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CRYPTIC SPECIATION IN DINARIC KARST WATERS: MOLECULAR APPROACHES REVEAL A NEW DIMENSION OF DIVERSITY

Peter TRONTELJ, Rudi VEROVNIK & Boris SKET
Department of Biology, University of Ljubljana, Slovenia

The aquatic fauna of the Dinaric karst area of the western Balkan Peninsula is renowned for its endemism and high diversity. This species richness is chiefly due to a high number of specialists inhabiting different types of subterranean waters, karst springs, resurgences, sinking rivers and other types of karst waters. Particularly rich groups with many endemic species can be found among crustaceans *(e.g.* amphipods, isopods, copepods), gastropods *(e.g.* Hydrobioidea), leeches (Erpobdellidae) and fishes (Cyprinidae). Many of these taxa live only in small, restricted areas, especially the subterranean or stygobitic species. The Dinaric region is known to hold the richest stygobitic fauna in the world. However, little is known about the processes and historical events that shaped such a rich fauna.

In contrast to many highly endemic, restricted-range-species, there are a number of widely distributed species occupying the same habitats. They are often morphologically homogeneous across their entire range. Examples of such taxa are the blind cave salamander *Proteus anguinus,* the cave shrimp *Troglocaris "anophthalmus",* the tube worm *Marifugia cavatica* and the leech *Dina lineata.* It has already been suggested that each of them might in fact represent a diverse species aggregate. The genetic patterns of their diversification might provide clues to the speciation process in karst waters.

Initial molecular genetic studies were conducted on *Dina lineata, Asellus aquaticus* and *Proteus anguinus.* Nuclear ribosomal ITS sequences, mitochondrial ribosomal genes and RAPDs have been employed as molecular markers. One of the most striking results of these studies was the high degree of isolation between neighboring populations. Adjacent populations of *D. lineata* differed from one another in their ITS2 sequences even more than some closely related erpobdellid species from each other. No gene flow could be detected between them. The most probable factor causing this isolation is the specific hydrography of karst waters. During the karstification process, rivers and other water bodies became fragmented in an island-like manner. The original dispersion must have taken place prior to extensive karstification, when the dispersion pathways were still present. The cryptic speciation of *D. lineata* can thus be explained by a series of vicariant events. A similar scenario may hold for most of the widely distributed genera with many endemic species and subspecies.

The case of troglomorphic forms of *Asellus aquaticus* demonstrates that the speciation processes can be more complex when it is linked to the invasion of caves. RAPD data have revealed that the stygobitic *A. a. cavernicolus* from Planina Cave does not interbreed with surface populations, with which they have potential physical contact. A second stygobitic population in the subterranean R. Reka some 30-km away clearly arose from an independent cave invasion. Moreover, a third, only partially troglomorphic population is known from the Postojna-Planina cave system. It arose from a more recent invasion and does not interbreed with the fully troglomorphic populations in the same system. The temporal, not the spatial component of isolation played the main role in producing this unique case of sympatric occurrence of two independent troglobitic descendants of the same surface species.

One can conclude that molecular genetic data have confirmed some predictions about the diversity patterns of aquatic animals of the Dinaric karst, like (1) the existence of cryptic species and (2) multiple independent steps of a single ancestral species towards a subterranean lifestile. They further provided evidence that vicariant habitat fragmentation events played a major role in the formation of species richness.

Key words: speciation, hypogean fauna, *Proteus, Asellus,* molecular biology