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A new *Nectonema* nematomorph species parasitic in an alpheid shrimp (Decapoda: Caridea)

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Abstract

We describe the marine horsehair worm *Nectonema shimadai* **sp. nov.**, which was parasitic in a snapping shrimp (*Alpheus* sp.) collected at 4 m depth from the coastal waters of Kikaijima Island, Japan, northwestern Pacific. The nematomorph was a female, 49 mm in length and 340 μm in width, salmon-pink when alive, with a translucent anterior body region and a distinct anterior chamber. The 18S sequence from *N. shimadai* **sp. nov.** was 12.8, 9.8, 9.6, and 9.3% divergent in Kimura 2-parametar (K2P) distance from *Nectonema* sp. parasitic in the isopod *Natatolana japonensis*, *Nectonema* sp. parasitic in the crab *Chionoecetes bairdi*, *N. munidae*, and *N. agile*, respectively. In an 18S tree, *N. shimadai* **sp. nov.** was the sister taxon to a clade comprising three decapod-parasitic species, and species sharing the same host group or ocean were not monophyletic. The tree may

reflect genetically distant warm-water and cold-water lineages within decapod-parasitic nematomorphs.

Introduction

Nectonema Verrill, 1879 is the sole marine genus in the phylum Nematomorpha (horsehair worms). *Nectonema* differs morphologically from other nematomorphs in having natatory bristles (present only in adults) and dorsal and ventral nerve cords, among other characters (Schmidt-Rhaesa 2013). Since the first discovery of *Nectonema* around 1870 (Ward 1892a), knowledge of this genus has accumulated through reports on detailed external and internal morphology (e.g., Ward 1892a), the parasitic nature of its juveniles (Ward 1892b), egg structure (Ward 1892a; Huus 1932), mating behavior (Huus 1932), development in the host (Huus 1932; Schmidt-Rhaesa 1996), species diversity (Verrill 1879; Nierstrasz 1907; Bock 1913; Brinkmann 1930; Poinar and Brockerhoff 2001), host diversity (e.g., Schmidt-Rhaesa *et al*. 2013, Kakui *et al*. 2021; Kakui 2024), and the chromosome-level genome (Cunha *et al*. 2023). *Nectonema* nonetheless remains enigmatic, with much of its biology—such as early development, true species and host diversity, and habitat use—remaining poorly known.

Nectonema nematomorphs have been reported as parasites in various decapod and isopod hosts (Supplementary Table S1). Although most of these records involved juvenile individuals lacking natatory bristles, some adults have been collected from inside host crustaceans (e.g., Brinkmann 1930; Schmidt-Rhaesa 1996) and likely represented individuals just before emergence. Some studies have suggested that *Nectonema* species exhibit high host specificity (Brinkmann 1930; Born 1967), although there are contradictory reports of several species occurring in a wide range of hosts (Supplementary Table S1). It remains unclear whether the latter species with apparently broad host utilization include cryptic species with narrow specificity, implying higher true species diversity than is presently known.

Nectonema is taxonomically challenging. Morphological differences among species are subtle, and the developmental stages and sexes of specimens used for species descriptions have not been consistent. There are currently five named species. Poinar and Brockerhoff (2001) distinguished them based on the coloration of the anterior body region (dark or translucent), presence or absence of an anterior chamber, arrangement of rows of natatory bristles in adults, and stoma shape in juveniles, among other characters. Males, females, and juveniles have been described only for *Nectonema agile* Verrill, 1879 (type locality: Massachusetts, USA, northwestern Atlantic) and *Nectonema munidae* Brinkmann, 1930 (type locality: near Bergen, Norway, northeastern Atlantic). For the other species, information is available only for males in *Nectonema melanocephalum* Nierstrasz, 1907 (type locality: Pulau Kawassang, Indonesia, eastern Indian Ocean), females in *Nectonema svensksundi* Bock, 1913 (type locality: Spitzbergen Island, Norway, Arctic), and juveniles in *Nectonema zealandica* Poinar and Brockerhoff, 2001 (type locality: South Island, New Zealand, southern Pacific). The latter three species have not been reported since their original descriptions.

