Genetic monitoring of *Proteus* populations

Genetski monitoring populacij človeških ribic

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The European cave salamander, or proteus, is a Slovenian national symbol. Endemic to the Dinaric Karst, it is the largest cave animal on our planet and the only European cave vertebrate (Parzefall et al. 1999). As a long-living top predator and charismatic species of karst underground waters it is of outstanding ecological and conservational importance. It is protected by several national and international regulations. Despite all this, proteus populations have not been monitored or surveyed in a way that is standard for most European amphibian species and other species protected under the EU Habitats Directive. From various scattered sources and anecdotic reports we can conclude that, over the last three decades or so, there has been a moderate to substantial decline at a number of sites with regular occurrence, and a decline in number of animals at some sites (Hudoklin 2011). These reports are mostly from the regions of Dolenjska and Bela krajina (southeastern Slovenia) as well as Istria (Croatia), and to a much smaller extent from other parts of the proteus range.

Nevertheless, at present we can hardly distinguish between true population decline on one side and periodic fluctuations, minor spatial shifts of habitat, or differences in observation effort on the other. There are three major reasons why reliable information on proteus population size, population density and frequency of occurrence is hard to obtain. The first and most important is the inaccessibility of its habitat. Karst groundwater and aquifers are accessible only through small »windows« in the form of caves or, sometimes, by cave diving. The most basic faunistic surveys are technically extremely demanding and expensive. At the same time, we do not know enough about the position, volume and extent of karst aquifers to infer indirect estimates of the populations' status and size. The second impediment is posed by several peculiarities in the life history and ecology of proteus that make most standard zoological research approaches inapplicable, even when the animals can be accessed in their natural environment. Common surface-dwelling amphibian species can be surveyed during seasonal migrations and mating, at different developmental stages, etc. None of these techniques can be applied to survey proteus populations. In theory, it is possible to count animals in cave lakes or along the few accessible stretches of subterranean rivers, but any interpretation of numbers would be completely arbitrary. It would be unclear whether fluctuations in numbers of counted individuals are caused by seasonal habitat shifts, random movements, disturbance avoidance, or by actual changes in population size. The third factor hindering effective monitoring and conservation management is the unresolved true taxonomic structure of the taxon. Unclear population and taxonomic boundaries hamper conservation decisions like setting of conservation priorities and management units.

The 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«, funded by the Slovenian Research Agency, the Ministry of the Environment and Spatial Planning and Centre for Cartography of Fauna and Flora, is aimed at overcoming these obstacles. Within the framework of this project we are developing methods and tools that will enable us to asses and monitor population sizes as well as establish their geographic and taxonomic structure. We have developed an efficient method for catching live proteus in open cave waters with the use of diving equipment and hand nets. This method allows capture rates of up to 80% of all observed individuals, which is much higher than any other means used so far. With it, we managed to catch about 800 animals from several Slovenian caves. The highest numbers were caught in the Postojna-Planina Cave System, followed by the subterranean Reka River in the South West and the Šica System in the South East. DNA is obtained non-invasively, by skin swabs (Prunier et al. 2012), and the animals are released immediately at the spot after their weighing and measuring. For genetic analysis we have developed a wide array of polymorphic tetranucleotide microsatellite markers, which will be used both for individual

genotyping within populations as well as for inferring the global population structure. A subset of 23 highly polymorphic loci is optimized for the Postojna-Planina Cave System, where we already recaptured some of the genetically »marked« individuals. Eventually, a full recapture is planned for the coming season in order to estimate the population by means of mark-recapture modelling (Amstrup et al. 2005). The developed loci have passed the tests of Hardy-Weinberg, linkage disequilibrium, probability of identity, and reliability of genotyping. Comparisons within and between hydrological systems revealed strong gene flow between the adjacent Postojna and Planina Caves (*Fst* = 0.006) and a strong differentiation between the Ljubljanica and other, more remote drainages, where populations had only few alleles in common. The strong differentiation at nuclear DNA level supports the previously established mitochondrial DNA lineages (Trontelj et al. 2009). In the following phases of the project, we plan to spatially delineate those lineages, to formalize their taxonomic status, and to provide for them first estimates of population size and conservation status.

References

Amstrup S.C., McDonald T.L., Manly B.F.J. (2005): Handbook of capture-recapture analysis. Princeton University Press, Princeton, 296 pp.

Hudoklin A. (2011): Are we guaranteeing the favourable status of the *Proteus anguinus* in the Natura 2000 network in Slovenia? In: Prelovšek M., Hajna N.Z. (Eds.), Pressures and Protection of the Underground Karst – Cases from Slovenia and Croatia. Inštitut za raziskovanje krasa ZRC SAZU, Postojna, pp. 169-181.

Parzefall J., Durand J.P., Sket B. (1999): *Proteus anguinus* Laurenti, 1768 – Grottenolm. In: Grossenbacher K., Thiesmeier B. (Eds.), Handbuch der Reptilien und Amphibien Europas. Band 4/I: Schwanzlurche I. Aula-Verlag, Wiesbaden, pp. 57-76.

Prunier J., Kaufmann B., Grolet O., Picard D., Pompanon F., Joly P. (2012): Skin swabbing as a new efficient DNA sampling technique in amphibians, and 14 new microsatellite markers in the alpine newt (*Ichthyosaura alpestris*). Mol. Ecol. Resour. 12: 524-531.

Trontelj P., Douady C.J., Fišer C., Gibert J., Gorički Š., Lefébure T., Sket B., Zakšek V. (2009): A molecular test for cryptic diversity in ground water: how large are the ranges of macro-stygobionts? Freshwater Biol. 54: 727-744.