



Integrating genetics and morphometrics in species conservation—A case study on the stone crayfish, *Austropotamobius torrentium*



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ABSTRACT

The stone crayfish, *Austropotamobius torrentium*, is a freshwater crayfish species native to Central and Southeast Europe. Due to various, mostly anthropogenic factors the species is facing a dramatic decline in many European countries. In order to plan and implement protection measures, it is essential to collect extensive data on the species, including biological aspects and the major threats. Therefore, in the frame of a species conservation program in western Austria, we assessed the genetic relationship of the local endangered stone crayfish populations to populations in eastern Switzerland and southern Germany as well as their genetic diversity using mitochondrial and nuclear DNA markers. Population differentiation was measured applying both genetic and morphometric tools in order to estimate the suitability of potential donor populations for future restocking and reintroduction measures. The results showed a high degree of genetic homogeneity at the lineage level in the alpine stretch of the Rhine valley. Despite a rather low genetic diversity, nuclear markers provided signs of genetic divergence among populations even at a local scale. Due to the inconsistencies found between genetic and morphometric differentiation patterns, we highlight the importance of integrating both tools for the identification of suitable crayfish stocking material.

1. Introduction

The stone crayfish, *Austropotamobius torrentium* (Schrank, 1803), is a freshwater crayfish species native to Central and Southeast Europe (Holdich et al., 2006; Kouba et al., 2014). The species typically inhabits small, fairly cold-temperate running waters, characterised by a moderate gradient and a high hydro-morphological and river bed heterogeneity (Streissl and Hödl, 2002; Pöckl and Streissl, 2005). Due to various anthropogenic influences, like habitat loss, water pollution, river regulation and the introduction of non-indigenous crayfish species, a dramatic decline of the species has been observed in most European countries (Füreder and Souty-Grosset, 2005; Füreder et al., 2010; Chucholl and Dehus, 2011; Berger and Füreder, 2013). Despite this alarming trend, the IUCN red list still classifies the species as data deficient (Füreder et al., 2010). However, as the species has become a subject of legislative protection via its listing in the Annexes II and V of the EU Habitats Directive (Council, 1992), EU member states are obliged to maintain this protected species at, or restore it to, the so called “favourable conservation status”. The same holds true for the Austrian Federal State Vorarlberg, where the stone crayfish is considered native

and a need for conservation actions has been recently identified (Berger and Füreder, 2013).

Various measures can be considered in freshwater crayfish protection, ranging from habitat restoration and enlargement, establishment of protected areas to restocking and reintroduction (Taugbøl and Peay, 2004; Souty-Grosset and Reynolds, 2009; Kozák et al., 2011). Studies contributing to a better understanding of the species’ ecology, the relationship between populations on the lineage level as well as the genetic structure are a prerequisite for the planning and implementation of conservation actions (Gouin et al., 2006; Klobučar et al., 2013; Bernini et al., 2016).

Molecular phylogenetics is a powerful tool for the identification of intraspecific lineages (phylogroups), some of which are of high conservation priority due to their uniqueness and high diversity (so called Evolutionarily Significant Units (ESU) sensu Moritz, 1994). In this regard, previous studies often provide evidence that present-day distributions of European freshwater organisms are mainly a consequence of Pre-Pleistocene paleohydrological and paleogeological events together with Pleistocene climatic shifts (Rögl, 1998; Verovnik et al., 2005). The occurrence of distinct phylogenetic lineages in Europe and

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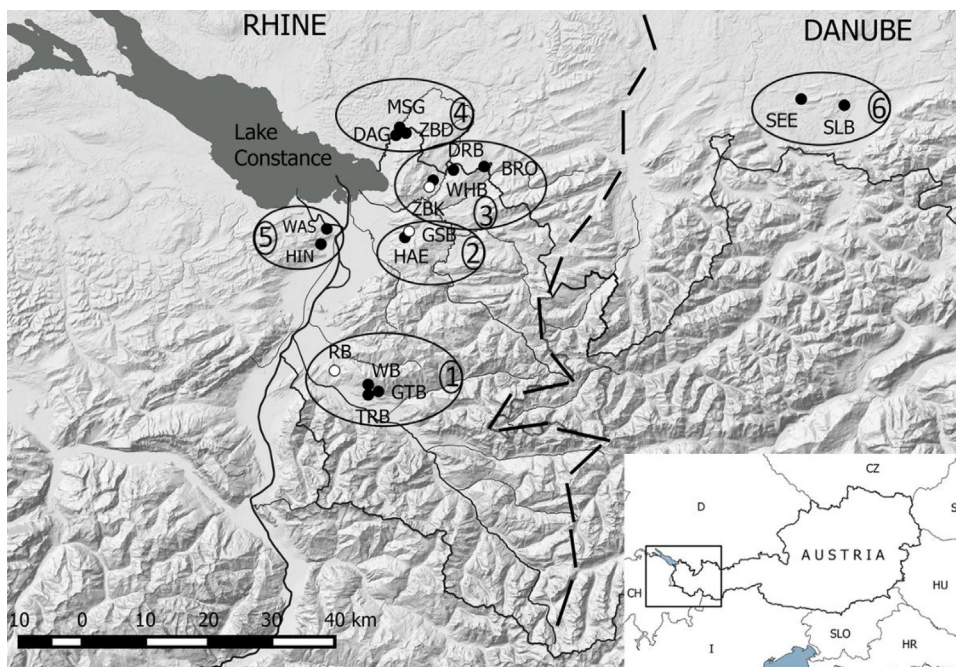


