

Endemism below the species rank: Population genetics of the European Mudminnow (*Umbra krameri*)

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Summary

Endemism is commonly handled on the level of species - an example is the theme topic of this session of the conference. However, biodiversity has an important sub-specific component, e.g. evolutionary significant units (ESUs) which are comprised of one or more populations. In fact, estimates of biodiversity loss due to population extinction are much higher compared to species loss (DICKMAN et al. 2007). Modern molecular genetic techniques shed new light on within-species diversity, starting with genetic variability of individuals, populations, ESUs and up to species (GEFFEN et al. 2007). Specifically population genetics and barcoding studies provide new information on the interplay of different levels of organization and contrast genetic with traditional, morphometric approaches (e.g. PALANDACIC et al. 2017). Here we provide an example of a population genetic study on the European mudminnow (*Umbra krameri*) analyzing the within-species genetic variability throughout the entire known range of occurrence (MARIC et al. 2017). The aim of the study was to test if the populations within the species' range are genetically uniform or if significant genetic structuring is present to warrant certain populations to be treated as unique and therefore endemic on a sub-specific level.

The European mudminnow (*Umbra krameri*) is a rare fish species with a relatively small total area of occurrence: It extends from Vienna in the west, through the Danube lowlands to the delta, including the Sava and Tisza River lowlands, and reaches the Dniester River in the east (WANZENBÖCK 1995). In Austria, the species occurs in only two habitats, one situated in the national park Donauauen (WANZENBÖCK & SPINDLER 1995). The species is specifically adapted to groundwater-fed waterbodies on the margins of floodplains and swamps and may serve as an indicator species of the ecological status of such critically endangered ecosystems (WANZENBÖCK 2004).

Mudminnows were sampled from 17 locations across the species natural distribution area (see Figure 1 in MARIC et al. 2017) and DNA extracted from fin clips. The mitochondrial protein-coding gene cytochrome b (1085 bp 3' - end) was PCR-amplified using GluF and ThrR primers and sequenced on an ABI Prism 3130xl machine. The genealogical relations between haplotypes were presented as a minimum spanning network (MSN) using the 95% statistical parsimony criterion in the TCS 1.2 program. Seven tetranucleotide microsatellite loci were amplified according to published protocols (WINKLER & WEISS 2009) and fragments analyzed on the ABI prism 3130xl machine. Software used to analyze the data included Gene-Mapper, Microchecker, Genetix 4.04, ADZE, SPAGeDI 1.3 and STRUCTURE (for details see MARIC et al. 2017).

The analysis of the mitochondrial cytochrome b gene revealed ten haplotypes. Their spatial distribution showed divergence of mudminnow populations according to the larger drainages: Populations in the Sava drainage showed exclusive haplotypes not found in the upper, middle or lower Danube (with one exception of the middle Danube close to the Sava mouth) or in the Tisza drainage. Similarly, populations in the Tisza drainage were composed of exclusive haplotypes not found anywhere else. The largest variety of haplotypes was found in the Danube delta (see Figure 1 in MARIC et al. 2017), however, most haplotypes dominating the populations in the upper and middle Danube were lacking in the delta.

Hierarchical STRUCTURE analysis of the microsatellite fragment length data revealed discrimination of a group consisting of populations in the upper and middle Danube, Drava, Mura and Lake Balaton from a group of populations of the lower Danube, Sava, Tisza and Dniester in a first division. Anyhow, already the second discrimination step allowed to distinguish populations of the Drava, Mura and Balaton area from populations of the middle and upper Danube, and within the latter group the population of Austria (Eckartsau) was clearly discernible from three other populations (see Figure 4 in MARIC et al. 2017). The third step of discrimination separated almost all the populations (with few exceptions).

In contrast to population genetic studies on a related species, the Olympic mudminnow (*Novumbra hubbsi*), which showed no genetic structure on the mitochondrial (mt) haplotype level, such structuring was clearly evident in the European mudminnow. Genetic structuring on the mt haplotype level coincided with the drainages of the Danube, Tisza and Sava i.e. the largest sub-drainages in the Danube system. Therefore, these three phyletic lineages could be considered potential evolutionary significant units (ESU). On the other hand, the uneven distribution of microsatellite polymorphism among the small sampled populations and high genetic structuring within each of the three phyletic lineages may not reflect a natural evolutionary process but rather random drift governed by recent habitat fragmentation as a result of human impact (e.g. damming).

Therefore, caution should be taken when delineating ESUs on the basis of microsatellites, as these markers known for their high mutation rate and neutral evolutionary history are likely to result in excessive splitting of populations and are generally inadequate for characterizing adaptive patterns. However, the Austrian population at Eckartsau, from the national park Donauauen, was found to be genetically different based on the STRUCTURE analysis very early in the hierarchical splitting procedure. This indicates that the Eckartsau population is unique on a sub-specific level and may be regarded as an endemic form or strain, warranting the status of a true biodiversity treasure in Austria.

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