

1 **First record of a mermithid nematode (Nematoda: Mermithidae) parasitizing**
2 **winged females of gall-forming aphids (Hemiptera: Aphididae: Eriosomatinae)**

3

4 Xin TONG^{1,2, *}, Natsumi KANZAKI³, Shin-ichi AKIMOTO¹

5

6 *¹Department of Ecology and Systematics, Graduate School of Agriculture, Hokkaido*
7 *University, Sapporo, 060-8589 Japan, ²Laboratory of Evolutionary Genomics, Center*
8 *for the Development of New Model Organism, National Institute for Basic Biology,*
9 *Okazaki, 444-8585 Japan, ³Kansai Research Center, Forestry and Forest Products*
10 *Research Institute, Kyoto, 612-0855 Japan*

11

12 *Correspondence:* Xin Tong, Department of Ecology and Systematics, Graduate School
13 *of Agriculture, Hokkaido University, Sapporo, 060-8589 Japan*

14 *E-mail:* sophiatone@hotmail.com

15

16

17 **Abstract**

18 Juvenile mermithid nematodes were found to parasitize winged females (sexuparae) of
19 *Erisoma auratum* and *Tetraneura radicolica*. The morphological characteristics of
20 mermithid nematodes are briefly described. The 18S rDNA and 28S rDNA extracted from
21 one nematode were sequenced and used to construct a Bayesian phylogenetic tree, on
22 which the host ranges of mermithid nematodes were represented. Our study indicated that
23 mermithid parasitism of sexuparae led to fewer and smaller sexual female embryos. This
24 is the first record of a mermithid in relation to eriosomatine aphids and the fourth record
25 with respect to Aphididae.

26

27 **Key words:** Mermithidae, nematode, aphid, parasite, Eriosomatinae

28

29 Mermithid nematodes (Nematoda: Mermithidae) are obligate parasites that have been
30 found in many invertebrates (Poinar 1975; Yeates & Buckley 2009; Kubo *et al.* 2016;
31 Watanabe *et al.* 2021). As with other parasitic nematodes, free-living mermithid
32 nematodes parasitize the hosts by actively penetrating the cuticles, either through natural
33 openings of the host body, or through ingestion of their eggs by host insects (Hajek 2004).
34 During our biological survey of eriosomatine aphids, a species of unidentified mermithid
35 nematode was found in the abdomens of aphids collected in Hokkaido, Japan.

36 Aphids of Eriosomatinae (Insecta: Hemiptera: Aphididae) induce leaf galls on the
37 primary host plants and parthenogenetically produce second-generation aphids within the
38 gall from early May to mid-June in Hokkaido, Japan, a cool temperate zone. Second-
39 generation aphids develop into winged adults, which migrate to the roots of secondary
40 host plants to form colonies. In autumn, winged females (sexuparae) appear on the roots
41 and migrate back to the primary host plants to produce sexual offspring. These offspring
42 (male and female embryos) develop inside the abdomens of the females at their nymphal
43 stage and are viviparously born on the trunk of the primary host plant. Sexual offspring
44 experience both underground and aboveground environments along with their mothers
45 from the embryonic stage until they are delivered.

46 After colonizing the roots of the secondary host plants, eriosomatine aphids live in
47 the soil environment from early summer to autumn, making them susceptible to infection
48 by soil-living parasites, such as nematodes and microbes. In the present study, we
49 examined the rate of parasitism of the unidentified mermithid and attempted to
50 molecularly characterize the species by employing the sequences of two ribosomal RNA
51 genes, 18S and 28S. Thereafter, the phylogenetic status of the species in the available
52 mermithid sequences was inferred based on the rDNA sequences.

80 TCGGAAGGAACCAGCTACTA–30 (Shih *et al.* 2019). The resulting templates were
81 purified using a QIAquick PCR purification kit (QIAGEN Inc.) and sequenced in both
82 directions using an ABI 3730xl Analyzer (Applied Biosystems). The resulting sequences
83 were deposited in GenBank, and the BLASTn algorithm (Altschul *et al.* 1990) was
84 applied to confirm the identity of the sequences.

