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Metapopulations in temporary streams – The role of drought–flood cycles in promoting high genetic diversity in a critically endangered freshwater fish and its consequences for the future

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ABSTRACT

Genetic factors have direct and indirect impacts in the viability of endangered species. Assessing their genetic diversity levels and population structure is thus fundamental for conservation and management. In this paper we use mitochondrial and nuclear markers to address phylogeographic and demographic data on the critically endangered *Anaecypris hispanica*, using a broad sampling set which covered its known distribution area in the Iberian Peninsula. Our results showed that the populations of *A. hispanica* are strongly differentiated (high and significant Φ_{ST} and F_{ST} values, corroborated by the results from AMOVA and SAMOVA) and genetically diversified. We suggest that the restricted gene flow between populations may have been potentiated by ecological, hydrological and anthropogenic causes. Bayesian skyline plots revealed a signal for expansion for all populations (t_{MRCA} between 68 kya and 1.33 Mya) and a genetic diversity latitudinal gradient was detected between the populations from the Upper (more diversified) and the Lower (less diversified) Guadiana river basin. We postulate a Pleistocenic westwards colonization route for *A. hispanica* in the Guadiana river basin, which is in agreement with the tempo and mode of paleoevolution of this drainage. The colonization of River Guadalquivir around 60 kya with migrants from the Upper Guadiana, most likely by stream capture, is also suggested. This study highlights the view that critically endangered species facing range retreats (about 47% of its known populations have disappeared in the last 15 years) are not necessarily small and genetically depleted. However, the extinction risk is not negligible since *A. hispanica* faces the combined effect of several deterministic and stochastic negative factors and, moreover, recolonization events after localized extinctions are very unlikely to occur due to the strong isolation of populations and to the patchily ecologically-conditioned distribution of fish. The inferred species distribution models highlight the significant contribution of temperature seasonality and isothermality to *A. hispanica* occurrence in Guadiana environments and emphasize the importance of stable climatic conditions for the preservation of this species. Given the strong population structure, high percentage of private haplotypes and virtual absence of inter-basin gene flow we suggest that each *A. hispanica* population should be considered as an independent Operational Conservation Unit and that *ex-situ* and *in-situ* actions should be conducted in parallel to allow for the long-term survival of the species and the preservation of the genetic integrity of its populations.

1. Introduction

Freshwater fish are amongst the most endangered groups of vertebrates (Duncan and Lockwood, 2001). It is estimated that 36–48% of the freshwater fish species assessed for the IUCN Red List are facing extinction in a near future (Carrizo et al., 2013). The family Cyprinidae comprises more than 2420 species, included in 220 genera (Nelson, 2006). In the Iberian Peninsula, located in the Mediterranean Basin hotspot (Myers et al., 2000), the native freshwater fish fauna comprises 11 cyprinid genera and more than 36 species, 31 of which are endemic (Doadrio et al., 2011a).

The Iberian fluvial network is characterized by a high number of Mediterranean-type Rivers, with autumn–winter floods and strong

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summer droughts (e.g. Gasith and Resh, 1999; Alvarez Cobelas et al., 2005). These very strong seasonal fluctuations in water volume and flow regime mean that many water bodies become transformed in a series of more or less disconnected pools, which act as summer refugia for fish until the next rains. In these pools, large numbers of fish are forced to congregate and are subjected to an increased probability of being predated, high competition for the limited space and food available, low concentration of oxygen, high temperature when the water level becomes very low, and a higher probability of being affected by infectious diseases (Magoulick, 2000; Blanco-Garrido et al., 2003; Mathews & Marsh-Mathews, 2003; Magoulick and Kobza, 2003; Dekar and Magoulick, 2007). Thus, summer droughts are often responsible for population fragmentation and massive kills which cyclically affect the structure of freshwater fish communities in Mediterranean-type Rivers (Prenda and Gallardo, 1996; Pires et al., 1999; Magalhães et al., 2002), with particularly dramatic consequences for already endangered and genetic depleted species. Moreover, Iberian freshwater fish face the direct and indirect effects of several anthropogenic mediated threats, namely pollution, damming, habitat destruction and proliferation of exotic species. As a consequence, native populations are suffering a generalized decline (e.g. García-Berthou and Moreno-Amich, 2000; Clavero et al., 2010; Doadrio et al., 2011a; Maceda-Veiga, 2013) and at least 64% of native Iberian cyprinids have been classified as vulnerable, endangered or critically endangered according with the IUCN criteria (Doadrio, 2001; Cabral et al., 2005; Doadrio et al., 2011a).

Anaecypris hispanica is one of the most threatened species of the Iberian Peninsula (Collares-Pereira and Cowx, 2001). This small size cyprinid (up to 8 cm TL) is endemic to some tributaries of the Iberian Guadiana and Guadalquivir Rivers (Fig. 1A) which are Mediterranean-type streams, with a typical intermittent regime, being reduced to pools during the summer (Alvarez Cobelas et al., 2005). *A. hispanica* prefers small and shallow streams, with low to medium flow waters, aquatic and riparian vegetation and coarse substratum (Collares-Pereira et al., 2000b; Ribeiro et al., 2000; Blanco-Garrido et al., 2009). It was never captured in reservoirs nor in the Guadiana main course, and generally occurs in the upstream sections of tributaries (Barrachina et al., 1989; Collares-Pereira et al., 2000a, 2000b; Filipe et al., 2002; Cabral et al., 2005). Its diet is mainly composed of detritus, filamentous algae and invertebrates (Rodriguez-Jimenez, 1987; Collares-Pereira et al., 1998). Some features of its life history seem to represent a "r" strategy of adaptation to the highly unstable environmental conditions of intermittent streams, namely, a short life cycle (three years maximum), small body size, rapid growth in the first year of life, early sexual maturation (at age 1) and fractional egg laying (Collares-Pereira et al., 2000a, 2000b; Ribeiro et al., 2000; Doadrio et al., 2011b; Carrapato and Ribeiro, 2012).

