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New records of the rare shrimp parasite *Zonophryxus quinquedens* Barnard, 1913 (Crustacea, Isopoda, Dajidae): ecological and phylogenetic implications

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Abstract The rare dajid, *Zonophryxus quinquedens* represents the only known isopod parasiting on shrimps in Antarctic waters. In contrast to the Bopyridae, which typically live in the gill cavity of their crab host, dajid isopods are normally attached to the carapace of the parasited shrimp. Four specimens of *Z. quinquedens* Barnard, 1913 were collected in the eastern and western Weddell Sea, Antarctica, during the expeditions ANT XXI/2 in 2003/2004 and ANT XXII/3 in 2005. Molecular phylogenetic analyses, based on small subunit rRNA gene sequences, indicate a close relationship of *Z. quinquedens* to the Bopyridae. Possible ecological and physiological aspects of the parasite–host interaction are discussed.

Introduction

The scarcity of decapod crustaceans is one of the most striking biodiversity characteristics of Antarctic waters when compared to other seas (Thatje and Arntz 2004), caused by physiological as well as ecological factors (e.g., Clarke 1983; Thatje et al. 2003). On the high Antarctic continental shelf only five benthic shrimp species are known (Gorny 1999). In contrast to the decapod crustaceans, the peracarid crustaceans, especially the Amphipoda and Isopoda, have flourished in terms of diversity in Antarctic waters (e.g., Brandt 1991, 2000). Among these, parasitic forms have received little attention in the past. Parasitic isopods with a highly modified morphology, strong sexual dimorphism, and

strange life cycles, occur in the taxon Cymothoidea Wägele, 1989 (e.g., the Gnathiidae Leach, 1814). In particular the Bopyridae are important isopod parasites of decapods. Although our knowledge of their life history and physiology is extremely limited, they are known to frequently infest lithodid crabs (Roccatagliata and Lovrich 1999, and references therein). Host–parasite interactions in the Lithodidae are known from subantarctic waters, albeit records of bopyrids are still lacking from high Antarctic lithodids.

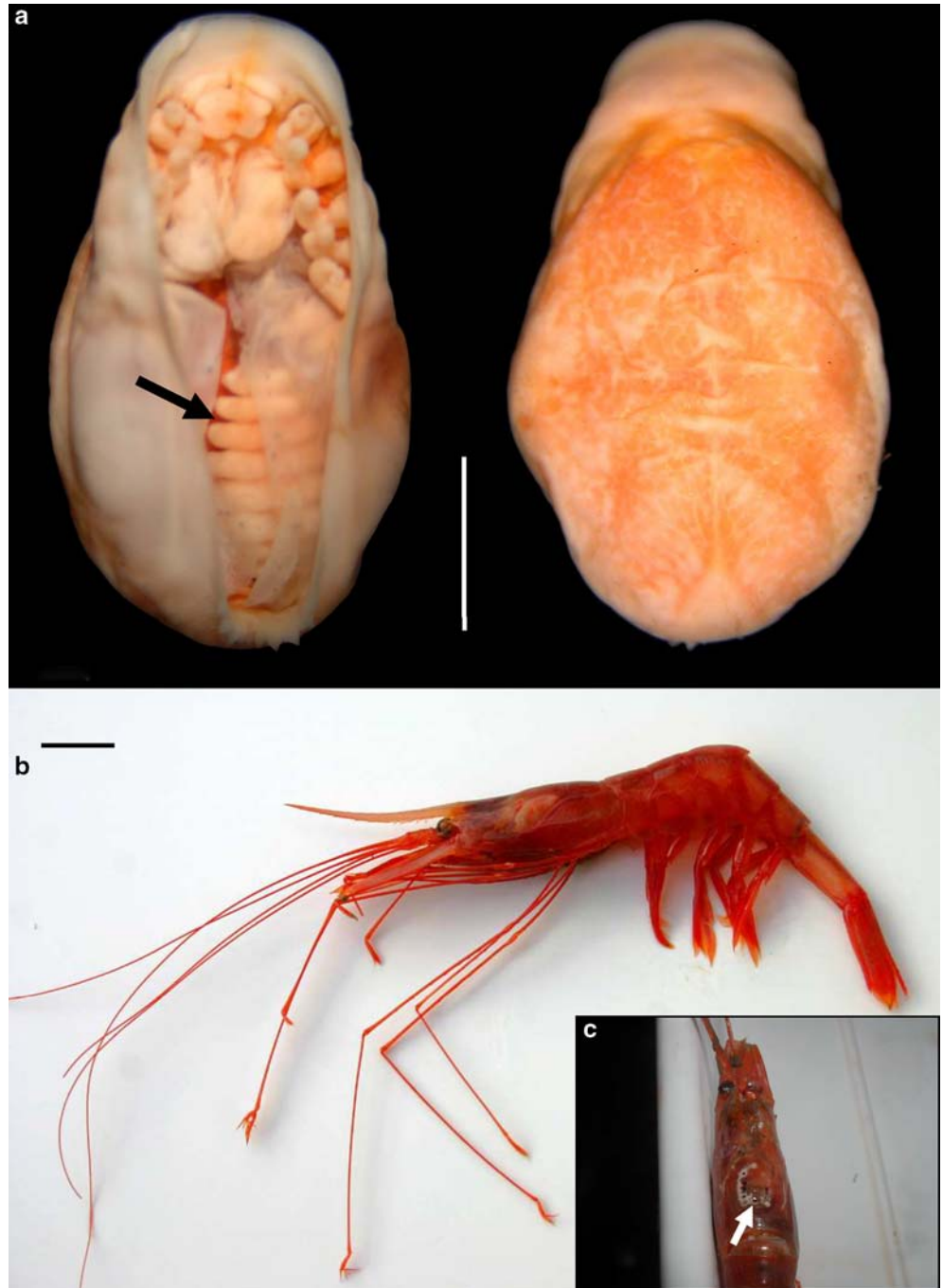
As for the Antarctic shrimp only the host–parasite interaction between the enigmatic isopod *Z. quinquedens* (Isopoda, Cymothoidea, Dajidae) (Fig. 1a) and *Nematocarcinus lanceopes* has been described so far (Brandt and Janssen 1994). Shrimps of the genus *Nematocarcinus* (see Fig. 1b) are common in Antarctic deep waters (Wägele and Sieg 1990), but specimens were found even in the Cape basin (see also Thatje et al. 2005a; Linse et al. 2005). Morphological studies indicate a close relationship of the Dajidae and Bopyridae (Wägele 1989), but the classification is still in discussion (Martin and Davis 2001; Brandt and Poore 2003). In contrast to the Bopyridae, which are known to infest their host in the branchial chamber below the carapace, the Dajidae are parasites typically attached to the carapace of euphausiids, mysids, and shrimps (see Fig. 1c), although some may also be found on the host gills or attached to the carapace of their hosts.

