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Phylogeny of the Paracalanidae Giesbrecht, 1888 (Crustacea: Copepoda: Calanoida)

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ABSTRACT

The Paracalanidae are ecologically-important marine planktonic copepods that occur in the epipelagic zone in temperate and tropical waters. They are often the dominant taxon – in terms of biomass and abundance – in continental shelf regions. As primary consumers, they form a vital link in the pelagic food web between primary producers and higher trophic levels. Despite the ecological importance of the taxon, evolutionary and systematic relationships within the family remain largely unknown. A multigene phylogeny including 24 species, including representatives for all seven genera, was determined based on two nuclear genes, small-subunit (18S) ribosomal RNA and Histone 3 (H3) and one mitochondrial gene, cytochrome *c* oxidase subunit I (COI). The molecular phylogeny was well supported by Maximum likelihood and Bayesian inference analysis; all genera were found to be monophyletic, except for *Paracalanus*, which was separated into two distinct clades: the *Paracalanus aculeatus* group and *Paracalanus parvus* group. The molecular phylogeny also confirmed previous findings that *Mecynocera* and *Calocalanus* are genera of the family Paracalanidae. For comparison, a morphological phylogeny was created for 35 paracalanid species based on 54 morphological characters derived from published descriptions. The morphological phylogeny did not resolve all genera as monophyletic and bootstrap support was not strong. Molecular and morphological phylogenies were not congruent in the positioning of *Bestiolina* and the *Paracalanus* species groups, possibly due to the lack of sufficient phylogenetically-informative morphological characters.

1. Introduction

1.1. Taxonomy of the Paracalanidae

The Paracalanidae is a family of small (0.36–1.36 mm; Razouls et al., 2005–2012) marine epipelagic calanoid copepods with a worldwide distribution throughout temperate and tropical waters. They often co-occur and can be very abundant in coastal, shelf, and oceanic regions (e.g. Araujo, 2006; Bowman, 1971; Bradford, 1978; Cornils et al., 2010; Hwang et al., 2006; McKinnon et al., 2008; Nishikawa et al., 2007; Siokou-Frangou, 1996). Despite their ecological importance, the evolutionary and systematic relationships within the family remain largely unknown, with contradictory results from numerous studies since Giesbrecht (1893) erected the family Paracalanidae, which included the genera *Acrocalanus*, *Calocalanus* and *Paracalanus* (Fig. 1).

Currently, the family comprises a total of 83 species in seven genera: *Acrocalanus*, *Bestiolina*, *Calocalanus*, *Delibus*, *Mecynocera*, *Paracalanus* and *Parvocalanus* (Razouls et al., 2005–2012). The genus *Bestiolina* (Andronov, 1991) was erected to rename *Acrocalanus inermis*, which differed from other *Acrocalanus* species in that the third endopodite segments of swimming legs 3 and 4 bear 6 (rather than 7) setae. The genus *Paracalanus* was separated by Sewell (1929) into two species groups differing in the segmentation of the antennules, the form of the spermatheca and the ornamentation of swimming legs; these are: (1) *Paracalanus aculeatus* group (*P. aculeatus* and *P. denudatus*); and (2) *Paracalanus parvus* group (all other *Paracalanus* species known at that time). Wellershaus (1969) introduced a third group (*Paracalanus crassirostris* group), which was elevated to the genus *Parvocalanus* by Andronov (1970) and included species with a short, blunt rostrum and short terminal setae on female P5. The genus *Delibus* (Andronov, 1972a) was erected to rename *Paracalanus nudus*, which differed from all other genera by the absence of the right female fifth swimming leg.

The genera *Calocalanus* and *Mecynocera* were briefly united in the family Calocalanidae, which was erected by Bernard (1958) and separated into three genera (Fig. 1). Andronov (1970) synonymised the Calocalanidae with the Paracalanidae, since they share unique characters of the male antennae (A2), e.g., the exopod

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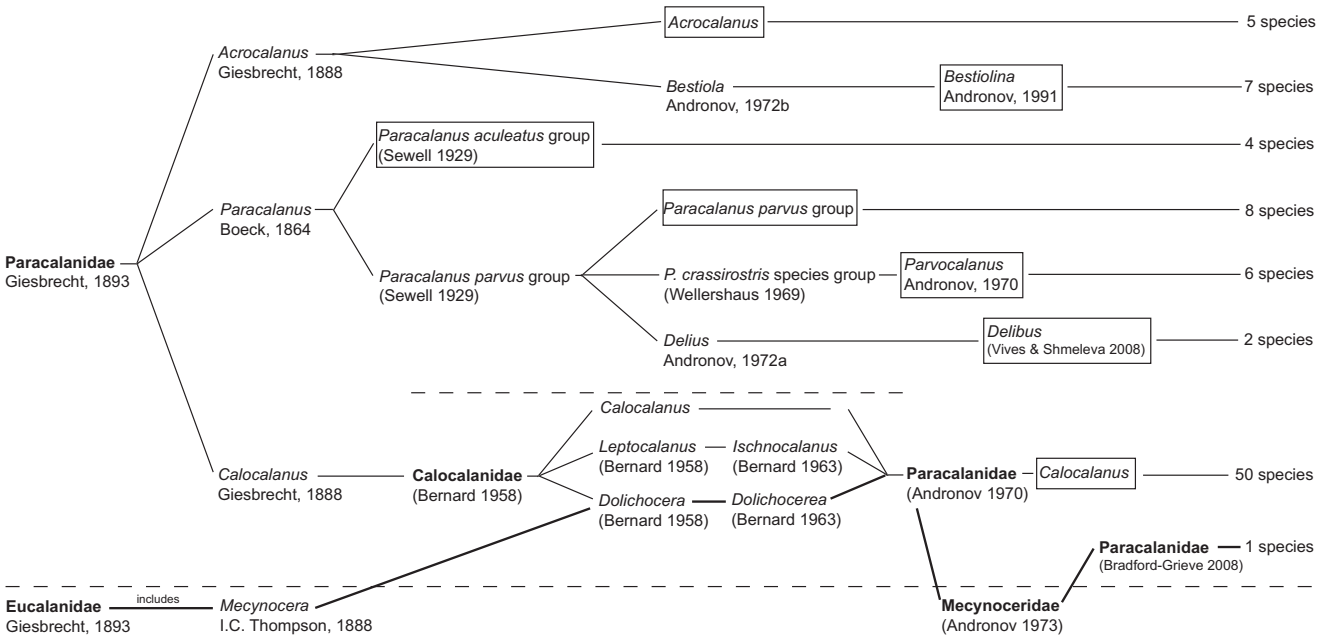