Although relatively abundant in the 1970s (Collares-Pereira and Almaça, 1979), *A. hispanica* has suffered a dramatic decline in its abundance and distribution range (Collares-Pereira et al., 1999, 2000a, 2000b, 2002) and is presently occasionally caught in some restricted stretches, usually in low numbers (Cardoso and Carrapato, 2008, 2010). This depletion has been more accentuated in the last twenty years, mainly due to the construction of several large sized reservoirs in the Guadiana river basin, receding water levels, habitat degradation, and increasing pollution and eutrophication (Collares-Pereira et al., 2000c). The proliferation of exotic species also represents a major threat to the survival of *A. hispanica* due to predation, competition for food and space, transmission of diseases, and hybridization (Collares-Pereira et al., 2000c; Collares-Pereira and Cowx, 2001; Doadrio, 2001; Blanco-Garrido et al., 2009; Hermoso et al., 2010). The potential hybridization with the phylogenetically close *Alburnus alburnus* (widely spread in the Iberian Peninsula), for instance, has been pointed by several authors as being highly probable, with dramatic consequences to the taxonomic integrity of *A. hispanica* (e.g. Robalo et al., 2006; Vinyoles et al., 2007; Perea et al., 2010; Almodóvar et al., 2012; Almeida et al., 2014). Under this scenario, *A. hispanica* is considered to be "Critically Endangered" according to the last version of the Portuguese Red Book (Cabral et al., 2005) and "Endangered" according to the Spanish Catalog of Endangered species (Law 139/2011; Doadrio et al., 2011b) and to the IUCN Red List (Crivelli, 2006). It

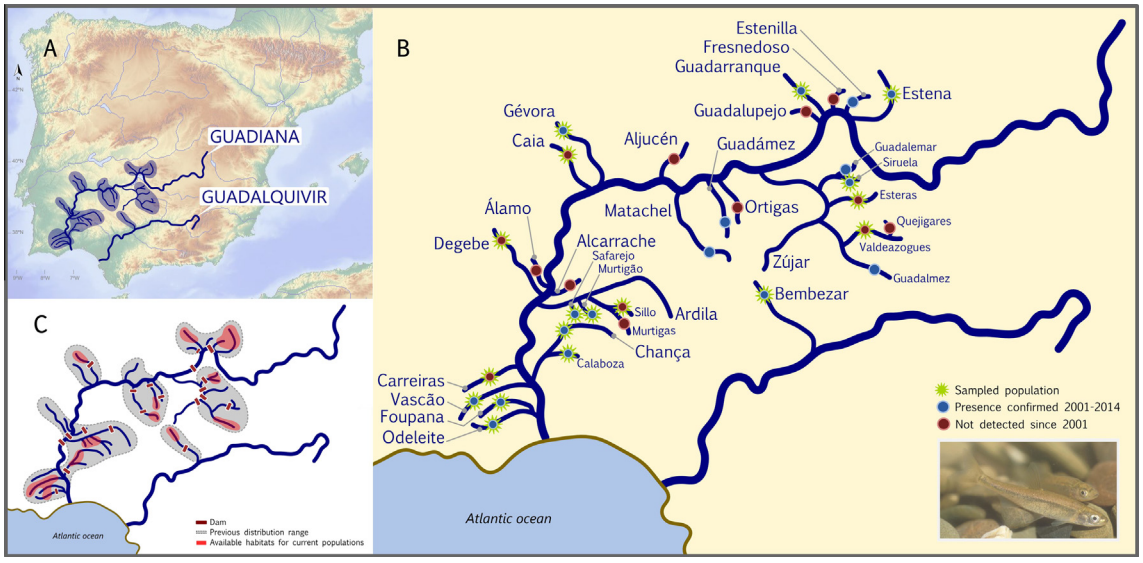

Fig. 1. (A) Distribution range of *A. hispanica*. (B) Location of the *A. hispanica* samples used in this study and status of all of the known populations of the species: populations for which the field work conducted between 2001 and 2014 confirmed the presence of the species and those for which no individuals were collected since 2001 (Cardoso and Carrapato, 2010; Doadrio et al., 2011b). (C) Currently available habitats for *A. hispanica* populations considering that this species is absent from deep water habitats, namely in main courses and dam reservoirs (Cardoso and Carrapato, 2010; Doadrio et al., 2011b). When compared to the previously known distribution range, the effective area occupied by the species is smaller due to the avoidance of main river stretches, dam reservoirs and river stretches downstream of the dam walls. Also, the effective area suffered a reduction in the last 15 years, with the virtual extinction of the species from several sub-basins (Carreiras, Degebe, Álamo, Alcarrache, Murtigas, Caia, Aljucén, Ortigas, Guadalupejo, Fresnedoso, Esteras and Valdeazogues).