During a faunal survey around Kikaijima Island, Japan, the second author collected one shrimp parasitized by a *Nectonema* nematomorph, which we here describe as a new species. This is the fifth published record of *Nectonema* from Japanese waters (Oku et al. 1983; Yoshida 2016; Kakui et al. 2021; Kakui 2024; this study). For phylogenetic inference, future DNA barcoding efforts, and accurate host–parasite pairing, we determined a partial sequence for the 18S rRNA (18S) gene from the nematomorph and a partial sequence for the cytochrome *c* oxidase subunit I (COI) gene from the host. To assess our species' taxonomic position, we conducted a phylogenetic analysis based on 18S from the new species and other *Nectonema* taxa for which 18S data were available.

Material and Methods

The infected shrimp was collected by snorkeling from coral rubble at 4 m depth at Keraji, Kikaijima Island, Ryukyu Islands, Japan (28.288667ºN, 129.975722ºE) on 27 May 2025. It was photographed

alive and then dissected to extract the nematomorph. The latter was photographed and video-recorded alive. The host and nematomorph were then fixed and preserved in 99% ethanol.

The fixed nematomorph was cut into several pieces. The anterior and posterior parts were mounted on a glass slide in glycerin and observed with an Olympus BX53 compound microscope. One of two subanterior fragments was used for DNA extraction; and another fragment was treated with hexamethyldisilazane (HMDS; Nation 1983), sputter-coated with gold, and observed at 15 kV accelerating voltage with a scanning electron microscope (SEM; S-3000N, Hitachi, Japan). The remaining part was preserved in 99% ethanol. The host was dissected with forceps, observed with a Nikon SMZ1500 stereoscopic microscope, and identified to genus based on Banner and Banner (1982), Poore and Ahyong (2023), Anker (2023), and Ashrafi *et al*. (2024). Illustrations were prepared with Adobe Illustrator CS6 from draft line drawings made with a drawing tube. The body length of the fresh nematomorph was measured from the anterior to posterior end, and body width at the widest portion. The carapace length of the host was measured from the orbital margin to the midpoint of the posterodorsal margin. All measurements were made from digital images by using ImageJ (Schneider *et al*. 2012). The studied material was deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan, under catalog number ICHUM9108.

Total DNA was extracted from part of the body of the nematomorph and from muscle tissue of the host by using a NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany). For COI, primers LCO1490 and HCO2198 (Folmer *et al*. 1994; Supplementary Table S2) were used for PCR amplification and cycle sequencing (CS); COI amplification was unsuccessful for the nematomorph. For 18S, primers SR1 and SR12 (Nakayama *et al*. 1996) were used for PCR amplification (18S amplification was unsuccessful for the host), and 18S-b3F, 18S-b3R, 18S-b4F, 18S-a4R, 18S-b5F, 18S-b6F, 18S-a6R, and 18S-b8F (Kakui *et al*. 2011, 2021; Kakui and Shimada 2017, 2022; Kakui and Hiruta 2022; Supplementary Table S2) for CS. PCR amplification conditions for COI and 18S with KOD ONE PCR Master Mix (Toyobo, Japan) were 45 cycles of 98 °C for 10 s, 56 °C (COI) or 60 °C (18S) for 5 s, and 68 °C for 1 s (COI) or 10 s (18S). All nucleotide sequences were determined with a

QuantumDye Terminator v3.1 Cycle Sequencing Kit (Quintara Biosciences, USA) and a 3130 or 3730 DNA Analyzer (Life Technologies, USA). Fragments were assembled by using MEGA7 (Kumar *et al*. 2016). The sequences were deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan (DDBJ). BLAST searches (Altschul *et al*. 1990) were conducted to determine the nucleotide sequences in the INSD most similar to those we determined.

The 18S dataset for phylogenetic analysis comprised the dataset from Kakui (2024) and the one nematomorph sequence we determined. The sequences were aligned (1710 positions in the aligned dataset; see Supplementary Files S1 and S2) as described by Kakui (2024). Methods for selecting the optimal Q mixture model (MIX{TIM3+FO,JC+FO}+R2), the maximum likelihood (ML) analysis, estimation of branch support (1000 pseudoreplicates for both Shimodaira-Hasegawa-like approximate likelihood ratio tests [SH-aLRT] and standard bootstraps [BS]), and tree drawing were as described by Katsuragawa *et al*. (2025). Kimura (1980) 2-parameter (K2P) distances among *Nectonema* species were calculated with MEGA7.

