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Dive into the sea: first molecular phylogenetic evidence of host expansion from terrestrial/freshwater to marine organisms in Mermithidae (Nematoda: Mermithida)

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Abstract

We report the first mermithid nematode found to be parasitic in a marine tanaidacean crustacean. Ten host tanaidaceans were collected from 52 m depth in Otsuchi Bay, Iwate, Japan, northwestern Pacific, and identified as a species in the tanaidid genus *Zeuxo* Templeton, 1840. Nematodes occurred in the host's body cavity; in one case, at least two individuals inhabited a single host. We provide a brief description and illustrations of the morphology of the nematode. In a phylogenetic reconstruction based on the 18S rRNA gene, the nematode nested in a clade otherwise containing mermithids from terrestrial or freshwater hosts, showing an expansion in host utilization in Mermithidae Braun, 1883 from terrestrial/freshwater hosts to a marine organism.

Keywords endoparasite · intermediate host · phylogeny · roundworm

Introduction

Species in Mermithidae Braun, 1883, one of two families in the nematode order Mermithida, have a pharynx posteriorly with a multicellular organ (stichosome) comprised of longitudinal rows of gland-like cells called stichocytes (Maggenti, 1981; Tchesunov & Spiridorov, 1993). The mermithid life cycle contains a parasitic juvenile stage and a free-living adult stage (see Poinar & Mullens, 1987). More than 500 mermithid species have been reported (Nemys eds., 2022), most of which use insects as their hosts, but with a few using non-insect hosts such as slugs (Mollusca: Gastropoda), nematodes, spiders (Chelicerata: Araneae), or crustaceans (e.g., Vandergast & Roderick, 2003; Ross *et al.*, 2010; Holovachov & Boström, 2013; Warren *et al.*, 2019). Nearly all mermithids have been reported from the terrestrial or freshwater environments; only two species are known from the marine environment: *Thalassomermis megamphis* Tchesunove & Hope, 1997 and a species in *Agamomermis* (Tchesunove & Hope, 1997; Dusto, 2020).

There is little information on the origin of marine mermithids. Like Mermithidae, the mermithidan family Tetradonematidae Cobb, 1919 likewise comprises mainly terrestrial/freshwater members. Recent phylogenetic studies have suggested a non-marine origin for Mermithida (e.g., Westerman *et al.*, 2021), but no molecular data from marine mermithids have been included there.

Here we report a mermithid species parasitic in a marine crustacean in the order Tanaidacea, which comprises about 1500 species (Anderson, 2020) and lacks any previous mermithid record. We provide an abbreviated description of the morphology of the nematode species, present nucleotide sequences for part of its nuclear 18S rRNA (18S) gene, and infer the species' phylogenetic position in the family Mermithidae based on 18S data.

Material and methods

Tanaidaceans were collected by the R/V *Yayoi* (The University of Tokyo) with a biological

dredge at 52 m depth in Otsuchi Bay (39°21.081'N 141°58.145'E), Iwate, Japan, northwestern Pacific on 28 April 2009, and preserved in 99% ethanol. Ethanol-preserved individuals were examined for parasite infection by ventral observation under an Olympus SZX9 stereomicroscope. The identification of hosts was based on Sieg (1980), Bamber (2005), Bamber & Boxshall (2006), Kakui *et al.* (2011), Larsen *et al.* (2015), and Chim & Tong (2019).

To extract parasites, seven infected tanaidaceans were dissected with chemically sharpened needles under an SZX9 stereomicroscope; three host individuals bearing parasites were kept intact for future nondestructive observation. The anterior and/or posterior regions of three extracted parasites were transferred into a 1:3:6 mixture of glycerin, absolute ethanol, and deionized water and placed in a thermostatic chamber at 40°C for 4 hours, after which they were mounted on glass slides in glycerin and observed with an Olympus BX51 microscope. Illustrations of parasites were prepared with Inkscape 1.0 (<https://inkscape.org>) from digital photomicrograph images captured with a PCM500 (AS ONE, Japan) digital camera and edited with GIMP ver. 2.10 (<https://www.gimp.org>). The cephalothorax width (CW) of the tanaidaceans, as an indication of specimen size, was measured at the widest portion of the cephalothorax. The body length of one nematode individual for which we successfully retrieved all body fragments (see below) was measured from the anterior to posterior tips of the body. Ten infected tanaidaceans and extracted nematodes have been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, under catalog numbers ICHUM8290–ICHUM8299. The other tanaidaceans remain in the first author's private collection.