Within the genus *Zonophryxus*, six different species have been described from polar, temperate, and tropical waters until now: *Z. dodecapus* Holthuis, 1949 (Canary Islands), *Z. grimaldii* Koehler, 1911 (Spain), *Z. quinquedens* Barnard, 1913 (South Africa), *Z. retrodens* Richardson, 1904 (Hawaii), *Z. similes* Richardson, 1914 (Hudson Bay), and *Z. trilobus* Richardson, 1910 (Philippines). *Z. quinquedens* is currently known only from three different locations, all located in the Southern Hemisphere: 18°29'E 34°21'S (Cape Point area) at 840–1,250 m (Barnard 1913), 48°W 62°S (Powell Basin) at unknown depth (Lopretto 1983) and 05°08'W 69°58'S (eastern Weddell Sea) at 665 m (Brandt and Janssen 1994).

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Fig. 1 **a** Female and dwarf male (*arrow*) of *Z. quinquedens* Barnard, 1913 (Crustacea, Isopoda, Dajidae) in ventral (*left*) and dorsal (*right*) view, scale bar = 5 mm; **b** *N. lanceopes* (Bates 1888), lateral view, scale bar = 1 cm; **c** drilling hole (*arrow*) of a female specimen of *Z. quinquedens* on the carapace of *N. lanceopes*



The present work gives insight into the phylogenetic position of *Z. quinquedens* within the Cymothoidea using ssu rRNA gene sequences and some possible ecological and physiological aspects of the parasite–host interaction.

Material and methods

Specimens

A single female specimen of *Z. quinquedens* was separated from an epibenthic sledge sample taken in the eastern Weddell Sea (PS65/232-1: 13°56'W 71°18'S) at

900–910 m water depth during the expedition ANT XXI/2 in December 2003. In addition, one female and one male were collected in the eastern Weddell Sea (PS67/074-7: 13°58'W 71°18'S, 1,030–1,040 m depth) and one female in the western Weddell Sea (PS67/151-1: 47°07'W 61°45'S) during ANT XXII/3 (ANDEEP III) in February 2005. The animals were immediately transferred into 96% ethanol for preservation. The benthos material obtained from the epibenthic sledge (ANT XXI/2) and Agassiz trawl (ANT XXII/3) contained 74, respectively, 84 specimens of the deep-sea shrimp *N. lanceopes* (Bate 1888), known as host of *Z. quinquedens* (Brandt and Janssen 1994).

