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Molecular systematics and biogeography of the western Mediterranean stonefly genus *Tyrrhenoleuctra* (Insecta, Plecoptera)

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Abstract

The stonefly genus *Tyrrhenoleuctra* includes species living in western Mediterranean temporary freshwater streams, sometimes also at sea level, a very unusual habitat for most Plecoptera. Traditional morphological approaches proved unsuccessful in drawing both taxonomic and phylogenetic patterns, thus hampering discussion of biogeographical patterns for this interesting group. We aimed at: (a) assessing the taxonomic status of populations of *Tyrrhenoleuctra* covering the geographic range of the genus; (b) studying the phylogenetic relationships among the recognized species; and (c) describing biogeographic patterns. We used phylogenetic analyses to infer the phylogenetic history of this group of stoneflies based on a combined data set of 1666 bp including fragments of the 12S ribosomal (*12S*) and cytochrome oxidase I (*CO-I*) mtDNA genes, with maximum likelihood and Bayesian methods. Two main clades have been identified: a Sardo-Corsican one, including *Tyrrhenoleuctra zavattarii*, and an Ibero-Maghrebian one including four lineages of unkown taxonomic rank from the Balearic Islands (Maiorca), from northern Africa (Ceuta) and southernmost Spain (Algeciras), and a complex preliminarily referred herein to *T. minuta* (Klapálek, 1901), which includes two lineages, one from Cordoba, and one from Sierra de Grazalema (El Cerro) and Portugal (Tellhares) respectively. Dating the nodes by fixing the split of the Ibero-Maghrebian clade from the Sardo-Corsican one at 29 million years ago (Mya), yielded dates referring to the major geological events in the Mediterranean basin. Estimated molecular evolutionary rates ranged from 0.02–0.09% per million years (my) in the *T. zavattarii* lineages, to 0.2–0.7% per MY in the Ibero-Maghrebian clade. The phylogenetic pattern emerged from the present study is congruent with the known paleo-history of the western Mediterranean basin, with the divergence of the two main *Tyrrhenoleuctra* lineages corresponding to the split of the Sardo-Corsican microplate from the Iberian block. Vicariance events have characterized the history of this stonefly group along its entire biogeographical history. Surprisingly low evolutionary rates, previously supposed by Fochetti (1991, 1994) and Fochetti et al. (2004) based on nuclear markers (allozymes), have been herein found also in mitochondrial markers.

Key words: Phylogeny – Leuctridae – cytochrome oxidase subunit-1 – 12S – evolutionary rates – west Mediterranean – palaeogeography

Introduction

The stonefly genus *Tyrrhenoleuctra* Consiglio, 1957, has a western Mediterranean distribution with populations occurring in temporary freshwater streams, sometimes occurring even at sea level, a very unusual habitat for most Plecoptera. Its monophyly is unquestionably supported by features of the larvae (e.g. separation of abdominal urites V–VII) and of the adult genitalia (e.g. peculiar epiproct) (Consiglio 1956; Sezzi 2001). Traditionally, it included three species (Fochetti 1994; Sánchez-Ortega and Tierno de Figueroa 1996): *T. minuta* (Klapálek, 1901) (Iberian Peninsula, Western Maghreb, Balearic Islands: the type species of the genus), *Tyrrhenoleuctra tangerina* (Navás, 1922) (Spain, North Africa), and *Tyrrhenoleuctra zavattarii* (Consiglio 1956) (Corsica and Sardinia).

These three species have been described on the basis of genitalia morphology, studied on a few samples from allopatric populations. However, genitalia analyses of individuals from a wider geographic area revealed the occurrence of extensive variation in nearly all traditional characters, with levels of intraspecific variability being as high as the interspecific ones (Sezzi 2001; Tierno de Figueroa and Fochetti 2001a). This finding has been further corroborated by the analysis of genetic data based on allozymes (Fochetti et al. 2004), which suggested that the population from Balearic Islands may deserve higher taxonomic rank.

The distribution of *Tyrrhenoleuctra* may be interpreted as a relict of a Messinian fauna (latest Miocene) whose distribution has been shaped by the complex geological events which occurred in the Mediterranean area during the Tertiary, such as the Oligo-Miocene rifting of the Mediterranean microplates and the Messinian salinity crisis (Alvarez 1972; Hsü and Bernoulli 1978).

Different genes have been used to evaluate the evolutionary histories of distant/closely related arthropod species or populations (Caterino et al. 2000). Highly conserved genes, such as most ribosomal RNAs, have been used successfully as molecular markers for distant-related taxa (Pelandakis and Solignac 1993), while more rapidly evolving genes have been used for comparisons of closely related taxa (Schlötterer et al. 1994; Malloch et al. 2001; Weekers et al. 2001; Young and Coleman 2003).

In the present study we have used sequences from two mtDNA genes, CO-I and 12S, with a final alignment of over 1600 nucleotide positions to:

(1) Assess the taxonomic and phylogenetic status of populations of *Tyrrhenoleuctra* covering the geographic range of the genus.
(2) Compare the phylogenetic patterns based on mtDNA data with the ones from morphology and allozyme data.
(3) Describe the biogeographic patterns and discuss the timing of origin of the different phyletic lineages in light of the two most likely vicariant hypothesis described above.

Materials and Methods

Taxon sampling and specimen collection

Specimens and source of sampling species are reported in Table 1 and Fig. 1, with acronyms used in the paper. We have included as far as possible specimens from topotypic populations or from populations very close to the topotypic one.