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Agamermis sp. (Nematoda: Mermithidae) parasitizing *Armadillidium vulgare* (Crustacea: Isopoda) in Argentina

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Abstract

The Mermithidae is a family of nematodes parasitic in many kinds of insects, spiders, leeches, crustaceans and other invertebrates throughout the world. While conducting an assay with entomopathogenic nematodes, we found *Armadillidium vulgare* (Crustacea: Isopoda) individuals to be infected with *Agamermis* sp., marking the fourth known discovery of a mermithid infection in the order Isopoda. In this work, we contribute with an 18S rDNA sequence of the isolated nematode and the morphological and morphometrical characterization of the juveniles.

Introduction

The Mermithidae is a family of nematodes parasitic in many kinds of insects, spiders, leeches, crustaceans and other invertebrates throughout the world (Poinar, 1975). In their life cycle, infective juveniles (pre-parasitic second-stage juveniles [J2]) hatch from eggs and actively seek and penetrate the host. The third-stage juvenile (J3) develops in the host, at which time the post-parasitic fourth-stage juvenile (J4) emerges, killing the host. The J4 develops into adults, which mate and lay eggs in the substrate. Juveniles hatched from these eggs will penetrate a new host and begin the cycle anew (Becnel & Johnson, 1998).

There have been three reports of mermitids parasitizing Isopoda Latreille, 1817 to date: Poinar (1975) found juveniles in *Armadillidium vulgare* Latreille, 1804 and *Porcellio scaber* Latreille, 1804 in San Diego (United States) as well as the nematode *Thaumamermis cosgrovei* Poinar, 1981 in soil from the surrounding area; Doucet & Cagnolo (1998) found *Agamermis decaudata* parasitizing *Castnia dedalus* in Córdoba, Argentina; and Yoshino & Waki (2021) recently discovered infected *Ligidium* sp. (Isopoda: Liigidae) with an undetermined species collected from forest areas of Kanagawa Prefecture, Japan. In the present work, we found *A. vulgare* (Crustacea: Isopoda) individuals to be infected with *Agamermis* sp. in Magdalena city, Buenos Aires, Argentina. To the best of our knowledge, this is the fourth record of a mermithid infection in the order Isopoda.

Material and methods

Specimens of *A. vulgare* were collected by hand and from compost in Magdalena city, Buenos Aires, Argentina (35° 05′ 00″ S 57° 31′ 00″ W) during the April–September period of 2022 (autumn–winter). Isopods were identified by the key of Pérez-Schultheiss (2010). Nematodes were obtained by the dissection of *A. vulgare* specimens in Petri dishes with distilled water under a stereomicroscope and measured using a Leica DM 500 microscope. Photographs were taken with an Olympus DP-71 camera and a Leica DM 500 microscope. In order to confirm the nematodes identification, a molecular approach was performed. Genomic DNA was extracted using 50 µl of a 5% suspension of Chelex (Bio-Rad) in deionized water and 2 µl of proteinase K (20 mg/ml), followed by overnight incubation at 56°C, boiling at 95°C for 10 min and centrifugation at 14.000 g for 10 min. Thirty-five µl of the supernatant was transferred to a new Eppendorf tube and 1.5 µl was utilized as the template for polymerase chain reaction (PCR). The 18S rRNA partial sequences were amplified using the primers MermF 18S (5′-CAAGGACGAAAGTTAGAGGTTC-3′) and MermR 18S (5′-GGAAACC TTGTTACGACTTTTA-3′) according to Kobylinski *et al.* (2012) with the GoTaq Master Mix (Promega Corporation, Madison, USA). The thermocycle conditions were as follows: 94°C for 5 min; 38 cycles of 94°C denaturation for 30 s; annealing 46°C for 40 s and extension 72°C for 80 s; and a single final extension period of 72°C for 3 min. PCR products were analysed by electrophoresis on 1% agarose gels and visualized by staining with ethidium bromide.

