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Original Investigation

Noninvasive genetic assessment of brown bear population structure in Bulgarian mountain regions

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ABSTRACT

The Balkans are one of the last large refugia for brown bear (*Ursus arctos*) populations in Europe, and Bulgaria, in particular, contains relatively large areas of suitable brown bear habitat and a potential population of more than 600 individuals. Despite this, the majority of brown bear research remains focused on bear populations in Central and Western Europe. We provide the first assessment of genetic population structure of brown bears in Bulgaria by analysing tissue samples ($n = 16$) as well as samples collected with noninvasive genetic methods, including hair and faecal samples ($n = 189$ and $n = 163$, respectively). Sequence analysis of a 248 base pair fragment of the mitochondrial control region showed that two highly divergent mitochondrial European brown bear lineages form a contact zone in central Bulgaria. Furthermore, the analysis of 13 polymorphic microsatellite markers identified 136 individuals and found substantial genetic variability ($H_e = 0.74$; $N_A = 8.9$). The combination of both genetic markers revealed the presence of weak genetic substructure in the study area with considerable degrees of genetic admixture and the likely presence of migration corridors between the two subpopulation in the Rhodope Mountains and Stara Planina as evidenced from the genetic detection of two male long-distance dispersers. A detailed assessment from densely collected samples in the Rhodope Mountains resulted in a population size estimate of 315 (95% CI = 206–334) individuals, indicating that not all available habitat is presently occupied by bears in this region. Efficient management plans should focus on preserving connectivity of suitable habitats in order to maintain gene flow between the two Bulgarian brown bear subpopulations.

Introduction

As a flagship species for European nature conservation, the genetic population structure of the brown bear (*Ursus arctos*) has been intensively studied throughout its western and central European range (Swenson et al. 2011). In Italy, for example, extensive long-term genetic monitoring has been conducted since 2002 in order to monitor population status and provide data for optimised bear management (DeBarba et al. 2010). In contrast, and only relatively recently, has research begun to focus on the genetic composition and structure of Eastern Europe bear populations (e.g. Romania, Straka et al. 2012; Croatia, Kocijan et al. 2011; Macedonia, Karamanlidis et al. 2013; Greece Karamanlidis et al. 2012; Caucasus, Murtskhvaladze et al. 2010; Slovakia, Graban et al. 2013; Serbia, Karamanlidis et al. 2014). This delay in rigorous scientific assessments of eastern European populations is somewhat surprising, considering that some of these bear populations number still above estimations for minimum viable population sizes and thus are of primary conservation relevance for the long-term preservation of the species in the remainder Europe. Given this and because genetic diversity is generally higher in eastern than in western bear populations (Swenson et al. 2011), the eastern populations of this species serve as valuable source populations for potential active future recolonisation (e.g., see Gutleb 1998) as well as donor regions for anthropogenic translocations (Kruckenhauser et al. 2009) of the largely bear-free western part of the continent.

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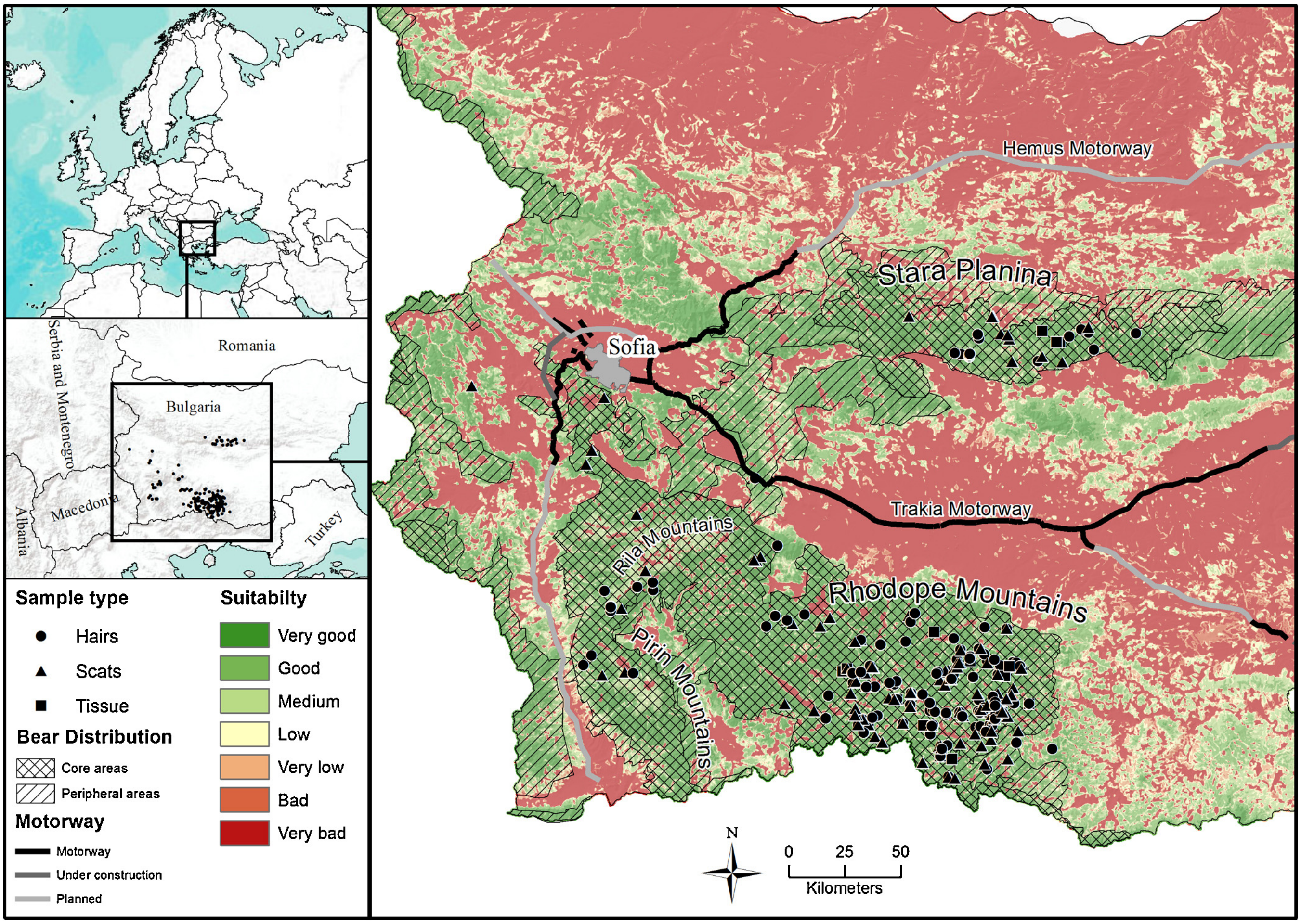

