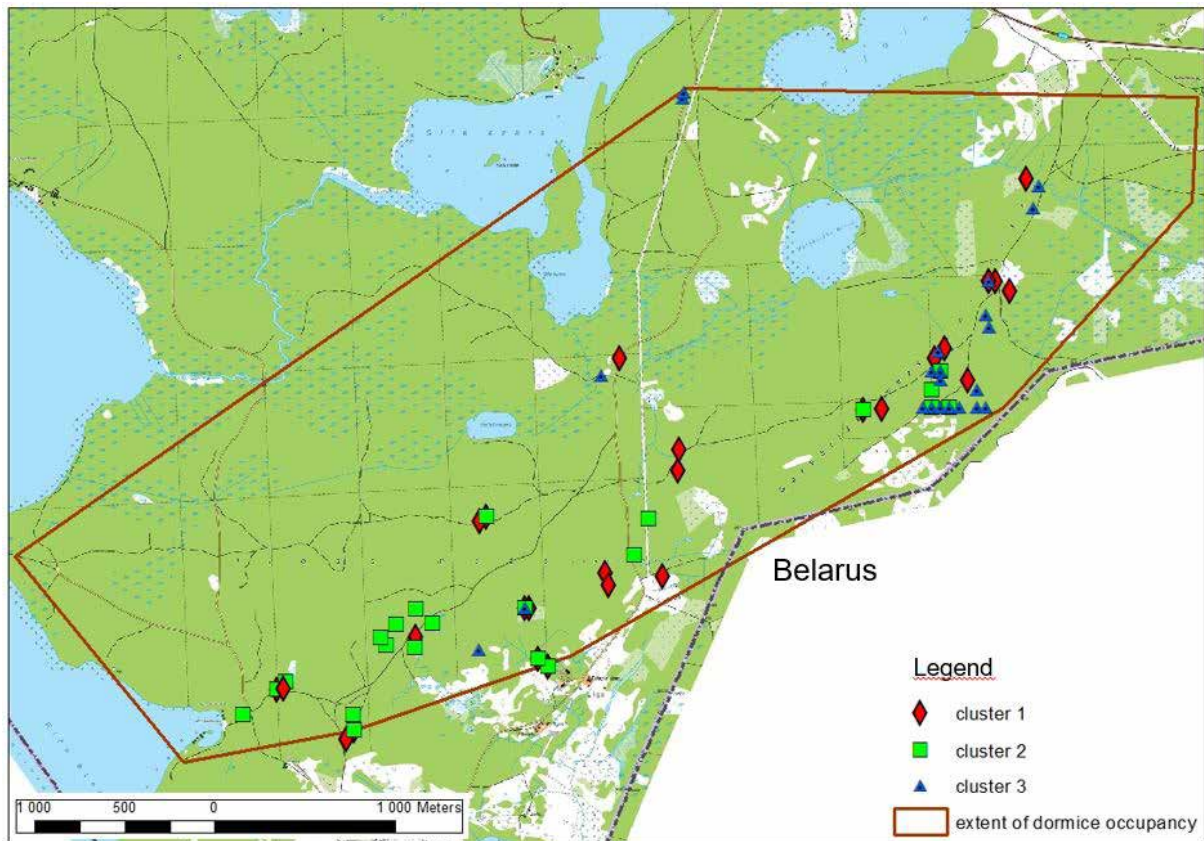


Fig. 3. Location of the three identified clusters in the sampling area of Latvia presented based of the location of the nest box, in which the corresponding dormouse was found.

the Latvian dormouse population. Gene exchange might also be limited because of uneven or mosaic type distribution of suitable habitats (forest stands rich with hazel in the understory) within the extent of dormouse occurrence. A study with radio-tagged forest dormice (PILĀRS et al. 2012) showed that usually they did not cross forest stands lacking a dense understory. Apparently, most dormice stay in one patch of suitable habitat during their whole life.

The Latvian forest dormouse population was discovered relatively recently (in the 1960s) and the history of this population is unknown. It is possible that some parts of the current population range were

isolated from the others (for longer or shorter periods), which might have resulted in the formation of the three identified genetic clusters.

