

Note

A morphological note on the pelagic polystiliferous hoplonemertean *Protopelagonemertes beebei* (Nemertea: Pelagica)

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Abstract: The three currently recognized species comprising the pelagic polystiliferous hoplonemertean genus *Protopelagonemertes* Brinkmann, 1917 are supposedly distinguishable chiefly with respect to the number of proboscis nerves: 29 (varying from 22 to 30) in *P. hubrechtii* (Brinkmann, 1917); 19–21 (varying from 19 to 22) in *P. beebei* Coe, 1936; and 36 in *P. joculari* Van der Spoel, 1988. A single specimen collected off the Pacific coast of Hokkaido, Japan, herein identified as *P. beebei*, was found to possess 19–23 primary proboscis nerves. In addition to the primary proboscis nerves, the material also possessed 12–16 secondary proboscis nerves, which are discernible from the primary nerves in that they coexist with putative glial cells. Although secondary proboscis nerves have been identified in some other species in the Pelagica, they have not previously been described for any members of the *Protopelagonemertes*. Our findings thus prompted us to question the validity of morphological species delimitation within this genus, given that the distinction between primary and secondary proboscis nerves has previously not been taken into consideration. Close examination of the specimen in the living state revealed that it is characterized by a pair of cephalic furrows, a structure commonly found in benthic representatives of the phylum, but herein confirmed for the first time among the Pelagica. A 658-bp partial sequence of the mitochondrial cytochrome *c* oxidase subunit I gene from the newly examined specimen was found to be identical to sequences derived from two other specimens previously collected in Sagami Bay, thereby indicating that *P. beebei* may occur commonly in Japanese meso- to bathypelagic waters.

Key words: COI, Hoplonemertea, marine invertebrate, Polystilifera, taxonomy

Specimens of pelagic nemerteans are rarely encountered in nature, and thus, their biology and systematics tend to be poorly characterized. Among the 1300 species in Nemertea (commonly known as ribbon or proboscis worms), approximately 100 have been reported from the epi- to bathypelagic zones within the water column, whereas the remainder are primarily benthic in distribution (Gibson 1995, Kajihara et al. 2008). With the exception of a few species belonging to the Monostilifera (Wheeler 1934, Korotkevitch 1961, Crandall & Gibson 1998, Chernyshev 2005, Crandall 2006, Chernyshev & Polyakova 2018, 2019), the majority of the pelagic nemerteans belong to the Pelagica within the suborder Polystilifera. Since the description of *Pelagonemertes rollestoni* Moseley, 1875 (the first representative of the Pelagica discovered during the *Challenger* expedition), three major taxonomic monographs have been published on this group of organisms

(Brinkmann 1917a, Coe 1926, Korotkevitch 1955). However, progress in the systematics of pelagic polystiliferans has been hindered by their rarity, with 52 of the 96 described species of Pelagica being known from a single specimen (Maslakova & Norenburg 2001, Kajihara & Lindsay 2010). Consequently, for many species the degree of intraspecific variation in taxonomic characters has yet to be established. Moreover, taxonomic descriptions have often been based on material that had become markedly distorted or damaged during retrieval from deep waters (reviewed by Maslakova & Norenburg 2001). As of writing, there are only four barcode-sequence entries for the Pelagica in the DDBJ/EMBL/GenBank databases to which species names have been tagged (Thollessen & Norenburg 2003, Bucklin et al. 2010, Kajihara et al. 2011, Chernyshev & Polyakova 2019). Accordingly, to ensure the correct application of names to pelagic nemerteans, larger amounts of data should be accumulated with respect to their precise morphology and DNA barcoding sequences, and for as many species/specimens as possible.

