UNIVERSITY OF CALIFORNIA SAN DIEGO

First record and description of a mermithid nematode infecting a marine decapod crustacean

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by

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University of California San Diego

2020

DEDICATION

To all of my family, friends, and mentors who have offered me unending support throughout the years. Thank you for always encouraging me to follow my dreams, no matter how big or small. I could not have done it without your constant love and support.

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ABSTRACT OF THE THESIS

First record and description of a mermithid nematode
infecting a marine decapod crustacean

by

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Juvenile mermithid nematodes are endoparasitic worms that infect and kill their arthropod hosts, typically insects. Here, we provide the first report of a mermithid infecting a marine decapod crustacean. In an initial parasitological survey in December 2018, we encountered juveniles of a new mermithid species infecting the hemocoel of *Hemigrapsus oregonensis* (Decapoda: Varunidae) at a single site in San Diego, CA. Prevalence at that time

was 15.5% (95% CI: 8.4-26.9%), and a latter survey in April 2019 yielded a lower prevalence of 5.6% (95% CI: 1.6-18.2%). Infection probability and the abundance of mermithids peaked in smaller crabs, and all infections were multiple infections (intensity ranged from 2-10). No new infections were found in subsequent surveys at the same or three other sites in San Diego County between June 2019 and February 2020 (n = 528 crabs), precluding efforts to rear worms to adulthood for thorough description. We examined parasitic juveniles using light microscopy to provide a morphological description of this stage. Preliminary phylogenetic analysis of 18S rDNA confirmed the nematode's placement in the family Mermithidae. Because only parasitic juveniles were available, we plan to assign this new species to the genus *Agamomermis*, a collective group for immature mermithids. Individuals of this new species, to be named elsewhere as *Agamomermis grapsifarciens* n. sp., are long (20-65mm), white, filiform worms with a discernible stichosome, a prominent trophosome, six cephalic papillae arranged hexagonally in a single plane, a terminal, centered mouth, a homocephalic head, and a bluntly rounded tail lacking a tail appendage.

Introduction

The present study reports the discovery and description[1] of parasitic juveniles of a new species of mermithid parasitizing an unusual host, the shore crab *Hemigrapsus oregonensis*. Warren et al. (2019) recently provided the first record of a mermithid infecting a decapod, the freshwater shrimp *Palaemon paludosus* (from freshwater lakes in central Florida, USA). That finding also represents only the second record of a mermithid species infecting a North American crustacean. In fact, mermithids are only known to infect thirteen species of crustaceans worldwide[2]. Additionally, although mermithids are well known to infect terrestrial and freshwater arthropods, they are poorly known in the marine environment. Accounts of marine mermithids are limited to two species: *Thaumamermis zealandica*, which infects the intertidal marine amphipod *Talorchestia quoyana* in New Zealand (Poinar et al., 2002), and *Thalassomermis megamphis*, which was discovered free-living in deep-sea marine sediments in the South Atlantic Ocean (Tchesunov and Hope, 1997). Here, I provide the first report of a mermithid infecting a marine decapod, the shore crab *H. oregonensis*. The description of the parasitic juveniles of this new species, to be named elsewhere as *Agamomermis grapsifarciens* n. sp., expands current knowledge of both the host taxon use of mermithids and of mermithids associated with the marine environment. Before proceeding with the description of this new mermithid species, I provide a general background on mermithid nematodes concerning systematics, life cycles and ontogenetic development, effects on hosts, and general issues concerning describing juvenile mermithids.

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[1] We disclaim any naming or other nomenclatural acts in this thesis for the purposes of formal zoological nomenclature (see ICZN Article 8.3). A more complete, formal taxonomic account is planned to follow.

[2] See Warren et al. (2019) for a summary table of mermithids infecting crustaceans.

Systematic and Biological Background

Phylum Nematoda represents an extremely diverse assemblage of roundworms with many free-living and parasitic forms, the latter of which are known to be parasites of vertebrates, invertebrates, and even plants (Poinar, 1975, 2010; Goater et al., 2014). Within this phylum, four parasitic nematode families, Mermithidae (order Mermithida), Tetradonematidae (order Mermithida), Marimermithidae (order Marimermithida), and Benthimermithidae (order Benthimermithida), are all similar in that members have an intestine which is modified into a nutrient storage organ called a trophosome (Chesunov, 1988; Petter, 1980; Rubzov, 1985; Rubzov and Platonova, 1974), exhibit reduction or absence of the rectum and anus (Rubzov, 1985; Rubzov and Platonova, 1974), and have a life cycle with a parasitic (typically parasitoid) stage followed by a non-feeding, free-living stage (Chesunov, 1988; Miljutin and Tchesunov, 2001; Petter, 1980). However, despite these similarities, the orders Mermithida, Marimermithida, and Benthimermithida may not be closely phylogenetically related. This idea is supported by a recent paper in which analysis of small subunit 18S rDNA indicated that a single genus of Benthimermithida is actually nested in the Order Plectida (class Chromadorea) (Leduc and Zhao, 2019), which is in a different branch of the nematode phylogeny than both Marimermithida and Mermithida (class Enoplea).

Further, the three groups have several distinct morphological differences. One of the most dramatic morphological differences between these groups concerns the structure of the esophagus. In marimermithids, the esophagus is well-developed and muscular, whereas in benthimermithids, the esophagus is highly reduced, non-muscular, and contains granular material, and in mermithids the esophagus is reduced, non-muscular, and is modified into a glandular structure called the stichosome (Petter, 1980; Rubzov, 1985). Members of the family

Marimermithidae are superficially the most similar to free-living marine nematodes of the order Enoplida (Petter, 1980; Rubzov and Platonova, 1974) in that they retain a well-developed, muscular esophagus (Petter, 1980; Rubzov, 1985; Tchesunov and Hope, 1997) that connects to the intestine, which connects to the rectum (Rubzov, 1985). In contrast, in mermithids, the trophosome and esophagus become disconnected during the course of development (Rubzov, 1985), and in benthimermithids, the esophagus, trophosome, and rectum are disconnected (Petter, 1980). Furthermore, while marimermithids and mermithids have a mouth, benthimermithids lack a mouth (Chesunov, 1988; Miljutin and Tchesunov, 2001; Rubzov, 1985), but instead have an apical pore (Chesunov, 1988). Lastly, the three groups differ in their distribution and host range. While both marimermithids and benthimermithids parasitize marine invertebrates as juveniles and are associated with deep-sea sediments as free-living adults (Chesunov, 1988; Tchesunov and Hope, 1997), mermithids parasitize mostly insects and are typically associated with freshwater or terrestrial environments (Poinar, 1975).

Members of the Mermithida are thought to be descended from predatory members of the nematode order Dorylaimida, from which they have retained an oral stylet to aid in penetrating the host body wall (Hominick, 1982; Poinar, 1983). Mermithida comprises two families: Mermithidae and Tetradonematidae. Members of both families are long, filiform, obligate endoparasitic worms that infect and kill both terrestrial and freshwater arthropod hosts (Nickle, 1972; Poinar, 1983). Members of Tetradonematidae are characterized by the presence of a four-celled glandular structure called the “tetrad” that is associated with the pharynx, are typically shorter and stouter than mermithids (Poinar, 1983), and reach sexual maturity and mate *inside* their invertebrate host’s body cavity before emerging (Poinar, 1975, 1983; Hominick, 1982; Tchesunov and Hope, 1997). In contrast, members of the Mermithidae usually have sixteen

glandular cells called stichocytes arranged along their esophagus and emerge from their hosts as juveniles, reaching the sexual adult stage in the external environment (Hominick, 1982).

Due to morphological and life history similarities, mermithids also resemble members of Phylum Nematomorpha, the hairworms. Both nematomorphs and mermithids are long, filiform, endoparasitic worms of invertebrates and are parasitoids, killing their host upon emergence (Goater et al., 2014). Additionally, both groups are parasitic only as juveniles and absorb nutrients from their host via a thin, metabolically active cuticle (Goater et al., 2014). The mesenchyme of nematomorphs is functionally equivalent to the trophosome of mermithids (Poinar and Brockerhoff, 2001) in that it serves as a food storage organ. Nematomorphs are commonly known for their water-driving behavioral manipulation, which draws infected hosts to water to facilitate emergence (Thomas et al., 2002; Poinar, 2010; Goater et al., 2014); a similar behavioral manipulation tactic occurs in some mermithids to drive their hosts to wet or moist environments for emergence (Leech, 1966; Poinar, 1987; Kaiser, 1991). But despite these similarities, nematomorphs have both morphological and developmental differences from mermithids. Nematomorphs are characterized by the presence of cuticular structures such as areoles, setae, or tubercles, a pseudocoelom that contains numerous mesenchymal cells, and a tail that may be multilobed or divided (e.g. bifurcate) (Poinar, 2010). Because nematomorphs are usually dark in color and the cuticle is opaque, observation of internal organs is difficult and diagnosis of emerged worms is largely based on external epicuticular structures (Poinar, 2010; Goater et al., 2014). Additionally, nematomorphs have unique J-shaped infective larvae that are morphologically very dissimilar from adults, bearing a spined, retractable proboscis used for host gut penetration (Poinar, 2010; Goater et al., 2014). Finally, unlike the mermithids whom most

often have a direct type of life cycle, nematomorphs typically have an indirect type of life cycle involving a paratenic or transport host (Schmidt-Rhaesa, 2001, 2005; Poinar, 2010).

In sum, mermithids are endoparasitic worms of the nematode order Mermithida and family Mermithidae. Mermithids exhibit what are likely convergent morphological and life history similarities to benthimermithids, marimermithids, tetradonematids, and nematomorphs. Here I have compared four taxonomic groups to the Mermithidae to highlight the distinguishing characteristics of mermithids and to clarify their relationships to nematomorphs and several parasitic nematode groups.