Further investigations are needed to analyse genetically other forest dormouse populations in order to compare directly genetic diversity between them, particularly in neighbouring populations. The analysis of additional genetic loci would improve the accuracy of the results obtained. RAPD markers are dominant and heterozygotes cannot be directly observed, thus reducing the information content. The use of alternative genotyping systems such as microsatellites or sequencing would provide an op-

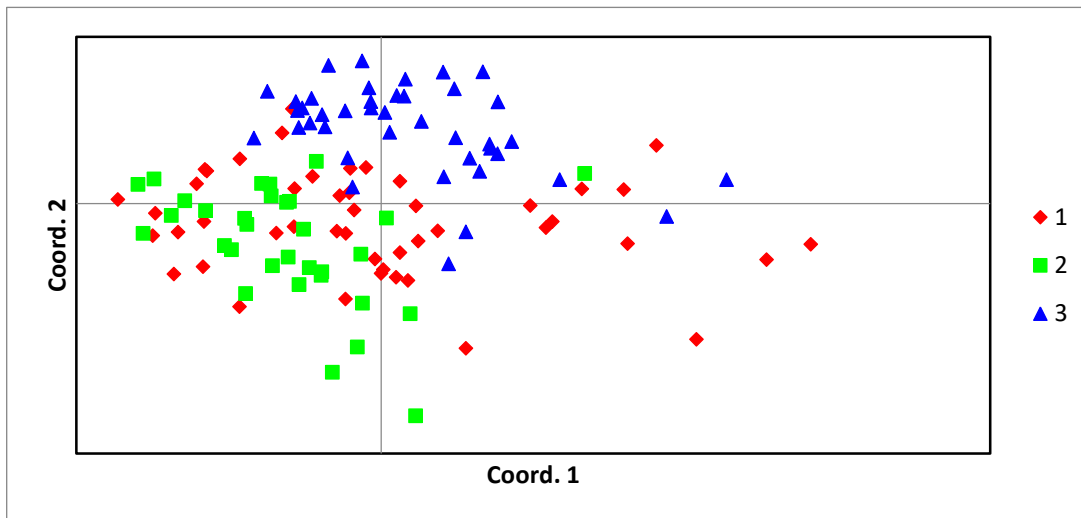


Fig. 4. PCoA of pairwise Nei genetic distances. Variation explained by axis 1 – 11.15%, axis 2 – 10.08%. Individuals were grouped according to the STRUCTURE results.

portunity not only to increase the number of loci but also to provide increased resolution of the analysed individuals and populations. In addition, better characterisation of individuals (e.g., with respect to sex or age) and changes in population structure over multiple years would provide additional information about population dynamics within the study area. Finally, detailed investigation in the trans-border region with Belarus would allow identification of the total size and area of this population as well as the possible connectedness with other forest dormouse populations.

Acknowledgements: The authors warmly thank volunteers who helped in molecular studies and collection of samples. The study was funded by the Latvian Environmental Protection Fund project “Meža susura populācijas ģenētiskās daudzveidības izvērtējums populācijas dzīvotspējas kontekstā” (Assessment of forest dormouse population genetic diversity in the context of population sustainability, No. 1-08/47/2017).

References

- ARIF I. A., KHAN H. A., BAHKALI A. H., AL HOMAIDAN A. A., AL FARHAN A. H., AL SADOON M. & SHOBRAK M. 2011. DNA marker technology for wildlife conservation. *Saudi Journal of Biological Sciences* 18: 219–225.
- BAHN V., O’CONNOR R. J. & KROHN W. B. 2006. Effect of dispersal at range edges on the structure of species ranges. *Oikos* 115: 89–96.
- BATSAIKHAN N., KRYŠTUFEK B., AMORI G. & YIGIT N. 2016. *Dryomys nitedula*. The IUCN Red List of Threatened Species 2016: e.T6858A115084761. <http://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T6858A22222806.en>
- CABALLERO A. 1994. Developments in the prediction of effective population size. *Heredity* 73: 657–679.
- CAUGHLEY G. 1994. Direction in conservation biology. *Journal of Animal Ecology* 63: 215–244.
- EUROPEAN COMMUNITIES. 1992. Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora. *Official Journal of the European Communities L 206*: 750.
- EVANNO G., REGNAUT S. & GOUDET J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14 (8): 2611–2620.
- FILIPPUCCI M. G., KRYŠTUFEK B., SIMSON S., KURTONUR C. & ÖZKAN B. 1995. Allozymic and biometric variation in *Dryomys nitedula* (Pallas, 1778). *Hystrix* 6 (1–2): 127–140.
- FRANKEL O. H. 1970. Variation, the essence of life. *Proceedings of the Linnean Society of New South Wales* 95: 158–169.
- FRANKHAM R. 2005. Genetic and Extinction. *Biological Conservation* 126: 131–140.
- GRIGORYEVA O., KRIVONOGOV D., BALAKIREV A., STAKHEEV V., ANDREYCHEV A. & ORLOV V. 2015. Phylogeography of the forest dormouse *Dryomys nitedula* (Gliridae, Rodentia) in Russian Plain and the Caucasus. *Folia Zoologica* 64 (4): 361–364.
- HERDEGEN M., RADWAN J., SOBCZYNSKA U., DABERT M., KONJEVIĆ D., SCHLICHTER J. & JURCZYNSZYN M. 2016. Population structure of edible dormouse in Poland: the role of habitat fragmentation and implications for conservation. *Journal of Zoology* 298 (3): 217–224.
- JUŠKAITIS R. 2015. Ecology of the forest dormouse *Dryomys nitedula* (Pallas 1778) on the north-western edge of its distributional range. *Mammalia* 79(1): 33–41.
- JUŠKAITIS R. 2021. Long-term abundance dynamics in four dormouse (Gliridae) populations in Lithuania. *Abstract book of 11th Baltic Theriological Conference*: 16.
- KING K. C. & LIVELY C. M. 2012. Does genetic diversity limit disease spread in natural host populations? *Heredity* 109: 199–203.
- KRYŠTUFEK B. & VOHRALIK V. 1994. Distribution of the forest dormouse *Dryomys nitedula* (Pallas, 1779) (Rodentia, Myoxidae) in Europe. *Mammal Review* 24(4): 161–177.
- KOPELMAN N. M., MAYZEL J., JAKOBSSON M., ROSENBERG N. A.

