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Redescription of wood-associated tanaidacean *Protanais birsteini* (Kudinova-Pasternak, 1970) and its relationship within the Tanaididae

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ABSTRACT

During the KuramBio Expedition on board the R/V *Sonne* in 2012 a sunken piece of wood debris was collected from a depth of over 5000 m. Among the recovered fauna twelve specimens of tanaidacean peracarid were identified as *Protanais birsteini* Kudinova-Pasternak. It is only the second finding of the species and the fourth record of the genus *Protanais*, whose distribution is restricted so far to the North Pacific. In this paper we redescribe the species and report on its stomach contents, which contain wood tissue crumbled in different degrees in posterior parts of the digestive system. This observation suggests that these tanaids might be xylophagous. In addition we report the presence of cells of unidentified protists attached to the mouthparts of *P. birsteini*, and filaments of bacteria, which densely covered the appendages of the largest specimens. Bayesian and maximum likelihood molecular analyses confirm the placement of *P. birsteini* within Tanaididae, and highlight some taxonomic questions requiring further research.

1. Introduction

Particulate organic matter (POM) produced in the process of photosynthesis is a substantial source of energy for abyssal organisms that is delivered to the oceanic floor in a regular quantity or in irregular pulses (Smith, 1985; Smith et al., 2006; West et al., 2011; McClain and Barry, 2014). The sinking particles of organic matter, which could be phytodetrital aggregates (Billett et al., 1983), sunken wood, or carcases of large marine vertebrates, change local food regimes on the generally low-resource abyssal plain and support a rapid development of distinct, diverse and often ephemeral assemblages of organisms. Numerous studies with experimental log deployments have documented patterns of temporal succession initiated by wood-boring and sulfide-metabolizing opportunistic colonizers (Kiel and Goedert, 2006; McClain et al., 2012; Bienhold et al., 2013). Xylophagous invertebrates physically modify solid wood tissue into three-dimensional habitat attractive to numerous benthic organisms and their predators (Bienhold et al., 2013, Bessette et al., 2014, McClain and Barry, 2014). These pioneer organisms consume oxygen and are responsible for the development of anaerobic chemoautotrophic conditions that in consequence support a growth of microorganisms involved in methane and sulfur cycles (Fagervold et al., 2012).

The list of organisms associated with wood-falls comprises representatives of most marine invertebrate groups (Becker et al., 2009; Hoyoux et al., 2009; Samadi et al., 2010). Peracarida of the order Tanaidacea are represented by only one genus associated with sunken wood – *Protanais*; however in studies by McClain and Barry (2014) these crustaceans were reported as the second most abundant and diverse group in wood-fall communities.

Tanaidacea associated with wood falls have so far been found only in the North Pacific – twice in the Western Pacific and once in the Eastern Pacific. Larsen (2006) has noted *Protanais ligniamator* Larsen, 2006 from the Cascadia Basin and Wuzza Bare Seamounts (47°47.085′N 127°41.478′W) at 2656 m depth, while McClain and Barry (2014) recorded an undescribed species from Station 'Deadwood' (36.1540988N, 122.408528W) at 3203 m depth. Both Larsen's and McClain and Barry's records of *Protanais* come from wood logs deployed in experimental approaches. In the Eastern Pacific e.g., the Kuril–Kamchatka Trench (44°07′N, 149°34′E) from 6090 m depth, *Protanais* was reported from one specimen only, although without knowledge of its association with wood falls (Kudinova-Pasternak, 1970).

During the KuramBio Expedition on board the R/V *Sonne* in 2012 a sunken piece of wood debris was collected from a depth of 5000 m. (Schwabe et al., 2015). Among the recorded fauna (Schwabe et al., 2015) twelve specimens of tanaidacean peracarid were

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identified as *Protanais birsteini* (Kudinova-Pasternak, 1970). In this paper we redescribe that species, report on examination of its stomach contents, and discuss its placement within Tanaididae.

2. Material and methods

During the joint German/Russian expedition KuramBio (Kuril–Kamchatka Biodiversity Study) on board the R/V *Sonne* (SO 223) to the Kuril–Kamchatka Trench and adjacent abyssal plain (Brandt and Malyutina, 2012) fragments of wood and piece of debris were collected by dredging the sea-bottom with an Agassiz Trawl at Station 12-5 (for details see Schwabe et al., 2015). All collected invertebrates were preserved in cold 96% non-denatured ethanol (EtOH), sorted on ice, and finally stored at 0–4 °C (Riehl et al., 2014).

2.1. Taxonomy

Three specimens of Tanaidacea (two mature and one immature) were dissected on slides and used for taxonomic studies. The figures were prepared with microscope-attached drawing tubes and digitally inked (Coleman, 2003). Pictures were made with a Nikon DSFi1 digital camera. Material has been lodged at A. V. Zhirmunsky Institute of Marine Biology in Vladivostok, Russia (MIMB) and the Zoological Museum of Hamburg, Germany (ZMH). Material other than types (unregistered) has been deposited at the Zoological Museum of Hamburg (Germany).

2.2. Genetic

Fragments (usually a cheliped and/or one pereopod) of five P. *birsteini* were used for DNA extraction, PCR, and sequencing. Initial tissue digestion was completed overnight at 55 °C in a 1.5 ml tube with 200 μl of Queen's lysis buffer containing 5 μl of proteinase K (20 mg ml^{-1}) (Seutin et al., 1991) and digested. DNA was isolated using a phenol–chloroform protocol (Hillis et al., 1996). DNA resuspended in 100 μl of TE buffer, pH 8.00, was stored at 4 °C until amplification. Approximately 670 base pairs (bp) of the mitochondrial cytochrome oxidase c subunit I (COI) gene were amplified using the primer pair LCO1490/HCO2198 (Folmer et al., 1994).

