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***Anomalomermis ephemerophagis* n. g., n. sp. (Nematoda: Mermithidae) parasitic in the mayfly *Ephemerella maculata* Traver (Ephemeroptera: Ephemerellidae) in California, USA**

George Poinar Jr. · Larissa Walder · Hiromi Uno

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Abstract A new nematode, *Anomalomermis ephemerophagis* n. g., n. sp. (Nematoda: Mermithidae) is described from the mayfly *Ephemerella maculata* Traver (Ephemeroptera: Ephemerellidae) in California. The new species is characterised by six cephalic papillae and four additional disk papillae located on the head between the cephalic papillae and stoma. Additional diagnostic characters are: a terminal mouth opening; 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 parasitised. 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, USA. 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 ×80 and a Nikon Optiphot microscope at ×1,000. Helicon Focus was used to stack photos for better depth of field. All measurements are in micrometres unless otherwise specified and are provided as the range followed by the mean in parentheses.

Order Mermithida Rubstov, 1978
Family Mermithidae Braun, 1883

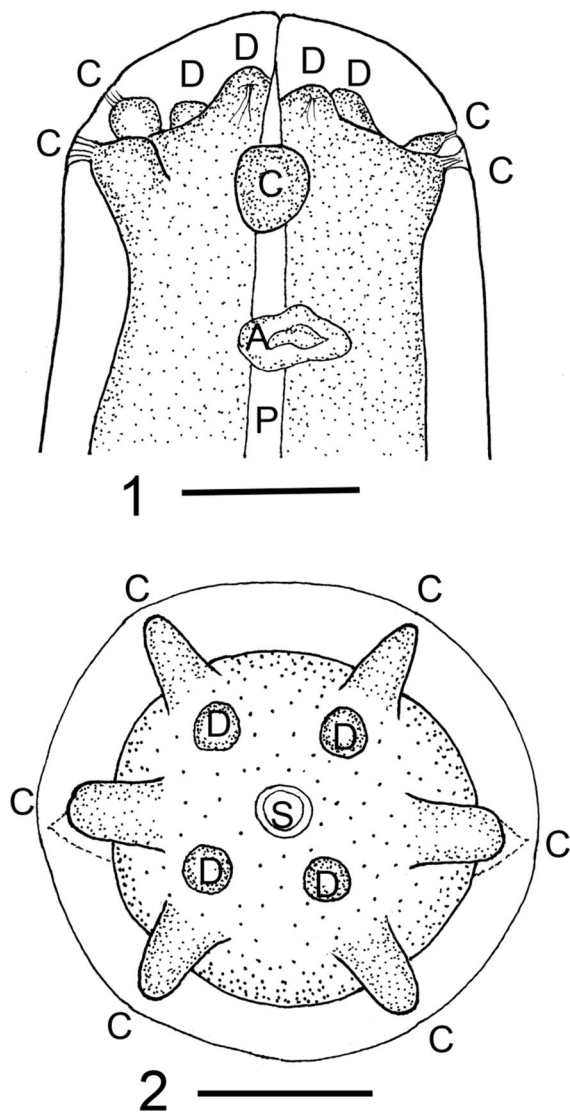
***Anomalomermis* n. g.**

Diagnosis

Medium-sized mermithids with six cephalic papillae and four disc papillae; disc papillae located on head