Life Cycle and Ontogenetic Development of Mermithids

Mermithids develop in either a direct or indirect type of life cycle, but the direct life cycle is typical for most mermithids (e.g., Nickle, 1972; Poinar and Hess, 1974; Poinar, 1975, 1983, 1987; Hominick, 1982; Platzer, 2007; Dong et al., 2014; Mazza et al., 2017). In the direct life cycle, adult mermithids deposit eggs in the external environment and, after a molt in the egg, a short-lived, non-feeding, second-stage pre-parasitic juvenile hatches from the egg, searches for a host, and penetrates the invertebrate's body wall using its oral stylet. Upon infection, the infective juvenile will enter the host hemocoel and begin its development, growing rapidly and eventually occupying the majority of the host body cavity, as well as packing the trophosome with food reserves acquired from the host's hemolymph. Once development is nearly complete and the trophosome is packed, the mermithid molts and emerges as a free-living, non-feeding, post-parasitic juvenile. The post-parasitic juvenile will molt and mature to the adult stage, mate, and deposit eggs in the external environment, completing the life cycle. Alternatively, in the indirect type of life cycle, eggs are deposited in the external environment and the infective

juveniles do not hatch until they are ingested by a "paratenic host"[3], at which point they hatch, penetrate the gut wall, and migrate to various host tissues where they remain dormant; only when the paratenic host is ingested by a suitable developmental host do they enter the hemocoel and complete their development (Poinar, 1983, 1985, 1987; Poinar and Ćurčić, 1992). In both types of life cycles, one molt occurs in the egg, one molt occurs during parasitic development, and two occur after the mermithid has emerged from its host- thus, mermithids have the same number of molts found in the typical nematode life cycle (Poinar and Otieno, 1974; Poinar, 1983; Sanad et al., 2013).

Ontogenetic changes in morphology and anatomy occur throughout the life cycle of mermithids, and the morphological features of each life stage correspond to that life stage's functional role in the life cycle. For example, pre-parasitic juveniles are the infective stage of mermithids (Poinar and Hess, 1974; Poinar, 1983; Sanad et al., 2013) and once in the host environment, pre-parasitic juveniles must find and infect a suitable invertebrate host before they perish. For this reason, many mermithid species have evolved specialized strategies for host finding and host selection and, further, these different host searching strategies are based on the ecology of their respective host environments. For example, infective juveniles of *Agamermis* and *Hexamermis* were found to be positively phototactic; juveniles ascended grass stems in the early morning light to facilitate encounter with their grasshopper nymph hosts (Robinson et al., 1990). Likewise, mosquito-parasitizing *Romanomermis iyengari* exhibited negative phototaxis to locate mosquito larvae along shaded edges of moving water environments, while *Strelkovimermis spiculatus* did not exhibit any phototaxis to locate mosquito larvae in temporary

[3] In this case, the initial host is usually referred to as a "paratenic host" rather than a "first intermediate host" because the mermithid does not undergo development within the host.

flood water environments where light was not a factor for host-finding (Dong et al., 2017). Different mermithid species may also differ in terms of penetration site selection and behavior during penetration (Sanad et al., 2013). However, in general, mermithid pre-parasitic juveniles use repeated sinuous movements of the stylet as a mechanical probe or chisel to bore an opening in the host cuticle (Poinar, 1968, 1983; Poinar and Hess, 1974; Poinar and Sanders, 1974; Rubzov, 1981; Kaiser, 1991; Sanad et al., 2013). The stylet likely also helps to direct glandular secretions onto the host's exoskeleton to aid in host penetration and paralysis during entry (Wulker, 1965; Poinar, 1968; Poinar and Hess, 1974; Poinar and Sanders, 1974; Rubzov, 1981). The presence of collapsed penetration glands immediately after host penetration provides evidence for the use of secretions in host infection (Gordon et al., 1974; Poinar and Sanders, 1974). Thus, different mermithid species have evolved different strategies for the all-important task of finding and penetrating a suitable host.

Once the pre-parasitic juvenile mermithid has infected a suitable invertebrate host, it is referred to as a parasitic juvenile. This stage undergoes substantial growth within the host, and parasitic juvenile mermithids often grow so large as to fill nearly the entire hemocoel of their host (Rubzov, 1981; Poinar and Ćurčić, 1992). The course of parasitic development can be classified into three distinct phases of growth: an initial phase of slow growth, where the mermithid increases in diameter, but not length, and the cuticle becomes thin and microvillous, a secondary phase of rapid growth and filling of the trophosome, and a final phase of cuticle thickening in preparation for the free-living post-parasitic and adult life stages (Kaiser, 1991). In mermithid pre-parasitic juveniles, the esophagus (sometimes referred to as a "pharynx" in mermithid literature) is connected to the intestine, but during the course of parasitic development, the esophagus separates from the intestine and becomes the structure known as the

stichosome, while the intestine becomes an enlarged and modified food storage organ known as the trophosome (Poinar and Sanders, 1974; Poinar, 1975, 1983, 2010; Batson, 1979b; Hominick, 1982; Poinar and Brockerhoff, 2001). Unlike most nematodes, mermithids do not feed using a mouth or a muscular pharynx. Instead, mermithids absorb nutrients directly from the hemocoel of their hosts and so have no need for a traditional intestine for the breakdown of complex food into simpler compounds (Steiner, 1933). Additionally, because mermithids are non-feeding and free-living after the parasitic juvenile stage, mermithids depend heavily on their hosts for nutrition and must rely on their accumulated food reserves for the energetically expensive processes of gametogenesis, energy metabolism, and sexual maturation later in life (Batson, 1979b; Gordon, 1981; Tchesunov and Hope, 1997). Due to this, the diet composition of the host drastically influences the development and survivorship of the mermithids it harbors. For example, in a laboratory study on the diet content of larval *Aedes aegypti* mosquito hosts parasitized by *Romanomermis culicivorax*, researchers found that mermithids that developed within nutritionally limited hosts had less densely packed trophosomes, took longer to complete parasitic development, and had fewer, smaller post-parasitic juveniles emerge compared to hosts that were fed a non-restricted diet high in protein content (Gordon et al., 1981).

Mermithids have a large body volume relative to their hosts and have thinner, longer, filiform bodies- a body shape that likely aids in increasing the surface area for the transcuticular uptake of nutrients (Kaiser, 1983; Rubzov, 1977). Evidence of transcuticular uptake includes the presence of endoplasmic reticulum and mitochondria in a metabolically active layer in the cytoplasmic region of the trophosome (Batson, 1979b; Webster and Gordon, 1974) through which nutrients are transferred, a thin cuticle with a sub-cuticular microvillous outer surface (Batson, 1979a), and the presence of cytoplasmic bridges within the pseudocoelom that connect

the trophosome to the hypodermal cords for nutrient transfer (Batson, 1979b; Gordon, 1981). Additionally, extra-intestinal feeding by mermithids is supported by a lack of esophageal musculature (Rubzov, 1977, 1981), disconnection of the esophagus and trophosome (Baylis, 1947; Poinar and Sanders, 1974; Poinar, 1975, 1983, 2010; Batson, 1979b; Rubzov, 1981; Hominick, 1982; Poinar and Brockerhoff, 2001), absence of a rectum or anus (Baylis, 1947; Rubzov, 1977, 1981; Batson, 1979b; Poinar, 1983; Camino, 1991; Kaiser, 1991), and overall reduction of the esophagus (Rubzov, 1981). Throughout the course of development, both the growth rate and the rate of nutrient absorption by mermithids are variable. For example, a laboratory study on transcuticular uptake in *Mermis nigrescens* parasitic juveniles found that younger worms incorporated glucose much more rapidly than older worms due to a combination of a thinner cuticle and the higher carbohydrate demand associated with their rapid development (Rutherford and Webster, 1974). Moreover, the rate of growth appears to be variable between different worms, as parasitic juveniles of different sizes can be found within a host even when their infection time was approximately the same (Baylis, 1947). Nonetheless, in late parasitic juveniles and newly emerged post-parasitic juveniles, the trophosome is a solid, opaque cytoplasmic cylinder (Batson, 1979b), is densely packed with a high proportion of fatty acid and proteinaceous food reserves (Chitwood and Jacobs, 1938; Rubzov, 1977), occupies nearly the whole length and width of the pseudocoelom (Batson, 1979b; Gordon, 1981; Hominick, 1982; Kaiser, 1991; Rubzov, 1977), and has almost no structural variation along its length, with the exception of a depression in the locality of the genital rudiment (Batson, 1979b; Kaiser, 1991).

Another structure that undergoes major development changes throughout ontogeny is the stichosome. The term stichosome refers to the posterior portion of the esophagus that contains the stichocytes (Steiner, 1933; Poinar and Hess, 1974; Rubzov, 1977). The stichosome is most

visible during early parasitic development, but is obscured by the developing trophosome later in development (Baylis, 1947; Gordon et al., 1974; Hominick, 1982; Rubzov, 1977) and after this overlap occurs, the structure can be seen ventral to the trophosome (Hominick et al., 1982). The stichocytes are large, unicellular esophageal glands that open into the lumen of the esophagus and have distinct nuclei and nucleoli (Chitwood, 1935; Hominick, 1982). The stichocytes are thought to be associated with nutrition (Gordon et al., 1974; Rubzov, 1977) and osmosis (Rubzov, 1977) during the parasitic phase. Immediately anterior to the stichosome is a pair of large, ovular cells called homorocytes (Hominick, 1982; Rubzov, 1977) that are proposed to have an excretory function (Steiner, 1933), but are also postulated to facilitate molting via mechanical pressure and rupturing of the sheath (Rubzov, 1981).

Before host emergence, the mermithid molts and gains a thicker, more protective cuticle suited for its free-living lifestyle (Batson, 1979a; Rubzov, 1981), the somatic musculature becomes more highly organized (Batson, 1979a), the hypodermis loses its microvillous nature (Batson, 1979a), and the stylet that was present throughout pre-parasitic and parasitic development is lost (Gordon et al., 1974; Rubzov, 1977, 1981; Hominick et al., 1982; Poinar, 1983; Camino, 1991). Additionally, the stichosome is reduced and the amphids become more complex (Rubzov, 1981). Once the trophosome is packed with nutrients and the mermithid has molted and gained a thicker cuticle, parasitic development is complete and the mermithid will emerge from its host. To facilitate emergence, an exit hole is created via the mechanical force of pushing without the aid of a stylet (Sanad et al., 2013) and, after emergence, post-parasitic juveniles aggregate into mating clusters in the host's habitat (Dong et al., 2014; Rubzov, 1981) and complete a double molt to the adult stage (Poinar and Otieno, 1974; Poinar and Sanders, 1974; Poinar, 1975, 1983; Hominick, 1982). In contrast to the parasitic juveniles, adult

mermithids have a thick, complex cuticle with a thin hypodermis and a non-microvillous sub-cuticular surface (Batson, 1979a). The trophosome of adults undergoes successive vacuolation or reduction with the depletion of nutrients (Batson, 1979b) and the nutrient reserves are nearly exhausted by the cessation of egg-laying (Rubzov, 1981).