85 The dataset of partial sequences of the nuclear 18S rDNA of mermithid nematodes
86 in GenBank was searched and aligned using the MEGA X software package (Kumar *et al.*
87 *al.* 2018). Host species were referenced to related publications and GenBank after
88 obtaining 18S rDNA sequences of the parasitic mermithid nematodes (Table S1).
89 Phylogenetic trees were constructed using Bayesian inference (BI) (Larget & Simon 1999)
90 and maximum likelihood (ML) (Felsenstein 1981). The best-fit evolutionary model K2 +
91 G + I was adopted by Mega X and used for all model-based methods (BI and ML). The
92 Bayesian tree was constructed by MrBayes 3.2.7 (Ronquist *et al.* 2012) using a Markov
93 chain Monte Carlo (MCMC) approach with 2 million generations, with tree sampling
94 every 500 generations. The 1000 replicates were run for maximum likelihood (ML)
95 bootstrap sampling using Mega X.

96 In total, 418 eriosomatine sexuparae, consisting of eight species of two genera,
97 *Tetraneura* and *Eriosoma*, were available for examination of parasitism. Five sexuparae
98 of *E. auratum* and one of *T. radicola* out of the 418 individuals were found to be
99 parasitized by a slender worm (Table 1). One parasite coexisted with embryonic sexual
100 offspring inside the abdomen of each parasitized aphid. One of the parasites was isolated
101 for molecular identification. The others were individually maintained with the host
102 sexuparae in the mounted specimens for morphological observation.

103 No significant difference was found in body size between adult mermithid-
104 parasitized and uninfected *E. auratum* sexuparae (ANOVA, $df = 1,32$, $F = 0.935$, $P =$
105 0.34). However, the number and body size of sexual female embryos were significantly
106 reduced in mermithid-parasitized sexuparae ($df = 1,32$, $F = 9.93$, $P = 0.0035$; and $df =$

107 1,32, $F = 16.87$, $P = 0.0003$, respectively) compared to uninfected sexuparae, whereas no
108 such significant associations were found in male embryos ($df = 1,32$, $F = 0.15$, $P = 0.70$;
109 and $df = 1,32$, $F = 0.26$, $P = 0.61$, respectively).

110 All nematodes were post-parasitic juveniles. One specimen that emerged from a *T.*
111 *radicicola* sexupara was in relatively good condition and was examined under a stereo
112 microscope for typological characters (Figure 1).

113 All isolated nematodes were juveniles without generic or species-specific
114 characters, and some parts of the morphological structures were vague, likely because of
115 the Hoyer fixation. Some morphological characteristics were confirmed in the specimens.
116 The body was slender, approximately 1.5 cm long, with a smooth surface. Anterior end
117 dome-shaped cephalic or labial papillae were not observed, possibly because of material
118 conditions. Stoma was conspicuous, and a stylet-like well-sclerotized stoma reached the
119 anterior end; the pharyngeal tube possessed a conspicuous lumen, connecting the stoma
120 and cardia, and at least two gland-like structures were observed on both sides of the stoma
121 and the anterior part of the pharyngeal tube. The cardia was funnel-shaped. Genital anlage
122 was not confirmed, possibly because of the material conditions. The posterior end of the
123 intestine was inconspicuous, and the anus and rectum were not observed, also likely due
124 to the material condition. A short and bluntly pointed spike-like projection was observed
125 at the tail tip.

126 The 18S rDNA and 28S rDNA gene fragments of the isolated parasite were
127 successfully sequenced from one individual, and the sequences were deposited in
128 GenBank under accession numbers MW649131 and MW653323. After alignment, 18S
129 rDNA of 42 taxa and 563 base pairs were available for phylogenetic analysis. The BLAST
130 search in GenBank indicated that the amplified sequence had the closest match and
131 formed a clade with a previously sequenced mermithid juvenile 18S sequence
132 (AY919185), which was collected from a grassland soil sample from Lincoln, Nebraska
133 (Posers, pers. comm.).

134 Although GenBank reference sequences are limited for mermithid nematodes, here,
135 the Bayesian-based phylogeny was constructed using currently available 18S rDNA
136 sequences with information on the host range. Mermithid nematodes have broad host
137 ranges, including 12 invertebrate genera, mainly Diptera and Hemiptera (Fig. 2). The
138 mermithid sp., which was isolated from an aphid (Insecta: Hemiptera) in the present study,
139 formed a clade with an environmental sample and was clearly separated from neighboring
140 hemipteran associates (Fig. 2).