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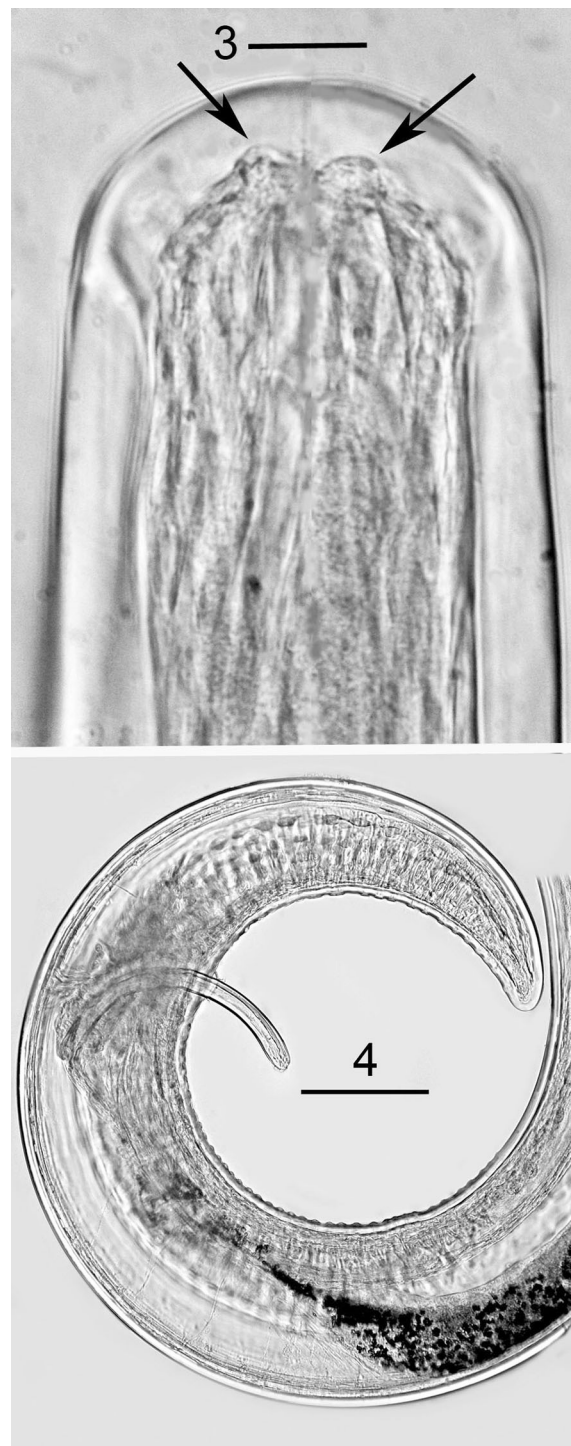
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Figs. 1–2 *Anomalomermis ephemerophagus* n. g., n. sp. 1, Lateral view of male head showing five cephalic papillae (C), four disk papillae (D), an amphid (A) and the pharyngeal tube (P). Fifth cephalic papilla is not visible; 2, “Enface” view of male head showing six cephalic papillae (C), four disk papillae (D) and stoma (S). Scale-bars: 1, 35 μ m; 2, 30 μ m

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.

Type-species: Anomalomermis ephemerophagus n. sp.



Figs. 3–4 *Anomalomermis ephemerophagus* n. g., n. sp. 3, Male head showing two disk papillae (arrows); 4, Male tail with partially exerted spicule. Scale-bars: 3, 20 μ m; 4, 115 μ m



Fig. 5 Mating of *Anomalomermis ephemerophagis* n. g., n. sp. Male spicules (arrow). Scale-bar: 100 μ m

Etymology: The generic name is from the Greek “anomalos” (abnormal) in reference to the four disc papillae and the Greek “mermithos” (cord, rope).

Anomalomermis ephemerophagis n. sp.

Type-host: *Ephemerella maculata* Traver (Ephemeroptera: Ephemerellidae).

