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## **SHORT COMMUNICATION**

FIRST RECORD AND GENETIC AFFILIATION OF THE BALKAN SNOW VOLE *DINAROMYS BOGDANOVI* (RODENTIA: CRICETIDAE) IN ALBANIA

Ivan Stolarik & Daniel Jablonski

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# FIRST RECORD AND GENETIC AFFILIATION OF THE BALKAN SNOW VOLE DINAROMYS BOGDANOVI (RODENTIA: CRICETIDAE) IN ALBANIA



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**Abstract:** One individual of the rare rodent *Dinaromys bogdanovi* (Martino, 1922) was trapped in Prokletije Mountains, northern Albania. This is the first record of this species in Albania, and morphological data and genetic affiliation are presented. Analysis of mitochondrial DNA (cytochrome *b*) places this individual within the reported central phylogenetic lineage of the species. This first record has impacts for future conservation activities.

**Keywords:** Balkan Peninsula, conservation, cytochrome *b*, new record, paleoendemics, phylogeography.

The southwestern Balkan Peninsula has a high number of endemic species, including *Drusus croaticus*, *Salmo letnica*, *Pelophylax shqipericus* or *Anguis graeca* (Džukić & Kalezić 2004; Kryštufek et al. 2009; Previšić et al. 2009; Jablonski et al. 2016). This endemism is likely linked to a complex paleogeography that resulted in topographic and climatic heterogeneity, producing strong contrasts between the eastern/western and northern/southern parts of the peninsula. In the west and south the Dinarides and Hellenides rise steeply from the coastal strip, and their geographic variation offers suitable conditions for historical local adaptations of populations (Jablonski et al. 2016). As a result, endemic species or cryptic lineages of species found in this region are often characterized by high levels of genetic

variability (see Sotiropoulos et al. 2007; Ursenbacher et al. 2008; Marzahn et al. 2016). This is not a rule, especially in mountain "sky-islands" species of the region with scattered distribution in current interglacial refugia (see Podnar et al. 2014; Mizsei et al. 2017).

One exception is the monotypic and endemic Balkan Snow Vole or Martino's Snow Vole Dinaromys bogdanovi (Martino, 1922), where molecular data suggest a species complex (Kryštufek et al. 2007). The Balkan Snow Vole is a rare palaeoendemic rodent of the mountain regions (found up to 2,200m, but is typically found over 1,500m and occasionally in lowlands) in the western Balkan area (Todorović 1956). It is a specialist of subalpine rocky regions, and the continuous system of the Dinarides and Hellenides provides ideal habitats. Despite this, populations of this species are probably highly fragmented, and potentially threatened (Kryštufek et al. 2007; 2009). Its relative abundance exceeds five individuals per 100 trap-nights (Kryštufek & Bužan 2008). Current distribution of the species is certainly less than 2,000km<sup>2</sup> and covers Croatia, Bosnia and Herzegovina, Kosovo/Serbia, Montenegro and western Macedonia. According to genetic and morphological data, three independent lineages were detected in this area (Kryštufek et al. 2007). While there are no known

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records from Albania and northern Greece, it is likely that the species range extends to these areas (Bego et al. 2008; Kryštufek & Bužan 2008) and the lack of observation is probably due to a lack of sampling effort.

#### **MATERIAL AND METHODS**

During the field work in northern Albania, an adult female of D. bogdanovi (Image 1) was trapped (29 September 2016; Prokletije Mountains, Dinarides; Image 2), measured and a piece of tissue collected for the Department of Zoology, Comenius University in Bratislava (voucher number DB1). The live traps (type Chmela) were used to catch with an oak flakes as a bait. The traps were exposed during one night and checked at the morning about 08:00hr. For identification based on morphology we used Petrov & Todorović (1982). The specimen's total genomic DNA was isolated from tail tips fixed in 96% ethanol. Isolation was carried out with a MACHEREY-NAGEL NucleoSpin® Tissue kit and complete mitochondrial DNA cytochrome b marker (cyt b) was amplified via polymerase chain reaction (PCR) using L14727-SP and H-15195-SP primers (Jaarola & Searle 2002). The PCR protocol consisted of an initial 3min denaturation step at 94°C, 30 cycles of denaturation at 94°C for 30s, annealing at 50°C for 30s and 3min long extension at 72°C and the final extension at 72°C for 10min. PCR product was sent to Macrogene Inc. (Amsterdam, the Netherlands) for purification and sequencing. Original material was compared with available GenBank sequences from Kryštufek et al. (2007) and Bužan et al. (2008). The novel

sequence (length 1143bp) was deposited in GenBank under accession number MF966150. The dataset of nucleotide sequences was aligned using Clustal W algorithm (Thompson et al. 1994) implemented in BioEdit 7.2.5 (Hall 1999) and edited by eye. Preliminary network analysis classified our sequence as "Central lineage" of the species (Kryštufek et al. 2007), and for the final dataset we used 30 sequences of this lineage (length 555bp). We used a network approach (Posada & Crandall 2001) to infer inter-individual relationships. For constructing a haplotype network we used the 95% limit of parsimony (Templeton et al. 1992) as implemented in TCS 1.21 (Clement et al. 2000) and the final network was visualized with tcsBU (dos Santos et al. 2016).