Fig. 1. Overview of sampling. A total of 368 samples were collected. Dots indicate hair samples ($n = 189$), triangles indicate scat samples ($n = 163$) and squares indicate tissue samples ($n = 16$). Colours from green to red indicate the degree of habitat suitability for brown bears (see Zlatanova et al. 2009). Lines in the main map show motorways (Kaphegyi et al. 2013), raster grid displays the current bear distribution (Action Plan for the Brown Bear in Bulgaria, 2008). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Bulgaria is located centrally within the south-eastern European bear range and still comprises large areas of near-natural montane habitat, including non-fragmented areas suitable for species such as the brown bear (Fig. 1). Additionally, anthropengic impacts in Bulgaria are relatively low due to the small populations size (66 inhabitants/km^2) and low level of infrastructure (road density of 0.293 km/km^2) (Kaphegyi et al. 2013). Perhaps due to these reasons, Bulgaria has one of the largest brown bear populations in the Balkan region, estimated at between 600 and 800 individuals (Kaphegyi et al. 2013). The population is restricted to mountainous areas, such as the Central Balkan Mountains (Stara Planina) and the Rilo-Rhodopean massif (Fig. 1). The amount of gene flow between the two Bulgarian mountain ranges and other adjacent bear regions in Greece, Serbia or Romania is virtually unknown, but at least the connection between the Bulgarian populations is under immediate threat due to increasing human pressure in the area with only few potential migration corridors (Zlatanova et al. 2009).

In this study we present the first genetic assessment of brown bear populations from the Bulgarian region of the Balkan Peninsula. We assessed population structure, genetic diversity, census size and migration routes within Bulgarian brown bear populations. As the presence of two highly divergent mitochondrial brown bear lineages is known to occur in Europe, with contact zones in nearby regions such as Romania (Zachos et al. 2008; Straka et al. 2012), we aimed to assess if this phylogeographic dichotomy also extends to Bulgaria. For this, we sampled hair, scat and tissue material from different Bulgarian regions and genotyped them with microsatellites and mitochondrial control region haplotype analysis. In particular we aimed to answer the following questions:

1. Are brown bears in Bulgaria regionally isolated or does gene flow occur among different mountain ranges, suggesting the existence of effective dispersal corridors?
2. Do genetic data support the official census population size estimation of brown bears in Bulgaria?
3. Is the degree of genetic diversity in Bulgarian brown bears comparable to other bear populations in Eastern Europe or is there evidence for genetic erosion?
4. Do both European brown bear mitochondrial lineages occur in Bulgaria and is there a geographic separation between these lineages?

Materials and methods

Sampling and DNA extraction

A total of 355 samples were collected between 2009 and 2012, and 13 samples were collected between 2004 and 2008 (Fig. 1). We analysed 16 tissue samples obtained from dead animals, legally killed following the derogation rules of EU directive 92/43 and animals trapped for GPS collaring, 189 non-invasively collected hair samples from barbed wire traps, scratch trees and fences, and