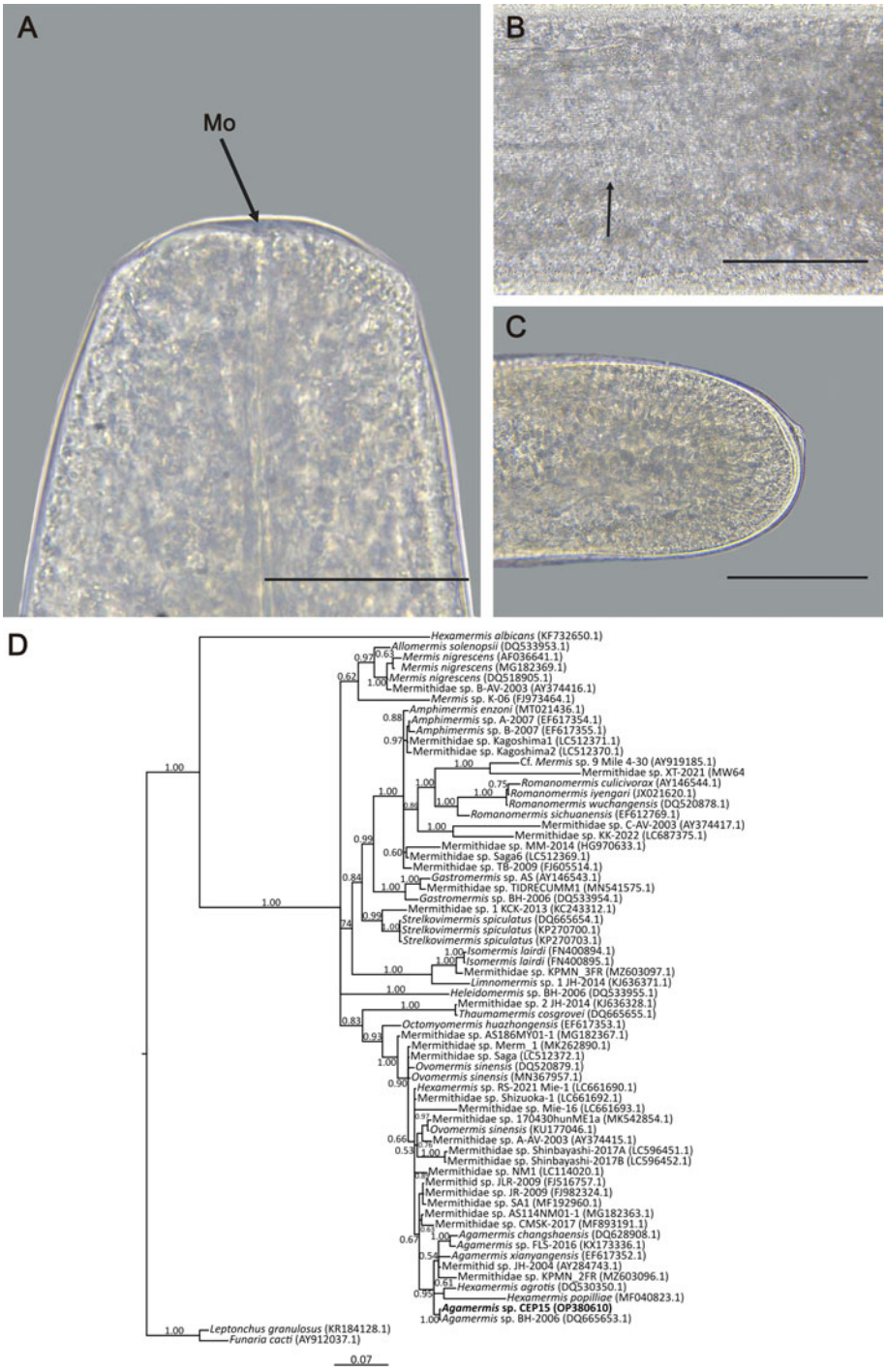

Fig. 1. *Agamermis* spp. (juvenile): (A) head showing the mouth opening (Mo); (B) arrow showing the striae on the surface of the body; (C) tail; and (D) Bayesian tree of the Mermithidae family based on 18S rDNA data including *Agamermis* sp. CEP15 (OP380610). Numbers above branches represent Bayesian posterior probabilities. Scale bars: 65 μm

The amplicons were sequenced in Macrogen Inc. (Korea) and edited with the Chromas software and the consensus sequence was obtained with Genedoc software (Kearse *et al.*, 2012). The obtained consensus sequences were compared with sequences in the BLAST tool available in the United States National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov). The resulting sequences were submitted to the NCBI GenBank database (http://www.ncbi.nlm.nih.gov) under the accession number OP380610.

Global multiple alignment was made by using the ClustalO approach (https://www.ebi.ac.uk/Tools/msa/clustalo/). The 18S gene sequences from species belonging to the Mermithidae family were selected and the outgroup formed with sequences from *Leptonchus granulosus* (KR184128.1) and *Funaria cacti* (AY912037.1) as included. The model selection (K2P + I + G4) was made with the IQTree server (http://iqtree.cibiv.univie.ac.at/), and was chosen under the Bayesian information criterion. The Bayesian inference was reconstructed using two parallel analyses of Markov chain Monte Carlo with the MrBayes software (version 3.2.7a) (Ronquist *et al.*, 2012). The number of generations was established at 6 million, and the posterior probability (PP) distribution was determined. The sample frequency was made every 200 generations and the average standard deviation of split frequencies was observed to be less than 0.05. In order to analyse the

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parameters across all runs, the burn-in was set at 10%. Then, Tracer software (version 1.7.1) was used to corroborate that the parameters reach an effective sample size ≥ 200. Finally, a consensus tree was reconstructed after applying a burn-in of 10%. The final trees were visualized in FigTree software v 1.4.4. The robustness of the clades was assessed using PP ≥ 0.95 criterion.

