

## FIRST RECORD OF A SPECIES OF MERMITHIDAE BRAUN, 1883 INFECTING A DECAPOD, *PALAEMON PALUDOSUS* (PALAEMONIDAE)

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### KEY WORDS ABSTRACT

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Pathology

The hemocoel of 26 of 30 (86%) eastern grass shrimps, *Palaemon paludosus* (Gibbes, 1850) (Decapoda: Palaemonidae), captured during June 2017 from several freshwater lakes near Leesburg and Lake Kissimmee, Florida, was infected by juveniles of a nematode species (Mermithidae sp.). Some infected eastern grass shrimps were preserved whole for histopathology, whereas others were dissected to excise parasitic juvenile nematodes, and still others were kept alive in glass aquaria such that post-parasitic (emerged) juvenile nematodes could be opportunistically observed alive and ultimately preserved. Parasitic and post-parasitic juvenile nematodes had cuticle cross-fibers, cephalic papillae, cup-shaped amphids, a horn-shaped vagina, a trophosome, and a caudal appendage, which collectively diagnosed them as Mermithidae sp. They differed from those of nematomorphs (Nematomorpha) by lacking 4 giant cells anteriorly, tegumental bristles, scale or plate-like areoles, a bifurcate or trifurcate posterior end, and an anus. A phylogenetic analysis of the small subunit rDNA (18S) that included all of the available mermithid sequences corresponding to morphologically diagnosed specimens recovered Mermithidae sp. within the clade of mermithids and sister to *Ovomermis sinensis* Chen, Jian, and Ren, 1991. This is the first record of a mermithid infection in a decapod and first report of a mermithid infection in an aquatic crustacean from North America (another mermithid infects a terrestrial isopod there). The high prevalence of infection and the multiple geographic localities harboring infected eastern grass shrimps indicated that these infections were not spurious. Because no other decapod is confirmed as a mermithid host, we suspect that these specimens likely represent a new species with a life cycle worth studying, since none for a mermithid involving a decapod nor a crustacean has been elucidated to date. We also provide a table of all mermithid and nematomorph infections in crustaceans.

Compared to our knowledge of the parasites of fishes and other aquatic vertebrates, our knowledge of the parasites of aquatic invertebrates is relatively poor (McElwain et al., 2016; Womble and Bullard, 2016; Warren et al., 2017). Aquatic mollusks and arthropods, for example, far outnumber aquatic vertebrates in species diversity and the proportion of endemic species (Johnson et al., 2013). However, the vast majority remains to be examined for parasitic infections. A large body of literature has explored digenetic-mollusk relationships (Ogelsby, 1961; Fraser, 1967; Wardle, 1979; Kraus et al., 2014), but the metazoan parasites of crustaceans remain underexplored.

The present work focuses on grass shrimps (Decapoda: Palaemonidae: *Palaemon* spp.) and parasitic species of Nematoda (nematodes) and Nematomorpha (horsehair worms) (Table I).

These infections in grass shrimps, which are relatively small, thin-shelled marine and freshwater decapods, are striking because the grass shrimp's shell is translucent and readily presents the parasite within the hemocoel of the living host. As such, infected hosts can be identified without dissection, and parasites can be observed within the living host without killing it, which is rare in parasitology. However, because nematodes and nematomorphs can appear grossly similar without microscopy and can be easily confused by the novice observer who may happen to notice them in the field, there is the possibility that some infections in grass shrimps can be, and have been, misidentified.

Few records of nematodes and nematomorphs infecting crustaceans exist. The mermithids infecting crustaceans total 9 nominal species assigned to 6 genera from Asia, Europe, North