- & MAYROSE I. 2015. CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources* 15 (5): 1179–1191.
- MILLER C.R. & WAITS L.P. 2003. The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): Implications for conservation. *PNAS* 100 (7): 4334–4339.
- MISHARA M., DUBEY N., TOTAY S.M., BATH K.V., BABU S., AWASTHI-KALIA M. & ANAND R. K. 2002. Phylogenetic relationships and genetic polymorphisms in wild Indian mice. *Biomolecular Engineering* 18: 281–288.
- MONTGOMERY M. E., WOODWORTH L.M., NURTHEN R.K., GILLIGAN D. M., BRISCOE D. A. & FRANKHAM R. 2000. Relationships between population size and loss of genetic diversity: comparisons of experimental results with theoretical predictions. *Conservation Genetics* 1: 33–43.
- MOSKA M., JAKUBIES J., WIERZBICKI H., STRZAŁA T. & KOZYRA K. 2016. Low genetic variability of the edible dormouse (*Glis glis*) in Stolowe Mountains National Park (Poland) – preliminary results. *Mammal Research* 61 (4): 409–415.
- NABHOLS B., MAUFFREY J. F., BAZIN E., GALTIES N. & GLEMIN S. 2008. Determination of mitochondrial genetic diversity in mammals. *Genetics* 178 (1): 351–361.
- PEAKALL R. & SMOUSE P. E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* 28: 2537–2539.
- PILĀTS V., PILĀTE D., ORNICĀNS, A. & KĀRKLIŅŠ A. 2012. Micro-habitat utilization by forest dormice (*Dryomys nitedula*) in boreo-nemoral forest-preliminary results. *Peckiana* 8: 77–85.
- PRITCHARD J. K., STEPHENS M. & DONNELLY P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.
- PLUESS A. R. & STÖCKLIN J. 2004. Genetic diversity and fitness in *Scabiosa columbaria* in the Swiss Jura in relation to population size. *Conservation Genetics* 5: 145–156.
- SELÇUK S. E., ÇOLAK R., KARACAN G. O. & ÇOLAK E. 2012. Population structure of edible dormouse, *Glis glis* (Linnaeus, 1766) in Turkey, inferred from RAPD-PCR. *Acta Zoologica Bulgarica* 64(1): 77–83.
- Ściński M. & BOROWSKI Z. 2006. Home ranges, nest sites and population dynamics of the forest dormouse *Dryomys nitedula* (Pallas) in an oak-hornbeam forest: a live-trapping and radio-tracking study. *Polish Journal of Ecology* 54: 391–396.
- TRACY L. N., WALLIS G. P., EFFORD M. G. & JAMIESON I. G. 2011. Preserving genetic diversity in threatened species reintroductions: how many individuals should be released? *Animal Conservation* 14: 439–446.
- WHITELEY A. R., HASTINGS K., WENBURD J. K., FRISSELL C. A., MARTIN J. C. & ALLENDORF F. W. 2010. Genetic variation and effective population size in isolated populations of coastal cutthroat trout. *Conservation Genetics* 11:1929–1943.
- WOODRUFF D. S. 2001. Populations, species and conservation genetics. *Encyclopaedia of Biodiversity* 4: 811–829.