This article is registered in ZooBank under: https://zoobank.org/References/A291194E-1583-4A0D-A3BD-2C57C8FD518A.

Results and Discussion

Host animal

The host shrimp, a female lacking both chelipeds, was identified morphologically as *Alpheus* sp. A BLAST search showed the 658-bp partial COI sequence we determined from the host was most similar to the COI sequence from *Alpheus edwardsii* H. Milne Edwards, 1837 (PP651964; query cover 100%, identity score 83.41%; McIlroy *et al*. 2024), which supported our generic identification based on morphology.

Taxonomy

***Nectonema* Verrill, 1879**

[New Japanese name: Oyogi-hariganemushi-zoku]

***Nectonema shimadai* sp. nov.**

[New Japanese name: Asahi-oyogi-hariganemushi]

Figs. 1–3, Supplementary Movies S1, S2

https://zoobank.org/NomenclaturalActs/E4F98AAF-7AB2-43F0-B77C-FABE40E541C0

Type host

Alpheus sp. (Decapoda: Caridea: Alpheidae).

Occurrence in host

Occupying host body cavity from anterior end of cephalothorax to posterior end of pleonite 5 (Fig. 1a, b).

Type locality

Keraji, Kikaijima Island, Ryukyu Islands, Japan (28.288667ºN, 129.975722ºE), 4 m depth.

Type material

Holotype. Adult female, ICHUM9108, one slide, one SEM stub, and one vial with nematomorph (one vial with host included under same catalog number); body length 49.0 mm, body width 340 μm, ex *Alpheus* sp. (female, carapace length 3.7 mm), collected at the type locality on 27 May 2025 by Shoki Shiraki.

Etymology

The specific name is a noun in the genitive case and honors Daisuke Shimada, a nematode taxonomist and long-standing collaborator with the first author in research on Nematoida.

Representative sequences

The 18S sequence (LC909668; 1894 bp) was determined from the holotype. The COI sequence (LC909669; 658 bp, encoding 219 amino acids) was determined from the host shrimp.

Description of female

Body (Fig. 1c; Supplementary Movie S1) elongate, nearly cylindrical but gradually tapering toward both ends, 144 times as long as wide. Anterior region translucent; remaining part salmon pink when alive. Fine transverse striations present from subanterior to subposterior region (Figs. 2, 3). Dorsal and ventral surfaces each with two rows of natatory bristles; many bristles lost, but observed from subanterior to subposterior regions (Fig. 2a, c, d). Anterior end (Figs. 1c', 2a, b; Supplementary Movie S2) covered with short bristles. Anterior margin bilobed in dorsal view, with shallow median furrow. Cephalic papillae not observed. Distinct anterior chamber (ca. 0.3 mm long) present, with four giant cells. Mouth opening anteroventrally. Oral cavity reduced. Sclerotized proboscis absent. Pharynx extending beyond septum, forming loop posteriorly. Intestine not observed. Developing gonads (?) in body cavity. Posterior end (Figs. 1c'', 2c, d) rounded; gonopore opening posteriorly.

Males and juveniles unknown.

Phylogenetic analysis and genetic divergence

The 18S sequence of *N. shimadai* **sp. nov.** was 12.8, 9.8, 9.6, and 9.3% divergent (K2P distance) from homologous sequences from *Nectonema* sp. parasitic in the isopod *Natatolana japonensis* (LC605988 and LC605989; Kakui *et al*. 2021), *Nectonema* sp. parasitic in the crab *Chionoecetes bairdi* (LC816087; Kakui 2024), *N. munidae* (JAUIRH010000294; Cunha *et al*. 2023), and *N. agile* (AF421767; Bleidorn *et al*. 2002), respectively. In the 18S-based ML tree (Fig. 4), the four decapod-

parasitic species form a highly supported clade (SH-aLRT/BS = 99.6%/100%), with *N. shimadai* **sp. nov.** the sister taxon to a highly supported (98.9%/99%) clade comprising the others. Pairwise K2P distances between *N. shimadai* **sp. nov.** and members of the latter clade (9.3–12.8%) was greater than those among the three species within the clade (0.3–2.3%).