**Table I.** Species of Mermithidae (Nematoda) and *Nectonema* (Nematomorpha) infecting crustaceans.

Parasite	Type host	Type locality	Reference
Nematoda			
Mermithidae Braun, 1883			
<i>Agamomermis gammari</i> von Linstow, 1892	<i>Gammarus pulex</i> (Linnaeus, 1758) (Amphipoda: Gammaridae)	Germany	von Linstow, 1892
	<i>Micruropus garjajewi</i> Bazikalova, 1945 (Amphipoda: Micruropodidae)	Germany	von Linstow, 1892
	<i>Micruropus semenowi</i> Bazikalova, 1945 (Amphipoda: Micruropodidae)	Germany	von Linstow, 1892
<i>Gammaromermis baikalensis</i> Rubstov and Bekman, 1979	<i>Echiuropus morawitzi</i> (Dybowsky, 1874) (Amphipoda: Crypturopodidae)	Russia	Rubstov and Bekman, 1979
<i>Gammaromermis carinogammari</i> Rubstov, 1976	<i>Asproogammarus rhodophthalmus</i> (Dybowsky, 1874) (as <i>Carinogammarus</i> ) (Amphipoda: Crypturopodidae)	Russia	Rubstov, 1976
<i>Gammaromermis langicaudata</i> Rubstov and Bekman, 1979	<i>A. rhodophthalmus</i> (Dybowsky, 1874) (as <i>Carinogammarus</i> ) (Amphipoda: Crypturopodidae)	Russia	Rubstov and Bekman, 1979
<i>Gastromermis</i> sp. Garcia and Camino, 1987	<i>Hyalella curvispina</i> Shoemaker, 1942 (Amphipoda: Hyalellidae)	Argentina	Garcia and Camino, 1987
<i>Limnomyrmis bonaerensis</i> Camino, 1989	<i>H. curvispina</i> (Amphipoda: Hyalellidae)	Argentina	Camino, 1989
<i>Pseudomermis cazanica</i> Bacesco, 1948	<i>Corophium</i> sp. Latreille, 1806 (Amphipoda: Corophiidae)	Romania	Băcesco, 1948
<i>Thaumamermis cosgrovei</i> Poinar, 1981	<i>Armadillidium vulgare</i> (Latreille, 1804) (Isopoda: Armadillidiidae)	USA	Poinar, 1981
<i>Thaumamermis zealandica</i> Poinar et al., 2002	<i>Porcellio scaber</i> Latreille, 1804 (Isopoda: Porcellionidae)	USA	Poinar, 1981
Mermithidae sp. (new host record)	<i>Bellorchestia quoyana</i> (H. Milne-Edwards, 1840) (as <i>Talorchestia</i> ) (Amphipoda: Talitridae)	New Zealand	Poinar et al., 2002
	Eastern grass shrimp, <i>Palaemon paludosus</i> (Gibbes, 1850) (Decapoda: Palaemonidae)	USA	Present study
Nematomorpha			
<i>Nectonema</i> Verrill, 1879			
<i>Nectonema agile</i> Verrill, 1879	Free living	USA	Verrill, 1879; Ward, 1892
	<i>Anapagurus hyndmanni</i> (Thompson, 1844) (Decapoda: Paguridae)	France	Pérez, 1927
	<i>Anapagurus laevis</i> (Bell, 1846) (Decapoda: Paguridae)	France	Pérez, 1934
	Small hermit crab, <i>Diogenes pugilator</i> (Roux, 1829) (Decapoda: Diogenidae)	France	Pérez, 1934
	Graceful blunt-tail prawn, <i>Gennadas elegans</i> (Smith, 1882) (as <i>Amalopenaeus</i> ) (Decapoda: Benthesicymidae)	Sierra Leone	Bate, 1888
	American lobster, <i>Homarus americanus</i> H. Milne Edwards, 1837 (Decapoda: Nephropidae)	Canada	Schmidt-Rhaesa et al., 2013
	Polar shrimp, <i>Lebbeus polaris</i> (Sabine, 1824) (as <i>Spirontocaris</i> ) (Decapoda: Thoridae)	Greenland	Nouvel and Nouvel, 1938
	Arch-fronted swimming crab, <i>Liocarcinus navigator</i> (Herbst, 1794) (as <i>Macropipus arcuatus</i> ) (Decapoda: Polybiidae)	France	Gallien, 1949
	<i>Liocarcinus pusillus</i> (Leach, 1816) (as <i>Macropipus</i> ) (Decapoda: Polybiidae)	France	Pérez, 1934
	<i>Macropodia rostrata</i> (Linnaeus, 1761) (Decapoda: Inachidae)	France	Pérez, 1934
	<i>Pagurus bernhardus</i> (Linnaeus, 1758) (Decapoda: Paguridae)	France	Mouchet, 1931
	Grass prawn, <i>Palaemon elegans</i> Rathke, 1837 (as <i>Leander squilla</i> ) (Decapoda: Palaemonidae)	France	Nouvel and Nouvel, 1938
	Common prawn, <i>Palaemon serratus</i> (Pennant, 1777) (as <i>Leander</i> ) (Decapoda: Palaemonidae)	France	Nouvel and Nouvel, 1934
	Marsh grass shrimp, <i>Palaemon vulgaris</i> Say, 1818 (as <i>Palaemonetes</i> ) (Decapoda: Palaemonidae)	USA	Born, 1967
	<i>Palaemon</i> sp. Weber, 1795 (as <i>Palaemonetes</i> ) (Decapoda: Palaemonidae)	USA	Ward, 1893
<i>Nectonema melanocephalum</i> Nierstrasz, 1907	Free living	Indonesia	Nierstrasz, 1907
<i>Nectonema munidae</i> Brinkmann, 1930	Arctic red prawn, <i>Eusergestes arcticus</i> (Krøyer, 1855) (as <i>Sergestes</i> ) (Decapoda: Sergestidae)	Norway	Greve, 1972
	Rugose squat lobster, <i>Munida sarsi</i> Huus, 1935 (Decapoda: Munididae)	Norway	Nielsen, 1969

**Table I.** Continued.

Parasite	Type host	Type locality	Reference
	Narrow-legged squat lobster, <i>Munida tenuimana</i> Sars, 1871 (Decapoda: Munididae)	Norway	Brinkmann, 1930
	<i>Pagurus cuanensis</i> Bell, 1846 (Decapoda: Paguridae)	Norway	Nielsen, 1969
	Downy hermit crab, <i>Pagurus pubescens</i> Krøyer, 1838 (Decapoda: Paguridae)	Norway	Brinkmann, 1930
	Norwegian shrimp, <i>Pontophilus norvegicus</i> (M. Sars, 1861) (Decapoda: Crangonidae)	Norway	Brinkmann, 1930
<i>Nectonema Svensksundi</i> Bock, 1913	Free living	Norway	Bock, 1913
<i>Nectonema zealandica</i> Poinar and Brockerhoff, 2001	<i>Hemigrapsus sexdentatus</i> (H. Milne-Edwards, 1837) (as <i>H. edwardsi</i> ) (Decapoda: Varunidae)	New Zealand	Poinar and Brockerhoff, 2001
<i>Nectonema</i> sp.	Jonah rock crab, <i>Cancer borealis</i> Stimpson, 1859 (Decapoda: Cancridae)	Canada	Leslie et al., 1981
	Atlantic rock crab, <i>Cancer irroratus</i> Say, 1817 (Decapoda: Cancridae)	Canada	Leslie et al., 1981
	Acadian hermit crab, <i>Pagurus acadianus</i> Benedict, 1901 (Decapoda: Paguridae)	Canada	Leslie et al., 1981
	Northern shrimp, <i>Pandalus borealis</i> Krøyer, 1838 (Decapoda: Pandalidae)	Canada	Leslie et al., 1981
	Pink shrimp, <i>Pandalus montagui</i> Leach, 1814 (Decapoda: Pandalidae)	Canada	Skaling and Mackinnon, 1988