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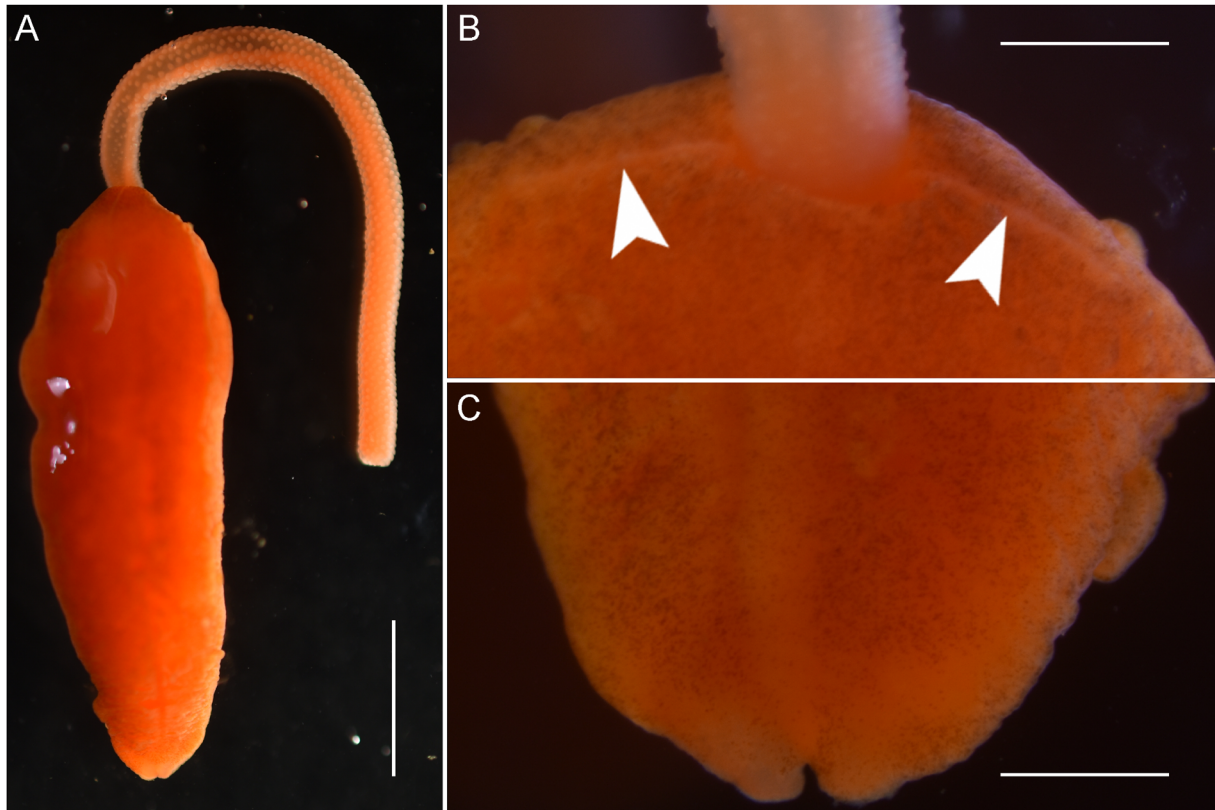


Fig. 1. Photographs of *Protipelagonemertes beebei* Coe, 1936 (ICHUM 6146), taken of the specimen in an anesthetized state. (A) Entire body, dorsal view. (B) Anterior end of the body, ventral view, showing cephalic furrows (indicated by arrowheads). (C) Posterior end of the body, dorsal view. Scale bars: 5 mm for (A); 1 mm for (B, C).

As with the case of other pelagic nemertean taxa, the taxonomy of the polystiliferan genus *Protipelagonemertes* Brinkmann, 1917b is problematic. Traditionally, the number of proboscis nerves has been one of the major taxonomic characters used to distinguish between the three species that constitute this genus (Coe 1936, Van der Spoel 1988): 29 in *P. hubrechtii* (Brinkmann, 1917a); 19–21 (varying within a single specimen) in *P. beebei* Coe, 1936; and 36 in *P. jocatorii* Van der Spoel, 1988. However, variations in the numbers of proboscis nerves have been subsequently documented for different specimens identified as *P. hubrechtii*: 22, 24, 25, and 26 nerves in four specimens collected in the Southeast Atlantic, off South Africa (Wheeler 1934); 26, 27–29, and 30 nerves in three specimens from the Northwest Atlantic, off Nonsuch Island, Bermuda (Coe 1936); and 30 nerves in a specimen from Antarctica (Wheeler 1940). In contrast, no further information has been forthcoming with regards to the variability of this character in two additional specimens identified as *P. beebei*, which were collected in the Northeast Pacific, off Baja California, and the Northwest Pacific, off the Kuril Islands, respectively (Coe 1954). However, the number of proboscis nerves in a specimen from Sagami Bay, Japan, identified as *P. beebei*, was found to vary from 19 to 22 (or, seemingly up to 25), thereby indicating that the variation in this character is completely continuous between that which has previously been reported for *P. beebei* and *P. hubrechtii* (Kajihara