An attempt was made to extract total DNA from part of body of each of five nematodes obtained from five different host individuals by using a NucleoSpin Tissue XS Kit (Macherey-Nagel, Germany), but only three of the five extracts allowed successful PCR amplification. To obtain a nearly complete 18S sequence, two partly overlapping fragments

were determined and then concatenated into a single fragment (cf. Holterman *et al.*, 2006). PCR primers for the first and second fragments were 1096F/1912R and 1813F/2646R (Holterman *et al.*, 2006), respectively; in addition, two primers, 18S-b3R (ATGTGGTAGCCGTTTCTCAGG; newly designed in this study) and 18S-b8F (Kakui *et al.*, 2011), were used in cycle sequencing. PCR amplification conditions with KOD FX Neo polymerase (Toyobo, Japan) were 94°C for 2 min; 45 cycles of 98°C for 10 s, 52°C (first fragment) or 55°C (second fragment) for 30 s, and 68°C for 30 s; and 68°C for 2 min. All nucleotide sequences were determined by direct sequencing with a BigDye Terminator Kit ver. 3.1 with a 3730 DNA Analyzer (Life Technologies, USA). Fragments were concatenated by using MEGA7 (Kumar *et al.*, 2016). The three 18S sequences we determined (which proved to be identical) were deposited in the International Nucleotide Sequence Database (INSD, 2022) through the DNA Data Bank of Japan, under accession numbers LC687375–LC687377.

The 18S dataset for a phylogenetic analysis included the unique sequence we determined and 58 sequences from 56 mermithidan species and two outgroup taxa (two species in Mononchida) taken from the INSD (table 1). The dataset was pre-aligned by using MAFFT ver. 7 (Katoh & Standley, 2013) with the “Auto” strategy, trimmed in MEGA7 to the shortest length among the sequences, and then realigned by eye according to the secondary structure of the honeybee 18S sequence (Gillespie *et al.*, 2006) and that of our used sequences predicted with the RNAfold WebServer (Gruber *et al.*, 2008; Lorenz *et al.*, 2011). The 687-bp aligned dataset used for phylogenetic reconstruction is presented as Supplementary File S1. The optimal substitution model determined under the corrected AIC (Akaike information criterion) option in ModelFinder (Kalyaanamoorthy *et al.*, 2017) was TPM2+F+I+G4. A maximum-likelihood (ML) analysis was conducted in IQ-TREE ver. 2.1.2 (Minh *et al.*, 2020); nodal support values were obtained from an ultrafast bootstrap analysis of 1000 pseudoreplicates under the “bnni” option (Hoang *et al.*, 2018). The ML tree was

drawn with FigTree v1.4.4 (Rambaut, 2022).

Results and Discussion

We examined 599 tanaidaceans for parasite infection and found 10 infected individuals (three males, CW 1.10–1.17 mm; seven females, CW 0.99–1.12 mm); some infected individuals may have been overlooked, as we did not perform dissections in our examination. We identified all tanaidaceans examined as the tanaidid *Zeuxo* sp. (fig. 1). Unfortunately, as the specimens had been preserved in absolute ethanol for more than 10 years, hosts and parasites were strongly dehydrated and deformed, and the latter had become very fragile, with the result that all parasites were fragmented into two or more pieces after extraction (several pieces were damaged and lost). It was thus impossible for us to count the number of parasites per host. We did obtain two posterior portions from one host individual, indicating that a single host individual can bear multiple parasites.

The nematodes were found in the host body cavity. All body fragments were successfully retrieved in only one nematode individual (fig. 1c–f; ICHUM8291). An abbreviated description of this nematode is as follows. Body thread-like, 7.19 mm long. Maximum body diameter 135 μ m. No transverse striation observed on cuticle. Dorsal, ventral, and two lateral chords with single rows of well-developed hypodermal cells. Head rounded. Anterior sensilla and amphids not observed, but four (?) gland cells present in cephalic region. Buccal cavity cup-shaped, without stylet. Pharynx non-muscular, with cuticularized lumen, passing through nerve ring, posteriorly not connected to trophosome. Stichosome consisting of two rows of large stichocytes. Two single stichocytes present apart from stichosome. Trophosome filled with numerous granular cells and without lumen. Anus and rectum absent. Tail rounded.