and South America, and New Zealand (Table I). No mermithid has been reported previously from a decapod, and only 1 mermithid reportedly infects a crustacean (a terrestrial isopod) in North America (Poinar, 1981). The nominal nematomorphs infecting crustaceans (all marine) comprise 5 species all assigned to *Nectonema* Verrill, 1879 from Africa, Europe, North America, Indonesia, and New Zealand (Table I). Hence, while no mermithid infection in a decapod has been reported previously, *Nectonema* spp. are typical parasites of decapods (Table I). No life cycle for a species of *Nectonema* is known, but it probably is similar to those of freshwater nematomorphs (Hanelt and Janovy, 2003; Hanelt et al., 2005). The only species of *Nectonema* reported from the United States is *Nectonema agile* Verrill, 1879, and only 3 original records of parasitic juveniles of *N. agile* exist (Ward, 1893; Born, 1967; Schmidt-Rhaesa et al., 2013): from an innominate grass shrimp, *Palaemon* sp. Weber, 1795; from a marsh grass shrimp, *Palaemon vulgaris* Say, 1818 (hereafter, "MGS"); and from the American lobster, *Homarus americanus* Milne Edwards, 1837 (Table I). None of these reports provided specific anatomical details of the parasite specimens themselves. Hence, any record of a nematode or mermithid infecting a crustacean, especially a species of *Palaemon*, is worthy of taxonomic investigation, because it can increase our knowledge of the life cycles of mermithids and nematomorphs infecting crustaceans in riverine, estuarine, and oceanic habitats.

The fortuitous discovery herein of numerous infected eastern grass shrimps, *Palaemon paludosus* (Gibbes, 1850) (hereafter, "EGSs"), from central Florida presented us with the opportunity to explore this host-parasite system by observing live, infected EGSs, studying well-fixed parasites for morphology, and obtaining molecular sequence data for the parasite. Because anatomical accounts of these parasites in grass shrimps are few, we thought it important to taxonomically identify the parasite with morphology and also examine museum specimens linked to nematode and

nematomorph infections in grass shrimps from North America. We were intrigued to explore this matter because mermithids infecting crustaceans are few, none has been reported from a decapod, and only 1 has been reported from a crustacean in North America (Poinar, 1981). Moreover, based on these facts, we suspect that the nematode species detailed herein likely represents a new species, with a life cycle worth studying because it is the only species of Mermithidae reported to infect a decapod.

## MATERIALS AND METHODS

### Collections and maintenance of infected EGSs

During June 2017, 30 EGSs were collected from the vicinity of several freshwater lakes near Leesburg (Lake Harris [28°45'46.60"N, 81°48'42.30"W], Lake Griffin [28°51'48.33"N, 81°50'59.13"W], Lake Yale [28°54'53.13"N, 81°44'24.99"W]), and Lake Kissimmee (27°55'4.81"N, 81°17'5.70"W), Florida. All specimens were shipped alive to Auburn University; 26 of 30 (86%) EGSs were infected. Of the 26 infected EGSs, 10 were immediately fixed whole (7 for histopathology; 3 for excision of the parasite and DNA extraction), and 16 were placed alive in an aquarium aside another aquarium holding 4 uninfected EGSs. The experimental setup consisted of 2 (40-L) glass aquariums (no substratum; equipped with aeration and filtration; kept in a climate-controlled [23 °C] room near a window for natural lighting) that were checked hourly during daylight hours for 21 days. Emerged post-parasitic juvenile nematodes were observed alive and ultimately preserved. Of the 16 aquarium-held EGSs, 8 shed 1 nematode (including 1 EGS that had a second parasitic juvenile nematode that did not emerge). Those 8 post-parasitic juvenile nematodes and the single parasitic juvenile nematode were parsed for morphology as whole-mounts ( $n = 4$ ), scanning electron microscopy (SEM) (2), and DNA extraction (3). After 21 days, the remaining 8 infected EGSs that had not shed their

nematode were dissected to excise each parasitic juvenile nematode, and those 8 parasitic juvenile nematodes were all whole-mounted. In total, we stabilized 27 nematode specimens, including 7 within intact EGSs for histopathology, 12 whole-mounted specimens, 2 SEM-prepared specimens, and 6 specimens available for DNA extraction (1 specimen was used for DNA extraction).

### Morphology (light and scanning electron microscopy)

Nematodes and nematomorphs intended as whole-mounts were photographed alive with the aid of a stereo-dissecting microscope and fiber optic light source, rinsed with distilled water, immersed in 95% glacial acetic acid for 5–10 min, fixed and cleared in 5 parts glycerin plus 95 parts 70% ethanol (EtOH) (“70 + 5”), mounted on glass slides using glycerin jelly, and studied with a Leica DM 2500 microscope (Leica, Wetzler, Germany) with differential interference contrast (DIC) optical components (Figs. 1–10). Nematodes for SEM were washed with deionized water, dehydrated through a graded EtOH series, critical point dried in liquid CO<sub>2</sub>, mounted on SEM aluminum stubs with double-sided carbon tape, sputter-coated with gold palladium (19.32 g/cm<sup>3</sup>; 25 mA), and viewed with a Zeiss EVO 50VP SEM. Morphological terms and nomenclature for nematodes follow Chitwood and Chitwood (1974), Poinar (1981), and Poinar et al. (2002). Measurements are reported in micrometers ( $\mu\text{m}$ ) as the range, unless otherwise specified, followed by the mean,  $\pm$  standard deviation, and sample size in parentheses. Voucher specimens were deposited in the National Museum of Natural History's Invertebrate Zoology Collection (USNM, Smithsonian Institution, Washington, D.C.). Morphological terms and nomenclature for Nectonematoidea (Nematomorpha) follow Verrill (1879), Ward (1892), and Poinar and Brockerhoff (2001).

### Histopathology

EGSs were preserved whole in 95% ethanol (EtOH), rinsed in deionized water for 2 hr, post-fixed by injection with and submergence in Davidson's fixative (1 part tissue:9 parts fixative) for 48 hr, processed routinely for histology by dehydration in an EtOH series, embedded under vacuum pressure in paraffin, sectioned at 4  $\mu\text{m}$ , mounted on glass slides, de-paraffinized, routinely stained with hematoxylin and eosin, cover-slipped, and examined and photographed with the aid of a compound light microscope.