141 For aphids and other herbivorous hemipteran insects that share a common
142 arrangement of sucking mouthparts, mermithid nematodes cannot enter host bodies
143 through mouthparts. In the present study, the unidentified mermithid nematode likely
144 parasitized the aphid by penetrating the cuticle or gaining entry through a natural opening
145 such as the anus. Root aphids are sedentary and susceptible to infection by nematodes and
146 other pathogens.

147 Mermithid parasitism of aphids is not commonly known and only three cases have
148 been reported (Guercio 1899; Davis 1916; Poinar 2017), although this could be due to
149 undersampling of the aphids for this condition. The most remarkable record is the
150 parasitism of an extinct aphid, *Caulinus burmitis* (Hemiptera: Burmitaphididae) by a
151 fossil mermithid, which was found in mid-Cretaceous Myanmar amber (Poinar 2017).
152 This example implies that the parasitic association between aphids and mermithid
153 nematodes has continued for more than 100-million years. In Italy, nymphs and winged
154 adults of the root aphid *Trama radices* Kaltenbach were found to be parasitized by an
155 unidentified mermithid in April and May 1899, which was dispersed and embedded in
156 the winged aphid (Guercio 1899). Davis (1916) conducted fieldwork to collect
157 mermithid-parasitized aphids in Indiana, USA between mid-September and October 1911,
158 and found mermithid-parasitized apterous viviparous and oviparous aphids of an *Anoecia*
159 sp. on October 16th and 19th on the roots of *Muhlenbergia*. This is also the first record of
160 mermithid parasitism in oviparous aphids.

161 The unidentified mermithid found in the present study was closest to a species
162 collected from grassland soil around the root system of Leadplant, *Amorpha canescens*
163 Pursh in the USA (Powers, pers. comm., also described in
164 <https://nematode.unl.edu/mermissp.htm>), which possibly contained herbivorous insects,
165 including aphids. However, the taxonomic status of the nematodes is unknown in both
166 cases since the samples were juveniles not closely aligned to any identified species. In
167 addition, although these two species formed a well-supported clade in the phylogenetic
168 analysis (Fig. 2), they were clearly separated from each other considering the branch
169 length between them. Therefore, they possibly represent separate, undersampled clades.
170 Further collections followed by phylogenetic analyses are required to understand their
171 relationships and taxonomic status.

172 The survival and performance of parasites can be largely affected by their hosts.
173 Nematodes receive nutrition from the host tissues and hemolymph, competing with the
174 host for nutrients that are important for its physiological development and reproduction
175 (Smith *et al.* 1985; Mcrae *et al.* 2015). Once mermithid nematodes parasitize host insects,
176 they can manipulate host behavior for their own benefits. For example, Allahverdipour *et*
177 *al.* (2019) reported that mermithid-parasitized female mosquitoes seek water three times
178 more than a blood source, whereas uninfected females were twice as likely to seek blood
179 than water. Moreover, parasitizing adult hosts could be a dispersal strategy for mermithid
180 nematodes (Campos & Sy 2003; Di Battista *et al.* 2015). In the present study, obvious
181 morphological or behavioral alterations were not confirmed in parasitized aphids and
182 parasitism was not detected until dissection. Nevertheless, our study indicated that
183 mermithid parasitism in sexuparae led to fewer and smaller female sexual embryos. It is
184 not clear whether the parasites negatively affect offspring fitness by competing for
185 nutritious resources directly or whether maternal investment changes in response to
186 parasitism. Thus, it is necessary to increase the sample size to investigate host
187 manipulation by mermithid nematodes in future studies.

188 Mermithid nematodes can infect a broad range of aquatic and terrestrial
189 invertebrates. However, because nematodes are often collected as juveniles, their
190 identification and host specificity are difficult to evaluate. *Mermis nigrescens*, a parasite
191 of grasshoppers, is reported to be found in other insect orders, such as Dermaptera,
192 Coleoptera, and Lepidoptera (Poinar 1979). However, because of the difficulty in
193 morphological identification, information on the host range needs to be confirmed by
194 molecular barcoding analyses. In the present study, although the species status is still
195 unknown, the molecular sequences can be regarded as a species-specific barcode for
196 taxonomic identification and evaluation of the host range in future studies.

