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Black bear poking its head out of the den during October 2022. Photo credit: Pam Talasco, SilverCloud Photography. See article on page 27.

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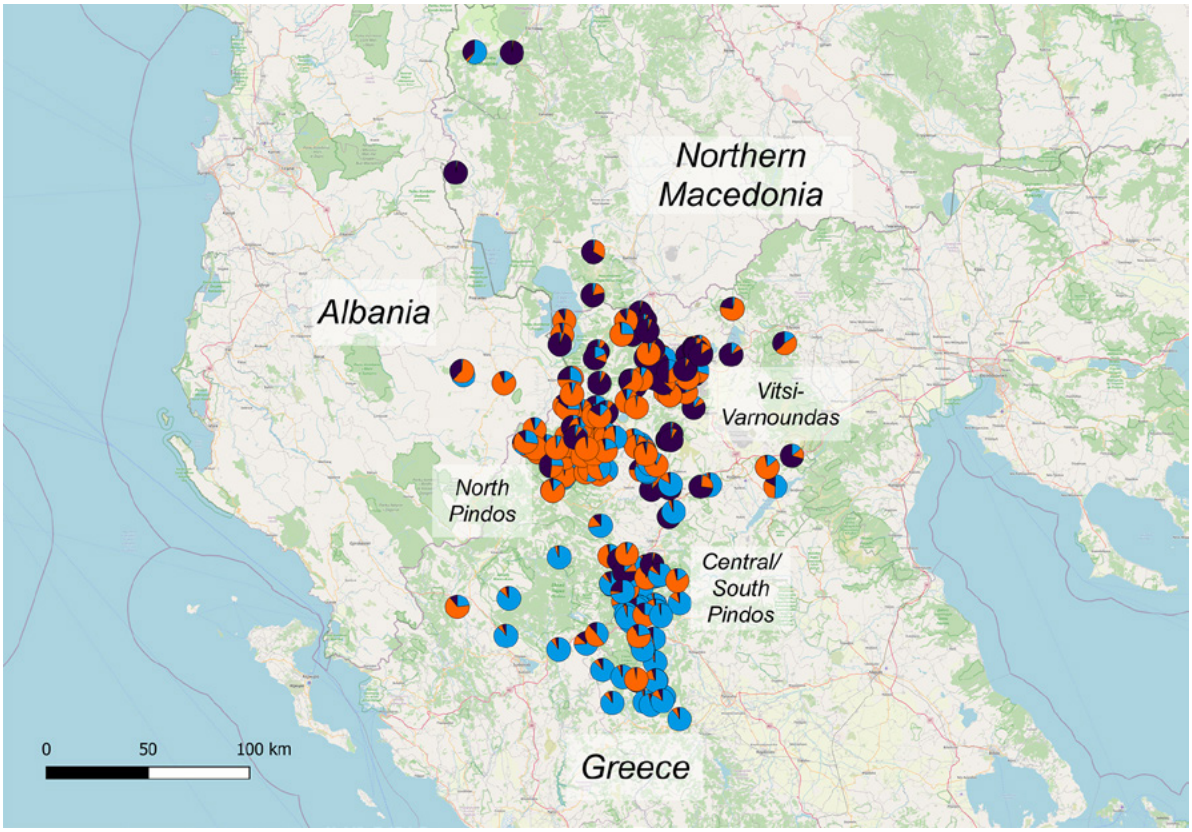
Conservation Status of Brown Bears in Greece: The "Hellenic Bear Register"-Phase II

Established in 2000 with the aim of initiating the first genetic study of brown bears (*Ursus arctos*) in Greece, the "Hellenic Bear Register" has been an important tool for the conservation of brown bears in the region. During the first phase of the project (2000 – 2010) 250 unique bears in Greece and throughout the southwestern Balkans were identified. This information has enabled a detailed understanding of the demographics, genetic diversity and gene flow and distribution of the brown bear population in Greece and its connection with bear populations in neighboring countries for the first time. Efforts for Phase I have been highlighted in over 10 scientific articles that have been published since (https://www.hellenicbearregister.com/publications).

After 23 years of continuous operation, the "Hellenic Bear Register" is now in the process of publishing the results of the second decade of the project's efforts (i.e., 2010 – 2020). During this time the project has continued genetic monitoring of the species in the country, but has also ventured into unknown territories through the study of hormone levels and heavy metal burdens of the hair samples that were collected, again, mainly relying on non-invasively collected biological material.

Selected results

The genetic study of brown bears during Phase II of the "Hellenic Bear Register" was based on 315 genotypes. We calculated expected and observed heterozygosity, as well as the number of alleles and assessed population structure with STRUCTURE. Brown bears in western Greece are structured into 3, geographically overlapping genetic clusters. In the northern part of the population (i.e., Vitsi-Varnoundas and North Pindos, for more information, see Karamanlidis et al. 2018) genotypes were assigned to all 3 clusters, indicating admixture of genotypes from the different genetic units. Genotypes from each cluster were also found in the southern part of the population, representatives of the southern cluster were in the majority, suggesting less connectivity and lower gene flow with the



Distribution and genetic STRUCTURE results of 323 individual bears identified throughout the southwestern Balkans during Phase II (2010 – 2020) of the "Hellenic Bear Project." Each circle represents a bear's genotype on their sample location, and the color their assignment to one of the three identified genetic clusters.

bears in the north or other regions. This result was corroborated with slightly higher heterozygosity and number of different alleles found in the genetic cluster in the north, perhaps due to assumed connectivity to the substantial Alps-Dinara-Pindos bear population further north. Genetic variation with *Ho*=0.69 (SE 0.015) and *He*=0.67 (SE 0.013) was overall reasonably high for the whole population and comparable with other bear populations in the Alps-Dinara-Pindos population.

The endocrine and toxicological study was performed on 206 hair samples that were comparatively evaluated for cortisol and 50 heavy metals. Whenever the available sample amount was not enough it was used for one single analysis: if below 10 mg, for heavy metals only, if between 10 and 50 mg, for cortisol only. Cortisol was extracted following standardized methods after washing to remove dirt and external cortisol (Davenport et al. 2006) and heavy metals were analyzed using Inductively Coupled Plasma-Mass Spectrometry. Analytical results showed some extremely high values in some heavy metal concentrations, which were sorted out by identifying outliers (2 to 3 samples maximum for each heavy metal). After sorting, results were statistically compared for sampling area (*Kruskal-Wallis* ANOVA) and for sex of bear (*Mann-Whitney* U test), and highlighted differences in some heavy metals by sampling area. These results confirm that these elements can be used to define the area of origin, as they create a unique fingerprint (Rapa et al. 2023). No difference was observed when sex was considered, and none as well was found in cortisol levels when compared by area and sex. High mean and median values for some toxic elements (i.e., lead, mercury, cadmium) were observed, raising questions as to the source of these high levels. A detailed analysis of the spatial distribution of these samples and the potential sources of this pollution will help clarify the reasons for such high concentrations, which might be indicators of a potential risk for the species.

Following the in-depth analysis of these data, the combination of all 3 information sources (i.e., genetic, endocrine, toxicological) will enable the identification of potentially new sources of threats for brown bears in Greece and the definition of effective management actions that should be incorporated in the conservation guidelines for the species in the country.

Acknowledgments

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