### DNA extraction and phylogenetics

Nematodes were rinsed in sterile water, lysed using tubes prefilled with lysing matrix B (0.1-mm silica spheres) and 500 ml of ddH<sub>2</sub>O, homogenized 3 times, placed on ice, and centrifuged at 7,168  $\times g$  for 1 min at room temperature. Amplification and sequencing of the small subunit rDNA (18S) used the set of primers described in Mazza et al. (2017), and PCR amplifications were performed according to Warren et al. (2017). Sequence assembly and analysis of chromatograms were performed with Geneious version 11.0.5 (<http://www.geneious.com>; Kearse et al., 2012). Nucleotide sequence data were deposited in GenBank (MK262890). A comparison of the new sequence utilized the NCBI BLAST tool (<https://blast.ncbi.nlm.nih.gov>). The phylogenetic analysis included the new nematode sequence plus the taxa

included in Mazza et al. (2017). Sequences were aligned using MAFFT (Katoh and Standley, 2013). JModelTest 2 version 2.1.10 (Darriba et al., 2012) was implemented to perform statistical selection of the best-fit models of nucleotide substitution based on Bayesian information criteria. Aligned sequences were reformatted (from .fasta to .nexus) using the web application ALTER (Glez-Peña et al., 2010) to run Bayesian inference (BI). BI was performed in MrBayes version 3.2.5 (Ronquist and Huelsenbeck, 2003) using substitution model averaging (“nst = mixed”) and a gamma distribution to model rate heterogeneity as indicated by JModelTest. Defaults were used for all other parameters. Three independent runs with 4 Metropolis-coupled chains were run for 5,000,000 generations, sampling the posterior distribution every 1,000 generations. Convergence was checked using Tracer v1.6.1 (Rambaut et al., 2014) and the “sump” command in MrBayes: All runs appeared to reach convergence after discarding the first 25% of the generation as burn-in. A majority rule consensus tree of the post-burn-in posterior distribution was generated with the “sumt” command in MrBayes. The inferred phylogenetic tree was visualized using FigTree v1.4.3 (Rambaut et al., 2014) and further edited for visualization purposes with Adobe Illustrator (Adobe Systems).

## RESULTS

### Live observations of infected EGSs and post-parasitic juveniles

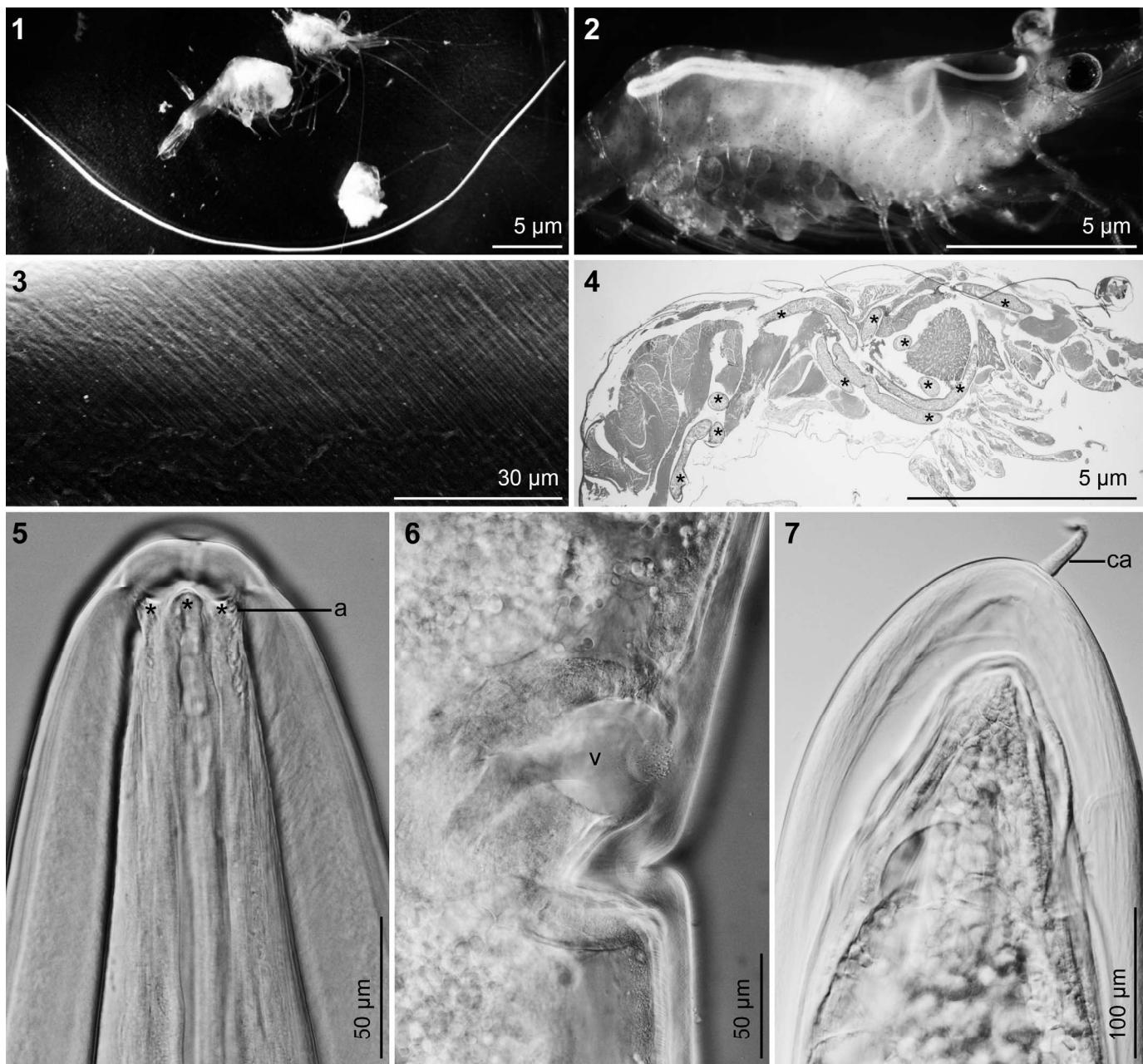
Emergence of post-parasitic juvenile mermithids occurred at night (mermithids were observed in the morning on the tank bottom) on days 1, 2, 3, 12, and 21. EGSs that shed nematodes died within 48 hr after mermithid emergence, and no post-parasitic mermithid survived >72 hr. Lack of substratum probably contributed to this, but EGS predation on post-parasitic juveniles also occurred: On 1 occasion, an EGS was observed to be eating the anterior end of a post-parasitic mermithid. Anecdotally, infected and uninfected EGSs were morphologically and behaviorally indistinguishable.