The three 18S sequences we determined were identical and 1581 bp long. In the ML tree (fig. 2), they are nested in a well-supported clade (98% ultrafast bootstrap support; BS)

along with 13 sequences from other nematodes that infect terrestrial or freshwater hosts. In the tree, the nematode is the sister group (94% BS) to a fully supported clade comprising two nematodes that parasitized a spider (AY374417) and alderfly (EU815285). This result shows a shift in host utilization in Mermithidae from terrestrial or freshwater species to a marine species.

This study provides the third example of mermithids from the marine environment and shows that tanaidaceans can serve as mermithid hosts. Knowledge of tanaidaceans as nematode hosts is currently somewhat limited. Only a few studies (Gardiner, 1975; Tchesunov & Rozenberg, 2011; Jakiel *et al.*, 2019) have reported tanaidaceans as hosts for nematodes; Martell & McClelland (1995) suggested that the anisakid nematode *Pseudoterranova decipiens* (Krabbe, 1878) uses tanaidaceans as an intermediate host. Except for the benthimermithid nematode *Trophomera granovitchi* Tchesunov & Rozenberg, 2011, none of the nematodes parasitic in tanaidaceans has been described morphologically. Future surveys for parasites in the small crustacean group Tanaidacea may provide additional examples of mermithid infections as well as those from other, previously unreported nematode groups.

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Conflict of interest declaration

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Figure and supplementary file captions

Fig. 1. Tanaidacean *Zeuxo* sp. and the infecting nematode parasite Mermithidae sp. a, b infected *Zeuxo* sp., dorsal and ventral views, ethanol-preserved specimen (ICHUM8290). c–f Mermithidae sp., ethanol-preserved specimen (ICHUM8291); c, photograph of anterior and posterior fragments; d–f, anterior (d, e) and posterior (f) portions, line drawings.

Abbreviations, *an*, anterior tip; *gcs*, gland cell of sensilla; *hc*, hypodermal cell; *me*, Mermithidae sp. inside the host; *nr*, nerve ring; *ph*, pharynx; *po*, posterior tip; *stc*, stichocyte; *sts*, stichosome; *tr*, trophosome. Scale bars: a, b, 1 mm; c, 0.5 mm; d, 0.2 mm; e, f, 0.05 mm.

Fig. 2. Maximum-likelihood (ML) tree for 18S sequences (dataset length 687 bp), including the sequence from Mermithidae sp., parasitic in *Zeuxo* sp. For the mermithid taxa, font color indicates the environment where the host and/or the nematode individual used for sequence determination was collected: black, no data; brown, terrestrial; light blue, freshwater (includes host species, such as mosquitos, having an aquatic phase in their life cycle); dark blue, marine. Information on the host group is presented in the right-hand column, with different groups differentiated by different shades of gray. Numbers near nodes are ultrafast bootstrap values > 80%; black circles indicate 100% ultrafast bootstrap support. The scale at the bottom indicates branch length in substitutions per site.

Supplementary File 1. Aligned 18S rRNA dataset used for phylogeny reconstruction.

Table 1. Information on the nematodes included in our phylogenetic analysis. OG, outgroup; nd, no data.

Species	Accession #	Habitat	Host group	Host name	Reference
<i>Actus salvadoricus</i> Baqri & Jairajpuri, 1974 (OG)	AB361035	Terrestrial	-	-	Olia <i>et al.</i> (2008)
<i>Anatonchus tridentatus</i> (de Man, 1876) (OG)	AJ966474	Terrestrial	-	-	Meldal <i>et al.</i> (2007)
<i>Agamermis changshaensis</i> Bao, Lou & Lou, 1992	DQ628908	nd	nd	nd	Poinar <i>et al.</i> (2007)
<i>Agamermis</i> sp.	DQ665653	nd	nd	nd	Unpublished
<i>Agamermis</i> sp.	KX173336	Terrestrial	Insecta	<i>Megacopta cribraria</i>	Stubbins <i>et al.</i> (2016)
<i>Agamermis xianyangensis</i> Xu & Bao, 1993	EF617352	nd	nd	nd	Wang <i>et al.</i> (2007)
<i>Allomermis solenopsii</i> Poinar, Porter, Tang & Hyman, 2007	DQ533953	Terrestrial	Insecta	<i>Solenopsis invicta</i>	Poinar <i>et al.</i> (2007)