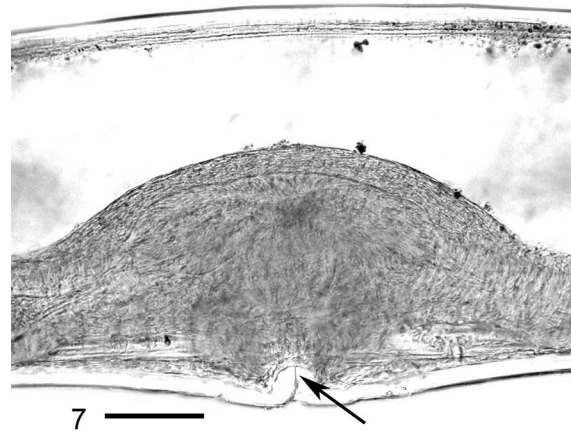
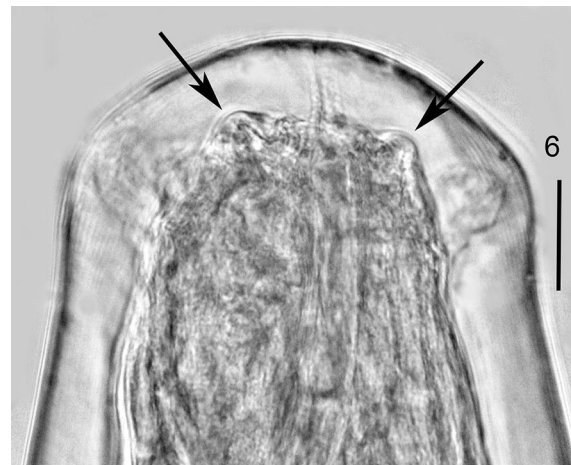
Type-locality: Fox Creek, a tributary of the South Fork Eel River in the Angelo Coast Range Reserve, Mendocino County, northern California, USA.

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 specific epithet is from the Greek “ephemerios” (short lived) and the Greek “phagos” (to eat).

Description (Figs. 1–10)

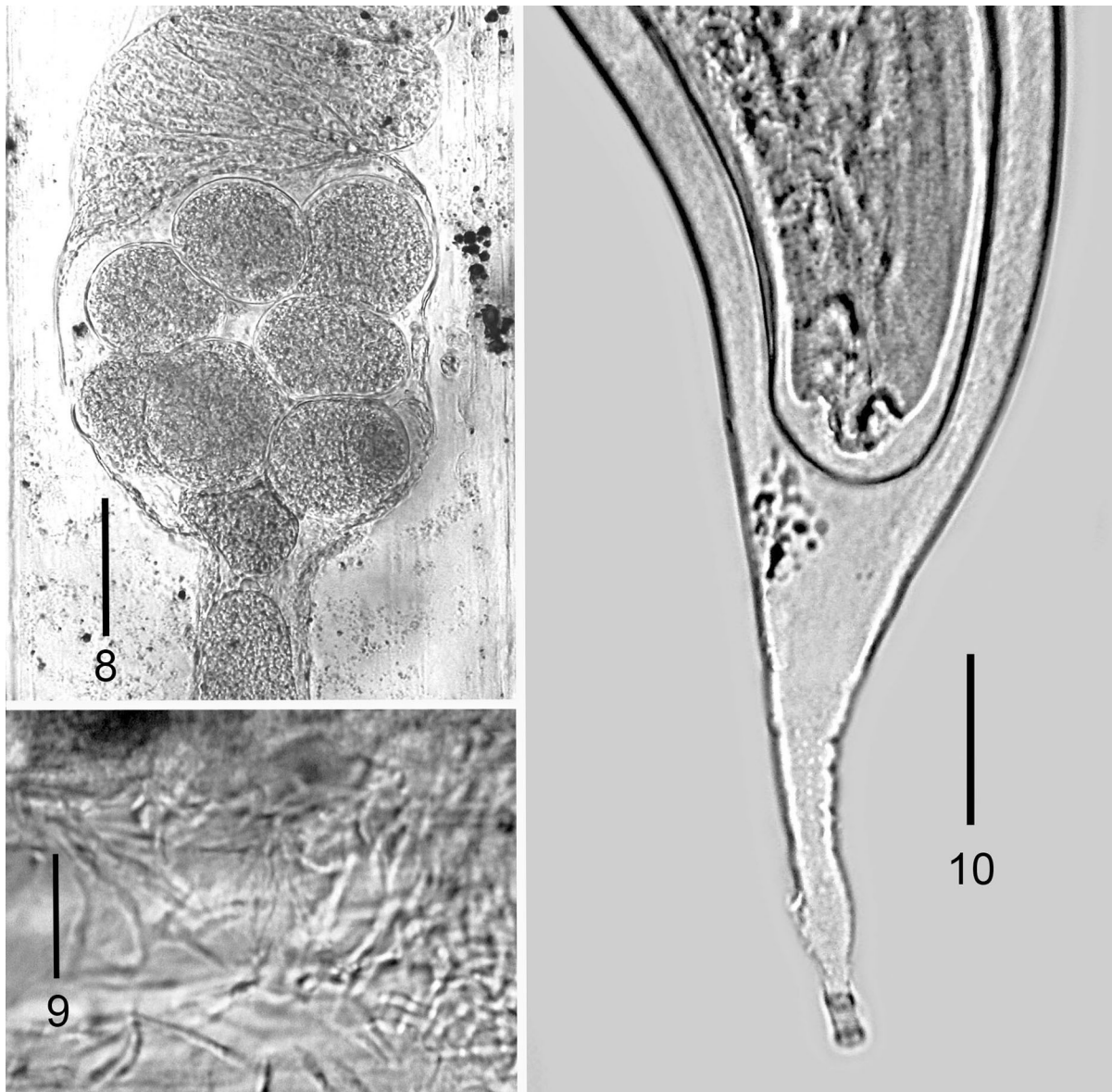
General. Medium-sized nematodes with cuticle of both adults and post-parasitic juveniles lacking cross fibers; adult trophosome with greenish-purplish tint; 6 large cephalic papillae arranged in a single plane; 4 smaller disk papillae arranged in submedial positions on head between cephalic papillae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