### Morphology of Mermithidae sp.

The 12 whole-mounted female specimens and 2 SEM-prepared female specimens (Figs. 1–7) (USNM 1531001–1531003) had the following anatomical features: white, body 63–74 mm ( $67 \pm 6.1$ , 4) long (Fig. 1), 125–435 ( $271 \pm 106$ , 11) wide, tapering anteriorly, posterior end bluntly rounded. Cuticle with fine cross-fibers (Fig. 3). Head with 6 cephalic papillae. Amphids 2 in number, in line with lateral papillae (Fig. 5). Mouth papillae lacking; oral stylet present. Vagina horn-shaped (Fig. 6), 133–200 ( $163 \pm 21$ , 8) long, 55–77 ( $69 \pm 7$ , 8) wide. Trophosome present, extending to posterior end. Caudal appendage 25–60 ( $39 \pm 15$ , 6) long, 3–4 ( $3.5 \pm 0.5$ , 6) wide (Fig. 7).

### Histopathology of infected EGSs

Histology of whole, infected EGSs resulted in 2 paraffin-embedded tissue blocks yielding 156 sections mounted on 52 glass slides. Sectioned mermithids had an intensely eosinophilic cuticle with distinct cross-fibers and infected the hemocoel (principally about the hepatopancreas, but also intertwining throughout the hemocoel in the cephalothorax and extending into the abdomen of the EGSs). Somatic muscle and viscera of the EGSs were



**Figures 1–7.** Mermithidae sp. infecting eastern grass shrimp, *Palaemon paludosus* (Gibbes, 1850) (Decapoda: Palaemonidae) (USNM 1531001–1531003), from central Florida. (1) Excised mermithid (infected eastern grass shrimp broken apart). Bar = 5 mm. (2) A single mermithid within hemocoel. (3) Scanning electron microscopy of cuticular cross-fibers. (4) Histological section of shrimp showing intertwined mermithid (\*). (5) Anterior end of mermithid; head papillae (\*), amphid (a). (6) Lateral margin of mermithid; vagina (v). (7) Posterior end of mermithid; caudal appendage (ca).

normal; no lesion was associated with the parasitic juveniles of Mermithidae sp. (Fig. 4).

#### 18S phylogenetic analysis

The 18S sequence of Mermithidae sp. from the infected EGS herein resulted in an alignment of 780 nucleotides. This 18S sequence had 7 (0.9%) and 9 (1.8%) nucleotide differences with those of *Ovomermis sinensis* Chen, Jian, and Ren, 1991, which infects a larval armyworm, *Mythimna separata* (Walker, 1865) (Lepidoptera: Noctuidae), from China. The phylogeny (Fig. 12)

recovered Mermithidae sp. sister to *O. sinensis*. The recovered phylogeny generally matched that of Mazza et al. (2017), except that it better resolved inter-generic relationships. *Gastromermis* Micoletzky, 1925 was sister to *Allomeris* Steiner, 1924 + *Mermis* Dujardin, 1842 (clade 1) (vs. grouping *Gastromermis* with *Amphimermis* Kaburaki and Imamura, 1932 + *Romanomermis* Coman, 1961). *Aranimermis* Poinar and Benton (1986) was sister to monophyletic *Romanomermis* (clade 3) (vs. sister to all other mermithid sequences), and *Hexameris agrotis* was within a polytomy (clade 4; including *Hexameris popilliae* Poinar, 2017,

*O. sinensis*, Mermithidae sp., and *Agamermis* spp. [vs. sister to a polytomy of those genera] (Fig. 12). Perhaps because we are beginners with mermithid morphology and because we know little about their comparative morphology in general, we were surprised that the Mermithidae sp. from EGSSs herein was sister to *O. sinensis* rather than to the crustacean-infecting mermithid *Thaumamermis cosgrovei* Poinar, 1981; i.e., crustacean mermithids are paraphyletic. Another noteworthy feature is that the mermithids in clade 4 infect hosts in 7 orders (Isopoda, Decapoda, Orthoptera, Diptera, Hemiptera, Lepidoptera, Coleoptera) and 2 classes (Insecta and Malacostraca) (Fig. 12), suggesting that many more mermithids should be sequenced to test the hypothesis that related mermithids infect related intermediate hosts.

The following taxa were excluded from our phylogenetic analysis because they were not morphologically diagnosed in their respective reports. Innominate mermithids infecting bumble bees, *Bombus pseudobaicalensis* Vogt, 1911 (Hymenoptera: Apidae), from Japan and another species infecting both common eastern bumble bees, *Bombus impatiens* Cresson, 1863, and brown-belted bumble bees, *Bombus griseocollis* (DeGeer, 1773), from Arkansas differed from Mermithidae sp. by 6, 10, and 16 nucleotides, respectively (Kubo et al., 2016; Tripodi and Strange, 2018). Another innominate mermithid infecting a jumping spider, *Habronattus signatus* Banks, 1900 (Araneae: Salticidae), from California differed by 10 nucleotides (Vandergast and Roderick, 2003). These sequences will likely add another order (Hymenoptera) and class (Arachnida) to the already diverse clade 4.

