Nectonema horsehair worms (Nematomorpha) parasitic in the Tanner crab *Chionoecetes bairdi*, with a note on the relationship between host and parasite phylogeny

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Running page head: *Nectonema* from *Chionoecetes bairdi*

ABSTRACT: *Nectonema* nematomorphs utilize marine crustacean hosts in their life cycle; 16 decapod and one isopod genera have been reported to date as host genera. This study reports the first case of *Nectonema* parasitic in the Tanner crab (*Chionoecetes bairdi*), adding another known host genus. A single nematomorph juvenile was recovered from the body cavity of each of two ovigerous female crabs. A nucleotide sequence for the 18S rRNA gene

(1854 bp) was determined from one *Nectonema* individual. The 18S sequence showed K2P distances of 10.0%, 2.0%, and 1.7% from 18S sequences from *Nectonema* sp. from an isopod host, *N. agile*, and *N. munidae*, respectively. In an 18S-based tree, the unknown species was the sister taxon to a clade comprising *N. agile* and *N. munidae*, both of which also utilize decapod hosts. The phylogenetic relationships among the three *Nectonema* species parasitic in decapods were not congruent with the phylogeny of the hosts, not supporting a hypothesis of nematomorph–host co-evolution.

KEY WORDS: Hairworm · Nectonematida · New host genus · Decapoda · Endoparasite · Japan

1. INTRODUCTION

With only five named species (Poinar & Brockerhoff 2001; Schmidt-Rhaesa 2005), the nematomorph genus *Nectonema* is relatively uncommon in marine environments. *Nectonema* juveniles are endoparasites and have been reported from the body cavity of approximately 30 crustacean species in 16 decapod and one isopod genera to date (Table S1 in Supplement 1 at www.int-res.com/articles/suppl/xxxxxxxx) (Nielsen 1969, Schmidt-Rhaesa et al. 2013, Yoshida 2016, Kakui et al. 2021, Cunha et al. 2023).

The host and species diversity of *Nectonema* have surely been underestimated. The infection rate is often low; for example, Yoshida (2016) found only one among 280 *Pagurus brachiomastus* (Thallwitz, 1891) to be parasitized, a sample prevalence of 0.36%. This low prevalence makes detection difficult in unreported host species. As a case in point, *Nectonema* was detected only recently in the American lobster (*Homarus americanus* Milne Edwards, 1837), a well-studied, commercially important decapod (Schmidt-Rhaesa et al. 2013). Because *Nectonema* shows a paucity of identifying morphological characters, conspecific adult–juvenile pairings are difficult, and *Nectonema* juveniles have typically been identified as *Nectonema* sp. or as one of two well-known species, *Nectonema agile* Verrill, 1879 or *Nectonema munidae* Brinkmann, 1930. It remains unclear, however, whether *N. agile* juveniles recovered from different host species are actually conspecific. Molecular tools can

48 resolve this question, but public databases presently contain only a few *Nectonema* DNA
49 sequences.
50 Here I report the first case of *Nectonema* parasitic in the Tanner crab, *Chionoecetes*
51 *bairdi* Rathbun, 1924, making *Chionoecetes* the 17th decapod genus in which nematomorphs
52 have been reported. For phylogenetic reconstruction and future DNA barcoding, I attempted
53 to determine nucleotide sequences for the three markers (18S rRNA gene [18S], cytochrome
54 *c* oxidase subunit I [COI] gene, and ITS-cluster) used in Kakui et al. (2021), but successfully
55 determined only one sequence for 18S. Based on 18S, I reconstructed phylogenetic
56 relationships among *Nectonema* sp. (this study) and other nominal taxa for which 18S data
57 were available.
58
59 **2. MATERIALS AND METHODS**
60 *Nectonema* nematomorphs were discovered in the body cavities of two fresh, ovigerous
61 female crabs bought at a supermarket in Tokyo, Japan on 27 April and 8 May 2024. The
62 labels on these crabs stated they were from “Hokkaido,” the northernmost prefecture in
63 Japan. Although more detailed information was unavailable, they were probably harvested
64 from the Pacific Ocean off the southern coast of Hokkaido, because unusual mass captures of
65 *Chionoecetes bairdi* have been observed only in this area since 2023 (Hokkaido Shinbun
66 Press, 30 May 2023; https://www.hokkaido-np.co.jp/article/853837/).