197

198 **ACKNOWLEDGEMENTS**

199

200 We thank Dr. Ryoji Shinya, Meiji University, for suggestions on designing the
201 experiments and Dr. Yuuki Kobayashi, National Institute for Basic Biology, for
202 suggestions on phylogenetic analyses. This research was supported partially by Grant-in-
203 Aid (19K06848) for Scientific Research from the Japan Society for the Promotion of
204 Science (to SA) and partially by a research grant from The Yanmar Environmental
205 Sustainability Support Association (to XT). XT is grateful to The Asahi Glass Foundation
206 for PhD scholarship.

207

208 **REFERENCES**

209

- 210 Allahverdipour HH, Talaei-Hassanloui R, Karimi J, Wang Y, Rochlin I, Gaugler R (2019)
211 Behavior manipulation of mosquitoes by a mermithid nematode. *Journal of*
212 *Invertebrate Pathology* **168**, 107273.
- 213 Altschul S, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment
214 search tool. *Journal of Molecular Biology* **215**, 403–410.

- 215 Campos RE, Sy VE (2003) Mortality in immatures of the floodwater mosquito
216 *Ochlerotatus albifasciatus* (Diptera: Culicidae) and effects of parasitism by
217 *Strelkovimermis spiculatus* (Nematoda: Mermithidae) in Buenos Aires Province,
218 Argentina. *Memórias do Instituto Oswaldo Cruz* **98**, 199–208.
- 219 Davis JJ (1916) A nematode parasite of root aphids. *Psyche* **23**, 39–40.
- 220 Di Battista CM, Fischer S, Campos RE (2015) Prevalence of parasitism and adult survival
221 time of *Aedes albifasciatus* (Diptera: Culicidae) parasitized by *Strelkovimermis*
222 *spiculatus* (Nematoda: Mermithidae). *Journal of Vector Ecology* **40**, 393–397.
- 223 Duan M, Xiong J, Lu D, Wang G, Ai H (2016) Transcriptome sequencing analysis and
224 functional identification of sex differentiation genes from the mosquito parasitic
225 nematode, *Romanomermis wuchangensis*. *Plos One* **11**: e0163127.
226 <https://doi.org/10.1371/journal.pone.0163127>
- 227 Felsenstein J (1981) Evolutionary trees from DNA sequences: A maximum likelihood
228 approach. *Journal of Molecular Evolution* **17**, 368–376.
- 229 Gordon R, Webster JM (1971) *Mermis nigrescens*: physiological relationship with its host,
230 the adult desert locust *Schistocerca gregaria*. *Experimental Parasitology* **29**, 66–79.
- 231 Gradinarov D (2014) The mermithid species *Isomermis lairdi* (Nematoda, Mermithidae),
232 previously only known in Africa, found in Europe. *Zookeys* **454**, 1–11.
- 233 Guercio DG (1899) Contribuzione allo studie delle forme e della biologia della *Trama*
234 *radicans* Kaltenbach. *Nuove Relazioni intorno ai lavori della R. Stazione de*
235 *Entomologia Agraria di Firenze* **1**, 195–206.
- 236 Hajek AE (2004) *Natural Enemies: An Introduction to Biological Control*. Cambridge
237 Univ. Press, Cambridge, UK.
- 238 Holterman M, Rybarczyk K, Van Den Elsen S *et al.* (2008) A ribosomal DNA-based
239 framework for the detection and quantification of stress-sensitive nematode families
240 in terrestrial habitats. *Molecular Ecology Resources* **8**, 23–34.