<i>Amphimermis</i> sp.	EF617354	nd	nd	nd	Wang <i>et al.</i> (2007)
<i>Amphimermis</i> sp.	EF617355	nd	nd	nd	Wang <i>et al.</i> (2007)
<i>Gastromermis</i> sp.	DQ533954	Freshwater	Insecta	<i>Prosimulium</i> sp.	Poinar <i>et al.</i> (2007)
<i>Heleidomermis</i>					
<i>magnapapula</i> Poinar & Mullens, 1987	DQ533955	Terrestrial	Insecta	<i>Culicoides variipennis</i>	Poinar <i>et al.</i> (2007)
<i>Hexamermis agrotis</i> Wang, Bao & Chen, 1986	DQ530350	nd	nd	nd	Poinar <i>et al.</i> (2007)
<i>Hexamermis popilliae</i>					
Poinar, 2017 in Mazza <i>et al.</i> (2017)	MF040823	Terrestrial	Insecta	<i>Popillia japonica</i>	Mazza <i>et al.</i> (2017)
<i>Hexamermis</i> sp.	LC661691	Terrestrial	Insecta	<i>Glaucias subpunctatus</i>	Watanabe <i>et al.</i> (2021)
<i>Hexamermis</i> sp.	LC661690	Terrestrial	Insecta	<i>Glaucias subpunctatus</i>	Watanabe <i>et al.</i> (2021)
<i>Isomermis lairdi</i> Mondet, Poinar & Bernadou, 1977	FN400892	Freshwater	Insecta	<i>Simulium squamosu</i>	Crainey <i>et al.</i> (2009)

<i>Isomermis lairdi</i>	FN400898	Freshwater	Insecta	<i>Simulium damnosum</i>	Crainey <i>et al.</i> (2009)
<i>Isomermis lairdi</i>	FN400896	Freshwater	Insecta	<i>Simulium damnosum</i>	Crainey <i>et al.</i> (2009)
<i>Isomermis lairdi</i>	FN400900	Freshwater	Insecta	<i>Simulium damnosum</i>	Crainey <i>et al.</i> (2009)
<i>Limnomermis</i> sp.	KJ636371	nd	nd	nd	Unpublished
<i>Mermis nigrescens</i> Dujardin, 1842	KF583882	Terrestrial	Insecta	<i>Forficula auricularia</i>	Presswell <i>et al.</i> (2015)
<i>Mermis nigrescens</i>	DQ518905	nd	nd	nd	Poinar <i>et al.</i> (2007)
<i>Mermis</i> sp.	FJ973464	Terrestrial	Insecta	<i>Culicoides obsoletus</i>	Unpublished (INSD, 2022)
<i>Octomyomermis</i> <i>huazhongensis</i> Luo, Bao, Chen, Wang, Luo & Li, 1996	EF617353	nd	nd	nd	Wang <i>et al.</i> (2007)
<i>Ovomermis sinensis</i> Chen, Jian, Ren & Pan, 1991	KU177046	Terrestrial	Insecta	<i>Cerapteryx graminis</i>	Unpublished (INSD, 2022)
<i>Ovomermis sinensis</i>	DQ520879	nd	nd	nd	Wang <i>et al.</i> (2007)

<i>Romanomermis culicivorax</i> Ross & Smith, 1976	DQ418791	Freshwater	Insecta	<i>Culex pipiens</i>	Wang <i>et al.</i> (2007) [†]
<i>Romanomermis iyengari</i> Welch, 1964	JX021620	Freshwater	Insecta	"mosquito"	Unpublished [†]
<i>Romanomermis sichuanensis</i> Peng, Song & Li, 1984	EF612769	nd	nd	nd	Wang <i>et al.</i> (2007)
<i>Romanomermis wuchangensis</i> Bao, Wang & Wu, 1985	DQ520878	nd	nd	nd	Wang <i>et al.</i> (2007)
<i>Strelkovimermis spiculatus</i> Poinar & Camino, 1986	KP270704	Freshwater	Insecta	<i>Aedes albifasciatus</i>	Belaich <i>et al.</i> (2015)
<i>Strelkovimermis spiculatus</i>	DQ665654	Freshwater	Insecta	<i>Aedes albifasciatus</i>	Unpublished [†]
<i>Strelkovimermis spiculatus</i>	KP270700	Freshwater	Insecta	<i>Aedes albifasciatus</i>	Belaich <i>et al.</i> (2015)
<i>Strelkovimermis spiculatus</i>	KP270703	Freshwater	Insecta	<i>Culex dolosus</i>	Belaich <i>et al.</i> (2015)