After the crabs had been boiled and their carapaces removed, two nematomorphs ("Worm A" and "Worm B") were found, one in the body cavity of each crab ("Host A" and "Host B"). Before they were cooked, Host A was alive but Host B was not. The nematomorphs and crabs were photographed and then frozen (Worm A and Host A) or refrigerated (Worm B and Host B). The worms were cut into several pieces; anterior, middle, and posterior portions of defrosted Worm A and posterior (presumably) and middle portions of Worm B were mounted on glass slides in glycerin and observed with an Olympus BX51 light microscope; the remaining parts of the nematomorphs and the hosts were preserved in 70% or 99% ethanol. The body length (BL) of Worm A was measured from the anterior to posterior ends of the body; BL could not be measured for Worm B, which was densely tangled and partly fused. As a body-size proxy for the crabs, I measured the distance between the bases of the pereopod-3 coxae (P3W), which is subequal to the width of abdominal segment 5, which was used as a size proxy by Fong & Dunham (2007). The animals examined were deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, under catalog numbers ICHUM8831 (Worm A and Host A) and ICHUM8832 (Worm B and Host B).

Total DNA was extracted from part of the body of the two nematomorphs and from muscle tissue of the two hosts by using a NucleoSpin Tissue XS Kit (Macherey–Nagel, Germany). The primers and methods for PCR-amplifying COI and ITS-cluster from the

nematomorphs were as described by Kakui et al. (2021), but the amplifications were unsuccessful. The primers and methods for sequencing 18S from a nematomorph and *C. bairdi* were as described by Kakui et al. (2021); those for *C. bairdi* COI were as described by Kakui (2024). The sequences I obtained were deposited in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan, under accession numbers LC816087–LC816090 (Worm-A 18S, Host-A COI, Host-B COI, and Host-B 18S, respectively).

An 18S dataset for phylogenetic analysis comprised the Worm-A sequence determined here and, from public databases, four *Nectonema* sequences (AF421767, *Nectonema agile*; LC605988, LC605989, *Nectonema* sp.; JAUIRK010000294, *Nectonema munidae*; Bleidorn et al. 2002, Kakui et al. 2021, Cunha et al. 2023), and two outgroup sequences (Y16914, *Enoplus meridionalis* Steiner, 1921; AF421765, *Gordionus wolterstorffii* [Camerano, 1888]; Kampfer et al. 1998, Bleidorn et al. 2002). To obtain 18S data of *N. munidae*, (1) I run a Blast (Altschul et al. 1990) against the whole genome dataset of *N. munidae* (JAUIRK010000000; Genome Assembly GCA_030549495.1; Cunha et al. 2023) by using LC605988 as a query sequence; (2) obtained the top-hit contig (JAUIRK010000294) in the Blast result; and (3) aligned the contig and the other *Nectonema* 18S-sequences to find the 18S region in the contig. The sequences were aligned by using MAFFT ver. 7 (Katoh & Standley 2013) with the "Q-INS-I" strategy (Katoh & Toh 2008) and trimmed in MEGA7

105 (Kumar et al. 2016) to the shortest length among them (Sequence Set 1 in Supplement 2 at
106 www.int-res.com/articles/suppl/xxxxxxxx). Alignment-ambiguous sites were then removed
107 with Gblocks ver. 0.91b (Castresana 2000) in NGPhylogeny.fr (Lemoine et al. 2019) under
108 the "relaxed" parameters described in Talavera & Castresana (2007). The final, aligned
109 dataset contained 1740 positions (Sequence Set 2 in Supplement 2 at www.int-
110 res.com/articles/suppl/xxxxxxxx). Methods for selecting the optimal substitution model
111 (TIM3+F+R2), the maximum likelihood (ML) analysis, estimating nodal support (1000
112 pseudoreplicates for both Shimodaira–Hasegawa-like approximate likelihood ratio tests [SH-
113 aLRT] and ultrafast bootstraps [UFBoot]), and drawing the tree were as described by
114 Shimada et al. (2023). Kimura (1980) 2-parameter (K2P) distances among *Nectonema*
115 species were calculated with MEGA7.
116

117 **3. RESULTS AND DISCUSSION**

118 Worm A (BL, 826 mm) (Fig. 1B) from Host A (P3W, 57 mm) (Fig. 1A) showed the
119 following morphology (Fig. 1B–D): anterior and posterior ends rounded, the latter narrower
120 than the former; cuticle light brown; cuticular natatory bristles lacking; posterior opening
121 (gonopore?) present; body filled with mesenchyme; and pharynx present. Its 18S sequence
122 (1854 bp, LC816087) was determined.

