

# GENETIC APPROACHES FOR ROMANIAN BROWN BEAR (*Ursus arctos*) CONSERVATION

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**Abstract:** *One of the largest populations of brown bear (*Ursus arctos*) is situated in the Romanian Carpathians, where more than 6000 individuals are distributed in suitable habitats. The aim of this paper is to conduct a review of the current state of knowledge on genetic studies of the Romanian brown bear. So far only a few studies with a limited number of samples from Romania have been carried out. The two main mitochondrial DNA lineages, which were described in Europe, are mt in the Romanian Carpathians. More data is needed about the contact zone and the degree of interference between the two lineages. Describing the genetic structure based on nuclear genetic markers will further help in developing appropriate conservation measures.*

**Key words:** *brown bear, mitochondrial DNA, microsatellites, conservation.*

## 1. Introduction

Brown bear has a high adaptability to different habitats, proved by its large distribution across the Eurasia and North America. Throughout the world, brown bear registers now a number of 200.000 individuals [24], [39].

In Europe, brown bear population was widespread and well represent in the past.

The species suffered a permanent persecution, due to the direct contact with humans. Livestock was the main reason for conflict interferences, and humans have conducted campaigns to eliminate the largest predator of Europe [25], [28], [30].

The elimination process of the brown bear in Western Europe has been almost completed, today only small populations are surviving. Populations, larger than those from the West, are concentrated in the Northern and Southeastern Europe [29], [30].

According to IUCN (International Union for Conservation of Nature and Natural Resources), the brown bear is on the red list of threatened species, in need of protection [24].

Starting with 1990, in order to ensure a long-term conservation of the species, a direct correlation between management and genetic studies has been proposed and established as a first priority [25].

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The first step in brown bear genetic studies was the research on mitochondrial DNA, in order to correlate geographic distribution with phylogeny, for identifying and establishing the conservation units [30], [16].

After almost three decades, molecular biology rapidly developed, and now it allows reading the bear's genotype using the last generation of DNA markers and visualizing the genotype on sequencer [3].

In the present di- and tetra-nucleotide microsatellites markers are validated and used in forensics, parentage and genetic population studies [9]. Extraction of DNA is performed from different kind of samples (tissues, hairs, bones and scat). This allows the use of non-invasive techniques and collection of samples without animal disturbing [8], [32], [25].

Nowadays, common guidelines for the genetic study of brown bears have been designed, in order to compare the genetic diversity and to differentiate populations in the Southeastern Europe. A common framework was proposed in order to have the possibility to compare the results obtained in different studies. This approach helps the new researchers, sharing the experience of genetic studies successfully conducted [7].

Knowing the genetic situation of the brown bear populations, valuable information will be provided (genetic diversity, existence of bottleneck and genetic drift, inbreeding, gene flow or isolation of the populations). In order to obtain population conservation, mitigation

measures fundamented by the genetic studies must be implemented [20].

## 2. Mitochondrial genetic analyses

In mammals, the mitochondrial DNA is maternally inherited [4]. More studies around the world were focused in studying mitochondrial DNA of the bears [37].

Two main mitochondrial DNA lineages have been revealed by the genetic analyses performed on the European brown bears: the Eastern lineage and the Western lineage [30].

Taberlet, P. and Bouvet, J. (1994) suggested that the Western lineage appears to be organized into two clades. The first clade includes bears from: Abruzzo, Trentino, Slovenia, Bosnia, Croatia, Greece and Bulgaria. The second clade is composed of bears from Cantabrian, Pyrenean, South of Sweden and Norway.

The Eastern lineage of mitochondrial DNA includes bear populations from Northern Scandinavia, Eastern Europe, Russia and Western part of Romania [11], [14], [30].

The brown bear population from Romania registered a special situation, due to the presence of both mitochondrial DNA lineages: the Western and the Eastern [9], [34]. The particularity registered in the mitochondrial DNA suggested the effect of brown bear females philopatry [31], [30], [26].

Thereby, samples from the Romanian brown bear (number ranged from 4 to 23) were analyzed in three mitochondrial DNA studies (Table 1).

Table 1

*The brown bear samples from Romania analyzed in mt DNA studies*

N	N <sub>1</sub> Eastern lineage	N <sub>2</sub> Western lineage	Reference
4	4 (Ro1, Ro2)	–	P. Taberlet and J. Bouvet, 1994
23	8 (lineage: 10, 26, 27, 28)	15 (lineage: 1, 5, 9, 10, 11)	M. Kohn et al. 1995
16	6 (R1, R2, R3, R4, R9)	10 (R3, R5, R6, R7, R8, R10)	F.E. Zachos et al. 2008

N-number of samples,

N1-number of individuals assigned to the Eastern lineage, N2-number of individuals assigned to the Western lineage

Among the Romanian brown bear population, an admixture between Western and Eastern lineage was found. Nevertheless, more individuals were assigned to the Western lineage of the mitochondrial DNA. The mitochondrial DNA phylogeographic break seems to appear between localities of Brasov and Sibiu (with a range of 100 km) [38].