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 μ m; 7, 50 μ m

with aperture circular to oval; 8 hypodermal cords at mid-body.

Free-living male (n = 11). Length 15–21 (17) mm; greatest width 158–202 (181); distance anterior extremity to nerve-ring 276–349 (314); distance anterior extremity to amphidial pouch 51–59 (56); diameter of amphidial openings 13–21 (16); spicules paired, separate but closely appressed, curved, 1.4–2 $\times$ body diameter at cloaca; length of spicules 164–284 (226); width at middle of spicules 13–19 (15); length of tail 321–441 (337); body width at cloacal opening 126–145 (136); 3 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 18–26 (22).



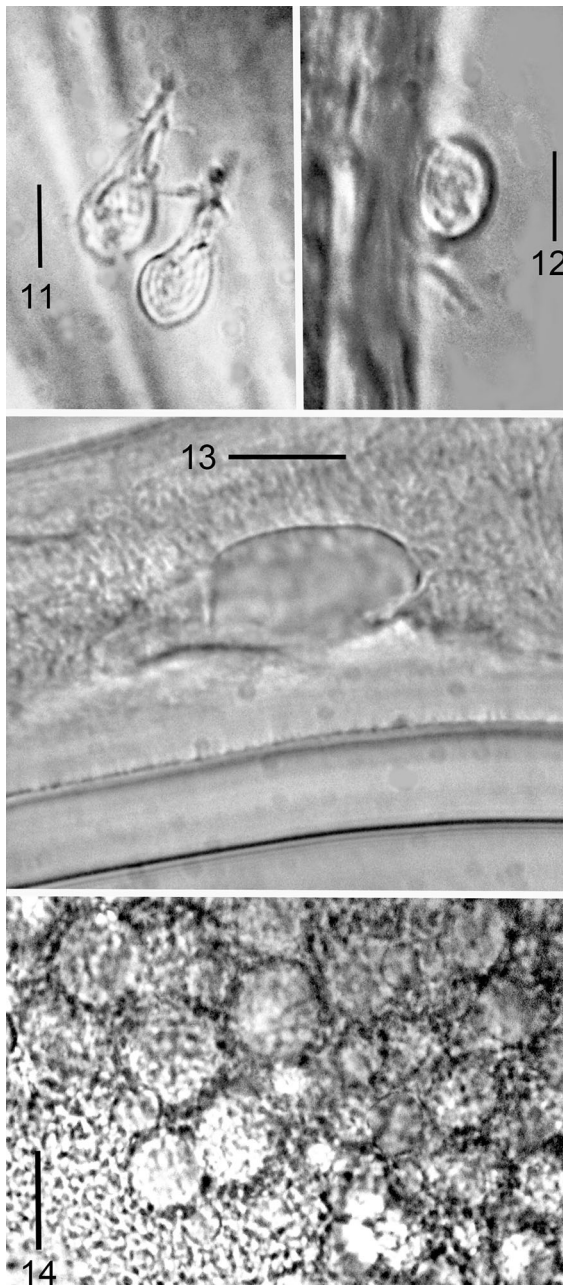
Figs. 8–10 *Anomalomermis ephemerophagis* n. g., n. sp. 8, Eggs at juncture of oviduct and uterus; 9, Sperm; 10, Tail of post-parasite juvenile. Scale-bars: 8, 65 μ m; 9, 18 μ m; 10, 40 μ m