Worm B (BL unavailable) (Fig. 1F) from Host B (P3W, 63 mm) (Fig. 1E) was strongly tangled and deformed, with several regions melted or fused together. Its 18S sequence was not determined. Considering that Host B was already dead before it was boiled and that Worm A retained its linear shape and was not partly melted or fused even after boiling, Worm B may have been dead for some time before being boiled.

The second infected crab (Host B) was one female of three female and five male crabs bought after the first infected crab had been found (Rieko Yamamoto personal communication, 8 May 2024). This suggests that the infection rate of *Nectonema* in *Chionoecetes bairdi* may not be low.

The 18S sequence from Worm A was 10.0%, 2.0%, and 1.7% divergent (K2P distance) from homologous sequences from *Nectonema* sp. (LC605988 and LC605989), *Nectonema agile* (AF421767), and *Nectonema munidae* (JAUIRH010000294), respectively (cf. Table S2 in Supplement 1 at www.int-res.com/articles/suppl/xxxxxxxx). A low K2P distance of 0.3% between *N. agile* and *N. munidae* suggests that the present *Nectonema* individuals are not conspecific with any of the three previously sequenced taxa included in the analysis. In the 18S ML tree (Fig. 2), my *Nectonema* sequence formed a fully supported clade with a strongly supported (SH-aLRT = 90.2 %; UFBoot = 100 %) *N. agile* + *N. munidae* clade. The hosts for these three *Nectonema* taxa were all decapods: Worm A from the brachyuran *C. bairdi*, the sequenced *N. agile* individual from a “shrimp” (very likely

142 *Pandalus montagui* Leach, 1814 but not sure; Andreas Schmidt-Rhaesa personal
143 communication, 13 May 2024), and the sequenced *N. munidae* individual from the anomuran
144 *Munida* sp.

145 The phylogenetic tree suggests that there might be at least two divergent groups in
146 *Nectonema*, one comprising species parasitic in isopods and the other in decapods. However,
147 the taxon sampling is too low (one taxon from an isopod and three from decapods) for the
148 tree to resolve the evolution of host utilization in *Nectonema*.

149 The phylogenetic relationships among the three *Nectonema* species parasitic in
150 decapods are not congruent with the phylogeny of the hosts, in which anomurans are more
151 closely related to brachyurans than to "shrimps" (Wolfe et al. 2019). The limited data
152 presented here thus do not support a hypothesis of nematomorph–host co-evolution.
153 However, the possibility of more local-scale host–parasite co-evolution, e.g., one between *N.*
154 *agile* lineages using different host groups and the corresponding hosts, is still conceivable.
155 Additional molecular data from *Nectonema* species and their hosts are needed to resolve this
156 question.

157

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159 photographs, and information on the infected crabs; Christoph Bleidorn and Andreas
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163