The Romanian brown bear population is situated in an important zone, thereby, together with Scandinavia represents a hybrid area between the Eastern and Western lineages, possibly resulted from the admixture of individuals in post-glacial recolonisation routes [11], [31], [36], [40].

The phylogeographic break observed in Scandinavia, presents itself differently from the Romanian one. Thereby, the Scandinavian phylogeographic break appears separated by 135 km, the Western lineage in the South and the Eastern lineage in the North.

For the Carpathians brown bear population, the break of distribution refers to the Eastern lineage [38].

Therefore it becomes necessary to perform mitochondrial DNA analysis, on a large number of brown bear samples (tissues, hairs, scats, bones) originating from all the Romanian territory, but mainly covering the lack of samples registered in previous studies [11], [38]. Samples from the Western part of Romania (between localities Brasov and Sibiu) must be examined at first [38].

Increasing the number of samples, in order to identify the location of the mitochondrial DNA phylogeographic break in the Romanian brown bear population will provide answers to questions linked to migration route of species, phylogenetic relationships of haplotypes, haplotype diversity and population expansion.

### 3. Nuclear genetic analyses

Microsatellites are known as Simple Sequence Repeats (SSRs) or short tandem repeats (STRs), representing a repeating sequence of 2-6 base pairs [4]. Today, all the genetic studies are based on them.

Microsatellite markers are usually used to assess the genetic structure and to estimate the level of genetic diversity and differentiation of populations. Using microsatellite markers, information about the population structure can be obtained, and also spatial distribution, parentage, and movements of brown bear individuals.

An important step is to select a suitable set of microsatellite markers for brown bear populations [6].

After 1994, when microsatellites markers for brown bears started to be developed [17], a great number of nuclear markers have appeared [1], [9], [33]. Today, multiplexes with 5-10 microsatellite markers designed for brown bear are applied in order to reduce genetic analysis costs [7].

When sets of microsatellite loci were used, higher expected heterozygosity and allelic diversity occurred in the largest populations, while low heterozygosity occurred in the smallest populations of brown bear [29].

The obtained results in genetic studies confirmed that microsatellites are great in studying fine-scale population structure [18].

Genetic studies were performed in all bear populations in the world. European brown bear populations as a whole registered a high genetic diversity [29].

A review of studies, regarding the obtained values of expected heterozygosity (HE) in world's bear populations, is represented in Table 2.

In insular and peninsular populations of black bear and brown bear (Kodiak brown bear and Newfoundland black bear) there was registered a reduction of genetic

variation. A similar study on brown bear of Hokkaido showed a low level of genetic diversity as well [35], [40].

In accordance with the studies done until now, the highest level of genetic diversity is present in the Russian bear population ( $H_E=0.83$ ), in Kirov Oblast region, while the lowest level of genetic diversity is present in

Kodiak Island bear population ( $H_E=0.27$ ). Romanian brown bear population has one of the highest levels of genetic diversity, occupying the third place, after Russia and the North of Finland ( $H_E=0.80$  for 109 samples analyzed). Studies performed on Romanian brown bear have used 9-13 microsatellites markers [26], [38].

Table 2

*A review of genetic analyses performed with variable DNA microsatellites markers -*