#### **RESULTS AND DISCUSSION**

The trapping site was in the southwestern part of the Albanian Prokletije Mountains near Qafe e Terthores (42.390 N & 19.726 E; 1,626m; coordinates and elevations acquired using a Garmin 60CSx Global Positioning System unit and referenced to map datum WGS84). The newly discovered population of *D. bogdanovi* is located (airline distances) 32km from the nearest locality of the species in Montenegro and 62km from the locality in Kosovo (Kryštufek et al. 2007; Kryštufek & Bužan 2008). The habitat represents subalpine/alpine grassland and sparse pine forest (*Pinus heldreichii* Christ, 1863) deposited on limestone, consisting of medium size rocks (>30cm in diameter). The trapping point was located on a steep slope with south orientation (Image 1). The specimen's body measurements were: weight 52g;



Image 1. An overview on the subalpine habitat of the species near Qafe e Terthores, Prokletije Mountains. Albania.

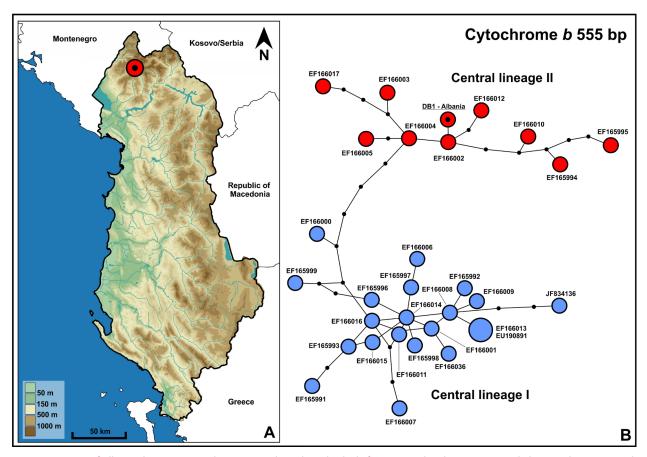


Image 2. A - Map of Albania showing geographic position where the individual of *Dinaromys bogdanovi* was recorded; B - Haplotype network of the Central lineage of the species defined by Kryštufek et al. (2007) and genetic affiliation of the individual from Prokletije Mountains (black dot, DB1). Small black circles represent missing haplotypes.

body length 123mm; length of hind foot 25mm; and tail length 92mm. Our record increases the number of small mammal species (Rodentia: Cricetidae) for Albania to 21 (see Bego et al. 2008; Stolarik et al. 2017).

According to our genetic data, the individual from Prokletije Mountains corresponds with Central lineage (Kryštufek et al. 2007). This lineage should be isolated by the Neretva River from the north and the Drin River from the south, and is formed by two main groups: Central lineage I and II, divided by five mutation steps. Our individual is part of the Central lineage II and has an unique haplotype that is closest to sequences with GenBank accession number EF166002 (one mutation step) and EF166004 (two mutation steps; Image 2B). These sequences are labeled as haplotypes C20 (voucher PMS998 from Prokletije on the Kosovo-Montenegro border) and C22 (voucher PMS1006 from Mt. Žljeb, Lučanski stanovi, Kosovo; see Kryštufek et al. 2007; 2012) and they are part of the group located from southern Bosnia and Herzegovina to southeastern Montenegro. Thus our individual corresponds with the genetic distribution pattern of the species, and is very close to populations in Montenegro and Kosovo (airline distance between pass Qafe e Terthores and Mt. Žljeb ca 60km). Haplotype uniqueness and number of missing haplotypes in the haplotype network (Image 2B) suggest historical fragmentation of species populations.

The species conservation status for the Balkan Snow Vole is Vulnerable (Kryštufek 2008; IUCN Red List). Understanding historical biogeography and distribution is essential in order to identify conservation management priorities (Kryštufek et al. 2007). The range of this species is probably decreasing (Kryštufek 2004; Kryštufek & Bužan 2008), thus distribution and phylogeographic knowledge might help to protect isolated populations. The species range covers several protected areas, but little conservation legislation is focused directly on its conservation. Albania should now join the conservation program for this endemic mammal species.

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