Host Effects

Mermithids often cause morphological abnormalities to their host's interna and externa. For example, mermithid-parasitized spiders frequently exhibit a swollen abdomen, shorter, stouter legs, or poorly-developed copulatory structures (Leech, 1966; Poinar, 1985, 1987; Meyer, 2014). Additionally, many mermithid-parasitized arthropod hosts exhibit a reduction of the digestive gland (Leech, 1966; Poinar, 1985, 1987), hemolymph (Rubzov, 1981), or fat body (Poinar and Sanders, 1974; Rubzov, 1981; Poinar, 1983), and often secondarily exhibit a reduction in gonadal tissue (Stiles, 1903; Leech, 1966; Poinar, 1975, 1983, 1987; Rubzov, 1981; Poinar and Ćurčić, 1992; Kubo et al., 2016), implying host castration. Mermithids have also been demonstrated to alter the behavior of their hosts. For example, mermithid-parasitized spiders, wasps, and ants have all exhibited migration towards water or moist substrate to facilitate emergence (Leech, 1966; Poinar, 1987; Kaiser, 1991) and mermithid-parasitized spiders often exhibit sluggishness and a cessation of feeding (Leech, 1966).

Emergence of the post-parasitic juvenile is lethal to the host (Dong et al., 2014; Nickle, 1972; G.O. Poinar Jr, 1975, 1985; Welch, 1965) due to damage to the host cuticle (Nickle, 1972; Poinar, 1975; Kaiser, 1991), loss of body fluids (Kaiser, 1991; Nickle, 1972), nutritional depletion (Poinar, 1975; Kaiser, 1991), and host susceptibility to secondary microorganism invasion (Poinar, 1975). According to Eggleton and Gaston (1990), a parasitoid is "an organism

which develops on or in another single ("host") organism, extracts nourishment from it, and kills it as a direct or indirect result of that development." Similarly, Lafferty and Kuris (2002) define a parasitoid as a type of parasite that attacks only one victim in a life stage, reduces the host's fitness to zero, and requires the death of the host to complete its life cycle. As such, mermithid parasitic juveniles are parasitoids, extracting nutrients from their host throughout development and eventually killing their host to reach the next stage of their life cycle.

Description of Juvenile Mermithids

The Mermithidae comprises over 65 nominal genera and over 500 species, but many of these species are considered to be inadequately described, with descriptions based only on a single specimen, a single sex, or juveniles (Nickle, 1972; Poinar, 1975; Kaiser, 1991). Descriptions based on juvenile mermithids presents a problem because the shapes and lengths of the sexual structures of adults- including the vulva and vagina of females and the spicules of males- are considered to be the most relevant features for both generic and specific diagnosis (Kaiser, 1991; Rubzov, 1977). However, several mermithid taxonomists have argued for the validity of the use of juvenile characters, such as the stichosome, tail, and trophosome, in species descriptions (Poinar and Sanders, 1974; Rubzov, 1977). Further, Rubzov (1977) suggests that many taxonomically informative characters of mermithids are stable between juveniles and adults, including the number of the longitudinal chords, the shape of the head, mouth, and amphids, the number and arrangement of cephalic papillae, the origination and cessation of the trophosome, the general structure of the stichosome and number of associated stichocytes, and the tail shape and presence of an appendage. As such, given sufficient material, juvenile mermithids may in fact provide taxonomically-useful information. However, the near-exclusive

focus on the adult stage for mermithid species descriptions prevents adequate assessment of the diagnostic value of juvenile characters for species descriptions (Hominick, 1982).

The lack of focus on immature mermithids in species descriptions is particularly problematic because juveniles are often the only specimens available for evaluation. Mermithids are often encountered opportunistically by parasitologists or entomologists during studies on their host, and therefore, mermithids are typically discovered either as parasitic juveniles infecting the host hemocoel or as post-parasitic juveniles emerging from the host. When a juvenile specimen is found, to be described using adult characters, it must be reared to adulthood (Poinar, 1975). This rearing usually requires first rearing of the host and then rearing of the emerged mermithid (usually in some appropriate 'benthic' substrate). However, attempts at rearing post-parasitic and adult life stages may not be successful or otherwise possible. Hence, in many cases, only juvenile specimens of a new mermithid species are available for description and the taxonomic neglect of juveniles precludes being able to assign them to genus.

However, such juveniles can be described and placed into the "collective group"[4] *Agamomermis* Stiles, 1903 (Hominick, 1982; Poinar, 2011; Poinar et al., 2020; Poinar and Welch, 1981; Poinar et al., 2006, 2002; Stiles, 1903). The use of *Agamomermis* is advantageous in that it provides the species with a formal name for immediate use, offers immediate recognition of the mermithid as being described from juvenile specimens, and permits the species to readily be assigned to an appropriate genus (keeping the trivial name) when and if adult stages are described (Poinar and Welch, 1981).

[4] A "collective group" is a name assigned to a certain taxonomic assemblage or group for convenience, and this name is treated as a genus-group name (see International Commission on Zoological Nomenclature Article 42.2.1).

Materials and Methods

Host Species

The yellow shore crab, *Hemigrapsus oregonensis* (Decapoda: Varunidae), naturally occurs along the west coast of North America from Resurrection Bay, Alaska to Bahía de Todos Santos, Baja California (Garth and Abbott, 1980). *H. oregonensis* is distributed along the mid and low intertidal zones of bays and estuaries beneath rock cover on mud or muddy sand, in bays covered with *Ulva*, and in burrows in *Salicornia* marshes (Garth and Abbott, 1980; Kuris et al., 2007). *H. oregonensis* is known to be final host to the entoniscid isopod *Portunion conformis* and the nemertean egg predator *Carcinonemertes epialti*, as well as intermediate host to larval acanthocephalans, trypanorhynch tapeworms, trematode metacercariae, and larval *Ascarophis* sp. nematodes (Kuris et al., 2007).

I morphologically identified *H. oregonensis* crabs by the presence of three teeth on the anterolateral margin of the carapace, hair on the legs, a distinct notch in the frontal margin of the carapace, and the absence of red spots on the chelipeds (the latter three features distinguish *H. oregonensis* from *H. nudus*) (Kuris et al., 2007). *H. oregonensis* can easily be distinguished from *Pachygrapsus crassipes* – a grapsid shore crab that frequently co-occurs with *H. oregonensis* – as *P. crassipes* has only two teeth on the anterolateral margin and has transverse ridges on the surface of the carapace (Kuris et al., 2007).

Collections and Dissections

Members of the Hechinger Lab initially discovered the new mermithid in December 2018, when they collected and dissected 58 *H. oregonensis* shore crabs from Liberty Station in San Diego, CA (32°43'44"N, 117°12'55"W) for a general parasitological survey. We

encountered the mermithid again in April 2019, when we collected and dissected 36 *H. oregonensis* crabs from the same locality. In both surveys, crabs were collected indiscriminately by hand during low tide from beneath riprap on the shoreline of this coastal bay site. As mermithids had not yet been reported to infect crab hosts (or even decapods at the time of discovery), we postulated that these worms were of a new species, and we aimed to rear crab hosts in the lab to facilitate both the capture of post-parasitic juveniles and the maturation of the mermithids to adulthood for description.

Between June 2019 and February 2020, I obtained an additional 528 *H. oregonensis* to rear for the collection of emerging post-parasitic mermithids. I obtained these crabs from a combination of four sites in San Diego County: Liberty Station (32°43'44"N, 117°12'55"W), Agua Hedionda Lagoon (33°08'52"N, 117°19'53"W), Tijuana River National Estuarine Research Reserve (32°33'35"N, 117°07'46"W), and Mission Bay Park (32°47'29"N, 117°12'37"W). These sites are all located in wave-protected estuaries or bays and were chosen because they either provided access to riprap on muddy substrate during low tide or permitted collection of *H. oregonensis* via minnow traps and seine nets.

At Liberty Station, Agua Hedionda Lagoon, and Mission Bay Park, I collected *H. oregonensis* crabs by hand at low tide from beneath riprap on muddy sand. In the initial survey, no crabs larger than 10.0 mm were found to harbor the mermithid juveniles. As such, in subsequent collections between April 2019 and February 2020, crabs with smaller carapace widths (<12.0 mm) were preferentially selected, but no size or sex was ever completely excluded from collection. In other words, crabs of all sizes were collected until the desired number of hosts was attained, but when a surplus of crabs was found, I preferentially retained the smaller crabs and released the larger crabs. In Fall 2019, field researchers working in the Tijuana River

National Estuarine Research Reserve collected *H. oregonensis* crabs from minnow traps and seine nets from multiple locations throughout the estuary during their routine monitoring program. I was fortunate to receive the *H. oregonensis* that they caught for use in my project. Due to the nature of this collection process, no size range or sex was preferentially selected.

I euthanized crabs before dissection and recorded the carapace width (to the nearest 0.1 mm), sex, female brood state (ovigerous or non-brooding), and parasite counts for each individual. I relaxed the recovered mermithids in hot 0.5% acetic acid in seawater and fixed them in hot 70% EtOH; I used this method because glacial acetic acid is known to be effective for relaxing and straightening out nematodes for examination (Berland, 1961). After fixation, I transferred the mermithids to a 50/50 EtOH-glycerine solution for clearing and allowed the EtOH to evaporate over the course of several days. Genetic vouchers were preserved in 70% EtOH.

Crab Husbandry

After our initial encounters with the mermithid juveniles in December 2018 and April 2019, I aimed to capture and rear *H. oregonensis* crabs long-term to collect and describe post-parasitic mermithids that emerged from the crab hosts. In June 2019, I created a small-scale laboratory crab-rearing setup in the water tables of our lab space in Hubbs Hall at Scripps Institution of Oceanography in La Jolla, CA. In this setup, I housed 48 *H. oregonensis* crabs individually in clear 90 ml urinalysis cups that were connected to a water flow system (see Appendix for images). Each water spigot was connected to a group of eight cups arranged in series, with water flow split between the cups via aquarium gang valves. Each cup had two holes drilled into the lid: one hole was connected to a continuously-flowing filtered-seawater system

via aquarium plastic tubing and the other hole allowed seawater to overflow out of the cup. I tested the water circulation of this setup using non-toxic food coloring before assigning crabs to their respective cups. For this test, I added a few drops of food coloring upstream of the crab cups, and I observed the circulation of the food coloring within each individual cup as well as the time it took the food coloring to completely dissipate from each cup. I adjusted the water flow levels until all the food coloring left the system in under ten minutes. Ultimately, this setup ensured that each cup received a continuously-renewing supply of oxygenated seawater. Water flow levels were checked and adjusted daily to confirm that sufficient water flow was maintained throughout the duration of husbandry. Additionally, each cup contained an approximately 1/4-inch thick piece of PVC pipe capped by 1000-micron polyester mesh, which created a platform within the cup that the crab could rest on. This platform would allow emerged mermithids to escape potential predation in the event that mermithids emerged before the death of the host, while simultaneously keeping the emerged mermithid coupled with its respective host. This setup was continuously recorded and I reviewed the video footage for any signs of parasite emergence whenever a deceased crab was found. The 48 crabs were kept in this setup from June 2019 until September 2019, and individual cups were removed and examined whenever a deceased crab was found. In September 2019, all remaining crabs were removed and dissected.