Remarks

Nectonema shimadai **sp. nov.** has a translucent anterior body region and a distinct anterior chamber, character states that distinguish it from adults of the congeners *N. melanocephalum* and *N. svensksundi*, which have a dark anterior body region and lack a distinct anterior chamber. Like *N. shimadai* **sp. nov.**, adult *N. agile* and *N. munidae* have a translucent anterior body region and a distinct anterior chamber, but *N. shimadai* **sp. nov.** differs from them in having a salmon-pink body color when alive (grayish or yellowish white in *N. agile*; whitish-yellow [females] or semi-transparent [males] in *N. munidae*; Verrill 1879; Brinkmann 1930). The 18S data showed that *N. shimadai* **sp. nov.** is highly divergent from these two species. In addition, the three species may parasitize different hosts, although *N. agile* and *N. munidae* have been reported from various decapod species (Supplementary Table S1; see also discussion below). The currently known host for *N. shimadai* **sp. nov.** is *Alpheus* sp. (Caridea: Alpheidae). The regular hosts of *N. agile* and *N. munidae* are considered to be *Palaemon vulgaris* Say, 1818 (Caridea: Palaemonidae) and *Munida tenuimana* G.O. Sars, 1872 (Anomura: Munididae), respectively (Brinkmann 1930; Born 1967).

Our female of *Nectonema shimadai* **sp. nov.** is 340 μm wide, which is narrower than juveniles (including “pre-adult” sensu Poinar and Brockerhoff [2001]) of *N. zealandica*, even when compared with juveniles that are shorter than our specimen. Poinar and Brockerhoff (2001: p. 151) gave the length of *N. zealandica* as 13 mm (parasitic juvenile) to 300 mm (pre-adult), and the maximum width as 472 μm (parasitic juvenile) to 737 μm (pre-adult). In addition, although it may be different between juveniles and adults, the color of fresh *N. zealandica* juveniles was white to cream rather than salmon pink as in *N. shimadai* **sp. nov.** *Nectonema zealandica* uses a host decapod in a different infraorder

from that of *N. shimadai* **sp. nov.** and was reported from far from Japan: it was parasitic in *Hemigrapsus sexdentatus* (H. Milne Edwards, 1837) (Brachyura: Varunidae) from South Island, New Zealand, southern Pacific. Given the various differences, we concluded that *N. shimadai* **sp. nov.** is not conspecific with *N. zealandica*.

Alpheidae, the family to which the host bearing *N. shimadai* **sp. nov.** belongs, is one of the most species-rich decapod families (De Grave *et al*. 2023), yet published reports of *Nectonema* utilizing species in this family are scarce. Prior to our study, Yamashita *et al*. (2023: fig. 1B) presented an image of an alpheid likely parasitized by a *Nectonema* nematomorph. Host surveys targeting alpheids will likely reveal additional cases of *Nectonema* infection.

Species sharing the same host group or ocean were not monophyletic in our 18S tree. *Nectonema shimadai* **sp. nov.**, a caridean parasite from the northwestern Pacific, was the sister group to a clade comprising *Nectonema* sp. (a brachyuran parasite from the northwestern Pacific), *N. munidae* (an anomuran parasite from the northeastern Atlantic) and *N. agile* (a caridean parasite from the northwestern Atlantic). In addition, *N. shimadai* **sp. nov.** showed a markedly larger genetic distance from members of the clade than the latter did among themselves. *Nectonema shimadai* **sp. nov.** was collected from warm, shallow water, whereas the other three were from cold, shallow to deep waters (cf. Spalding *et al*. 2007). The inferred tree might thus reflect genetically distant warm- and cold-water lineages within decapod-parasitic nematomorphs.

The true species diversity of *Nectonema* may be much higher than currently recognized. Information is lacking for the early stages in the life cycle of *Nectonema*, i.e., from hatching to the stage preceding infection of the definitive host. We thus cannot rule out the possibility that this group uses a broadly distributed planktonic paratenic host, which would enable passive long-distance dispersal. However, given that *Nectonema* appears to have a short adult lifespan (Brinkmann 1930) and high host specificity (Brinkmann 1930; Born 1967), it likely contains many locally endemic species. The broad trans-Atlantic distribution and high host diversity reported for *N. agile* suggest that its species boundaries merit closer examination, especially in regard to whether it represents a single

species or a multiple-species complex. *Nectonema* remains taxonomically challenging, but continued accumulation of molecular data from both juvenile and adult stages, together with the formal description of clearly distinguishable adults, represents a steady and important approach to elucidating the taxonomy.

