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Anomalomermis ephemerophagis n. g., n. sp. (Nematoda: Mermithidae) parasitic in the mayfly, Ephemerella maculata Traver, 1934 (Ephermeroptera: Ephermerellidae), in California

George Poinar, Jr.¹, Larissa Walder² and Hiromi Uno²

¹Department of Integrative Biology, Oregon State University, Corvallis, OR 97331, USA: email: poinarg@science.oregonstate.edu

²Department of Integrative Biology. University of California, Berkeley, CA 94720, USA

Abstract

A new nematode, *Anomalomermis ephemerophagis* n. g., n. sp. (Nematoda: Mermithidae) is described from the mayfly, *Ephemerella maculata* Traver,1934 (Ephermeroptera: Ephermerellidae), in California. The new species is characterized by 6 cephalic papillae and 4 additional disk papillae located on the head between the cephalic papillae and stoma. Additional diagnostic characters are: mouth opening terminal, absence of X-fibers in the cuticle of both postparasitic juveniles and adults, paired, curved, medium-sized spicules, a straight barrow-shaped vagina and large eggs. Two infectious agents were present in some specimens. This is the first description of an adult nematode from a mayfly.

Introduction

During a study on biological and physical factors affecting the distribution of the mayfly *Ephemerella maculata* Traver, in northern California, a mermithid nematode parasite was discovered. Both nymphal and adult mayflies were parasitized. A study of the morphological features of the mermithid showed it to represent a new genus and a description follows below. This is the first description of an adult nematode from a mayfly.

Materials and methods

Free-living adult and post-parasitic juvenile mermithids were collected from Fox Creek, a tributary of the South Fork Eel River in the Angelo Coast Range Reserve in Mendocino County, northern California. The nematodes were transferred to 95% ethanol and processed to glycerin by the evaporation method. Examination and photographs were made with a Nikon stereoscopic microscope SMZ-10-R at 80x and a Nikon Optiphot microscope at 1000x. Helicon Focus was used to stack photos for better depth of field. In the following description all measurements, including the average value and those of the range (in parentheses), are in micrometers unless otherwise specified.

Order Mermithida Rubstov 1978

Family Mermithidae Braun 1883.

Anomalomermis Poinar, Waldo & Uno, n. gen.

Type species: Anomalomermis ephemerophagis Poinar, Waldo & Uno

Diagnosis: Medium-sized mermithids with 6 cephalic papillae and 4 disc papillae; disc papillae located on head between cephalic papillae and stoma; eight hypodermal cords; absence of X-fibers in the cuticle of both postparasitic juveniles and adults; paired, curved, separate, medium-sized spicules; straight barrow-shaped vagina and large eggs.

Anomalomermis ephemerophagis n. gen., n. sp.

Type-host: Ephemerella maculata Traver, 1934 (Ephermeroptera: Ephermerellidae).

<u>Type-locality:</u> Fox Creek, a tributary of the South Fork Eel River in the Angelo Coast Range Reserve, Mendocino County, northern California.

Type material: Holotype male (T-670t) and paratype female (T-6321p) deposited in the Beltsville Nematology Collection, Beltsville Maryland, USA. Paratypes deposited in the authors' collection.

Etymology: The generic name is from the Greek "anomalos" = abnormal in reference to the 4 disc papillae and the Greek "mermithos" = cord, rope. The specific epithet is from the Greek "ephemeros" = short lived and the Greek "phagos" to eat.

Description (Figs. 1-10)

General

Medium-sized nematodes with cuticle of both adults and post-parasitic juveniles lacking cross fibers; adult trophosome with greenish-purplish tint; 6 large cephalic papillae arranged in a single plane; 4 smaller disk papillae arranged in submedial positions on head between cephalic papillae and stoma. Mouth opening terminal. Amphids positioned

below cephalic papillae, with aperture circular to oval; eight hypodermal cords at midbody.