## DISCUSSION

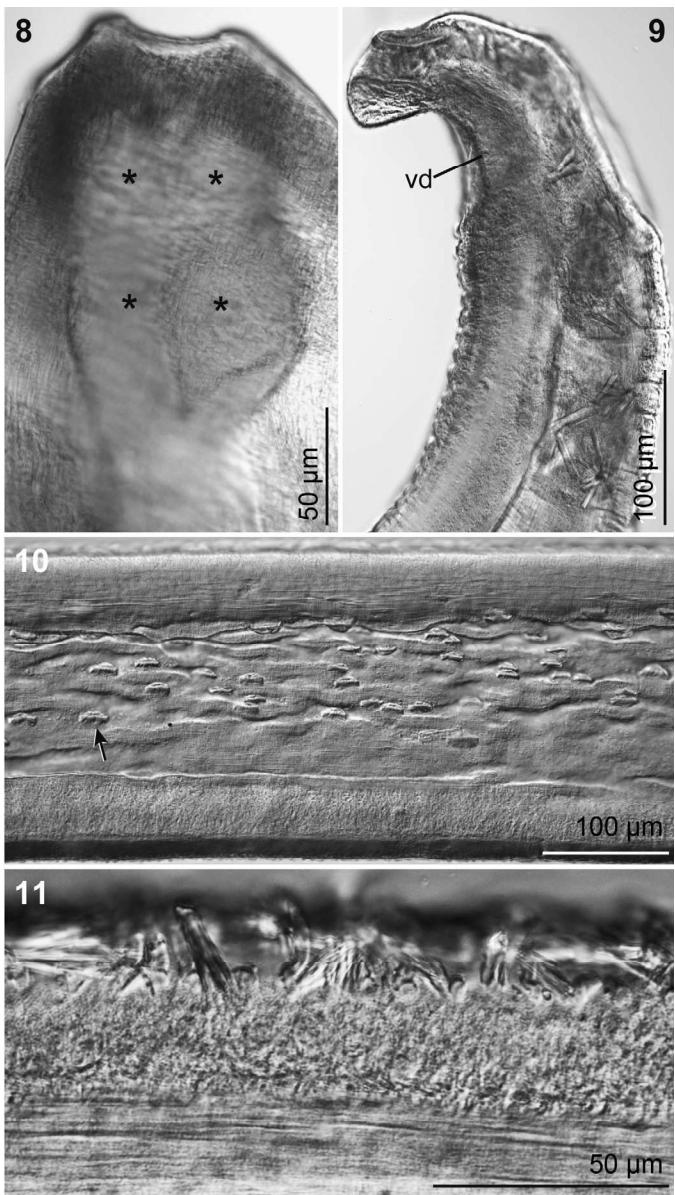
Most mermithid species descriptions are based on free-living mature adults. Several of these descriptions detail parasitic and post-parasitic specimens, including measurements of body length, body width, and caudal appendage length/width as well as presence/absence of cuticle cross-fibers (Fig. 3), cephalic papillae, and amphids (Poinar, 1981; Camino, 1991; Poinar et al., 2007; Mazza et al., 2017). We include those details as well as the number of cephalic papillae (6), number of amphids (2) (Fig. 5), and shape of the vagina (horn-shaped) (Fig. 6). Based on the presence of a cuticle with cross-fibers (Fig. 3), 6 cephalic papillae (Fig. 5), and a terminal mouth, our specimens could be assigned to *Hexamerism* Steiner, 1924, *Thaumamermis* Poinar, 1981, or *Ovomermis* Rubstov, 1976 (Poinar, 1981; Poinar et al., 2002). However, species of *Thaumamermis* have a “modified” S-shaped vagina (vs. a horn-shaped vagina in our specimens) (Poinar, 1981; Poinar et al., 2002) (Fig. 6). Without a male, we cannot further diagnose our post-parasitic juveniles because the length, shape, and configuration of the spicules, cephalic papillae, amphids, and caudal papillae in the male (and the caudal papillae of the female) differentiate species of these genera (Poinar, 1981; Chen et al., 1991; Poinar et al., 2002; Mazza et al., 2017).

Little information is available on histopathological alterations to host tissue associated with mermithid infections, but some studies have documented infection site and growth of mermithids within the arthropod host. Bailey and Gordon (1973) reported that high-intensity infections (4–6 mermithids) of *Reesimermis nielseni* Tsai and Grundmann, 1969 in the larvae of yellow-fever mosquitos, *Aedes aegypti* Linnaeus, 1762, caused deterioration of body tissues. Denner (1975) asserted infections by *Mermis nigrescens* Dujardin, 1842 in red-legged grasshoppers, *Melanoplus*

*femurrrubrum* (DeGeer, 1773), were associated with compressed or atrophied mandibular adductor muscle fibers, compressed, atrophied, or destroyed dorsal musculature fibers of the body wall, atrophied or misshapen regions of the brain (protocerebrum and deutocerebrum), reduced salivary glands, destruction of outer layers of the gut, and compression and partial closure of the gut lumen due to crowding of the parasite. Unlike other mermithid infections in other arthropods wherein the host shows behavioral or anatomical abnormalities (Leech, 1966; Poinar, 1985), no morphological or behavioral abnormality was observed in the infected EGSSs in our study. However, all EGSSs died soon after nematode emergence (while infected conspecifics survived in the same aquarium). Our procedure herein was inadequate to robustly determine if this infection can affect grass shrimp behavior or health; however, based on our observation that no EGSS survived long after mermithid emergence, we suspect that these infections are lethal to EGSSs upon emergence of the mermithid (perhaps due to mechanical damage and resulting osmotic challenge upon parasite transit from the hemocoel [Fig. 4] to the external environment). If so, this mermithid species could be regarded as a parasitoid. Other mermithids have been proposed as biological controls (Cobb et al., 1923; Christie, 1937; Mongkolkitti and Hosford, 1971; Poinar, 1981; Hominick et al., 1982; Poinar et al., 2007; Rusconi et al., 2017) but are not referred to as parasitoids or obligate pathogens of their arthropod intermediate hosts. We do not understand why, aside from the fact that nematodes are not insects. Eggleton and Gaston (1990; p. 418) defined ‘parasitoid’ as “an organism which [sic] develops on or in another single (“host”) organism, extracts nourishment from it, and kills it as a direct or indirect result of that development.”