- 241 Iryu T, Tanaka R, Yoshiga T (2020) Mermithid nematodes isolated from the shield bug
242 *Parastrachia japonensis*. *Nematological Research* **50**, 1–7.
- 243 Kobylinski KC, Sylla M, Black W *et al.* (2012) Mermithid nematodes found in adult
244 *Anopheles* from southeastern Senegal. *Parasites Vectors* **5**, 131.
- 245 Kubo R, Ugajin A & Ono M (2016) Molecular phylogenetic analysis of mermithid
246 nematodes (Mermithida: Mermithidae) discovered from Japanese bumblebee
247 (Hymenoptera: Bombinae) and behavioral observation of an infected bumblebee.
248 *Applied Entomology and Zoology* **51**, 549–554.
- 249 Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary
250 genetics analysis across computing platforms, *Molecular Biology and Evolution* **35**,
251 1547–1549.
- 252 Larget B, Simon DL (1999) Markov Chasin Monte Carlo algorithms for the Bayesian
253 analysis of phylogenetic trees, *Molecular Biology and Evolution* **16**, 750.
- 254 Li FC, Nie GF, Feng Y, Xia ZM (1993) Study on *Hexamermis agrotis* Wang *et al.* – an
255 important natural enemy of cutworms. *Entomological Knowledge* **30**, 40–42. (in
256 Chinese.)
- 257 Mazza G, Paoli F, Strangi A *et al.* (2017) *Hexamermis popilliae* n. sp. (Nematoda:
258 Mermithidae) parasitizing the Japanese beetle *Popillia japonica* Newman
259 (Coleoptera: Scarabaeidae) in Italy. *Systematic Parasitology* **94**, 915–926.
- 260 McRae KM, Stear MJ, Good B, Keane OM (2015) The host immune response to
261 gastrointestinal nematode infection in sheep. *Parasite Immunology* **37**, 605–613.
- 262 Neilson R, Ye W, Oliveira CMG *et al.* (2004) Phylogenetic relationships of selected
263 species of Longidoridae (Nematoda: Longidoridae) from North America inferred
264 from 18S rDNA gene sequence data. *Helminthologia* **41**, 209–215.
- 265 Nickle WR (1972) A contribution to our knowledge of the Mermithidae (Nematoda).
266 *Journal of Nematology* **4**, 113–146.

- 267 Peng Y, Song J, Lee Z, Xiong X (2002) Bionomics of *Romanomermis sichuanensis*, a
268 Mermithid Parasite of *Anopheles sinensis*. *Journal of Invertebrate Pathology* **61**,
269 107–109.
- 270 Poinar GO, Camino NB (1986) *Strelkovimermis spiculatus* n. sp. (Mermithidae:
271 Nematoda) parasitizing *Aedes albifasciatus* Mac. (Culicidae: Diptera) in Argentina.
272 *Journal of Nematology* **18**, 317–319.
- 273 Poinar GO (1975) Description and biology of a new insect parasitic rhabditoid,
274 *Heterorhabditis bacteriophora* n.gen., n.sp. (Rhabditida; Heterorhabditidae n.fam.).
275 *Nematologica* **21**, 463–470.
- 276 Poinar GO (1979) *Nematodes for Biological Control of Insects*. CRC Press, Boca Raton.
- 277 Poinar GO (2017) A mermithid nematode, *Cretacimermis aphidophilus* sp. n. (Nematoda:
278 Mermithidae), parasitising an aphid (Hemiptera: Burmitaphididae) in Myanmar
279 amber: a 100 million year association. *Nematology* **19**, 509–513.
- 280 Poinar GO, Latham ADM, Poulin R (2002) *Thaumamermis zealandica* n. sp.
281 (Mermithidae: Nematoda) parasitising the intertidal marine amphipod *Talorchestia*
282 *quoyana* (Talitridae: Amphipoda) in New Zealand, with a summary of mermithids
283 infecting amphipods. *Systematic Parasitology* **53**, 227–233.
- 284 Presswell B, Evans S, Poulin R, Jorge F (2015) Morphological and molecular
285 characterization of *Mermis nigrescens* Dujardin, 1842 (Nematoda: Mermithidae)
286 parasitizing the introduced European earwig (Dermaptera: Forficulidae) in New
287 Zealand. *Journal of Helminthology* **89**, 267–276.
- 288 Ronquist F, Teslenko M, Mark PVD *et al.* (2012) MrBayes 3.2: efficient Bayesian
289 phylogenetic inference and model choice across a large model space, *Systematic*
290 *Biology* **61**, 539–542.
- 291 Ross JL, Ivanova ES, Spiridonov SE *et al.* (2010) Molecular phylogeny of slug-parasitic
292 nematodes inferred from 18S rRNA gene sequences. *Molecular Phylogenetics and*
293 *Evolution* **55**, 738–743.