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Conflict of interest

The authors declare none.

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Figure legends

Fig. 1. *Nectonema shimadai* **sp. nov.** parasitic in *Alpheus* sp. a, b *Alpheus* sp. containing the holotype of *Nectonema shimadai* **sp. nov.**, dorsal and left views, fresh specimen. c *Nectonema shimadai* **sp. nov.**, holotype, partly damaged fresh specimen. c', c'' same, enlarged views of the anterior and posterior ends, respectively. Arrowhead, giant cell; arrow, female gonopore.

Fig. 2. *Nectonema shimadai* **sp. nov.**, holotype, ethanol-fixed, deformed specimen in glycerin. a, b anterior portion, right dorsal view, drawing (a) and light micrograph (b); ventral rows of natatory bristles not shown in (a). c, d posterior portion, lateral(?) view, drawing (c) and light micrograph (d). In (a), internal structures (pharynx and giant cells) are drawn in gray lines. Abbreviations: *ac*, anterior chamber; *gc*, giant cell; *gp*, gonopore; *mo*, mouth; *nb*, natatory bristle; *ph*, pharynx.

Fig. 3. *Nectonema shimadai* **sp. nov.**, holotype, fragment of laterally flattened portion, lateral view, SEM image showing fine transverse striations on body.

Fig. 4. Maximum-likelihood tree for *Nectonema* reconstructed from 18S sequences (1710 positions); numbers near nodes are Shimodaira–Hasegawa-like approximate likelihood ratio test (SH-aLRT)/standard bootstrap (BS) values as percentages; scale at bottom indicates branch length in substitutions per site.

Supplementary files

Table S1. Reported hosts of *Nectonema* species, updated from Kakui (2024).

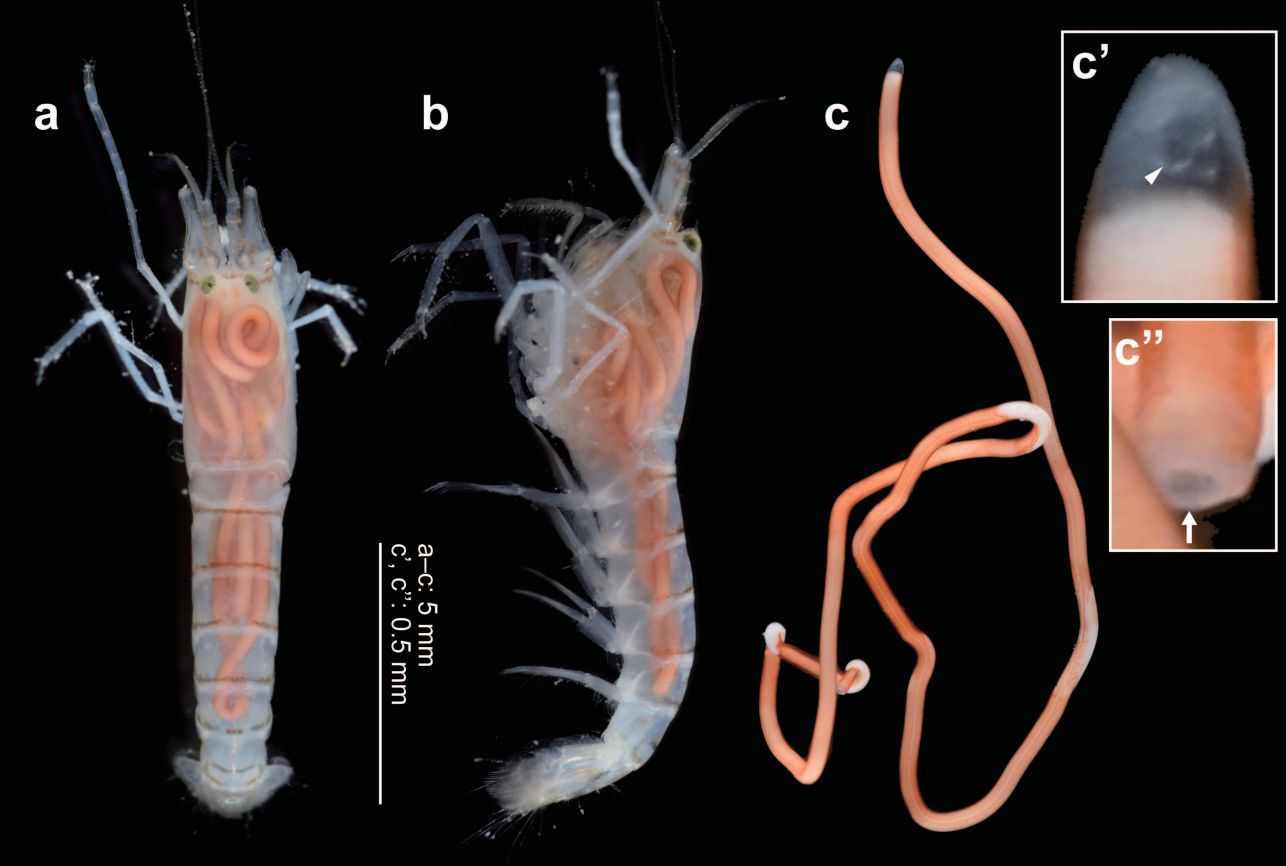
Table S2. List of PCR and cycle sequencing (CS) primers used in this study.