Additional sequences included in the DNA analyses were obtained from GenBank and included all available cymothoids except *Paragnathia formica* (AF255687), which is being known as being a long-branched taxon (see Dreyer and Wägele 2001 for details). The following sequences were used for outgroup comparison: Decapoda: *Astacus astacus* (AF235959), Stomatopoda: *Squilla empusa* (X01723), Isopoda (Anthuridea): *Cyathura carinata* (AF332146), and *Paranthura nigropunctata* (AF279598).

DNA extraction, PCR, and sequencing

Methods for DNA extraction, amplification (including primers), and sequencing are given elsewhere (Raupach et al. 2004). Total genomic DNA was extracted from one leg of one specimen (female, ANT XXI/2). The new ssu rDNA sequence can be retrieved from GenBank (DQ008451).

Alignment and phylogenetic analyses

All 14 ssu rDNA sequences used were aligned using CLUSTAL X on default parameters (Thompson et al. 1997), generating an alignment of 3,546 base pairs (bp). Variable regions within ribosomal RNAs, especially in isopods, can greatly vary in length, which makes it almost impossible to establishing the base homology between distantly related species (e.g., Choe et al. 1999; Dreyer and Wägele 2001). Highly variable and non-alignable regions within the alignment were identified using the secondary structure of the ssu rRNA in the decapod *A. astacus* (Wuyts et al. 2002). Therefore most parts of the expansion segments V4, V7, V9 and some other helices were excluded from further phylogenetic analyses (definitions in parentheses refer to the helix numbering of ssu rRNAs): 76–86 [E_6], 133–151/183–447 [E_8–E_11], 821–1974 [E_23/1–E23/17 = V4], 2505–3011 [E_43/1–E_43/4 = V7], and 3336–3460 [E_49 = V9]. The final alignment used for this study had 1,464 bp; both alignments are available from the authors.

The homogeneity of base frequency versus taxa was tested using the χ^2 -test implemented in PAUP*4.0b10 (Swofford 2002). Sequences were analysed using a Bayesian approach with the program MrBayes 3.1 (Huelsenbeck and Ronquist 2001), with clade support assessed by posterior probability, on default parameters. Trees were sampled every 100 generations, yielding 9,000 samples of the Markov chain after a “burn in” of 1,000 generations. The appropriate model of nucleotide substitution for the Bayesian analyses was determined by using the Akaike Information Criterion (Akaike 1974), implemented in MODELTEST version 3.7 (Posada and Crandall 1998), which has several important advantages over the hierarchical likelihood ratio test (hLRT) (see Posada and Buckley 2004 for details).

Results

All analysed sequences deviate somewhat from the expected base frequencies (A:C:G:T = 0.26:0.23:0.27:0.24), but there are no significant differences in base composition (χ^2 test: $df=39$, $P=0.99$). Plots of transitions and transversions versus evolutionary distances indicated no substitution saturation (not shown). The Akaike Information Criterion suggests the use of the general time-reversible model (Tavaré 1986) with gamma-distributed rates for the ssu rRNA gene dataset (alpha = 0.68, Pinvar = 0.40, $R_{(AC)}=1.05$, $R_{(AG)}=2.73$, $R_{(AT)}=1.46$, $R_{(CG)}=0.72$, $R_{(CT)}=4.17$, $R_{(GT)}=1.00$) as parameters for the Bayesian analyses.