In September 2019, I created a second, larger-scale crab-rearing setup. This setup was located in the Kaplan Experimental Aquarium at Scripps Institution of Oceanography in La Jolla, CA. Between September 2019 and March 2020, I housed *H. oregonensis* crabs in flow-through seawater aquaria using modified parts boxes (see Appendix for images). For each parts box, I drilled holes in the top and sides of every compartment to allow adequate water flow through the box, and I inverted the box and replaced the lid with a 1000-micron polyester mesh through

which emerging worms could escape into a plastic reservoir tub that was situated beneath the box. Within each tub, the parts box was elevated from the floor of the tub using plastic egg crate and was kept submerged using stainless steel ball weights. I directed the spigot into the front of the reservoir tub and drilled holes in the back wall of the tub just above the height of the parts box. This design allowed continuously-flowing seawater to flow into the front of the tub, enter the box through the mesh-lined bottom and sides of the box, and overflow out the back wall, while simultaneously retaining any emerged worms in the bottom of the tubs. Again, before any crabs were added to the setup, I tested the water circulation through each parts box using a few drops of non-toxic food coloring; I ensured that the dye had entirely dissipated from within every individual compartment in under ten minutes. Each parts box accommodated up to 34 crabs housed in separate compartments, and between a total of six modified parts boxes, a maximum of 204 crabs could be housed at any given time. Further, each compartment of the parts box was 4.5 x 3 x 3.5 cm, but most walls of the parts box could be removed to create compartments that were up to 9 x 8.8 x 3.5 cm, if needed. I checked the reservoir bins and parts boxes for emerged worms daily. In this setup, crabs were held for a minimum of three weeks to allow for the development of parasitic juveniles and the natural emergence of post-parasitic juveniles; after a minimum of three weeks passed with no emergence, crabs were euthanized and dissected.

In both husbandry setups, crabs were kept submerged at all times except during daily checks and feedings. I fed all crabs a mixed diet of packaged crustacean nutrition pellets and crushed algae wafers. I performed autopsies on deceased crabs to check for any morphological abnormalities or signs of emergence, such as damage to the carapace or the abdomen of the crab.

Illustrations & Measurements

I mounted all fixed mermithids in a drop of glycerin and viewed them using light microscopy. All measurements were taken using the ocular micrometer under 40x, 100x, or 400x magnification - body length measurements were repeated under both 40x and 100x magnification, while body width measurements and finer measurements, such as the length of the stylet and the distance from the head to the nerve ring, were measured under 400x magnification. I examined and measured a total of eight fixed parasitic juveniles of varying lengths to provide both morphometric data and a morphological description of the parasitic juvenile stage. I created vector-based illustrations from photographs of two live parasitic juveniles using InkScape 1.0 software (see Figures 1 and 2). Photographs were enhanced in Adobe Photoshop v. 21.2.1 according to the Zootaxa Digital Imaging Guide Version 1.2 (Geiger, 2012).

Statistical analyses of parasite prevalence and abundance

Prevalences are presented with 95% confidence intervals (CIs) calculated using the Wilson score method (Newcombe, 1998).

We examined whether and how the probability of being infected varied with host carapace width, sex (male or female), and collection date using logistic regression (Quinn and Keough, 2002). We included a quadratic term for carapace width to permit examining the possible peak of infection probability at intermediate-sized small crabs. Width was centered when used in the interaction term. We started off with a global model with the above main effects, and their two and three-way interactions (and, technically, a four-way interaction for the carapace width quadratic term). However, we had to simplify the global model because all

parameter estimates were unstable when the model included the highest order interactions terms between size and date and sex. Then, we further simplified the new global model by sequentially removing other non-significant terms, first size*sex and date*sex, and then sex (all $p > 0.46$).

We examined the relationship between worm abundance and host size using a negative binomial regression with a log link (Myers et al., 2010), including size as described above. Given the low amount of infections, we ran this model on all the data from the December 2018 and April 2019 surveys pooled.

We used JMP Pro v. 13 (SAS Institute, Inc.) for logistic regression and R v. 3.5.2 (R Foundation for Statistical Computing) for negative binomial regression. We assessed model adequacy by examining plots of Pearson and Studentized Deviance residuals versus predicted values, and by comparing model-predicted values to observed data. We created logistic regression graphs in Excel 2019 v. 16.0 (Microsoft Corporation).

Results

Taxonomic account

Mermithida Hyman, 1951

Mermithidae Braun, 1883

Agamomermis Stiles, 1903

Stiles (1903) established *Agamomermis* as a collective genus for immature mermithids.

***Agamomermis grapsifarciens*, n. sp.**

Diagnosis

Medium to large-sized white, filiform nematodes infecting *Hemigrapsus oregonensis* crabs (Figure 1); head homocephalic; mouth terminal and centered; mouth papillae lacking; cephalic

papillae six in one plane; esophagus non-muscular; stichosome with stichocytes and homorocytes; trophosome prominent; tail bluntly rounded, lacking appendage.

Description

Measurements are given in micrometers unless otherwise indicated. Values are reported as 'average (range)' (see Table 1).

Parasitic Juveniles. (N=8) total body length 45.3 mm (19.6-65.0 mm), body width at head 83.1 (66.2-96.2), body width at midbody 158 (105-210), body width at tail anterior 92.7 (58.8-129); (N=3) distance from head to nerve ring 203 (180-240); (N=2) length of oral stylet 8.75 (7.50-10.0).

The parasitic juveniles are medium to large (~2-7 cm long), white, filiform worms with a terminal, centered mouth, no mouth papillae, a head that is symmetrically rounded (homocephalic), six cephalic papillae arranged hexagonally in a single plane, and a tail that tapers to a bluntly rounded point, but with no tail appendage (Figures 2 and 3). No anus was present. A slightly protruding oral stylet was observed in two of the eight examined individuals. The genital structures were not developed or visible in the immature worms.

A trophosome was present in all examined worms. In longer worms, the trophosome was larger and originated closer to the anterior of the worm than it did in shorter worms; in other words, longer, more-developed worms had a longer trophosome relative to their body length than did shorter, less-developed worms (Figure 2). In the longest, most developed worms, the trophosome originated anteriorly at or around the nerve ring and extended posteriorly almost to the end of the tail. The trophosome appeared to be filled with spherical granules of varying sizes.

Longer worms with relatively longer trophosomes appeared to have more densely packed granules within their trophosomes than did smaller worms with relatively shorter trophosomes.

The stichosome was discernible in all parasitic juveniles, but was most apparent in shorter, less-developed worms. In shorter, likely less-developed worms, the trophosome is not yet well-developed in the esophageal region (Figure 2, top). In longer, likely more-developed worms, the trophosome was well-developed in the esophageal region and obscured much of the stichosome (Figure 2, bottom). While the nuclei and nucleoli of some of the stichocytes could be seen in our photographs of live worms, the stichocytes could not be accurately enumerated.

Figure 3 illustrates the basic morphological features that were discernible on live mermithid parasitic juveniles, including a terminal, centered mouth that leads into a non-muscular esophagus, the modification of the posterior region of the esophagus into a glandular structure (stichosome), the presence of two large ovular cells (homorocytes) visible at the anterior end of the stichosome, the absence of a tail appendage or a functional anus, and the presence of a trophosome that lays adjacent to the stichosome in the anterior region of the worm and extends posteriorly for nearly the entire body length of the worm.

Remarks

Agamomermis grapsifarciens n. sp. is different from all other known mermithids in that it parasitizes a marine decapod crustacean, the shore crab *Hemigrapsus oregonensis*.

Measurements of fixed worms (see Table 1) revealed that the shortest parasitic juvenile (measuring 19.6 mm in total body length) had the shortest trophosome length relative to body length (89.8%) and the largest distance from the mouth to the anterior origination of the trophosome (1890 μm). However, the longest worms did not necessarily have the largest overall

percentage of trophosome length relative to total body length; for example, a mermithid measuring 54.4 mm in body length had a higher percentage of trophosome relative to body length (99.1%) compared to a 65.0 mm worm that had a slightly lower percentage of the trophosome relative to body length (98.5%). Nonetheless, the trophosome filled an average of 97.4% (range: 89.8-99.2%) of the total length of the worm, making the trophosome the largest and most recognizable structure in mermithid parasitic juveniles.

The most parsimonious explanation for the observation of the oral stylet on only two of eight observed individuals is that the oral stylet was present on all worms, but was only protruded and discernible in two of the eight individuals.

Taxonomic Summary

Type Host. *Hemigrapsus oregonensis* Dana, 1851 (Decapoda: Varunidae)

Type Locality. Liberty Station, San Diego, CA (32°43'44"N, 117°12'55"W)

Site of infection: Host hemocoel

Etymology. The specific epithet is formed from the New Latin *grapsus* (a crab; which itself is derived from the ancient Greek *grapsaios*), the Classical Latin *farcire* (to stuff or to fill up), and the standard Latin connecting vowel *i*. The epithet is used as the singular present active particle in the nominative case, and is taken to match the feminine gender of the genus. The epithet therefore means “crab-stuffing” or “crab-filling”, referring to the large volume of the host hemocoel taken up by the parasite.

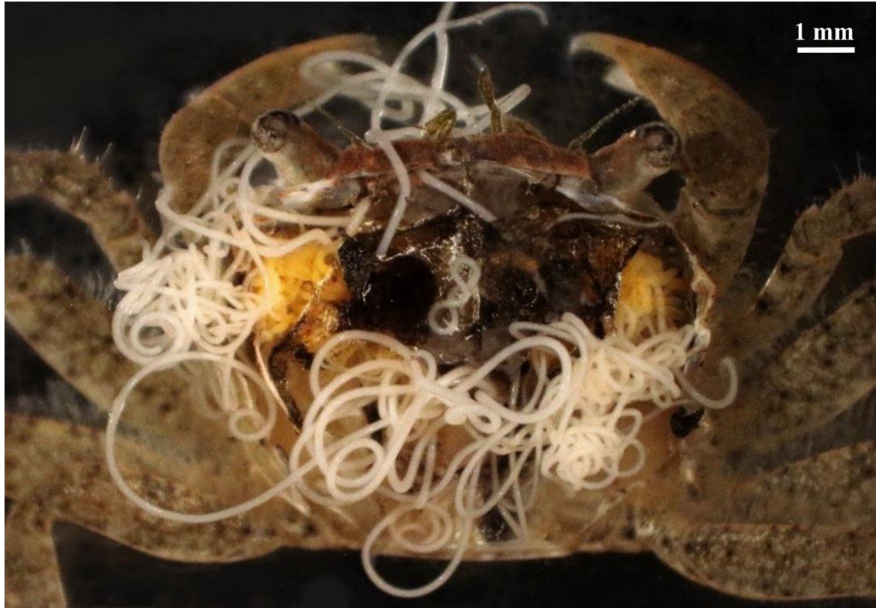


Figure 1. High intensity infection of *Agamomermis grapsifarciens* n. sp. in a *Hemigrapsus oregonensis* host (carapace width 9.1 mm). Photo by Daniel Metz, enhanced by Jennifer Dusto.