Free-living male (n= 11). Length, 17 (15-21) mm; greatest width, 181 (158-202); head to nerve ring, 314 (276-349); L top of head to amphidial pouch, 56 (51-59); diameter amphidial opening, 16 (13-21); L spicule, 226 (164-284); W at middle of spicule, 15 (13-19); L tail, 337 (321-441); W at cloacal opening, 136 (126-145); spicules paired, separate but closely appressed, curved, between 1.4 and 2 times body diameter at cloaca; male with three rows of genital papillae extending past cloaca nearly to tail tip; with 35-50 genital papillae per row; middle row bifurcates around cloacal opening; tail pointed; sperm thin, elongate, 22 (18-26).

Free-living female (n=13). Length 26 (19-35) mm; greatest width, 280 (239-346); head to nerve ring, 333 (285-348); length top of head to amphidial pouch, 61 (32-72); diameter amphidial opening, 10 (6-14); vagina straight, barrow-shaped, with cuticular (vagina vera) and tissue (vagina uterina) portions; L vagina vera, 58 (41-88); length vagina uterina, 63 (32-95); % vulva, 48 (42-53); width vagina, 123 (101-152); diameter eggs in uteri, 64 (51-70); tail pointed.

Postparasitic juvenile (n=10), Body cuticle thinner than that of adults; body length, 16 (15-10) mm; tail with extended cuticular appendage 120 (88-170) in length.

Remarks

Very few mermithids are known with a combination of 6 cephalic and 4 disk papillae.

Neomermis macrolaimus Linstow, 1904 was described as having 10 cephalic papillae. In 1978, Rubstov synonymized Neomermis with his newly erected genus Decamermis

Rubstov 1978. Besides the original N. macrolaimus, Rubstov included N. khartschenkoi

Artykhovsky & Kharchenko that had previously been described in the genus Neomermis

(Artykhovsky & Kharchenko, 1965). The genus Decamermis was defined by Rubstov

(1978) as mermithids possessing 2 hypodermal cords, cross fibers in the cuticle and 10 head papillae. All of the papillae are relatively the same size and all are arranged in the same plane around the head. The amphids in Decamermis are located slightly above the level of the cephalic papillae and the males have 2 nearly straight spicules. The females and post-parasitic juveniles are unknown. Decamermis differs significantly from Anomalomermis ephemerophagis that has 8 hypodermal cords, lacks cuticular cross fibers, possess 6 cephalic and 4 disk papillae of different sizes and in different positions and has curved spicules.

Regarding the structure and position of the head papillae, the nematode originally described as *Mermis viridipenis* Coman (1960) comes closest to the present species. In this nematode, there are 6 large cephalic papillae and 4 additional papillae, however the latter are closely appressed against the stoma. Also *M. viridipenis* has cuticular cross fibers, 6 longitudinal chords, long, partially fused spicules and the female has an S-shaped vagina. Because of its unique characters, Rubstov was not sure where to place

M. viridipennis and first transferred it into the genus *Comanimermis* Rubstov (1974) and later to the genus *Aquaemermis* Rubstov, 1973 (Rubstov, 1978).

Infection rate: Out of a total of 304 adult mayflies examined in 2012 and 2013, the infection rate was 55%. The mermithids sterilized the female mayflies and reduced their body size. Additional studies on the effect of this mermithid on the population biology of the mayfly are in progress.

Discussion

While mermithids have previously been reported from Ephemeroptera (Poinar, 1975)(Shimazu, 1996), none have been described up to the present. However, mermithid diversity in mayflies may be greater than thought since now at least two genera are known to parasitize Ephemeroptera, the present species and a species of *Gastromermis* (Vance & Peckarsky, 1996). The only other nematode group known to occur in the body cavity of mayflies is the spirurids (Spirurina). Spirurid nematodes utilize mayflies as intermediate hosts and complete their development in vertebrate hosts, which are usually freshwater fish (Shimazu,1996).