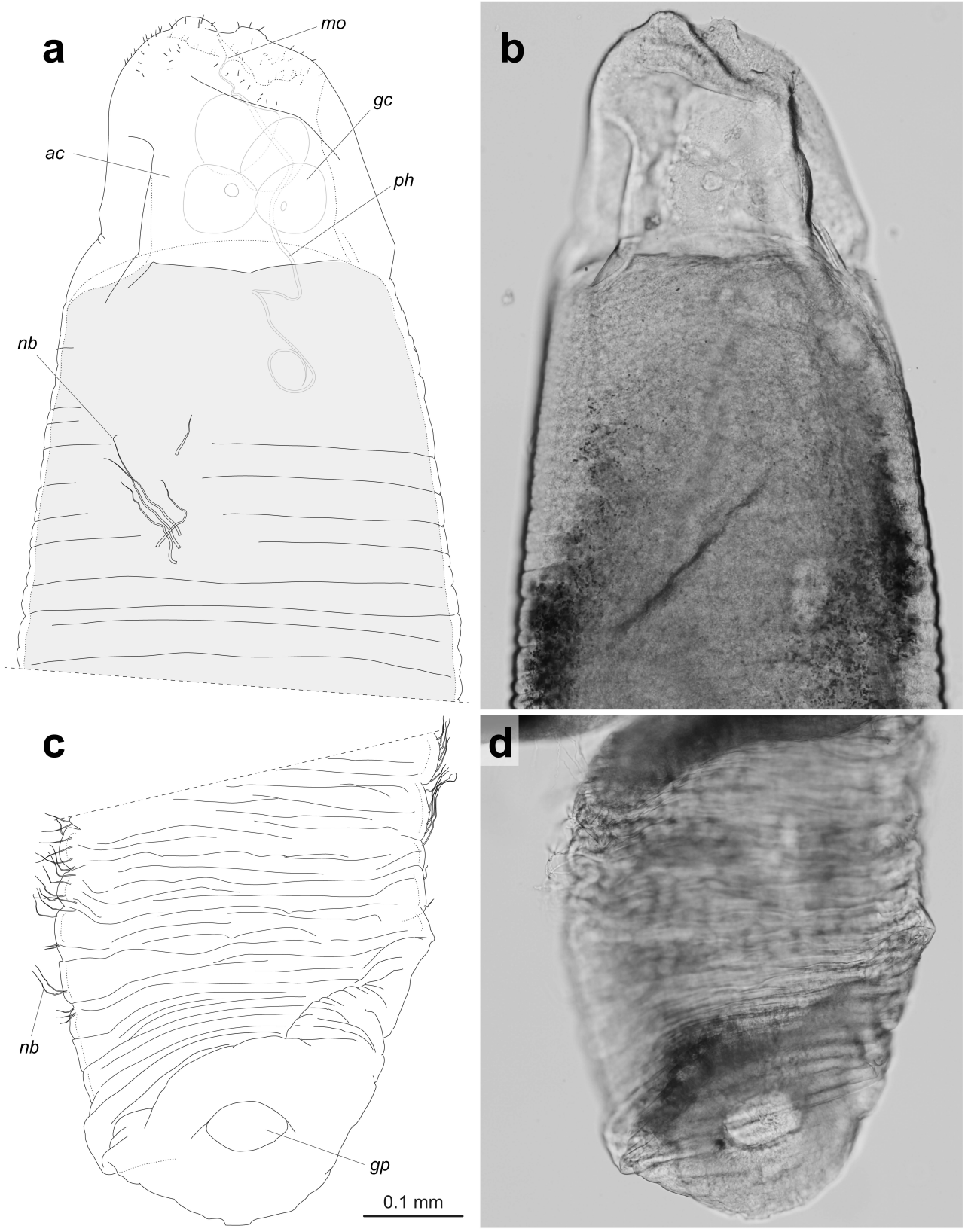
File S1. Aligned 18S sequences of Nematoida, before trimming to the shortest length among them and removing alignment-ambiguous sites.