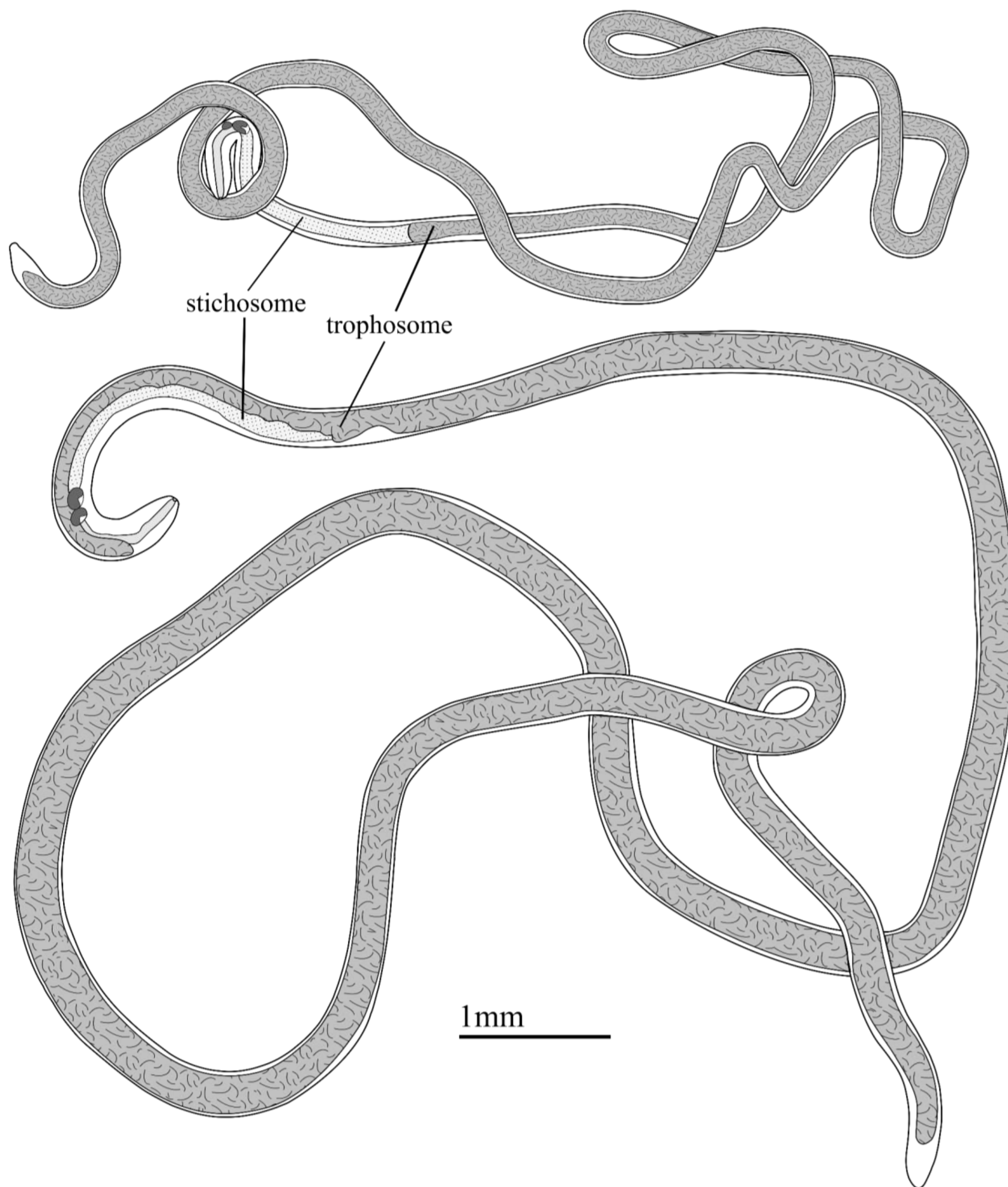


Figure 2. Full body illustrations of two mermithid parasitic juveniles of *Agamomermis grapsifarciens* n. sp. measuring 22.3 mm (top) and 32.1 mm (bottom) in total body length. Note the anterior extension of the trophosome in the bottom (larger, likely more-developed) worm relative to the top (smaller, likely less- developed) worm.

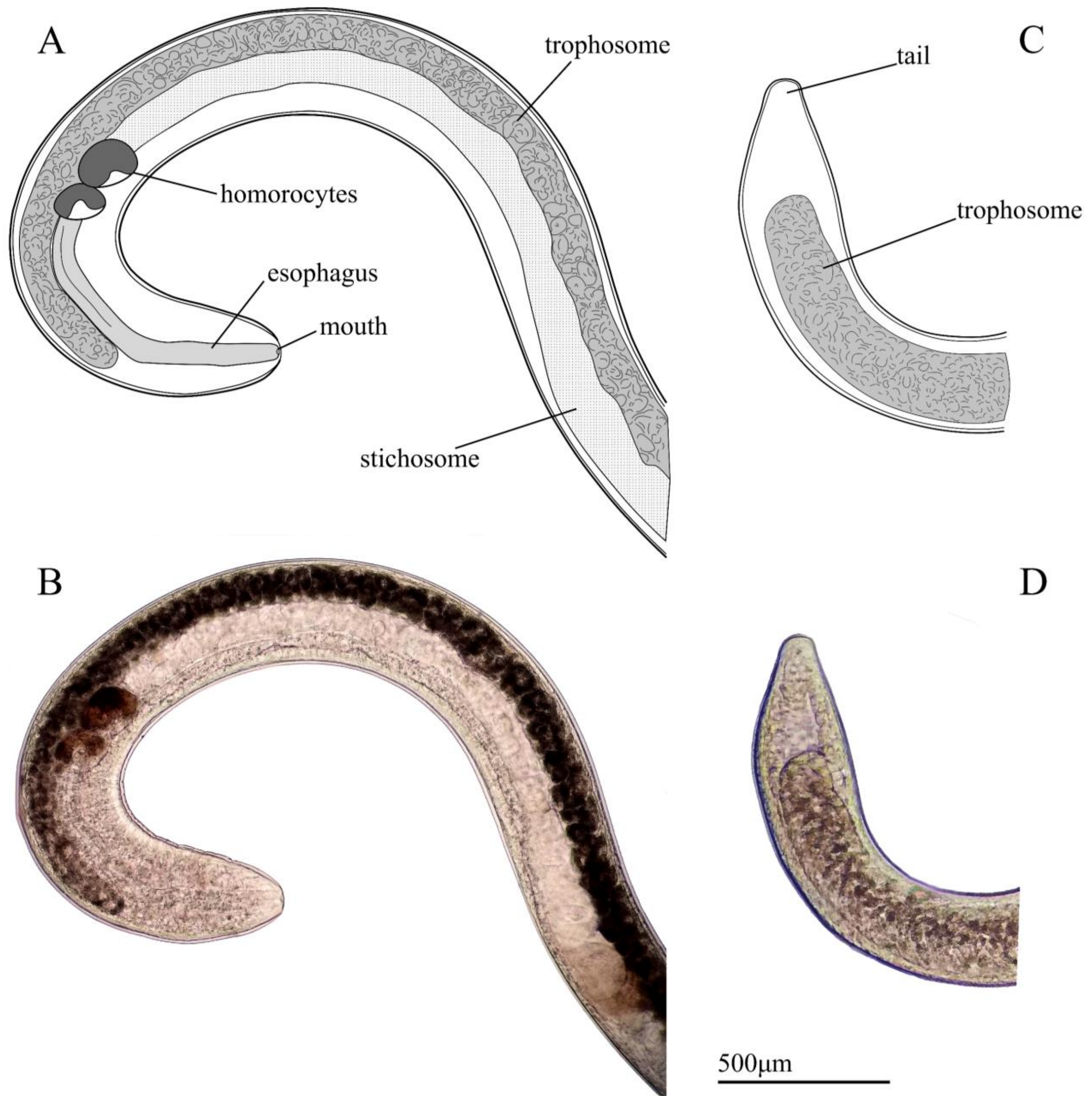


Figure 3. Anterior and posterior regions of live mermithid parasitic juveniles of *Agamomermis grapsifarciens* n. sp.: (A) illustration of anterior region, (B) image of anterior region, (C) illustration of posterior region, (D) image of posterior region. The mermithid in (A) and (B) measures 32.1 mm in total body length while the mermithid in (C) and (D) measures 22.3 mm in total body length.

Table 1. Morphometric data of fixed parasitic juveniles of *Agamomermis grapsifarciens* n. sp. from December 2018 and April 2019. Mermithids #1-6 were recovered from a 10.1 mm female *H. oregonensis* host collected in April 2019. Mermithids #7-8 were recovered from *H. oregonensis* host(s) in December 2018: the carapace width and sex of the host(s) are unknown for these two individuals.