Fig. 1. Study area and sampling sites. Black dots indicate the sites included into both analysis of COI gene sequence and microsatellites, white dots indicate the sites included into analysis of microsatellites only. Dashed line indicates border between Rhine and Danube catchment. Circles indicate the 6 population clusters used for AMOVA based on local river catchments. Abbreviations for populations are given in Table 1. Small map: Austria with surrounding countries, CH = Switzerland, D = Germany, CZ = Czech Republic, SK = Slovakia, HU = Hungary, HR = Croatia, SLO = Slovenia, I = Italy.

their distribution are assumed to be shaped by the historic cycles of warming and cooling periods and subsequent recolonizing processes (Hewitt, 1999). Central European stone crayfish populations show a low lineage diversity consisting of a few dominating haplotypes (Schubart and Huber, 2006; Petrusek et al., 2017), whereas Southeast Europe is considered the hotspot of diversity with several deeply divergent phylogroups (Trontelj et al., 2005; Klobučar et al., 2013). Nevertheless, in Central Europe local patches of increased inter-population diversity have been detected and are assumed to reflect potential micro-refugia during one or more glaciation events and thus deserve special attention concerning their conservation (Schubart and Huber, 2006). However, based on these local findings no conclusions can be drawn with regard to other regions in the European Alps. In fact, the diversity and differentiation of the remaining stone crayfish populations in western Austria as well as their genetic relationship to populations in neighbouring regions is still completely unknown.

Apart from phylogenetics, the genetic diversity within and differentiation among populations is pivotal for the planning of conservation measures on the local and regional level. As most of the remaining Central European stone crayfish populations are restricted to small and isolated running waters at high altitude (Bohl, 1999; Füreder et al., 2006; Chucholl and Schrimpf, 2015), they can display signs of genetic structure and inbreeding depression due to genetic drift and a lack of gene flow. While inbreeding depression can negatively affect fitness, the artificial mixing of populations can cause outbreeding depression (Moritz, 1999). Thus, the identification of regional management units is of high relevance for conservation measures such as reintroduction or restocking. In contrast to ESUs, which require reciprocal monophyly of mtDNA alleles, such management units represent a population or group of populations with significant divergence of allele frequencies at nuclear or mitochondrial loci on which conservation actions are applied on the regional scale (Moritz, 1994). Within such management units, highly potential populations in terms of population size and gene pool characteristics are to be identified as suitable donor populations for reintroduction and restocking (Begg et al., 1999; Souty-Grosset and Reynolds, 2009; Kozák et al., 2011). However, we lack understanding of the population genetic structure of *A. torrentium* and thus the identification of such suitable stocks is highly challenging.

Despite the ongoing advance in molecular techniques, traditional morphometric methods are advantageous not only for the distinction of

different crayfish species (Sint et al., 2006), but also subspecies (Bertocchi et al., 2008) and populations (Grandjean et al., 1997; Sint et al., 2006; Maguire et al., 2017). Comparison between phenotypic and genetic differentiation of crayfish populations can reveal inconsistencies (Bech et al., 2016; Mijošek et al., 2017). Knowing that phenotypic traits are to some degree determined by genetic information (Vogt et al., 2008), but can also exhibit phenotypic plasticity linked to environmental factors (Begg and Waldman, 1999), such a discordance of genetics and morphometrics is not unexpected. Therefore, combining genetic and morphometric tools in the evaluation of population relatedness is encouraged (Scalici and Bravi, 2012).

The aim of our study was thus to assess the genetical and phenotypic relationship of the remaining endangered stone crayfish populations in the Austrian Federal State of Vorarlberg to populations of neighbouring regions in eastern Switzerland and southern Germany as well as to evaluate their genetic diversity and population differentiation. Due to their fragmented distribution across the northern Alps, characterised by numerous valleys separated by mountain ranges, we hypothesised that 1) this region bears micro-refugia of high relevance for future conservation plans, 2) populations show signs of genetic and phenotypic differentiation due to their prolonged isolation, and 3) due to potential differences in the habitats, inconsistencies between phenotypic and genetic differentiation will be detected. With the presented study, we attempt to provide a valuable guidance of how phylogeographic, population genetic and morphometric analysis can be integrated to provide guidance for urgently needed species conservation plans.

2. Materials and methods

2.1. Study area, study sites and sampling

The study was conducted in the Austrian Federal State Vorarlberg, in the Swiss Canton Sankt Gallen and in the German Free State Bavaria as part of a stone crayfish conservation program in Vorarlberg (Fig. 1). All sampled populations inhabited small to mid-size headwater streams located in a mountainous to hilly landscape.

Analyses of mtDNA (COI) were performed in order to assess the phylogenetic position of *A. torrentium* populations from Vorarlberg. Sampling was conducted in Vorarlberg (10 sites, 2009), Sankt Gallen (2

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