Region	N	$H_E$	Source
Russia, Kirov Oblast	13	0.83	Tammeleht et. al 2010 [34]
Finland N	26	0.82	
<b>Romania</b>	<b>16</b>	<b>0.81</b>	<b>Zachos et. al 2008 [38]</b>
<b>Romania</b>	<b>109</b>	<b>0.80</b>	<b>Straka et. al 2012 [26]</b>
Russia W	32	0.80	Swenson et. al 2011 [29]
Russia Arkhangelsk Oblast	16	0.79	
Alaska, Alaska Range	28	0.78	Waits et. al 2000 [40]
Finland S	44	0.77	Tammeleht et. al 2010 [34]
Balkans S	49	0.77	Karamanlidis et. al 2010 [8]
Kluane, Yukon	50	0.76	Waits et. al 2000 [40]
Albania, Greece, Macedonia	49	0.76	Karamanlidis et. al 2010 [8]
Richardson Mountains NWT	119	0.75	Waits et. al 2000 [40]
Alaska, Brooks Range	148	0.75	Waits et. al 2000 [40]
Croatia, Dinaric Mountains	156	0.75	Kocijan 2011 [10]; Straka et. al 2012 [26]
Italian Alps	2793	0.74	De Barba et. al 2010 a [5]
Sweden	380	0.71	Waits et. al 2000 [40]; Kindberg et. al 2010
Slovakia N	71	0.71	Straka et. al 2012 [26]
Slovakia C	96	0.70	
Scandinavia NS	108	0.70	Waits et. al 2000 [40]
Scandinavia NN	29	0.69	Waits et. al 2000 [40]
Flathead River BC/MT	40	0.69	Waits et. al 2000 [40]
Scandinavia S	156	0.68	Waits et. al 2000 [40]
Estonia	62	0.68	Tammeleht et. al 2010 [34]; Swenson et. al 2011
Alaska, Kuskokim Range	55	0.68	Waits et. al 2000 [40]
Scandinavia—M	88	0.67	Waits et. al 2000 [40]
East Slope, Alberta	45	0.67	Waits et. al 2000 [40]
Slovakia E	16	0.65	Straka et. al 2012 [26]
Alaska, Admiralty Island	30	0.63	Waits et. al 2000 [40]
Coppermine NWT	36	0.61	Waits et. al 2000 [40]
Austria	379	0.61	Kruckenhauser et. al 2009 [13]
Yellowstone, MT/WY	57	0.55	Paetkau et. al 1998 [19]; Waits et. al 2000 [40]
Pakistan	28	0.55	Bellemain et al. 2007 [2]
Alaska Baranof/Chicago	35	0.49	Waits et. al 2000 [40]
Spain, W	39	0.45	Perez et al 2010 [22]
Italy Appenines	30	0.44	Lorenzini et al 2004 [15]
France W	5	0.40	Taberlet et. al 1997 [33]
Spain E	71	0.25	Perez et. al 2009 [21]
Kodiak Island Alaska	34	0.27	Paetkau et. al 1998 [19]; Waits et. al 2000 [40]

N-Sample size,  $H_E$  – expected heterozygosity; BC, British Columbia; Is, Island; MT, Montana; NWT, Northwest Territories; WY, Wyoming, S-South, N-North, E-East, W-West, C-Central

Increasing the number of samples and markers, in order to obtain a genetic profile of brown bear will lead to genetic mapping of Romanian population and will help us to take the best mitigation measures for conservation.

#### **4. Genetic conservation and management of brown bear populations**

The maintenance of genetic diversity represents a priority for all populations and for all species. Once lost, the genetic diversity is very hard to recover. Particularly, small populations are the most threatened. Even if it is a large population, after it passed through a bottleneck effect, a low level of genetic variation is often registered. Usually evolutionary implications of such reduction are having consequences.

If the Scandinavian brown bear population is analyzed, where independent genetic drift existed followed by a rapid population growth and gene flow among the four previously isolated populations, a high level of genetic diversity has been preserved. These results are particularly relevant in the conservation biology and suggest that gene flow among populations has a significant role.

For brown bear conservation it is recommended to preserve a big number of individuals and to ensure sustainable flow between populations [40].

Straka et al. (2012) suggested that human caused isolation and fragmentation of bear populations in the Western and Eastern Carpathians resulted in evident genetic differentiation. Future management efforts were proposed in order to secure and restore the connectivity of populations. Those are aimed to preserve the genetic variation of Carpathian brown bear subpopulations and to support the gene flow between them.

In order to preserve a high genetic diversity of Carpathian brown bear, it is necessary to use the result of the genetic studies before taking other conservation measures [1].

#### **5. Conclusions**

Regarding the few studies conducted on Romanian brown bear population, results indicated a unique genetic make-up, including two mitochondrial lines and one of the highest values for expected heterozygosity.

Increasing the number of analysed samples, in order to identify the location of the mitochondrial DNA phylogeographic break, in the Romanian brown bear population, will provide answers to questions linked to migration route of species, phylogenetic relationships of haplotypes, haplotype diversity and population expansion. Genetic conservation status will be determined, a long-term conservation ensured through the proposal of the conservation measures.

The main objective is to obtain a genetic mapping of Romanian brown bear population, to determine the presence of subpopulations and the existence of natural borders between them.

In the context of rapid development of infrastructure (highway construction), it is necessary to obtain the genetic mapping before their construction. Interrupted migration of individuals between habitats, caused by highways, might be easily observed and mitigation measures adopted in order to ensure a high genetic diversity.

The goal in future conservation of the Romanian brown bear is to preserve a high genetic diversity with efforts in maintaining the connectivity between subpopulations.

A combination of the nuclear and mitochondrial DNA analyses will provide useful data for Romanian brown bear conservation.

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