Mermithid #	Total body length (mm)	Body width at head (µm)	Body width at midbody (µm)	Body width at tail (µm)	Distance from mouth to anterior trophosome (µm)	Distance from posterior trophosome to end of tail (µm)	Total length of trophosome (mm)	Distance from mouth to nerve ring (µm)	Length of oral stylet (µm)	Percentage of trophosome length relative to total body length
1	54.4	96.2	208	120	290	216	53.9	240	-	99.1%
2	25.1	71.2	120	92.5	312	154	24.6	-	-	98.0%
3	60.5	87.5	191	97.5	212	250	60.0	-	-	99.2%
4	60.8	92.5	210	129	650	160	60.0	-	-	98.7%
5	19.6	66.2	105	58.8	1890	182	17.6	188	-	89.8%
6	25.9	70.0	111	67.5	312	160	25.4	-	-	98.1%
7	50.8	87.5	150	96.2	950	105	49.7	180	10.0	97.8%
8	65.0	93.8	166	80.0	815	138	64.0	-	7.50	98.5%
average (range)	**45.3 (19.6-65.0)**	**83.1 (66.2-96.2)**	**158 (105-210)**	**92.7 (58.8-129)**	**679 (212-1890)**	**171 (105-250)**	**44.4 (17.6-64.0)**	**203 (180-240)**	**8.75 (7.50-10.0)**	**97.4% (89.8-99.2%)**

Dashes (-) indicate that the feature was not discernible and was therefore not recorded

Parasite prevalence, intensity, and abundance

In our initial parasitological survey of *H. oregonensis* in December 2018, the prevalence of mermithid parasitic juveniles was 15.5% (9/58) (95% CI: 8.4-26.9%), the recorded intensity of infection ranged from 2-10 mermithids (4.7 ± 2.9 SD), and the carapace widths of mermithid-infected crabs ranged from 6.0-9.7 mm. In the second survey in April 2019 at the same locality, prevalence was 5.6% (2/36) (95% CI: 1.6-18.2%), the intensity of infection ranged from 2-6 mermithids (4.0 ± 2.8 SD), and the carapace widths of mermithid-infected crabs ranged from 10.1-10.9 mm. Subsequent surveys at Liberty Station between Summer 2019 and Winter 2019-2020 (involving 204 crabs) failed to detect mermithid infections in *H. oregonensis* 0% (95% CI: 0-1.9%), as did the surveys of 324 crabs from X other San Diego County localities between Fall 2019 and Winter 2019-2020, 0% (95% CI: 0-1.2%). Additional collection-specific, crab demographic, and parasitological information is provided in Table 2, and size frequency distributions of crabs collected from different localities are provided in A7 of the Appendix.

The final logistic regression model indicated that the probability of infection exhibited a peak in smaller crabs on both dates (Figure 4, Table 3). Further, the influence of size varied by date, reflecting that the infection probability peak shifted to slightly larger crabs as the overall crab size-distribution shifted towards larger sizes (Figure 4).

The peak in infection probability at intermediate sizes was mirrored by a peak in mermithid abundance, as confirmed by the negative binomial regression on the pooled crabs from surveys at Liberty Station in December 2018 and April 2019 (Figure 5, Table 4).

Table 2. *Hemigrapsus oregonensis* collection data.

Site	Date	No. crabs	Carapace width (mm) mean (range)	No. female	No. male	No. infected	No. uninfected	Prevalence (95% CI)	Intensity mean (range)	Total no. mermithids in sample
LS	Winter 2018-2019	58*	9.2 (5.0-17.0)	30	20	9	49	15.5% (8.4-26.9%)	4.7 (2-10)	>33
LS	Spring 2019	36	11.1 (7.8-18.0)	21	15	2	34	5.6% (1.6-18.2%)	4.0 (2-6)	8
LS	Summer 2019	56	10.9 (7.1-16.9)	29	27	0	56	0% (0-6.4%)	-	0
TJE	Fall 2019	219†	15.2 (6.7-27.1)	87	130	0	219	0% (0-1.7%)	-	0
AH	Fall 2019	40	12.0 (8.0-17.8)	20	20	0	40	0% (0-8.8%)	-	0
LS	Fall 2019	38	9.8 (6.2-13.4)	20	18	0	38	0% (0-9.2%)	-	0
LS	Winter 2019-2020	110	12.8 (7.9-19.0)	53	57	0	110	0% (0-3.4%)	-	0
MBP	Winter 2019-2020	16	17.6 (14.3-23.0)	7	9	0	16	0% (0-19.4%)	-	0
AH	Winter 2019-2020	49	15.3 (10.1-21.4)	31	18	0	49	0% (0-7.3%)	-	0
	TOTAL	**622**		**298**	**314**	**11**	**611**			

For site: LS = Liberty Station, TJE= Tijuana River National Estuarine Research Reserve, AH= Agua Hedionda Lagoon, MBP= Mission Bay Park.
Carapace width is presented as mean (min-max) and prevalence is presented as a percentage with 95% confidence interval.
* sex was not recorded for 8 crabs, † sex was not recorded for 2 crabs

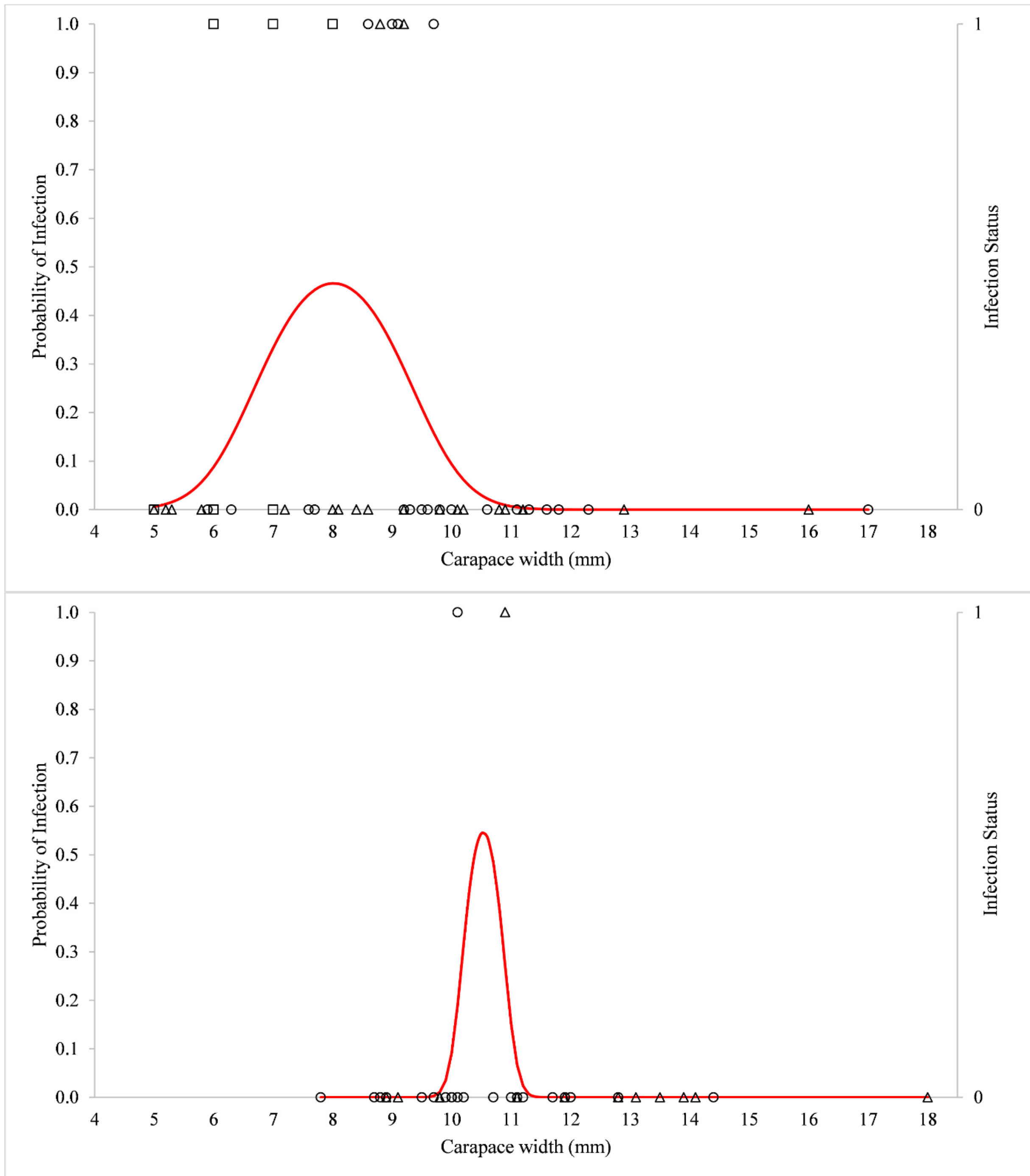


Figure 4. Probability of infection by mermithids vs *Hemigrapsus oregonensis* size in December 2018 (top) and April 2019 (bottom) at Liberty Station in San Diego, CA. Plotted points represent the observed infection status of our hosts, where triangles represent male *H. oregonensis*, circles represent female *H. oregonensis*, and squares represent juvenile *H. oregonensis* for which sex was not recorded. On the secondary y-axis, a value of 1 indicates that the crab was infected by at least one mermithid, while a value of 0 indicates that the crab was not infected by mermithid(s). The line represents the expected probability of infection originating from the logistic regression model.

Table 3. Parameter estimates from logistic regression model (n = 94).

December 2018		
Effect	**Estimate**	***p*-value**
carapace width	-1.27	**0.03**
carapace width * carapace width	-0.54	**0.02**

April 2019		
Effect	**Estimate**	***p*-value**
carapace width	-10.3	0.26
carapace width * carapace width	-8.76	0.24

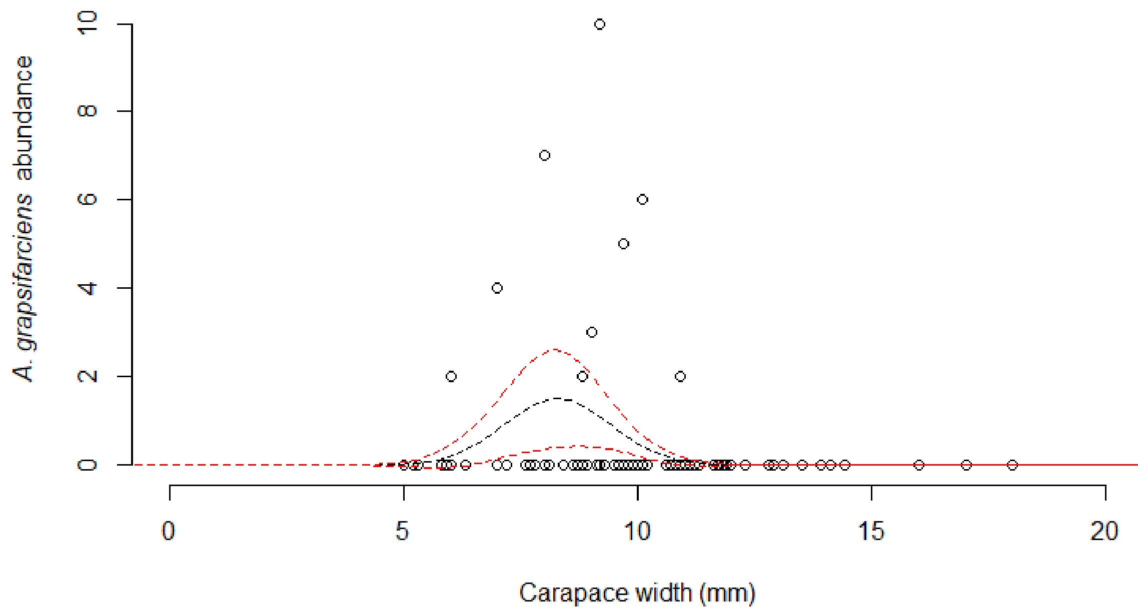


Figure 5. Mermithid abundance vs. *Hemigrapsus oregonensis* host size. The curves are the expectations from the negative binomial regression: the black dashed line represents the predicted mean and the dashed red lines indicate its upper and lower 95% confidence limits.