Results and discussion

While conducting an assay with entomopathogenic nematodes, we serendipitously found a specimen of *Agamermis* sp. when dissecting a dead *A. vulgare*. In total, two *Agamermis* sp. (juveniles) were recovered from the cavity of 556 *A. vulgare* (prevalence of 0.36%). The mean intensity of infection was one (one parasite specimen per infected host). Of the 556 isopods dissected only eight were dead. Mermithids were found on two of these dead hosts.

Agamermis sp. was white in colour and had a long and cylindrical body that measures 4.6 cm in length and 0.027 cm width. Smooth cuticle with lateral lines that run on the body surface. Head of 76.5 µm in diameter, with six cephalic papillae of 63 µm long that surrounds the mouth opening. Amphids present. The nerve ring was situated at 292.3 µm from the anterior end and the body width at that level was 153 µm. Trophosome visible, extending three-quarters the length of the body. Tail length of 369 µm and anus width of 243 µm. Caudal appendage lacking (fig. 1A–C). These juveniles oriented themselves longitudinally in the host and could be seen with the naked eye. The absence of tail appendage and presence of a tail end ring provided robust evidence for identification of the genus (Kaiser, 1991).

The obtained 18S gene sequence of *Agamermis* sp. named CEP-15 (accession number OP380610) was approximately 800 base pairs (bp) and, as it was expected, showed a high similarity with members of the Mermithidae family, being *Agamermis* sp. BH-2006 isolate 'Riverside' (accession number: DQ665653.1) the most similar (percentage identity: 100%) with a sequence of 735 bp and not the reported host. As it is seen in fig. 1D, the Bayesian inference showed a close relation between these two species (PP: 1.00). Also, they seem to be related to other species non-totally identified: a clade comprising *Agamermis* sp. FLS-2016 (KX173336.1) with length fragments of 755 bp, found in nymphs and adults of agricultural pests bugs in the south-eastern United States: *Chinavia hilaris*, *Euschistus servus*, *Euschistus* sp. (Hemiptera: Pentatomidae and Plataspidae, respectively) and *Megacopta cribraria* (Hemiptera: Plataspidae) (Stubbins *et al.*, 2016); Mermithid sp. JH-2004 (AY284743.1) (1728bp, host not reported); Mermithidae sp. KPMN_2FR (MZ603096.1) (820 bp) isolated from *Simulium nigrogilvum* Summers, 1911 (Diptera: Simuliidae) in central Thailand and *Agamermis changshaensis* (DQ628908.1) (759 bp); and *Agamermis xianyangensis* (EF617352.1) (817 bp), both with not reported hosts; The other clade consisted of *Hexamermis agrotis* (DQ530350.1) (757 bp) (host not reported) and *Hexamermis popilliae* (MF040823.1) (762 bp) parasitizing the Japanese beetle *Popillia japonica* Newman (Coleoptera: Scarabaeidae) in Italy (Mazza *et al.*, 2017) where PP: 95.

Worldwide, six species of the genus *Agamermis* have been described: *A. decaudata* Cobb, Steiner & Christie, 1923; *Agamermis unka* kaburaki & Imamura, 1932; *Agamermis cobbi* Schuurmans, Stekhoven & Mawson, 1955; *Agamermis sinuosa* Kaiser, 1977; *A. changshaensis* Bao, Lou & Lou, 1992; and *Agamermis catadecaudata* Baker and Poinar, 1995. In Argentina, only *A. decaudata* was cited in representatives of two families of the order Orthoptera (i.e. Acrididae and Gryllidae) and in the isopod *Castnia dedalus* (de Doucet & Cagnolo, 1998; Camino & Achinelly, 2011; Rusconi *et al.*, 2017). According to de Doucet & Cagnolo (1998), the host specificity of *A. decaudata* is considered relatively narrow and related to physiological and ecological factors. Unfortunately, we could not reach species level due to the very low prevalence obtained and the fact that the nematodes died immediately after dissecting the pill bugs. In addition, this nematode was unable to complete its life cycle within the host since we only found it in dead isopods making the breeding impossible.

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Conflicts of interest. The authors declare that they have no conflicts of interest.

Ethical standards. The authors state that all procedures contributing to this work comply with the ethical standards of relevant national and institutional guidelines on the care and use of laboratory animals.

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