Fig. 1. Historical and present taxonomy of the Paracalanidae. Bold names show the family names. Bold lines refer to the taxonomic history of *Mecynocera*; rectangles emphasize the actual genera and in case of *Paracalanus* the species groups.

segments 1 and 2 are devoid of setae (Giesbrecht, 1893; Bradford-Grieve, 1994). Recently, *Mecynocera* was added to the Paracalanidae (Bradford-Grieve, 2008) due to shared unique characters of the male second antenna, e.g., the terminal segment of the exopod has the form of a small knob and bears no terminal setae). Previously, *Mecynocera* had been placed with the Eucalanidae and the Calocalanidae, and also in a separate family, the Mecynoceridae, due to its short female terminal antennal exopod segment and lack of one inner seta of endopod segment 2 of leg 2–4 (Andronov, 1973).

1.2. Molecular systematic approaches

Mitochondrial marker genes have been used regularly to investigate the phylogeny of marine crustaceans, including copepods (Blanco-Bercial et al., 2011; Bucklin and Frost, 2009; Goetze, 2003; Machida et al., 2006; Taniguchi et al., 2004). These genes are easy to extract from tissues due to their high copy number; however, they are haploid and maternally inherited, and thus represent only one quarter of the population diversity (Moritz et al., 1987). The addition of independent nuclear genes may lead to better interpretation of phylogenetic relationships. Nuclear genes typically evolve more slowly than mitochondrial genes, due to a lower substitution rate (Moriyama and Powell, 1997). Nuclear ribosomal genes have been widely used by themselves or combined with other genes or morphological characters for phylogeny reconstruction in arthropods in general and copepods in particular (Blanco-Bercial et al., 2011; Braga et al., 1999; Figueroa, 2011; Jørgensen et al., 2010; Marrone et al., 2013; Marszalek et al., 2009; Thum, 2004; Wyngaard et al., 2010). Nuclear, protein-coding genes have also been used recently for the same purpose with high success (Meusemann et al., 2010; Regier et al., 2008, 2010; von Reumont et al., 2012).

The combined use of many genes (nuclear ribosomal, nuclear protein coding and mitochondrial) has been in debate, because the phylogenies of different marker genes are not always congruent (Cunningham, 1997; Leigh et al., 2011; Moore, 1995). However, this approach has proven successful in many cases. The addition of genes that evolve rapidly does not hide the phylogenetic signal provided by more conserved ones (Fisher-Reid and Wiens, 2011; Wenzel and Siddall, 1999). Furthermore, additional genes may improve the general support for phylogenies, even when some nodes are not supported in the single-gene analyses (Cameron et al., 2004; Fisher-Reid and Wiens, 2011; Gatesy et al., 1999). Multigene phylogenetic approaches have been successfully applied in previous studies of copepods (e.g. Blanco-Bercial et al., 2011; Huys et al., 2007; Thum and Harrison, 2009; Wyngaard et al., 2010).

Recently it has been shown that the comparison of molecular and morphological characters (Adamowicz et al., 2007; Böttger-Schnack and Machida, 2011; Bucklin and Frost, 2009), as well as the combination of both kinds of characters in a single phylogenetic reconstruction (Bradford-Grieve and Blanco-Bercial, 2011; Bucklin and Frost, 2009; Schnabel et al., 2011), have resulted in plausible and robust phylogenies improving, in many cases, the results obtained with each set of data independently.

This study aims to establish a comprehensive molecular phylogeny of the Paracalanidae with both nuclear and mitochondrial genes. The resulting molecular phylogeny of the Paracalanidae is compared to a phylogeny based on a morphological character set based on 14 diagnostic characters that were used by Andronov (1973) to place *Mecynocera* in a separate family and by Hiromi (1987) to distinguish between genera.

2. Methods

2.1. Molecular taxon sampling

Specimens for genetic analysis were identified from samples preserved in 95% ethanol collected from various locations (Table 1). Only females were used, because males are less abundant, harder to identify, and have not been described for all species. The samples and DNA were obtained from archived collections resulting from the Census of Marine Zooplankton (CMarZ) at the University of Connecticut (USA) or the Alfred-Wegener-Institute for Polar and Marine Research (Germany). DNA was analyzed from 24 species of all seven genera of the Paracalanidae, including *Mecynocera*. For three species, specimens were analyzed from different geographic