et al. 2011). *Protipelagonemertes jocatorii* was described based on a single specimen (Van der Spoel 1988), and accordingly, there is no indication as to the intraspecific variation of this character in this species. Moreover, it seems highly probable that a proportion, if not all, of the previous studies on these specimens have failed to make a distinction between primary and secondary proboscis nerves. Secondary proboscis nerves were first mentioned by Coe (1926) for *Planktonemertes agassizii* Woodworth, 1899; *Pelagonemertes joubini* Coe, 1926; *Plionemertes plana* Coe, 1926; and *Proarmaueria pellucida* Coe, 1926, and have also been referred to as “small” (маленький) proboscis nerves for *Neoarmaueria laticeps* (Korotkevitch, 1955) and *Neoarmaueria tenuicauda* (Korotkevitch, 1955) (Korotkevitch 1955); *Balaenanemertes parvula* Korotkevich, 1964; *Chuniella compacta* Korotkevich, 1964; *Nectonemertes tenuis* Korotkevich, 1964; *Obnemertes solida* Korotkevich, 1964; and *Obnemertes maximovi* Korotkevich, 1960 (Korotkevitch 1964, 1966). Chernyshev (1992) also described 7–8 “large” (крупный) and 6–8 “small” (маленький) proboscis nerves in *Zinarmaueria platonovae* Chernyshev, 1992, and subsequently presented a histological photomicrograph of secondary [“false” (ложный)] proboscis nerves in *Planktonemertes* sp. (Chernyshev 2011, pl. XX, fig. 5). More recently, Chernyshev & Polyakova (2018) have presented a confocal laser scanning micrograph image (labeled with phalloidin, 5-HT, and α -tubulin antibodies) for *Nectone-*

mertes cf. *mirabilis* Verrill, 1892, depicting primary (larger) and secondary (smaller) proboscis nerves. Despite the findings of these previous studies, the distinction between the primary and secondary nerves is not necessarily obvious. In this regard, Coe (1926, p. 40) reasoned that the primary nerves represent the true proboscis nerves, given “the fact that their branches divide the longitudinal muscular layer into the same number of separate bundles”; however, this can equally apply to secondary nerves. Later, Chernyshev (2011) remarked that the primary nerves can be distinguished from secondary nerves based on the fact that the former invariably come into contact with the inner circular muscle layer of the proboscis.

Very recently, on June 9, 2020, during a research cruise of the training vessel *Ushio-Maru*, a single specimen of an orange pelagic polystiliferan, approximately 2 cm long and 7 mm wide in the anesthetized state (Fig. 1A), herein identified as *Protopelagonemertes beebei*, was collected between 15 : 35 and 16 : 10 (JST), using a vertical tow of a 80-cm ring net with 63 μm mesh, from a depth of 830 m (20 m above the sea floor) to the surface at 41°58.13'N, 141°39.79'E, off the Pacific coast of Hokkaido, northern Japan. On board, the specimen was acquired by Atsushi Yamaguchi, maintained at 3°C in a 500-mL bottle, and subsequently sent to Hiroshi Kajihara on June 13, 2020. On arrival at the laboratory, the specimen was anesthetized in MgCl_2 solution isotonic to seawater, during which the proboscis was partially protruded, and subsequently photographed (Fig. 1A, B). The posterior end of the body, which was slightly notched along its medial portion in dorsal view (Fig. 1C), was cut for a few millimeters and fixed in 99% EtOH for DNA extraction. The remainder of the body was fixed in Bouin's fluid for 24 h, dehydrated in 99% EtOH, cleared in xylene, embedded in paraffin, serially sectioned at a thickness of 7 μm , stained using Mallory's trichrome method (Gibson 1994), and embedded in Entellan New (Merck). This voucher specimen has been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), with the catalogue number 6146.

Histological observation of the proboscis of ICHUM 6146 revealed that the secondary proboscis nerves in this taxon are accompanied by cell bodies, putatively glial cells (or otherwise neuronal perikarya). In contrast, the primary proboscis nerves are devoid of cell bodies and appear to consist exclusively of axonal fibers (Fig. 2A, B). We counted 19–23 primary proboscis nerves and 12–16 secondary proboscis nerves in the anterior proboscis chamber in the present material (Fig. 2A). The number of the primary proboscis nerves in ICHUM 6146 overlaps with that reported for *P. beebei*, at 19–21 (Coe 1936). However, when combined, the number of the primary and secondary nerves in certain portions add up to 36, thereby encompassing the numbers reported for *P. hubrechtii* (29, varying from 22 to 30) and *P. joculari* (36). Accordingly, *P. beebei*, and possibly *P. joculari*, may be synonymous with *P. hubrechtii*. Our observations thus prompted us to question the validity of morphological species delimitation in *Protopelagonemertes*, given that no distinction has been made between primary and secondary proboscis nerves in the original