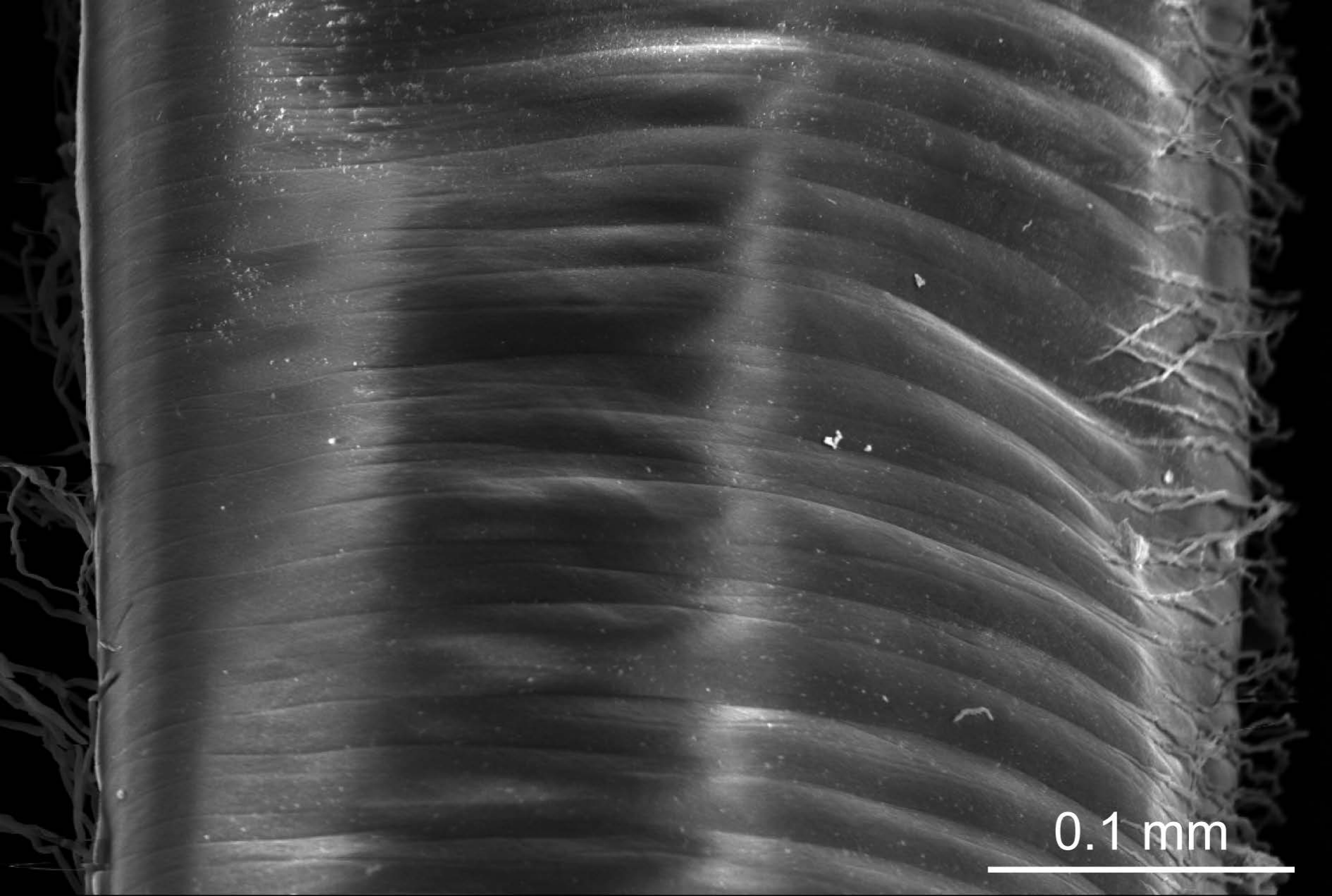
File S2. Aligned 18S sequences of Nematoida used for the maximum-likelihood analysis, after removing alignment-ambiguous sites with Gblocks under “relaxed” parameters and trimming in MEGA7 to the shortest length among them.