As previously indicated, records of nematomorphs and mermithids infecting grass shrimps are rare (Table I): comprising 1 nominal nematomorph (*N. agile*) reported from North America (Ward, 1893; Born, 1967; Schmidt-Rhaesa et al., 2013) and Europe (Nouvel and Nouvel, 1934, 1938) plus the innominate mermithid reported herein from North America. Because of the rarity of collecting these parasites from grass shrimps, because no mermithid had been reported previously from a decapod (including grass shrimps), and because morphological diagnoses of parasitic juveniles of *N. agile* are rare, we thought it was important to examine type material (free living) from Verrill (1879) and vouchers (parasitic in grass shrimps) from Ward (1893) of *N. agile*. We also wanted to be sure that they were distinct from our mermithid specimens from a congeneric grass shrimp (comprising the only record of a mermithid infecting a grass shrimp and second crustacean in North America).

Regarding the morphological features of specimens identified as *N. agile* from North America, we borrowed syntypes from Verrill (1879) (as *agilis*; USNM coll. no. 13203; 19 syntypes comprising 5 intact specimens plus 14 specimen fragments) and examined them to confirm specific morphological features (Figs. 8–11). Verrill (1879) described *N. agile* based on free-living specimens only. He diagnosed the species principally based upon the presence of 4 giant cells (“roundish bodies”) in the translucent anterior end (Fig. 8), a papilliform posterior end (Fig. 9), posterior verrucae (Fig. 10), and tegumental bristles along the mid-lateral body margin (Fig. 11). Verrill’s syntypes, however, are morphologically heterogeneous: 1 of 5 intact syntypes had giant cells, tegumental bristles, posterior verrucae, and a papilliform



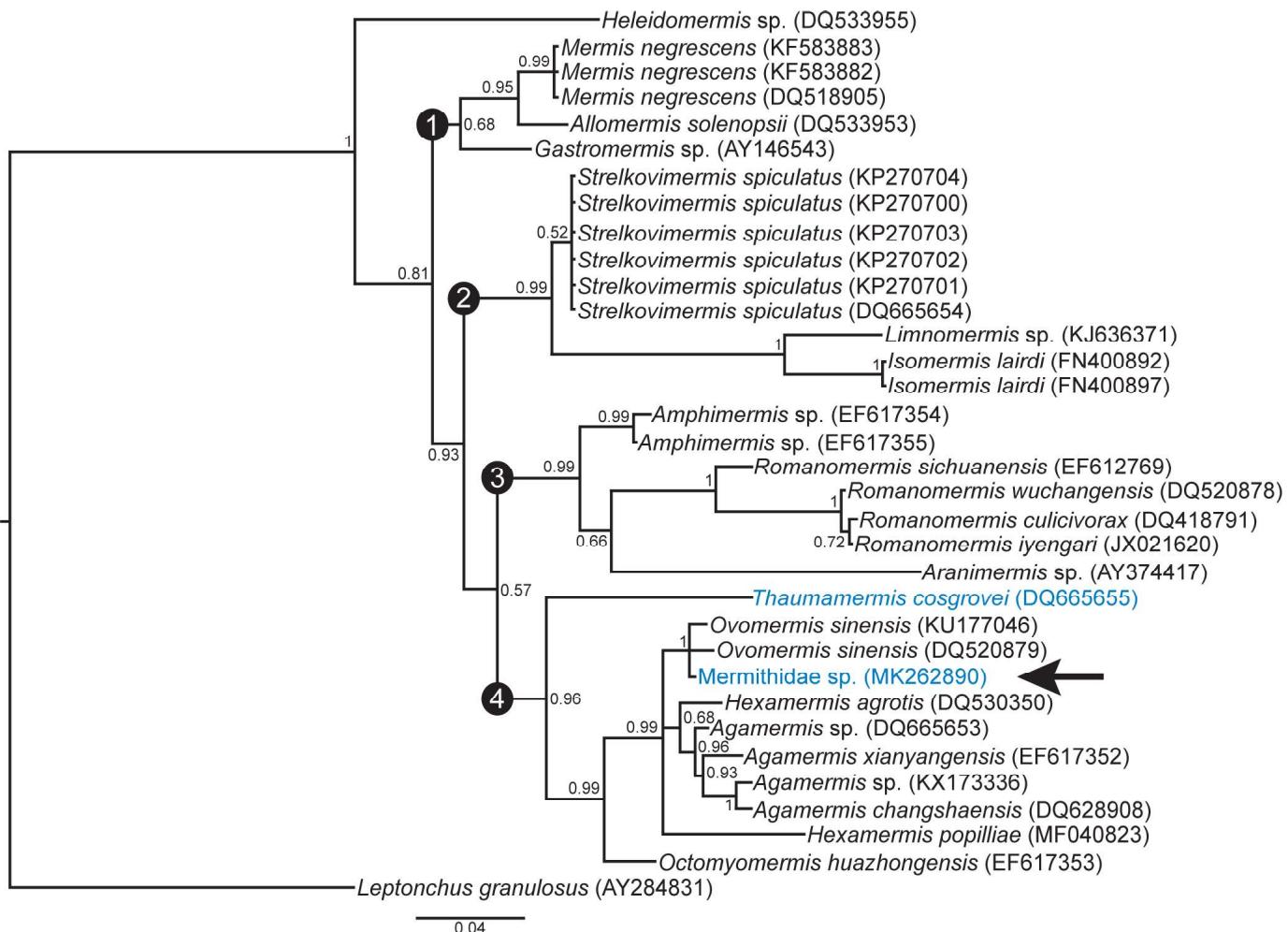
**Figures 8–11.** *Nectonema agile* Verrill, 1879 (Nematomorpha; USNM 13203) from Vineyard Sound, Massachusetts. (8) Anterior end; 4 giant cells “roundish bodies” (\*) in head region. (9) Posterior end (“papilliform posterior end”); vas deferens (vd). (10) Posterior verrucae (arrow). (11) Tegumental bristles in posterior end of body.

