

## ITS2 in calanoid copepods: reconstructing phylogenetic relationships and identifying a newly introduced species in the Mediterranean

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(Received 5 July 2016; accepted 18 December 2016)

### Abstract

Phylogenetic inference and molecular taxonomy are becoming increasingly important approaches to classical morphological systematics and marine ecology. The number of molecular markers suitable for such goals is quite high, but general use restricts the list to a few of them, mainly mitochondrial (namely cytochrome c oxidase subunit I, COI and Cytochrome b), especially in copepods. The ribosomal cistronic regions have been widely used for broad phylogenetic analyses in different taxa. Among them, the internal transcribed spacers (ITS rDNA) are powerful tools for phylogenetic reconstructions at the different taxonomic levels, although not yet extensively used for copepods. In the present work, we tested the suitability of ITS2 rDNA marker to reconstruct the phylogenetic relationships of calanoid copepods using sequences retrieved from GenBank, complementing the phylogenetic positions of the species studied with their morphological and ecological traits. Through ITS2 rDNA we provided the first molecular evidence for the invasive calanoid *Pseudodiaptomus marinus* from the Mediterranean Sea (Lake Faro, Sicily, Italy), and compared it with the GenBank ITS2 sequences for *P. marinus* from Korea and other calanoid species. The divergence of the sequences of our *P. marinus* from those of Korean specimens was quite prominent (4.4%) and allowed us to hypothesise either a new *forma* living in the Mediterranean or a cryptic species. This study highlights the appropriateness of ITS2 for phylogenetic reconstructions and species identification, as well as for barcoding, meta-barcoding and phylogeographic approaches, and evidences the need for a more thorough knowledge of ribosomal regions in copepods from different sites.

**Keywords:** Mediterranean Sea, phylogenetic reconstruction, *Pseudodiaptomus marinus*

### Introduction

The “insects of the sea”, as copepods are colloquially called (Huys & Boxshall 1991), are the most abundant metazoans on Earth (Hardy 1970; Wiebe et al. 1992), outnumbering insects, which outdo copepods only in species number (Schminke 2007). Recent estimates set the number of species at 14,710 (Walter & Boxshall 2016). They inhabit any aquatic environment, from deep-ocean trenches to mountain lakes (Huys & Boxshall 1991), and are a key component of the pelagic food web (Bradford-Grieve et al. 1999).

Owing to their ecological role, it is thus of paramount importance to properly identify copepod species and investigate their phylogenetic relationships.

However, due to the abundance and extreme diversity of this class, and considering the possibility of a large number of cryptic species (Blanco-Bercial et al. 2014 and references therein), the morphological identification is often difficult to accomplish. Classical taxonomic identification analyses are now being increasingly supported by molecular techniques, providing an invaluable contribution to the exact assessment of species boundaries (e.g. Bucklin et al. 2010, 2011; Blanco-Bercial et al. 2014). To date, the integration of these two methods represents the most complete approach for taxonomical identification (Blanco-Bercial et al. 2014).

Copepod phylogeny and species identification have been proficiently carried out using molecular markers

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