- 294 Rusconi JM, Di Battista CM, Balcazar D, Rosales M, Achinelly MF (2020) *Amphimermis*
295 *enzoni* n. sp. (Nematoda: Mermithidae) parasitizing damselflies and dragonflies in
296 Argentina. *Journal of Nematology* **52**, 1–9.
- 297 Shamseldean MM, Platzer EG (1989) *Romanomermis culicivorax*: penetration of larval
298 mosquitoes. *Journal of Invertebrate Pathology* **54**, 191–199.
- 299 Shih PY, Lee JS, Shinya R *et al.* (2019) Newly identified nematodes from mono lake
300 exhibit extreme arsenic resistance. *Current Biology* **29**, 3339–3344.
- 301 Smith WD, Jackson F, Jackson E, Williams J (1985) Age immunity to *Ostertagia*
302 *circumcincta*: comparison of the local immune responses of 4 1/2- and 10-month-old
303 lambs. *Journal of Comparative Pathology* **95**, 235–245.
- 304 Stubbins FL, Agudelo P, Reay-Jones FP, Greene JK (2016) *Agamermis* (Nematoda:
305 Mermithidae) infection in south Carolina agricultural pests. *Journal of Nematology*
306 **48**, 290–296.
- 307 Suman DS, Brey CW, Wang Y, Sanad M, Shamseldean MSM, Gaugler R (2013) Effects
308 of insect growth regulators on the mosquito-parasitic nematode *Romanomermis*
309 *iyengari*. *Parasitology Research* **112**, 817–824.
- 310 Sun B, Li F, He X *et al.* (2020) First report of *Ovomermis sinensis* (Nematoda:
311 Mermithidae) parasitizing fall armyworm *Spodoptera frugiperda* (Lepidoptera:
312 Noctuidae) in China. *Journal of Nematology* **52**, 1–7.
- 313 Tang S, Hyman BC (2007) Mitochondrial genome haplotype hypervariation within the
314 Isopod parasitic nematode *Thaumamermis cosgrovei*. *Genetics* **176**, 1139–1150.
- 315 Tong X, Akimoto SI (2019) Female-female competition leads to female-biased sex
316 allocation and dimorphism in brood sex composition in a gall-forming aphid.
317 *Functional Ecology* **33**, 457– 466.
- 318 Vandergast AG, Roderick GK (2003) Mermithid parasitism of Hawaiian *Tetragnatha*
319 spiders in a fragmented landscape. *Journal of Invertebrate Pathology* **84**, 128-136.

- 320 Villemant C, Zuccon D, Rome Q, Muller F, Poinar GO, Justine JL (2015) Can parasites
321 halt the invader? Mermithid nematodes parasitizing the yellow-legged Asian hornet
322 in France. *PeerJ* **3**, e947.
323 <https://doi.org/10.7717/peerj.947>
- 324 Wang JY, Xu F, Liu XS, Wang GX (2007) Molecular phylogeny of entomopathogenic
325 nematodes (Mermithidae) inferred from DNA sequences of 18S rDNA, 28S rDNA
326 and COI genes. *Acta Zoologica Sinica* **53**, 835–844. (in Chinese.)
- 327 Watanabe S, Tsunashima A, Itoyama K, Shinya R (2021) Survey of mermithid nematodes
328 (Mermithida: Mermithidae) infecting fruit-piercing stink bugs (Hemiptera:
329 Pentatomidae) in Japan. *Applied Entomology and Zoology* **56**, 27–39.
- 330 Xu F, Wang GX, Zha Y, Liu P, Cao W, Liu X (2005) Genetic relationships among five
331 species of Mermithidae based on RAPD. *Zoological Research* **26**, 294–299.
- 332 Yeates GW & Buckley TR (2009) First records of mermithid nematodes (Nematoda:
333 Mermithidae) parasitising stick insects (Insecta: Phasmatodea). *New Zealand*
334 *Journal of Zoology* **36**, 35–39.

335

336 SUPPORTING INFORMATION

337

338 Additional Supporting Information may be found online in the Supporting Information
339 section at the end of the article.

340 **Table S1.** List of GenBank accession numbers, sample species, and references for host
341 information included in the phylogenetic tree. DS: direct submission to GenBank.

342

343 **Figure legends**

344

345 **Figure 1** Typological characters of nematode isolated from *T. radicicola*. **A:** Whole body;
346 **B:** Anterior region; **C:** Close-up of anterior end (“C” in subfigure B) in five different focal
347 planes showing stoma and glands; **D:** Close-up of pharynx-intestinal junction region (“D”
348 in subfigure B) in six different focal planes showing funnel-shaped cardia and body
349 surface structure; **E:** Posterior end of body; **F:** Close-up of tail tip (“F” in subfigure F)
350 showing tail spike (appendage).

351

352 **Figure 2** Bayesian phylogenetic tree inferred from the 18S rDNA sequences of mermithid
353 nematodes. Values on nodes represent posterior probabilities for Bayesian inference and
354 bootstrap support for maximum likelihood, respectively. The orders of the hosts
355 parasitized by mermithid nematodes are listed on the right of the tree in accordance with
356 the record of parasites.

