Oral Session: The Ecological Theatre

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Searching for black *Proteus (Proteus anguinus parkelj)* in karst groundwater with the help of eDNA.

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The black *Proteus (Proteus anguinus parkelj)* forms a very small and endangered population of the only European subterranean amphibian. Since its discovery in 1986 until the most recent sighting in 2012, the black *Proteus* has been documented at only four sites in extreme Southeastern Slovenia. They are situated within a 2 by 2.5 km maximum aerial distance from each other. Nearby in the same geographic area, but presumably in a different geological formation, the nominate troglomorphic white Proteus (Proteus anguinus anguinus) has been spotted at about 10 sites (springs and caves), several of which have never been verified by experts. Since the habitat of *Proteus* is inaccessible to man, we developed an environmental DNA-based assay of spring water samples to test for its presence. Employing TaqMan qPCR chemistry with specific probes and primers we (1) conducted a systematic inventory of *Proteus* presence in the region, (2) verified certain historic records of white *Proteus*, (3) determined the maximum span of the black *Proteus* range to the East and South, and (4) tested for possible cooccurrence of the two subspecies. We detected black *Proteus* eDNA at five new sites. In one of these we found both black and white *Proteus* eDNA syntopically. These results are consistent with known hydrogeological patterns, and suggest a possible contact or parapatry of the subspecies. eDNA data can therefore, when used together with information from phylogeographic and population genetic analyses, broaden our knowledge of species evolution and systematics, as well as help establish efficient conservation measures through updated distribution maps of the species.

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