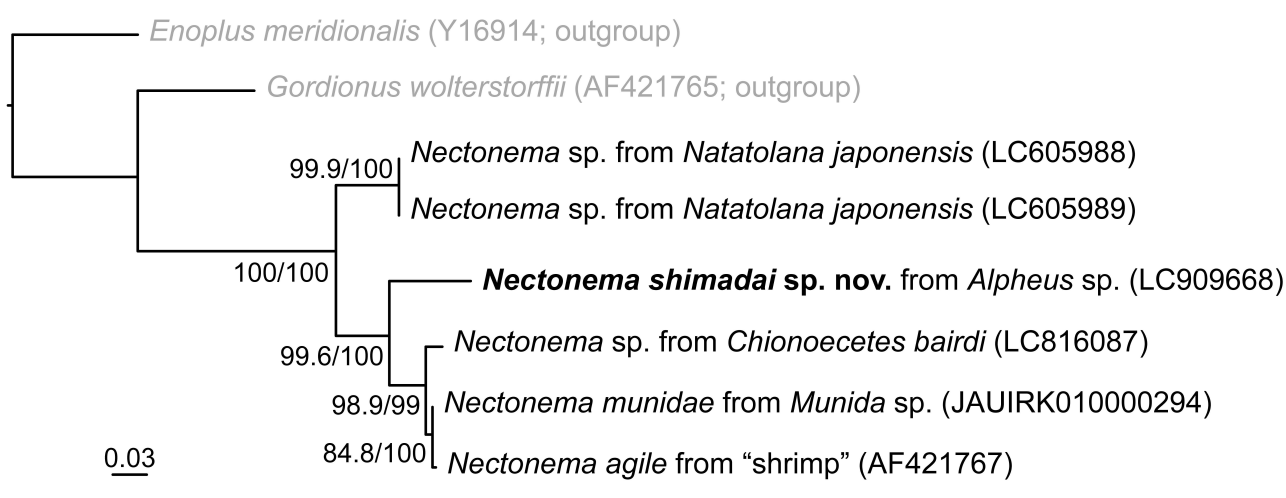
Movie S1. Swimming behavior of a partly damaged individual of *Nectonema shimadai* **sp. nov.**

Movie S2. Anterior region of the ethanol-fixed *Nectonema shimadai* **sp. nov.** observed with light microscopy.


a
b
c
c’
c’’
a–c: 5 mm
c’, c’’: 0.5 mm


a
mo
gc
ac
ph
nb
b
c
nb
gp
0.1 mm
d


0.1 mm


Enoplus meridionalis (Y16914; outgroup)
Gordionus wolterstorffii (AF421765; outgroup)
99.9/100
Nectonema sp. from *Natatolana japonensis* (LC605988)
Nectonema sp. from *Natatolana japonensis* (LC605989)
100/100
***Nectonema shimadai* sp. nov.** from *Alpheus* sp. (LC909668)
99.6/100
Nectonema sp. from *Chionoecetes bairdi* (LC816087)
98.9/99
Nectonema munidae from *Munida* sp. (JAUIRK010000294)
84.8/100
Nectonema agile from “shrimp” (AF421767)
0.03