357

358

359

360

361

362

363

364

365

366

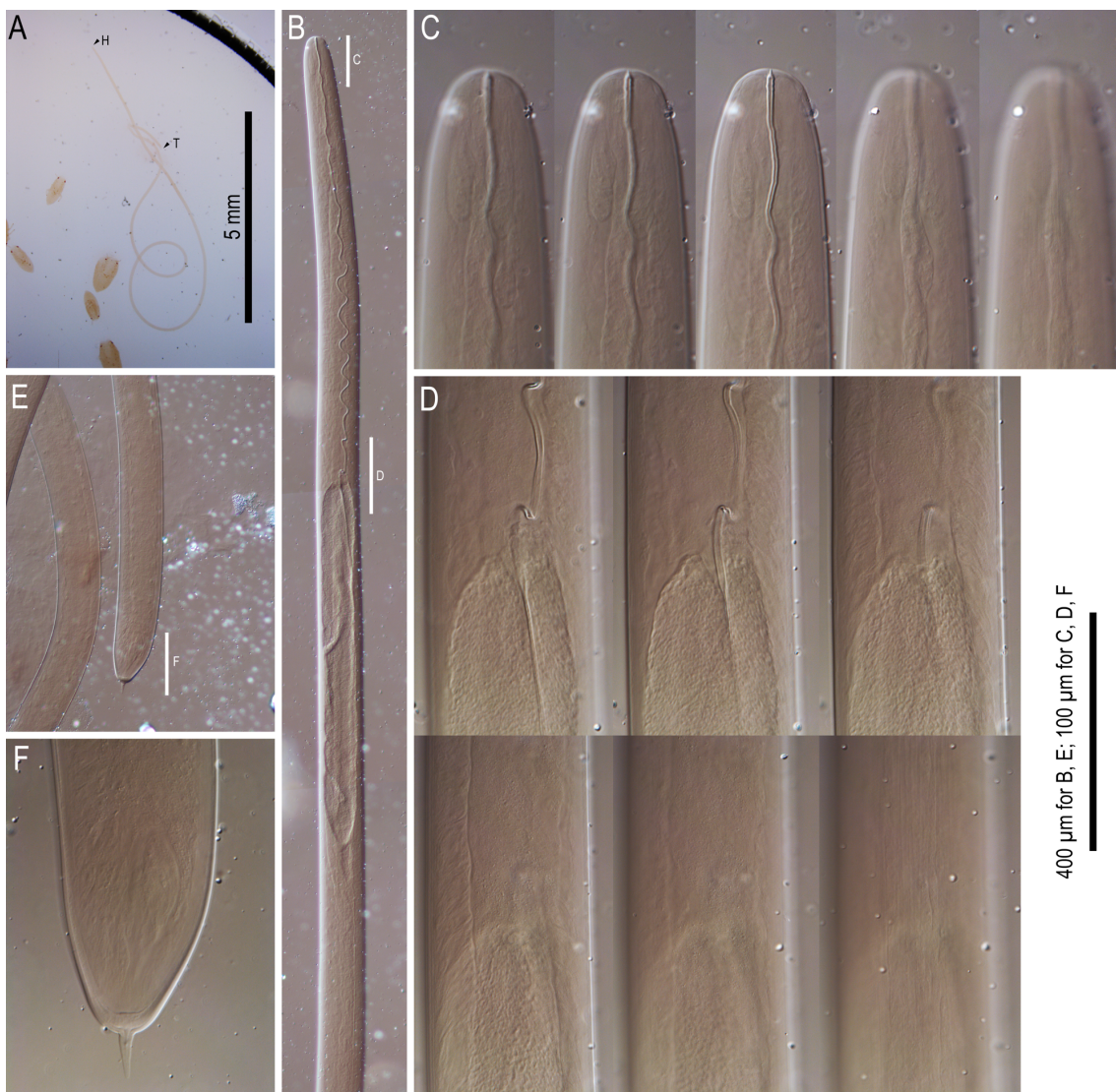
367

368 **Table 1** Proportion of mermithid parasitism in eriosomatine aphids collected in 2017

