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Cryptic speciation of Greek populations of the freshwater shrimp genus *Atyaephyra* de Brito Capello, 1867 (Crustacea, Decapoda), evidence from mitochondrial DNA

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Abstract

The freshwater shrimp genus *Atyaephyra* de Brito Capello, 1867 is a complex of cryptic species with morphologically overlapping features, which has led to invalid identifications until recently. The genus is distributed around the entire Mediterranean Basin and surrounding areas. In 2009 we used a molecular approach with 16S and Cox1 genes to identify the European and African populations and to show the existence of different species and groups of haplotypes (García Muñoz *et al.* 2009). Christodoulou *et al.* (2012) recently identified four species in Greece (*Atyaephyra stankoi*, *A. thyamisensis*, *A. strymonensis* and *A. acheronensis*) based on morphology and some partial sequences of Cox1. In the present study we analyse several Greek populations of *Atyaephyra* spp. using morphology and two mitochondrial markers (16S and Cox1). Our molecular data confirm the taxonomic validity of *A. stankoi*, *A. thyamisensis* and *A. strymonensis* but show that *A. acheronensis* should be considered a synonym of *A. desmarestii*. The limited distribution of *A. desmarestii* in only a few rivers in the Ionian region is probably the result of a posterior introduction from neighbouring European areas. After the genetic identification and as a consequence of the high variability observed within species and between rivers, mainly in *A. thyamisensis*, we re-describe this species and provide morphological data for the other species. We also give information on the chromatophore pattern in larvae, which could be very useful in the future for recognizing species and their distribution. The distribution of Greek *Atyaephyra* species seems to be related to the very complex paleogeographical history of the Paratethys and Mediterranean Sea in the Balkan Peninsula. Three areas can be recognized in relation to the distribution of these species: a global region, a western region separated from the eastern region by the Pindos Mountains, and a Macedonia-Thracian region.

Key words: *Atyaephyra* complex, Atyidae, 16S, Cox1, cryptic species, Greece

Introduction

Taxonomic difficulties in the identification of the freshwater monotypic shrimp genus *Atyaephyra* de Brito Capello, 1867 are well documented (García Muñoz *et al.* 2009). They are related to the high intrapopulational variability in the morphological characters used for identifying the species and subspecies (Bouvier 1913, 1925; Holthuis, 1961; Kinzelbach and Koster, 1985; Al-Adhub, 1987; Gorgin, 1996; Anastasiadou *et al.* 2004), such as the form of the endopod of the first male pleopod, the shape and denticulation of the rostrum, and the pterygostomian angle, among others. There are morphologically different populations of a particular species in the same area that seem to be the result of different geographical, hydrological and environmental conditions (e.g. Dhaouadi-Hassen & Boumaiza 2005, Dhaouadi-Hassen *et al.* 2006, for Tunisian rivers).

Five subspecies or species have been distinguished for the Mediterranean and surrounding areas: *A. d. desmarestii* (Millet, 1831), *A. d. orientalis* Bouvier, 1913 (raised to full species rank by Christodoulou *et al.* 2012), *A. d. stankoi* Karaman, 1972, *A. d. mesopotamica* Al-Adhub, 1987 and *A. rosiana* de Brito Capello, 1867. The last, resurrected as a valid species by Anastasiadou *et al.* (2008), was invalidated by a posterior molecular study (García Muñoz *et al.* 2009).

desmarestii. Therefore, this species coexists with *A. thyamensis* in this river. The presence of *A. desmarestii* in these northwestern locations is probably due to a later introduction from northern European areas, considering that the same haplotypes of *A. desmarestii* have been found in the specimens from Slovenia and Croatia. *Atyaephyra stankoi* is also a Greek endemism but with a wider distribution; it has been collected in the western area of the Macedonia-Thessali or Thrace region (Richios River), the Vadar region (Axios, Loudias and Pineios Rivers), and the Ionian Drainages or South Adriatic Ionian (lakes Amvrakia, Oxeros and Lissimákia, and Evino and Alfeios Rivers), with two different haplotypes separated by the Pindos Mountains. Finally, *A. strymonensis* is confined to a well-defined area (Nestos and Strymonas Rivers) in the Thracian-East Macedonia division.

Three regions can be recognized in relation to the distribution of the genus *Atyaephyra*, a wide north-south distribution, a western region separated from the eastern region by the Pindos Mountains, and a Macedonia-Thracian region (Fig. 1). The comparison of the above distributions with those of some other Greek freshwater decapod species, such as the *Potamon* species (Maurakis *et al.* 2004), shows that there are some similarities; for example, *P. ibericum* has a similar distribution to *A. strymonensis*, while *P. fluviatile* has a wider distribution in the east and west, which is perhaps comparable to that of *A. stankoi*.

There are numerous biogeographical studies of fish from Greece (Doadrio & Carmona 1998, Durand *et al.* 1999, Zardoya *et al.* 1999, Durand *et al.* 1999, Freyhof *et al.* 2006) that show different distributions for the species and subspecies. Some of these are quite similar to distributions we determined in this study for the genus *Atyaephyra*. These and other studies (Bianco 1990, Economidis & Banarescu 1991, Durand *et al.* 1999, Reyjol *et al.* 2007) state that the connection between the Balkan Peninsula and Anatolia and its separation from central and eastern Europe by the Parathetys (until the late Pliocene) could have led to closer ties with the eastern fauna than with the European fauna. The differentiation and dispersion could have occurred in the late Miocene - Pliocene in relation to the Pindic Cordillera, the compressional tectonic period, the Messinian crisis and Pleistocene glaciations. However, in order to reach a conclusion on the origin of the species of the genus *Atyaephyra* within the Balkan region, it is first necessary to carry out a more exhaustive molecular study to obtain more information on the distribution of these species.

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