PCR was performed in a total volume of 20 μl containing 10 μl of DreamTaq Master mix (2 ×), first primer – 1.6 μl, second primer – 1.6 μl (concentration for both primers – 5 μM), ddH2O – 4.8 μl, and 2 μl of DNA, according to protocol: 94 °C for 3 min.; 35 cycles of 94 °C for 30 s, 48 °C for 45 s, 72 °C for 1 min.; 72 °C for 3 min. Purified products were sequenced by Macrogen (Netherlands). Sequences were checked against contamination and sequence errors using BLAST and the GenBank database (Altschul et al., 1997). Alignment was performed using the Clustal algorithm (Larkin et al., 2007) in BioEdit to check for proper amino acid encoding.

The sequence of *P. birsteini* was compared to a recently obtained database of tanaidacean COI sequences collected from the waters around Iceland (manuscript in prep), augmented with all sequences for Tanaididae available from GenBank (several of which were recently published by Larsen et al., 2014); these consisted of *T. dulongii* (Audouin, 1826), *Zeuxo exsargasso* Sieg, 1980, *Z. holdichi* Bamber, 1990, *Z. normani* (Richardson, 1905), and *Zeuxo* sp. With Sphyrapodidae from the Icelandic collection

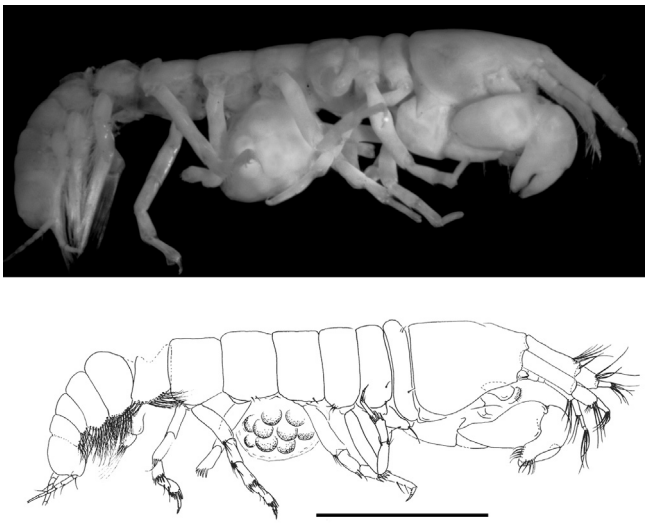

Fig. 1. *Protanais birsteini*, ovigerous female, a photograph and a drawing of the same specimen. Scale line = 1 mm.

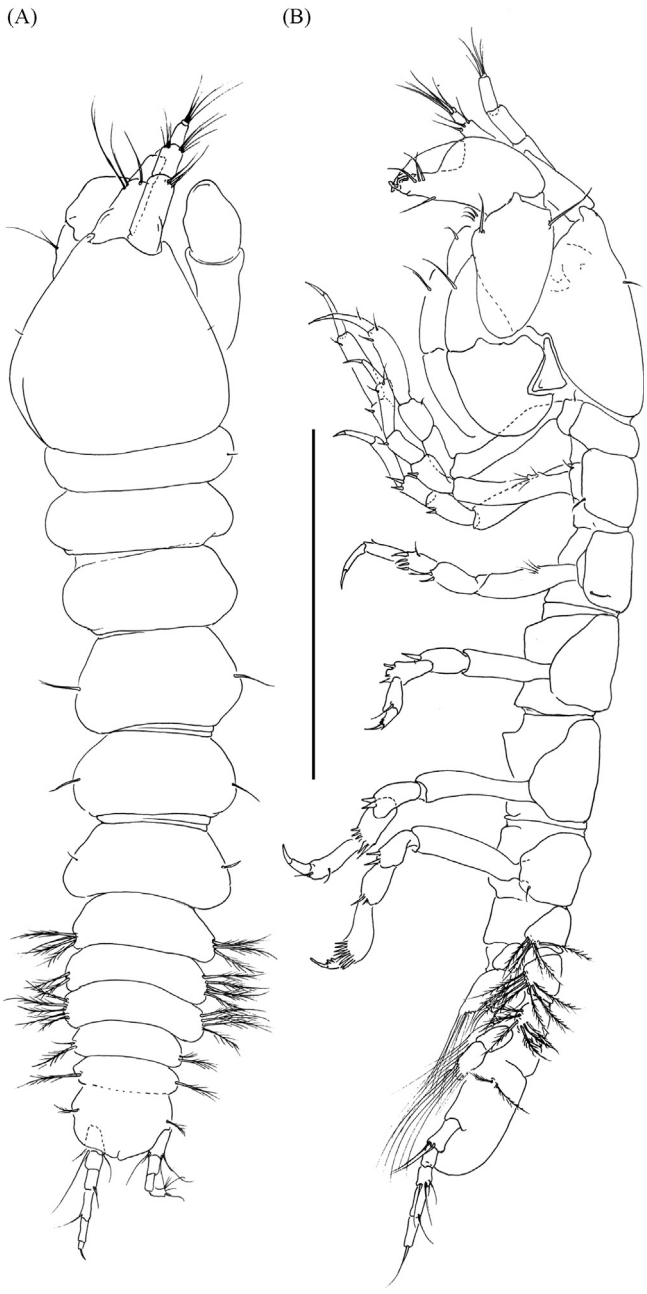

Fig. 2. *Protanais birsteini*, juvenile, (A) dorsal; (B) lateral. Scale line = 1 mm.