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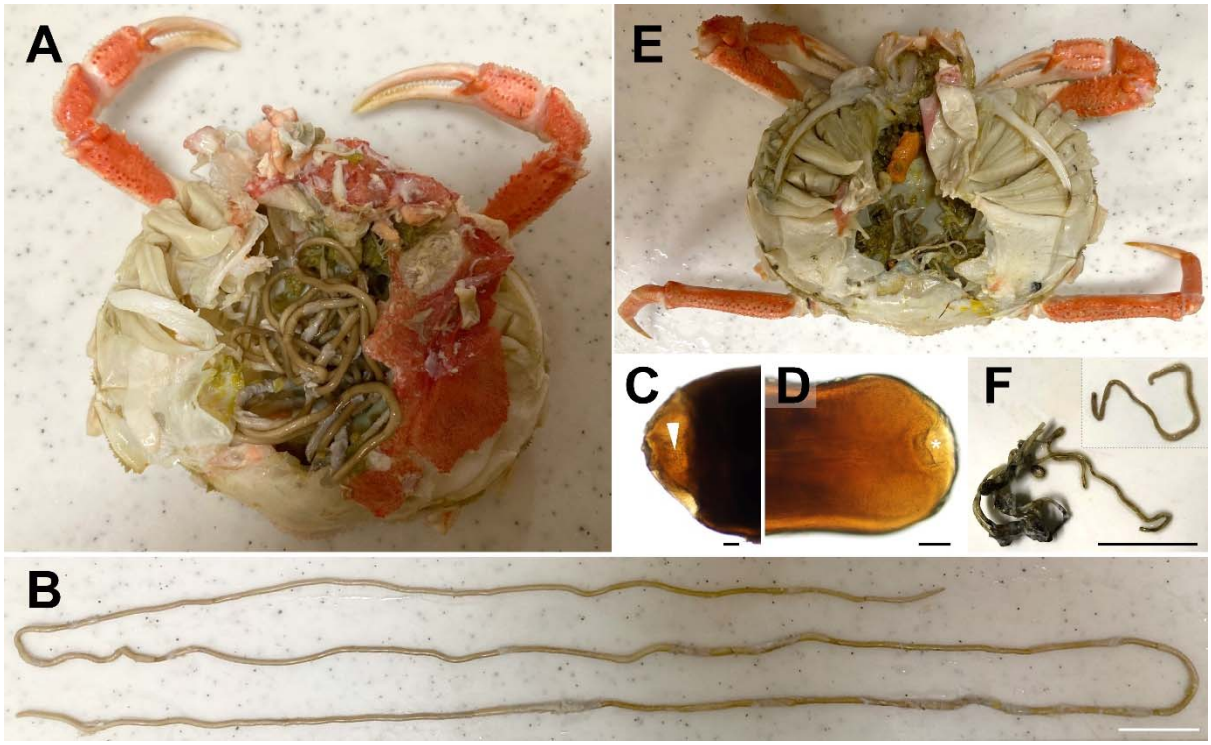
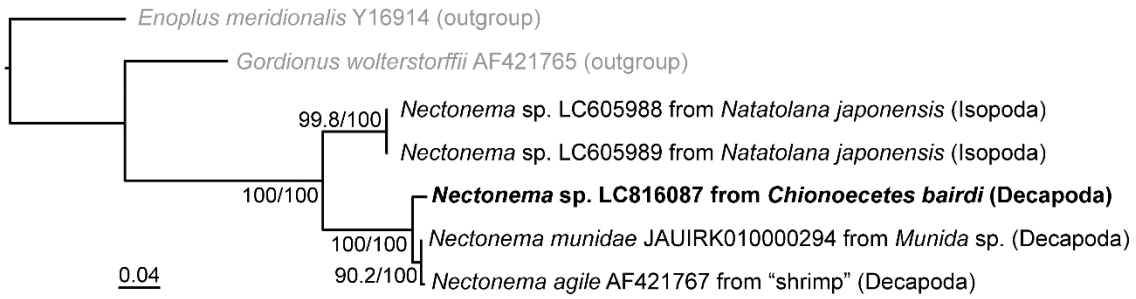


Fig. 1. *Nectonema* sp. parasitic in *Chionoecetes bairdi* females. (A) Worm A within Host A (ICHUM8831). (B) Worm A extracted from the host. (C) Anterior end of Worm A, with an arrowhead indicating pharynx. (D) Posterior end of Worm A, with an asterisk indicating posterior opening. (E) Worm B within Host B (ICHUM8832). (F) Two parts of Worm B extracted from the host. Scale bars: 2 cm (B, F), 0.1 mm (C, D). The photographs in (A), (B), and (E) were taken by Rieko Yamamoto



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232 Fig. 2. Maximum-likelihood tree for *Nectonema* reconstructed from 18S sequences (1740

233 positions); numbers near nodes are Shimodaira–Hasegawa-like approximate likelihood ratio

234 test [SH-aLRT] / ultrafast bootstrap [UFBoot] values as percentages; the scale at the bottom

235 indicates branch length in substitutions per site.

Supplementary Files

Supplement 1

Table S1. Reported hosts of *Nectonema* species, updated from Schmidt-Rhaesa et al. (2013).

Table S2. K2P distances among 18S sequences from nematode and nematomorph species (1666 positions; all positions containing gaps and missing data were eliminated).

Supplement 2

Sequence Set 1. Aligned 18S sequences, trimmed in MEGA7 to the shortest length among the sequences.

Sequence Set 2. Aligned 18S sequences used for the maximum-likelihood analysis, reduced to 1740 positions by removing alignment-ambiguous sites.