Table 4. Parameter estimates from negative binomial regression (n = 92).

Effect	**Estimate**	***p*-value**
carapace width	-1.28	**0.03**
carapace width * carapace width	-0.38	**0.03**

Discussion

Morphological and Molecular Support of New Species

In December 2018, members of the Hechinger Lab opportunistically discovered long, white worms infecting the hemocoel of *H. oregonensis* crabs from Liberty Station (San Diego County) while performing a general parasitological survey. We initially identified these worms as parasitic juveniles of the nematode family Mermithidae based on morphological characteristics such as the filiform body shape, presence of a stichosome and trophosome, and absence of a functional anus. We determined that the worms did not belong to the family Tetradonematidae (also within the Order Mermithida) based on the absence of a four-celled glandular "tetrad" in the pharynx, and because none of the observed worms had reached sexual maturity within the host as occurs in tetradonematids (Poinar, 1975, 1983; Hominick, 1982; Tchesunov and Hope, 1997). However, the timing of sexual maturation of this new mermithid could not be confirmed as we lacked post-parasitic juveniles and sexually mature adult worms. Nonetheless, no signs of a developed vulva, vagina, or spicules were observed in any of our mermithid specimens, and this further supported that the worms are juvenile mermithids rather than tetradonematids. Further, we confirmed that the worms did not belong to Phylum Nematomorpha based on the presence of a clear pseudocoelom with a trophosome, the absence of epicuticular features such as setae, areoles, or tubercles, and the absence of a multilobed or divided tail as occurs in nematomorphs (Poinar, 2010).

Because mermithids are not known to infect marine decapod crustaceans, it is likely that these worms represent a new species. We performed initial sequencing of 18S rDNA from three individuals; our preliminary analysis of the sequences indicates that the samples group within the

nematode family Mermithidae (unpublished data), and we are currently getting more, and longer, sequences to conduct more robust molecular phylogenetic analysis of rDNA.

Because we lacked post-parasitic and adult stages of the new mermithid species, we are unable to assign our mermithid juveniles to an appropriate genus. Instead, we plan to assign this new species to the collective group *Agamomermis* Stiles, 1903, a genus-group that is designated for mermithids described from juveniles for which adult specimens are not available (Stiles, 1903; Poinar and Welch, 1981). We plan to name this new species *Agamomermis grapsifarciens* n. sp., the trivial name means “crab-stuffing”, as the juveniles grow to fill the host’s body cavity.

Mermithid infection intensity

Although we only encountered 11 infected crabs, we note that each infected crab harbored more than one mermithid. Multiple infection (intensity > 1) is rare for parasitoids in general, where single infections are usually most common (Kuris, 1974). Mermithids generally follow this trend of single infections being most common, including for 3 of the 4 crustacean hosts for which adequate intensity information is available (Poulin and Latham, 2002b; Rubzov and Bekman, 1979; Warren et al., 2019), and for many insect mermithids (e.g. mosquitoes and blackflies, see Ezenwa and Carter, 1975; Peterson, 1972; Wise de Valdez, 2006). Although single infections are typically most common for mermithids, multiple infections are frequently reported in mermithid-parasitized hosts (e.g. Peterson, 1972; Poulin and Latham, 2002b; Welch, 1965). Multiple infections may reflect some sort of packeting of infectious propagules (pre-parasitic juveniles) or strong variability in host susceptibility, and certainly impacts several aspects of mermithid biology [e.g., within-host competition and sex determination (see Peterson, 1972; Poinar, 1983; Poulin and Latham, 2002b; Welch, 1965)].

Mermithid infection and crab size

When the parasite was present, both infection probability and the abundance of mermithids peaked in intermediate-sized smaller crabs (that is, smaller-sized crabs, but not the smallest-sized crabs). Such a pattern is consistent with smaller crabs being most susceptible to mermithid infection, and that mermithid infections are fatal to the host. Small crabs have a certain daily risk of infection, which explains the initial cumulative increase in infection probability as small crab size increases. The subsequent decrease in infection probability is likely explained by infected small crabs being killed upon the completion of parasitic development and the mermithid's emergence.

Although we did not have the opportunity to observe the process of host emergence, we expect that juveniles of *Agamomermis grapsifarciens* n. sp. are parasitoids. By definition, parasitoids typically kill the host as part of their life cycle (Lafferty and Kuris, 2002). Infection by juvenile mermithids is typically fatal to the host (Nickle, 1972; Poinar and Sanders, 1974; Poinar, 1975, 1981, 1985; Kaiser, 1991; Poinar et al., 2002; Dong et al., 2014), and mermithids, like parasitoids in general, are generally large relative to their hosts (compared to other types of parasites) (Lafferty and Kuris, 2002).

Smaller hosts being most susceptible to infection is consistent with another crab parasitoid, the parasitic platyhelminth *Fecampia erythrocephala*, which infects only very small green crabs (*Carcinus maenus*) (see Kuris et al., 2002 and references therein). The parallel pattern of juvenile crabs belonging to two different superfamilies being susceptible to parasitoids belonging to different phyla highlights the possibility that parasitoid-ism of young settled crabs is much more common than appreciated.

The size range of infected crabs shifted toward slightly larger crabs between December 2018 and April 2019. This shift may reflect a simple shift in the crab population's size distribution associated with seasonal recruitment (followed by crab growth), as, despite our targeting smaller crabs, crabs were larger in April 2019 than in December 2018. Careful crab demographic study, which is lacking for the host in California (Morris et al., 1980), combined with parasitological investigation, would shed light on whether seasonal shifts in the patterns of infection with crab size are typical, and whether they are explained by temporal changes in susceptibility, infection rates, and/or growth of crabs after infection.

Parasite prevalence, intensity, and abundance in space and time

Between December 2018 and February 2020, my colleagues and I collected and dissected a total of 622 *H. oregonensis* crabs from four sites throughout San Diego County. Despite the fifteen-month collection period and multiple-site sampling, collections at Liberty Station in December 2018 and April 2019 were the only surveys that yielded infections by the mermithid. We did not detect the parasite at this site afterwards, in Summer 2019, Fall 2019, or Winter 2019-2020, when we dissected an additional 204 *H. oregonensis* crabs (of which 88 were smaller than 11.0 mm carapace width – the maximum observed size infected). Further, we did not detect the parasite at Agua Hedionda Lagoon, Mission Bay Park, or Tijuana River National Estuarine Research Reserve between Fall 2019 and Winter 2019-2020, when we dissected 324 *H. oregonensis* crabs (of which 67 were smaller than 11.0 mm). Based on the observed prevalence of infection in collected crabs measuring less than 11.0 mm from Liberty Station [which was 7.1% (11/154) (95% CI: 4.0-12.3%)], and our collection of 67 crabs measuring less than 11.0 mm from other sites in San Diego County, there was only a 0.7% chance of not detecting the

parasite in these 67 other crabs if the parasite had the same prevalence in the population. Therefore, it is very unlikely that our sampling would have missed infections in other collection sites, had they been present at the same overall prevalence characterizing the locality from which they were detected. Further, the 95% confidence limits for the 'zero prevalence' samplings indicate that the mermithid, if present at all, was extremely rare, having a maximal prevalence of 1.8% (upper 95% CI) at Liberty Station, and a maximal prevalence of 1.2% (upper 95% CI) for the other three sites (pooled) in San Diego County. These prevalences are well below the lower 95% CI of 8.4% when it was detected in December 2018, and similar to the lower 95% CI of 1.6% when it was detected in April 2019, further indicating the parasite was not present or much rarer after its initial detection.

After our first few surveys revealed that the mermithid's prevalence dropped from 15.5% in Winter to 5.6% in Spring and to 0% in Summer and Fall 2019, we recognized that there might be strong seasonal cycling of infections in *H. oregonensis* hosts. We posited that the period of parasitic development of the mermithids in *H. oregonensis* occurs from winter until springtime and that the mermithids are free-living in the environment as post-parasitic juveniles, adults, and eggs until the next winter when the infective pre-parasitic juveniles hatch and infection occurs. Under this hypothesis, we predicted that mermithid infections would return in Winter 2019-2020. However, we did not re-encounter mermithid juveniles in *H. oregonensis* in any of our surveys during the following winter season, despite 27 of the 110 crabs collected from Liberty Station in Winter 2019-2020 measuring between 6.0 - 10.9 mm in carapace width (the size range in which we had found mermithid infections in the previous winter season). Assuming the prevalence was similar to the 15.5% (95% CI: 8.4-26.9%) observed in December 2018 when we initially encountered the parasite, we would expect around 17 (between 9-30) of the 110 crabs to be

infected with the parasite. But, although the size range of collected crabs included intermediate-sized smaller crabs, we did not re-encounter the parasite in Winter 2019-2020. Ultimately, the lack of a recurrence of infections in Winter 2019-2020 does not support the hypothesis of a simple seasonal trend in infection.

Due to the overall low observed prevalence of the mermithid and possible lack of a recurring seasonal cycle of mermithid infections in *H. oregonensis* hosts, it is possible that this new mermithid either infects *H. oregonensis* only sporadically or that *H. oregonensis* is an unusual host for the mermithid. Sampling other potential arthropod hosts that live in the same ecosystems as *H. oregonensis* could help to determine if mermithid infection results from some "spillover" infection from a more typical arthropod host. However, mermithids are not well known in the marine environment as a whole, and, to date, there are no reported host-mermithid associations in marine environments in North America. Further, there are only two other mermithid-crustacean associations in North America- a freshwater decapod and a terrestrial isopod. Therefore, we cannot postulate as to other potential hosts of marine crustacean-associated mermithids in San Diego County, CA.