	<i>T. sorini</i>	<i>T. radicola</i>	<i>T. triangula</i>	<i>T. nigriabdominalis</i>	<i>E. harunire</i>	<i>E. auratum</i>	<i>E. yangi</i>	<i>E. parasiticum</i>	Total
No. examined	273	49	15	8	29	41	2	15	432
No. parasitized	0	1	0	0	0	5	0	0	6
Parasitized rate (%)	0	2.04	0	0	0	12.20	0	0	1.39

369

370 **Figure 1**

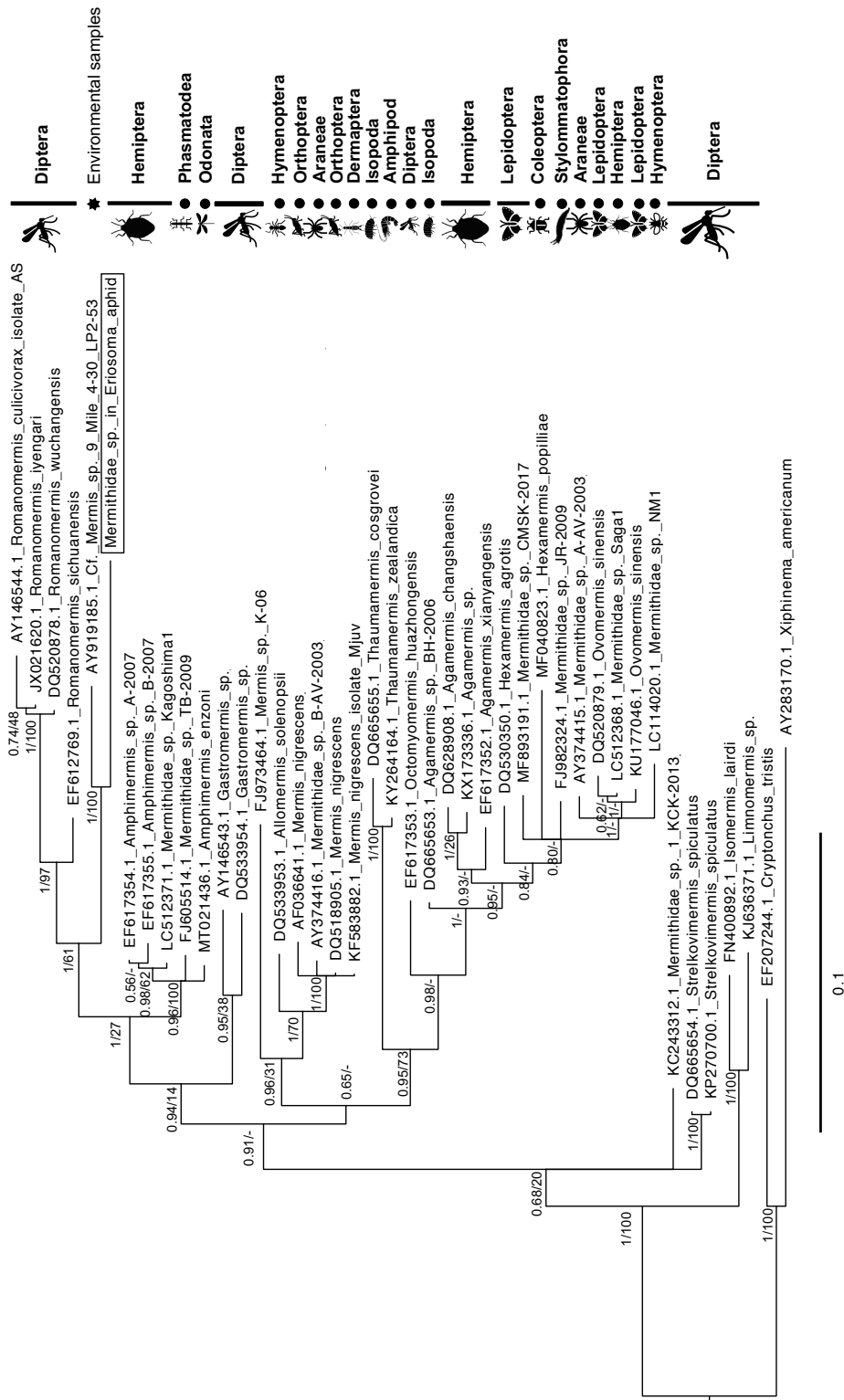


371

372

373 **Figure 2**

Mermithid-parasitized hosts



374