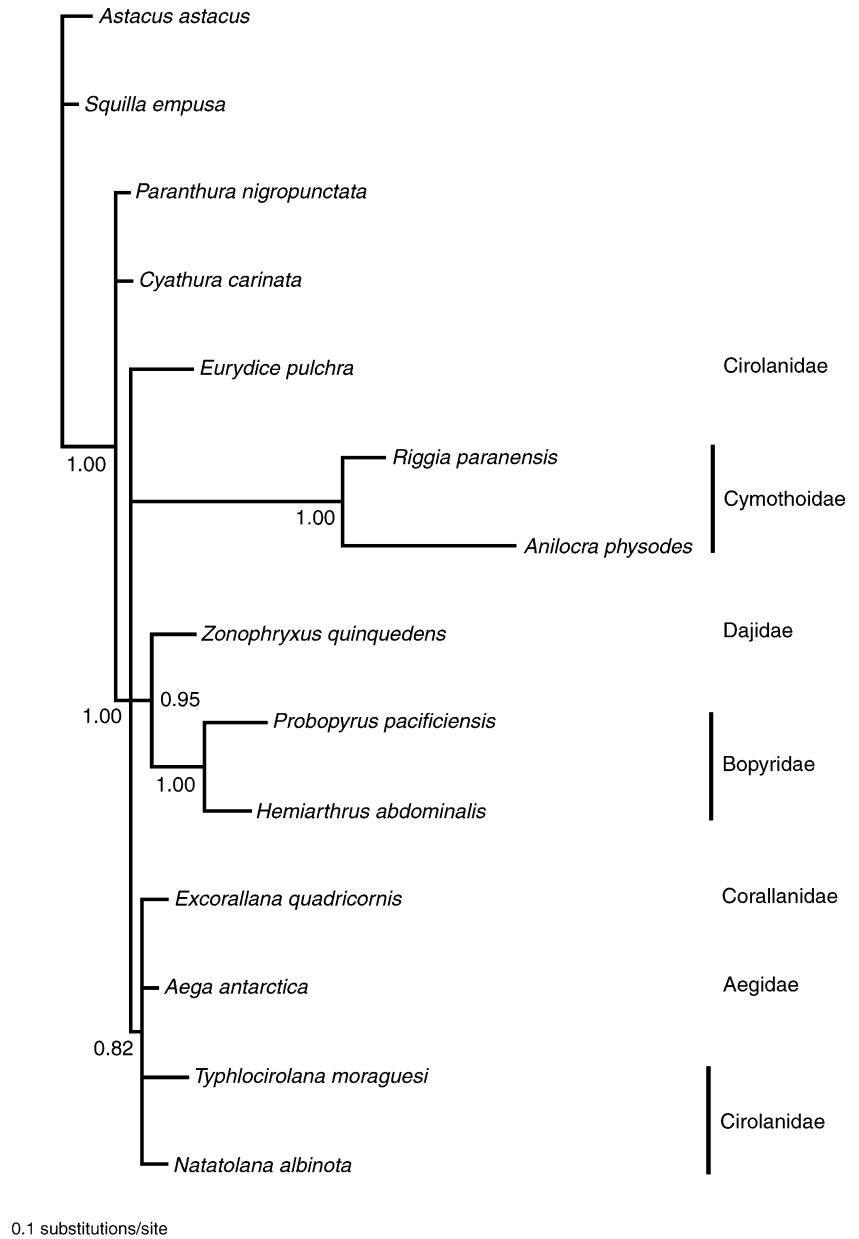
The tree resulting from the Bayesian analysis is provided in Fig. 2. The stomatopod *S. empusa* and the decapod *A. astacus* were used to root the trees. The topology supports the monophyly of the Cymothoidea (1.00). Further groups recovered with high support are the Cymothoidea (1.00) and Bopyridae (1.00). *Z. quinquedens* appears as the sister taxon of the Bopyridae (0.95). The monophyly of the Cirolanidae is not supported.

Discussion

Despite the small number of cymothoid sequences, these analyses reveal some insights into the phylogeny of this taxon. Nevertheless, additional sequences are urgently needed to reconstruct the phylogeny of the Cymothoidea. The Bayesian analyses strongly support the monophyly of the Cymothoidea and Bopyridae, but the results do not support a sister-group relationship of these two taxa as suggested by other molecular studies (Dreyer and Wägele 2001, 2002). In addition, no evidence was found for the monophyly of the Cirolanidae. However, ssu rDNA data support the hypothesis that *Z. quinquedens* is closely related to the Bopyridae, as it is also suggested by some morphological evidence (Wägele 1989; Brandt and Poore 2003).

Mobility represents an important trait for the female *Z. quinquedens* because the parasitic isopod has to move from the cast exuvia to the fresh carapace of its host when it moults. Moulting in adult Antarctic shrimp has only been described in *Chorismus antarcticus* from the Weddell Sea shelf (Thatje et al. 2005b). The moulting in one female of *C. antarcticus* observed in the laboratory, from rupture of the carapace to leaving the exuvia, surprisingly lasted only about 3 min (Thatje et al. 2005b). A few abdominal flappings, as typically found in escape attempts of shrimps, were performed to leave the exuvia. If this moulting pattern is similar in *N. lanceopes*, the moult of the host should be a crucial moment in the life history of the isopod. This may be determined by changes in the host's steroid levels in the haemolymph. As an ectoparasite, *Z. quinquedens* should use its reduced but functional pereiopods to move from the

Fig. 2 Bayesian consensus tree based on an alignment of 1,464 bp from the ssu rRNA gene. Model choice based on the Akaike Information Criterion: Six substitution types (GTR model) with gamma-distributed rates ($\alpha = 0.68$) and invariant positions (Pinvar = 0.40). Numbers at the nodes represent posterior probabilities; values below 0.50 are not shown



cast exuvia to the fresh carapace of the host during moult; all this has to take place within a very short time period. The fresh and still soft carapace should facilitate the infestation of the host by the isopod, and it is remarkable to observe that the reduced and modified legs of *Zonophryxus* do indeed superficially penetrate the carapace of *Nematocarcinus* (Fig. 1c), which is a strong hint for a continuous infestation after moult. However, most aspects of this enigmatic isopod are still unknown and thus remain in speculation, and biochemical analyses are urgently needed to understand the physiology of this remarkable parasite.

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