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

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

Remarks

Very few mermithids are known with a combination of six cephalic and four disk papillae. *Neomermis macro-laimus* Linstow, 1904 was described as having ten



Figs. 11–14 Pathogens of *Anomalomeris 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 μ m; 12, 11 μ m; 13, 15 μ m; 14, 10 μ m

cephalic papillae. In 1978, Rubstov synonymised *Neomermis* Linstow, 1904 with his newly erected genus *Decamermis* Rubstov, 1978. Besides the original

N. macrolaimus, Rubstov included *N. khartschenkoi* Artyukhovsky & Kharchenko, 1965 that had previously been described in the genus *Neomermis* (see Artyukhovsky & Kharchenko, 1965). The genus *Decamermis* was defined by Rubstov (1978) as mermithids possessing two hypodermal cords, cross fibers in the cuticle and ten 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 two nearly straight spicules. The females and post-parasitic juveniles are unknown. *Decamermis* differs significantly from *A. ephemerophagis* n. sp. that has eight hypodermal cords, lacks cuticular cross fibers, possesses six cephalic and four 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 viridipennis* Coman, 1961 comes closest to the present species. In this nematode, there are six large cephalic papillae and four additional papillae, however the latter are closely appressed against the stoma. Also *M. viridipennis* has cuticular cross fibers, six longitudinal chords, long, partially fused spicules and the female has an S-shaped vagina (Coman, 1961). Because of its unique characters, Rubstov was not sure where to place *M. viridipennis* and first transferred it into the genus *Comanimermis* Artyukhovsky, 1969 (see Rubstov, 1974) and later to the genus *Aquaemermis* Rubstov, 1973 (see Rubstov, 1978).

Out of a total of 304 adult mayflies examined in 2012 and 2013, the infection rate was 55%. The mermithids sterilised 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.

Pathogens

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 & 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* n. sp. (Fig. 13). Also in the body cavity of several adults there 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 (see Poinar & Hess, 1986) and unidentified sporozoans in the marine nematodes *Alaimus primitivus* De Man and *Prismatolaimus dolichurus* De Man (see Dollfus, 1946). How these pathogens affect the life-cycle and population dynamics of the mermithid and its mayfly host is unknown.

Discussion

While mermithids have previously been reported from Ephemeroptera (see 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 parasitise Ephemeroptera, the present species and a species of *Gastromermis* Micoletzky, 1923 (see Vance & Peckarsky, 1996). The only other nematode group known to occur in the body cavity of mayflies is the spirurids (Spirurina). Spirurid nematodes utilise 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 mainstream. The post-parasitic juveniles of *A. ephemerophagus* 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 Fox Creek, but have not yet been found in the South Fork Eel River. Thus,

it appears that the new species moves between the mainstream and tributaries of the river network as its host migrates. Further studies on the life-cycle of *A. ephemerophagus* n. sp. and its interactions with the mayfly host are in progress.

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