Another possibility is that the new parasite is more common in *H. oregonensis* populations, but that our sampling of crabs missed the bulk of infected crabs in the population. Specifically, the majority of crabs that we collected were on the surface under riprap in the intertidal zone, but *H. oregonensis* can both bury in the substrate and extend into the subtidal zone (Jensen, 2014). Hence, if mermithid-infected crabs tend to inhabit subtidal waters or tend to burrow deeper in the substrate, our sampling would have inadequately detected the mermithids. The possibility of infected crabs burying is suggested by the finding that mermithid-infected *Talorchestia quoyana* amphipods burrow deeper into the sand than uninfected amphipods

(Poulin and Latham, 2002a). A study that includes subtidal and buried crabs could ascertain whether the mermithid is truly more common, and if our sampling skewed our findings toward low prevalence and rarity of infection.

Crab Husbandry for Mermithid Rearing

Between June 2019 and February 2020, I reared all captured crabs for a minimum of three weeks before euthanization and dissection. This rearing was performed to allow post-parasitic mermithids to emerge naturally from any infected crabs. I planned to keep any emerged post-parasitic juveniles in separate aquaria until the juvenile mermithids molted to the adult stage and both adult sexes could be described. However, none of the 528 crabs I collected and dissected during this period revealed any sign of the emergence of mermithids, nor were any infected with parasitic juveniles. The lack of obtaining post-parasitic juveniles or adults would not seem to be due to any demonstrated shortcomings in my experimental setups, but rather due to the low observed prevalence of the mermithid species in my samples. We have no reason to believe that the setups would have been unsuccessful in containing emerged post-parasitic mermithids had they been there, and we would use the same or similar designs in any future attempts of accommodating crabs in an effort to catch emerging mermithids. Further, each of the experimental setups had unique advantages and disadvantages, and our choice of which setup to use in the future will reflect our primary goal in crab husbandry- either video recording a small sample, or mass-rearing a large sample of crabs.

The initial laboratory crab-rearing setup provided the ability to continuously video record *H. oregonensis* to attempt to observe parasite emergence. Additionally, the use of individual collection cups was successful in keeping crabs separated, which granted us the ability to track

host molting, eliminate any chance of crab combat or post-molting predation, and definitively correlate any emerged worms to their host by retaining any emerged mermithids in the host cup. However, this initial setup had a few major difficulties. Most notably, the collection cup setup involved the use of six separate water spigots, each of which was connected to an aquarium gang valve that split the water flow from the spigot into eight cups arranged in series. Although this design worked well during the trial period, over the course of ongoing husbandry (June 2019-September 2019), it proved difficult to maintain adequate water flow to each of the cups, ultimately resulting in the mortality of several crabs due to poor water circulation and resultant anoxia. Essentially, the use of other adjacent spigots in the water table affected the water pressure in the spigots designated for our experimental setup, causing the flow rate into the gang valves to change over time. Although the water flow into each cup was checked on a daily basis, it was impossible to maintain sufficient water flow to allow adequate replacement of water into all 48 cups for a prolonged period of time, and we were eventually forced to eliminate cups with poor water flow. As this setup was arranged in the water tables of our lab space, only a maximum of 48 crabs could be accommodated at any time, and the elimination of cups with poor water flow further reduced our maximum capacity of crab hosts. Hence, although this experimental design was highly effective over a short time period, it ultimately suffered due to both poor water flow and in limiting total sample size. To improve this experimental setup, I recommend limiting the number of cups in series to a maximum of four (instead of eight) and ensuring the underlying continuously-flowing seawater system is capable of maintaining high water flow to each spigot over an extended period of time.

Our demonstrated difficulties with the initial setup and the low prevalence of infection led us to create a more efficient and larger-scale crab-rearing design to provide a better chance of

obtaining emerging mermithids. By housing crabs in modified parts boxes instead of individual cups, we increased the number of crabs we could accommodate at any time, while simultaneously removing the requirement for water flow splitting. Additionally, by separating crabs into individual compartments, we were once again able to prevent crab combat and post-molting predation events, while maintaining the ability to track the host molting and growth of individual crabs. By drilling holes into the walls of the parts boxes, replacing the floor with polyester mesh, and maintaining a high rate of water flow into each reservoir tub, we were able to maintain sufficient water flow throughout the duration of husbandry, and we did not experience any significant mortality of crabs using this setup. Additionally, as space and water flow pressure were not limiting factors in this setup, we could accommodate a substantially larger number of crabs at any one time (i.e., up to 204 vs 48 max). The only perceived disadvantages of this setup compared to the initial setup were the inability to continuously record crabs, and the inability to easily definitively correlate the host crab to any emerged worms, as the worms would fall through the mesh floor into the shared reservoir tub. However, even here, if only worms from one host were to emerge since the last reservoir tub check, it should be possible to connect an emerged worm to its host if such hosts are dead or moribund (the default expectation for a mermithid-infected host). For this reason, given the rarity of infection, we decided that the overall increase in maximum capacity and ease of water circulation of this setup outweighed the benefit of video recording, and we used this new design for the remaining duration of the project.

Conclusion

Here, I have contributed a description of the parasitic juvenile stage of a new species of mermithid that we plan to name *Agamomermis grapsifarciens* n. sp., with information on the parasite's patterns of infection with host size and observed prevalence in San Diego County. In addition to being the first mermithid known to infect a marine decapod, the new species is only the third mermithid known from the marine environment, and the eleventh known to infect a crustacean worldwide (the third from North America). If and when we reencounter the mermithid in the host population with a high enough prevalence to permit study of the parasite, we aim to once again lab-rear crab hosts, capture and raise emerging post-parasitic juveniles, and describe adult specimens to place *Agamomermis grapsifarciens* n. sp. in an appropriate genus based on adult characters. Further, we are currently conducting molecular phylogenetic analysis of rDNA to confirm the placement of *Agamomermis grapsifarciens* n. sp. in the nematode family Mermithidae, and reveal its relationship to other sequenced mermithid genera.

The material in this master's thesis is currently being prepared for submission for publication. Dusto, Jennifer A.; Metz, Daniel C.G.; Hechinger, Ryan F. The thesis author was the primary investigator and author of this material.

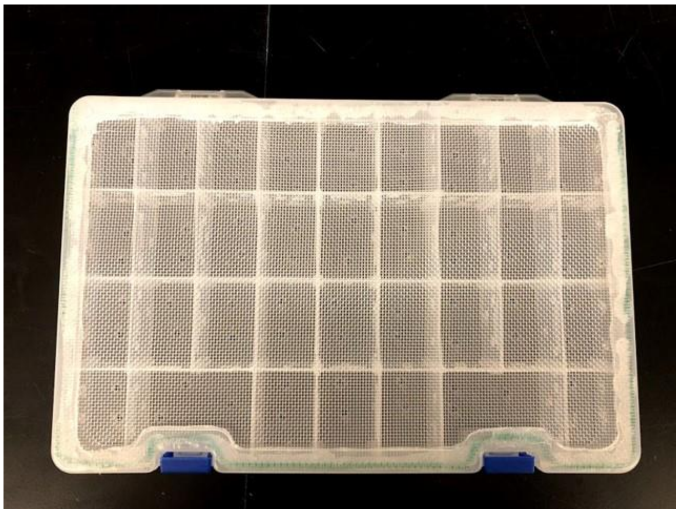
Appendix

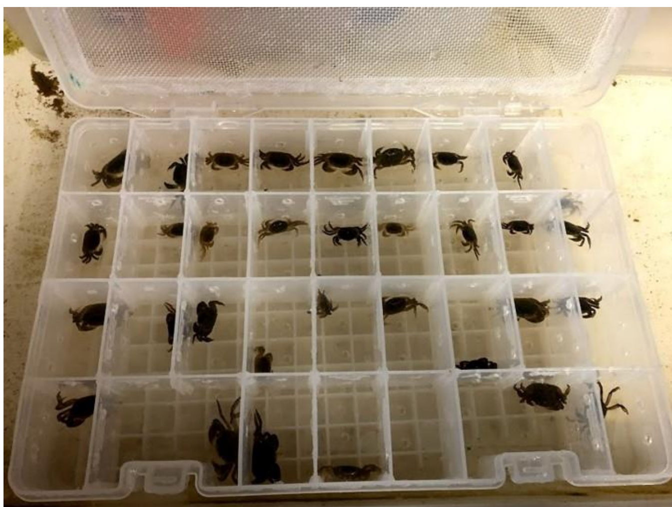
A1. "Crab cup" from initial crab husbandry setup

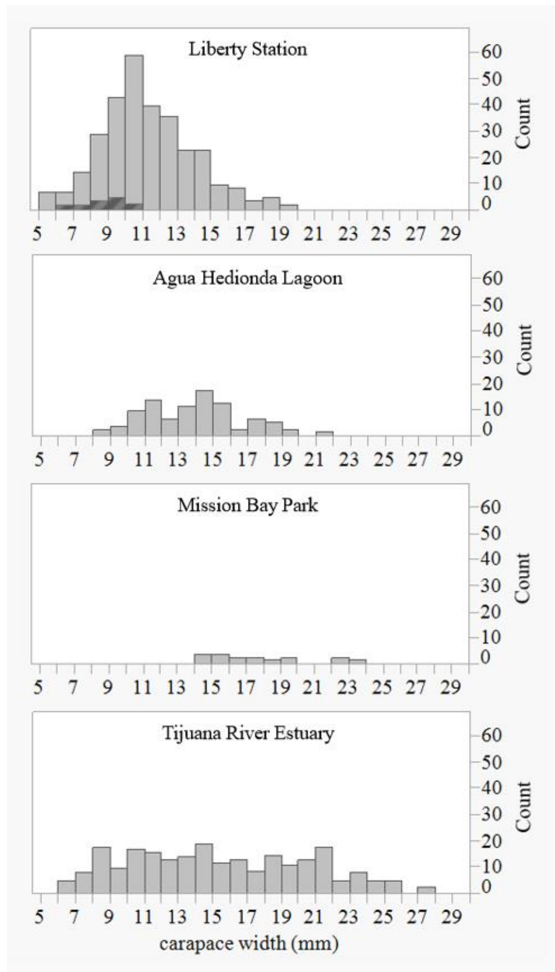
A2. Overview of initial crab husbandry setup

A3. "Crab cups" during water flow dye test, initial crab husbandry setup

A4. Overview of second crab husbandry setup


A5. Modified parts box from second crab husbandry setup


A6. Modified parts box from second crab husbandry setup, with *H. oregonensis*



A7. Size-frequency distributions of *Hemigrapsus oregonensis* by collection site. The dark shaded region on the Liberty Station distribution (top) represents hosts infected by *Agamomermis grapsifarciens* n. sp.

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