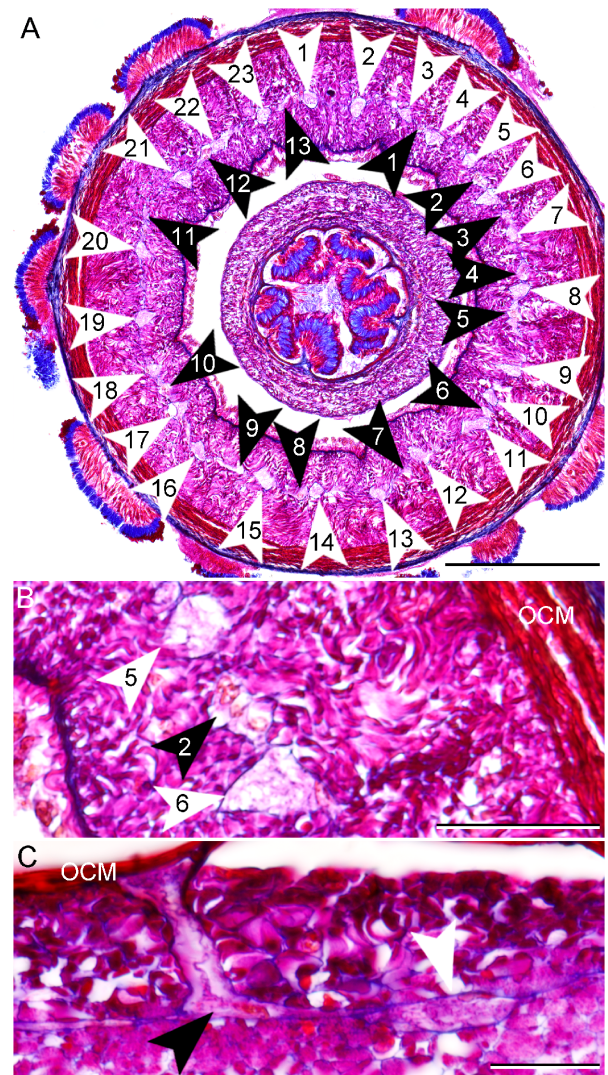


Fig. 2. Transverse sections of the proboscis anterior chamber of *Protopelagonemertes beebei* Coe, 1936 (ICHUM 6146) showing primary (indicated by white arrowheads) and secondary (indicated by black arrowheads) proboscis nerves. (A) Twenty-three primary nerves and 13 secondary nerves. (B) Magnification of #5 and #6 primary and #2 secondary nerves in (A), the latter of which contains putative glial cells. (C) A secondary nerve extending to the outer circular muscle layer. Abbreviation: OCM, outer circular muscle layer of the proboscis. Scale bars: 200 μm for (A); 50 μm for (B); 20 μm for (C).

descriptions of the three congeners *P. hubrechtii*, *P. beebei*, and *P. joculari* (Brinkmann 1917a, b, Coe 1936, Van der Spoel 1988). In addition, a connection between what is probably a secondary proboscis nerve and the outer circular muscle layer is observed in ICHUM 6146 (Fig. 2C), which is inconsistent with the criterion proposed by Chernyshev (2011). Hence, with a view toward a more systematic classification of this genus, morphological and molecular data from reliably identified material, ideally from type localities, should be accumulated for each of the nominal species *Bathynemertes hubrechtii* Brinkmann, 1917b (type locality: North Atlantic),

Protopelagonemertes beebey Coe, 1936 (type locality: off Nonsuch Island, Bermuda), and *Protopelagonemertes joculari* Van der Spoel, 1988 (type locality: Banda Sea).

We confirmed the presence of cephalic furrows in ICHUM 6146, which is probably the first time this feature has been described in the Pelagica (Fig. 1B). Although the function of these furrows is uncertain, it can be speculated that they may act as chemical or physical receptors, conceivably by perceiving changes in water pressure attributable to vibrations/waves generated by prey and predator species. Cephalic furrows are commonly found in benthic nemerteans and may also be present in pelagic types. However, in pelagic nemerteans, the epidermis is often dislodged during collection (e.g., Coe 1926), thereby rendering the detection of cephalic furrows difficult or impossible.

Protopelagonemertes beebey appears to be common in Japanese meso- to bathypelagic waters; for this species, we propose a new Japanese name, *dai-dai-oyogi-himomushi*. A megablast search (Zhang et al. 2000) at the DDBJ website (<http://blast.ddbj.nig.ac.jp>) based on the LC565011 sequence (658 bp, cytochrome *c* oxidase subunit I [COI] gene) derived from ICHUM 6146 resulted in a 100% match with AB587263 (Kajihara et al. 2011) and HQ848618 (Andrade et al. 2012) which are sequences derived from two specimens collected at a depth of ~1300 m in Sagami Bay (Kajihara et al. 2011). To determine the sequence of LC565011, total DNA was extracted from the EtOH-preserved tissue using a DNeasy Blood & Tissue Kit (Qiagen). PCR amplification was performed using the primer pair LCO1490/HCO2198 (Folmer et al. 1994) under the following thermal cycling conditions: an initial denaturation at 98°C for 1 min; followed by 35 cycles of 98°C for 10 s, 45°C for 30 s, and 72°C for 1 min; with a final extension at 72°C for 4 min. Nucleotide sequencing was carried out using the same primer pair with an ABI BigDye Terminator ver. 3.1 Cycle Sequencing Kit and a 3130 DNA Analyzer (Applied Biosystems).

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