Searching for the black *Proteus* with the help of eDNA

Iskanje črnega močerila s pomočjo okoljske DNA

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Proteus is a specialized, blind and depigmented subterranean salamander inhabiting groundwaters of the Dinaric Karst. In Bela krajina, Slovenia, a unique black population is known from only four springs in the proximity of a closely related white, troglomorphic population (Aljančič et al. 1986, Sket 1997, Ivanovič 2012, Škedelj Petrič et al. 2014). In the face of the scarcity of the distributional information, potentially very limited distribution and impending threat due to heavy fertilizer load, establishing the detailed distribution of these populations was the focus of our work. Because its subterranean habitat is inaccessible, several techniques were employed at springs, including traps and visual observation in the dark using night goggles, but the survey predominantly aimed to introduce a novel approach – forensic analysis of traces of *Proteus* DNA released in water (environmental DNA or eDNA).

To detect *Proteus* eDNA in samples of spring water and to discriminate between the black and white populations, we developed specific TaqMan probes and PCR primers, homologous to variable regions of *Proteus* mtDNA (Gorički 2006, Gorički & Trontelj 2006). Of the 19 spring water samples collected and filtered, six were positive for *Proteus* DNA, five of which were also positive for black *Proteus*. All five are new localities, where *Proteus* has neither previously nor during the survey been sighted or otherwise detected. Along with detection of *Proteus* eDNA in spring and cave water in southern Herzegovina and Montenegro (Aljančič et al. 2014, Stanković et al. 2016), this survey represents the first successful application of the eDNA approach in detection of a subterranean organism. The methodology developed and tested herein is the only quick and accurate method available for determining its presence and is fully appropriate for monitoring the distribution of this rare and endangered stygobiont in Bela krajina.

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