Application of environmental DNA for detection of *Proteus*

Uporaba okoljske DNA pri iskanju človeške ribice

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The rare and highly endangered olm (*Proteus anguinus*) is the only obligate subterranean vertebrate of Europe. It inhabits subterranean waters of the Dinaric Karst in the north-western Balkan Peninsula (Sket 1997). As only small fragments of its subterranean habitat are accessible, the basic but important question of its exact distribution has been difficult to address. Its presence can only rarely be confirmed by classical survey methods such as trapping and visual encounters. For this reason, alternative methods are required to test for its presence in new potential localities. Detection of species-specific DNA released into the aquatic environment (environmental DNA or eDNA) has already been shown as an appropriate approach to monitoring the distribution of vertebrates from surface waters. In aquatic environments rapid diffusion of eDNA from its source means that the presence of a specific animal could be detected anywhere within such water body and not just at its point of origin, thus making this approach particularly useful for those species that are difficult to detect using conventional methods or are very rare (Rees et al. 2014). We developed an adjusted eDNA approach for filtering water samples from karst springs, wells and caves and provide two specific primer sets that can be used to amplify short conserved fragments of *P. anguinus* mitochondrial DNA (Gorički 2006, Gorički & Trontelj 2006) by real-time PCR based on SYBR chemistry. The specificity of the assay was first tested on trout, crested newt and human DNA. In controlled conditions at the Tular Cave Laboratory the minimum density at which its DNA could still be detected corresponded to one animal per 256 m^3 of standing water, when sampling 20 L of water. The method, tested at three Slovenian field test sites occupied by different lineages of *P. anguinus*, was 100% effective. Subsequently, a pilot survey of its distribution was conducted along the southern limit of its known range in Herzegovina and Montenegro. Using DNA-based identification, we unequivocally established the presence of *P. anguinus* at four sites, and found its likely traces at additional eight sites – most of them new localities for this species. Even though the SYBR chemistry-coupled real-time PCR approach was shown to be very successful and time-efficient method for detection and monitoring of *P. anguinus* that can be applied with fidelity anywhere within its known range of occurrence, detectability can still be increased using TaqMan probes (Gorički et al. 2016).

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