



UNIVERSITY OF BIALYSTOK

Faculty of Biology

K. Ciołkowskiego 1J, 15-245 Białystok, Poland
tel. 85 738 8383 • e-mail: biologia.dzieskanat@uwb.edu.pl • biologia.uwb.edu.pl



Prof. dr hab. Mirosław Ratkiewicz
ermi@uwb.edu.pl

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Information on the procedures and genetic analyzes performed for the project "The Return of Lynx to Northwestern Poland" implemented by the Western Pomeranian Nature Society (ZTP)

The Eurasian lynx as top predator regulates the number of its prey and thus assures better ecosystem functioning. Its restoration in Europe contributes to reducing the amount of damage caused by roe deer in forests and farmlands. The greater number of natural populations and the large number of individuals in lynx populations, the larger chances of survival of the species. Previous scientific studies have shown that the populations of this predator are characterized by low intra-population genetic variation and large divergence between isolated populations in Europe (Ratkiewicz et al. 2012, 2014). Moreover, recent genomic studies have confirmed these results and indicated that the Carpathian lynx population is a separate genetic unit from the so-called lowland population (Lucena-Perez et al. 2020).

Therefore, for the reintroduction of lynxes in northwestern Poland, all specimens were subjected to genetic testing at the Department of Zoology and Genetics, Faculty of Biology of the University of Białystok in order to assign lynx individuals to genetically distinct populations and to establish the degree of genetic relatedness between lynxes from zoos. These studies were supposed to provide information that would allow the reintroduction of only lynxes from the so-called "lowland" population. On the other hand, when it is found that two individuals of different sex are close relatives - releasing them away from each other, in distant forest complexes, so that relatives will likely not mate in the wild. These goals were achieved by analyzing the control region sequence of mitochondrial DNA and genotyping lynx at carefully selected 20 microsatellite loci (whose results are compatible with genomic data) along with genetic identification of their sexes in ca. 120 *Lynx lynx* individuals. Since hair samples were used, they were treated as non-invasive samples and each analysis was repeated up to 4-6 times to ensure that the correct genotype was obtained and to eliminate genotypic errors. DNA isolation controls and negative PCR controls were used to prevent contamination with foreign DNA. Both cr mtDNA strands were sequenced. High-end reagents and kits (Qiagen, Germany, BDT 3.1, Thermo Fisher) were used for molecular biology analyzes and operated on a GenAmp 9700 thermocycler (ABI) and ABI3130 sequencer to ensure high-quality results. The obtained sequences were compared to the cr mtDNA sequence of lynx deposited in GenBank database and to the database of several hundred Eurasian lynxes, owned by those carrying out genetic research. Haplotypes of individual lynxes were assigned to previously defined mtDNA genetic variants, and in the case of new ones - their phylogenetic relationship with known genetic variants was determined. All genotypes and sequences were made available to ZTP on an ongoing basis. The microsatellite analyzes allowed to assign each studied lynx to the source population by means of PCA (Principal Component Analysis) and DAPC (Discriminative Analysis of Principal Components) analyzes. Both analyzes gave very similar results and, for example, it turned out that 8 of the studied individuals undoubtedly belonged to the Carpathian line, and as a result they were not recommended for reintroduction (for



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example Goliath and his sister Gotha). Analysis of microsatellites revealed several parent-child, sibling and cousin pairs. Such individuals were not recommended for breeding and were not released in the same area. The analyzes were carried out by Dr Maciej Matosiuk and Prof. Mirosław Ratkiewicz who both have several years of experience in research in the field of population genetics, molecular biology and phylogenetics of mammals, including lynx, and in the last 10 years have participated in significant research in the field of genetics and genomics of this predator (listed below). These researchers have used reference samples that allowed to determine the genetic identity of the individuals. For the purposes of the project, 103 samples from 95 individuals provided by ZTP were genetically tested, 12 individuals from zoos selected for the project in 2017-2018 by the coordinator from EAZA and their offspring, another 82 were lynx from private breeding centers, wildlife parks and zoos, while 1 individual was born in the wild. Out of the 91 examined individuals born in various breeding centers, 83 individuals were qualified for reintroduction or further breeding as not significantly different from the population of the Baltic lowland line, while 8 individuals as genetically deviating from the lowland line were eliminated from the project.

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Sincerely yours

Prof. dr hab. Mirosław Ratkiewicz

*Mirosław
Ratkiewicz*