

Conservation genetics of proteus in the Postojna-Planina Cave System

Varstvena genetika močerila v Postojnsko-planinskem jamskem sistemu

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Proteus is the largest obligate cave animal in the world. It inhabits subterranean waters of the Dinaric Karst. It is an endangered species protected under the European Habitats Directive and national legislations. During the last reporting period under the EU Habitats Directive in 2013, the conservation status of *proteus* in Slovenia was estimated and reported as unfavourable (Hudoklin 2011, 2016). In recent years, considerable effort has been invested to improve conservation-relevant knowledge of the species, e. g. new approaches to monitoring presence using environmental DNA (Gorički et al. 2017), monitoring of habitat quality parameters (Hudoklin 2016), and genetic monitoring of quantitative population parameters (Trontelj & Zakšek 2016).

Here we present our progress on the conservation genetic research conducted within an applied research project under the title »Toward the conservation of the European cave salamander (*Proteus anguinus*): monitoring guidelines, current status estimation and identification of evolutionarily significant units«. In this project, we are estimating population parameters (e.g. heterozygosity, gene flow, possible population bottlenecks, effective population size, migration rates), genotyping individuals, and evaluating the taxonomic and conservation status of *proteus* populations. The greater part of the population-level work has been carried out in the Postojna-Planina Cave System, a large subterranean system, which hosts high numbers of *proteus* that can be accessed without the need of deep sump technical diving. In Planina Cave, we sampled *proteus* from the inner 1.8 kilometres of the subterranean Pivka River. In order to have reference points for the geographic position of the caught animals, the

studied part of the cave was divided into 23 sections that were between 50 and 100 m long (Fig. 1). In Postojna Cave, *proteus* were caught in a 200 m long sidearm of the same river in the part called Črna jama.

For the purpose of genetic monitoring, we developed and characterized the first microsatellite markers for *proteus* (Trontelj & Zakšek 2016, Zakšek et al. accepted). We amplified 23 microsatellites from populations sampled in the caves of Postojna and Planina. The number of alleles per locus varied from 3 to 9. The loci were largely unlinked and conformed to Hardy-Weinberg frequencies. Genetic equilibrium and an F_{ST} value of only 0.0024 suggest a nearly panmictic population in both caves, even though they are separated by some 10 kilometres of subterranean river course. Bayesian clustering detected a weak genetic structure, but failed to unambiguously assign individuals to either of the two caves. These microsatellites will be applied in a genetic mark-recapture study and population monitoring in the near future.

In the summers of 2015 and 2016, 775 and 824 animals, respectively, were caught, using hand nets during snorkelling and scuba-diving. Skin swabs were taken to obtain DNA. In both years a very similar number of animals were caught per individual sections (Fig. 2). Large-scale genotyping and genetic identification of individuals is still in progress, but we already have data on 73 individuals that were genetically marked in 2015 and recaptured the following year. Out of the 73 recaptured animals, most (77%) were found strictly in the same cave section in both years, 16% in adjacent sections, and only 7% were recaptured in non-contiguous cave sections, although never more than 200 m away. These data suggest that at least some animals are strongly territorial and do not change their positions more than some tens of meters within one year. The finding is in agreement with Balázs et al. (2015) who reported extreme philopatry of visually tagged *proteus* over several years in southern Herzegovina. Further support of a constant territorial structure is the similarity between patterns of caught animals by section (Fig. 2). On the other hand, the near lack of genetic structure between Postojna and Planina Cave means that there must be substantial migration going on over a distance of about 10 kilometres. It is possible that only some individuals

are territorial, while others, perhaps sexually immature ones, are more mobile and inclined to disperse. Genotyping of additional animals and reconstructing their relatedness will hopefully give more conclusive answers.

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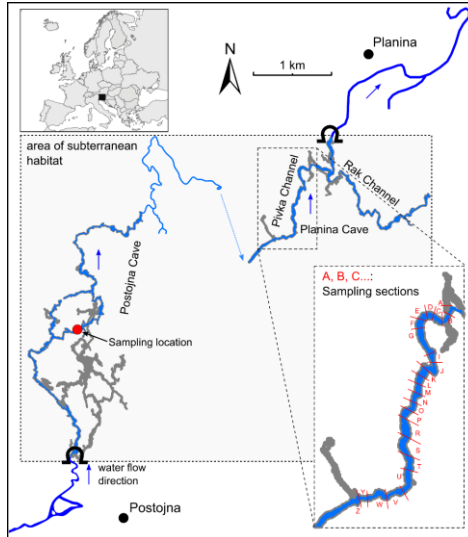


Figure 1. Map of the study area with the two sampling areas (marked by red dot: Črna jama and by red letters: Pivka River in Planina Cave) of proteus populations within the Postojna and Planina Cave System.

Slika 1. Shematski prikaz vzorčenja močerila v Postojnsko-planinskem jamskem sistemu. Rdeči krogec označuje območje vzorčenja v Črni jami, rdeče črke pa odseke v Pivškem rokavu v Planinski jami.

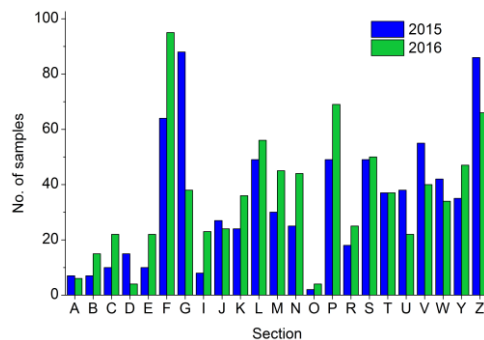


Figure 2. Number of caught and genetically marked animals by sections of the subterranean Pivka River in Planina Cave in 2015 and 2016.

Slika 2. Število ujetih in genetsko označenih močerilov v posameznih odsekih vzdolž podzemeljske Pivke v Planinski jami v letih 2015 in 2016.