The host mayfly, *Ephemerella maculata* exhibits migratory life cycles in the type locality (Uno and Power unpublished). The nymphal stage of *E. maculata* develops in open reaches of the South Fork Eel River. After emergence and mating, females migrate into nearby small tributaries, oviposit, and die. The eggs complete development in these tributaries but undergo a diapause before hatching. After eclosion, the young instars drift down to the mainstem. The post-parasitic juveniles of *Anomalomermis*

ephemerophagis n. gen., n. sp. were found in both *E.maculata* nymphs in the South Fork Eel River and *E.maculata* adults in Fox creek. Free-living adult nematodes have been found in the South Fork Eel River, but have not yet been found in Fox Creek. Thus, it appears that *Anomalomermis ephemerophagis* n. gen., n. sp. moves between the mainstream and tributaries of the river network as its host migrates. Further studies on the life cycle of *Anomalomermis ephemerophagis* n. gen., n. sp. and its interactions with the mayfly host is in progress.

At least two infectious agents were discovered within the adult mermithids during the present study. The first was a species resembling members of the fungal genus *Haptoglossa* Drechsler, 1940 (Oomycota: Haptoglossaceae)(Glockling and Beakes, 2000). Several developmental stages of this parasite were present, including spores attached to the adult cuticle (Fig.11), a spore in the act of penetrating the cuticle (Fig. 12) and a thallus developing in the hypodermal cord of *A. ephemerophagus* (Fig. 13). Also in the body cavity of several adults were numerous spores and developing pansporoblasts of a possible microsporidian parasite (Fig. 14). The stages resembled those of *Microsporidium rhabdophilum* Poinar & Hess (1986) developing in the free-living nematode, *Rhabditis myriophila* Poinar and unidentified sporozoans in the marine nematodes, *Alaimus primitivus* De Man and *Prismatolaimus dolichurus* De Man (Dollfus, 1946). How these pathogens affect the life cycle and population dynamics of the mermithid and its mayfly host is unknown.

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Figures

- Figs. 1-2. *Anomalomermis ephemerophagis* n. g., n. sp. 1, Lateral view of male head showing 5 cephalic papillae (C), 4 disk papillae (D), an amphid (A) and the pharyngeal tube (P). Fifth cephalic papilla is not visible; 2, "Enface" view of male head showing 6 cephalic papillae (C), 4 disk papillae (D) and stoma (S). Scale-bars: 1, 35 μm; 2, 30 μm.
- Figs. 3-4. *Anomalomermis ephemerophagis* n. g., n. sp. 3, Male head showing two disk papillae (arrows); 4, Male tail with partially exserted spicule. *Scale-bars*: 3, 20 μm; 4, 115 μm.
- Fig. 5. Mating of *Anomalomermis ephemerophagis* n. g., n. sp. Arrow shows male spicules. *Scale-bar*: 100 μm.
- Figs. 6-7. *Anomalomermis ephemerophagis* n. g., n. sp. 6, Head of female showing disc papilla (arrows). 7, Vulva and vagina. *Scale-bars:* 6, 20 μm; 7, 50 μm.
- Figs. 8-10. *Anomalomermis ephemerophagis* n. g., n. sp. 8, Eggs at juncture of oviduct and uterus. 9, Sperm. 10, Tail of post-parasite juvenile. *Scale-bars:* 8, 65 μm; 9, 18 μm; 10, 40 μm.

Figs. 11-14. Pathogens of *Anomalomermis ephemerophagis* n. g., n. sp. 11, Two possible haptoglossacean spores attached to the cuticle. 12, Possible haptoglossacean spore penetrating the cuticle. 13, Developing thallus of a possible haptoglossacean in the hypodermal cord. 14, Developing pansporoblasts and isolated spores of a possible microsporidian parasite. *Scale-bars:* 11, 10 μm; 12, 11 μm; 13, 15 μm; 14, 10 μm.