<i>Thaumamermis cosgrovei</i> Poinar, 1981	DQ665655	Terrestrial	Crustacea: Isopoda	<i>Armadillidium vulgare</i>	Unpublished [†]
<i>Thaumamermis zealandica</i> Poinar, Latham & Poulin, 2002	KY264164	Terrestrial	Crustacea: Amphipoda	<i>Bellorchestia quoyana</i>	Tobias <i>et al.</i> (2017)
Mermithidae sp.	AY374417	Terrestrial	Chelicerata: Araneae	<i>Tetragnatha</i> spp.	Vandergast & Roderick (2003)
Mermithidae sp.	MK262890	Freshwater	Crustacea: Decapoda	<i>Palaemon paludosus</i>	Warren <i>et al.</i> (2019)
Mermithidae sp.	LC596451	Terrestrial	Crustacea: Isopoda	<i>Ligidium</i> sp.	Yoshino & Waki (2021)
Mermithidae sp.	LC114020	Terrestrial	Insecta	<i>Bombus</i> <i>pseudobaicalensis</i>	Kubo <i>et al.</i> (2016)
Mermithidae sp.	LC512372	Terrestrial	Insecta	<i>Parastrachia</i> <i>japonensis</i>	Iryu <i>et al.</i> (2020)
Mermithidae sp.	LC512368	Terrestrial	Insecta	<i>Parastrachia</i> <i>japonensis</i>	Iryu <i>et al.</i> (2020)

Mermithidae sp.	FJ516757	Terrestrial	Mollusca	"slugs"	Unpublished (INSD, 2022)
Mermithidae sp.	FJ982324	Terrestrial	Mollusca	<i>Deroceras caruanae</i>	Ross <i>et al.</i> (2010)
Mermithidae sp.	MF192960	Terrestrial	Mollusca	<i>Deroceras panormitanum</i>	Unpublished (INSD, 2022)
Mermithidae sp.	AY284743	Terrestrial	nd (coll. from soil)	nd	Holterman <i>et al.</i> (2006)
Mermithidae sp.	AY374416	Terrestrial	Chelicerata: Araneae	<i>Tetragnatha</i> spp.	Vandergast & Roderick (2003)
Mermithidae sp.	FJ605514	Terrestrial	Insecta	<i>Clitarchus hookeri</i>	Yeates & Buckley (2009)
Mermithidae sp.	LC512371	Terrestrial	Insecta	<i>Parastrachia japonensis</i>	Iryu <i>et al.</i> (2020)
Mermithidae sp.	LC512370	Terrestrial	Insecta	<i>Parastrachia japonensis</i>	Iryu <i>et al.</i> (2020)
Mermithidae sp.	LC512369	Terrestrial	Insecta	<i>Parastrachia japonensis</i>	Iryu <i>et al.</i> (2020)
Mermithidae sp.	KC243312	Freshwater	Insecta	<i>Anopheles</i> spp.	Kobylinski <i>et al.</i> (2012)

Mermithidae sp.	KJ636328	nd	nd	nd	Unpublished
Mermithidae sp.	FJ040480	Terrestrial	nd (coll from soil)	nd	van Megen <i>et al.</i> (2009)
Mermithidae sp.	AY374415	Terrestrial	Chelicerata: Araneae	<i>Tetragnatha</i> spp.	Vandergast & Roderick (2003)
Mermithidae sp.*	EU815285	Freshwater	Insecta	<i>Sialis californica</i>	Winterton <i>et al.</i> (2010)
Mermithidae sp.**	JQ894731	Terrestrial	Insecta	<i>Kosciuscola tristis</i>	Umbers <i>et al.</i> (2015)
Mermithidae sp.**	JQ894732	Terrestrial	Insecta	<i>Kosciuscola tristis</i>	Umbers <i>et al.</i> (2015)
Mermithidae sp.	LC687375	Marine	Crustacea: Tanaidacea	<i>Zeuxo</i> sp.	This study

* This sequence was deposited as "*Sialis californica*," but we regard it as coming from a mermithid nematode, based on the high identity score in BLAST searches with our sequence (query cover 90%; percent identity 91.30 %).

** These sequences were deposited as "Nematoda sp.," but we regard them as coming from mermithid nematodes, based on high identity scores in BLAST searches with our sequence (query cover 93–94%; percent identity 91.84–94.41 %).

† Although no host data are presented in the INSD, one of the authors who deposited this sequence (Bradley Hyman) indicated this nematode species as extracted from this host species in other work(s) (see Hyman *et al.*, 2005; Pérez-Pacheco *et al.*, 2015).