posterior end; 3 intact syntypes lacked giant cells but had tegumental bristles, posterior verrucae, and a papilliform posterior end; and 1 intact syntype lacked giant cells and tegumental bristles but had posterior verrucae and a papilliform posterior end. Only 3 of 14 specimen fragments had tegumental bristles. Based on these observations, we suspect that the tegumental bristles became dislodged or damaged in some of Verrill’s specimens. Missing bristles could also be explained by life stage differences (i.e., whether or not the specimens are all mature adults), which could also explain why giant cells are not evident in the anterior end of all intact syntypes (Schmidt-Rhaesa, 2013). We are skeptical that these specimens are not conspecific, but we

think it is noteworthy that they are morphologically variable. To our knowledge, no other worker since Ward (1893) has described these syntypes.

Verrill’s (1879) original description of *N. agile* (type species of *Nectonema*) was basic, including a short generic diagnosis and short species description unaccompanied by a figure. This description was based on free-living specimens only, and Verrill (1879) refrained from speculating on the life cycle. Later, H. B. Ward re-described *N. agile* based on Verrill’s entire collection (35 free-living adults) from Vineyard Sound, Massachusetts, 5 free-living adults collected by Dr. E. A. Andrews and 1 by Dr. W. M. Woodworth from Woods Hole, Massachusetts, and his own newly collected free-living adults (14) from Newport, Massachusetts (Ward, 1892). We borrowed Ward’s (1892) free-living adults of *N. agile* (USNM 1349723), which had tegumental bristles but lacked an anterior end or posterior end such that we could not confirm giant cells, verrucae, or a papilliform posterior end. Subsequent to Ward (1892; p. 261), J. P. McMurrich sent Ward 2 specimen fragments of *N. agile* that were collected from a species of “*Palaemonetes*” (we presume a species of *Palaemon*) from Rhode Island. McMurrich’s specimens comprised the first parasitic specimens of *N. agile* known, and they remain only 1 of 2 (Schmidt-Rhaesa et al., 2013) extant museum specimens of a nematomorph infecting a crustacean in North America. Based on those specimens, Ward (1893; p. 261) concluded that *N. agile* used the grass shrimp as an intermediate host, stating that, “the discovery of this individual seems to establish beyond question the parasitic nature of the young *Nectonema*, and indicates *Palaemonetes* as its host.” We borrowed and examined specimens that we believe are McMurrich’s specimens (USNM 1349724; now comprising numerous specimen fragments), but their identity could not be objectively confirmed because no anterior end nor posterior end was present and because no fragment had a tegumental bristle.

Regarding the only other record of a nematomorph infecting a North American palaemonid, Born (1967) reported an infection of *N. agile* from the type host (marsh grass shrimp) close to the type locality (estuarine localities near Woods Hole). He reported no morphological feature of the parasite, nor did he deposit a voucher specimen, and the image he provided did not show a morphological feature that would definitively diagnose a nematomorph infection. We do not doubt that the nematomorph *N. agile* infects marsh grass shrimp, but neither are we certain that Born (1967) was not reporting a mermithid infection. Because mermithids may be pathogenic to grass shrimps and because there is a hobby aquarium industry focused on grass shrimps, the identity of these infections matters. For example, a casual internet search quickly led us to a blog for palaemonid hobbyists, including images of EGSSs infected with probable mermithids. These hobbyists identified these infections as horsehair worms, assumedly based upon published taxonomic works that list the nematomorph *N. agile* as a common parasite of grass shrimps (Verrill, 1879; Ward, 1892, 1893; Born, 1967). This confusion is understandable and certainly not restricted to hobbyists; initially, we and other experienced parasitologists assumed our specimens of Mermithidae sp. to be nematomorphs based on preliminary photographs of infected hosts. Another noteworthy observation is that Born (1967) erroneously stated that Ward (1893) himself observed a single specimen of *N. agile* infecting a grass shrimp, but, in fact, McMurrich sent excised parasitic specimens of *N.*



**Figure 12.** Phylogenetic relationships of mermithids reconstructed using Bayesian inference with the small subunit ribosomal DNA (18S) gene. Numbered clades correspond to text. Numbers beside nodes indicate posterior probability. Arrow indicates the sequence representing *Mermithidae* sp. infecting eastern grass shrimp in central Florida. The color blue represents the only 2 sequences (*Thaumamermis cosgrovei* [DQ665655]; *Mermithidae* sp. [MK262890]) from species that infect crustaceans. Color version is available online.

*agile* (USNM 1349724) to Ward. Ward did not observe the infection in a grass shrimp. Hence, the only people that have reportedly observed an infection of *N. agile* in a grass shrimp in North America are McMurrich in Ward (1893) and Born (1967) (Table I).

Regarding the materials of *N. agile* from Europe, Nouvel and Nouvel (1934, 1938) characterized the gross appearance of the parasites they identified as *N. agile* in grass shrimps (Table I). No specified voucher specimen exists from these studies, and the parasitic juvenile specimens detailed therein were not morphologically diagnosed. Given their geographic locality, these infections should be re-examined, because they may represent a new species of *Nectonema* or of *Mermithidae*.

The mermithid reported herein likely represents a new species, with a life cycle worth studying, as it is the only species of *Mermithidae* reported to infect a decapod. Our anecdotal observations of EGSSs in aquaria indicate that this mermithid could be an obligate pathogen (parasitoid) of *P. paludosus*.

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