Journal of Biological Control, 33(3): 217-221,2019, DOI: 10.18311/jbc/2019/22801

Research Article

Report of a mermithid nematode infecting *Amyna axis, Chrysodeixis* spp. and *Spodoptera* spp. from India

S. RAMESH BABU[1], VICTOR PHANI[2], P. K. MEENA[1], KHUSHBU CHAUHAN[2], M. R. KHAN[2] and V. S. SOMVANSHI[2*]

[1]*Agriculture Research Station, Maharana Pratap University of Agriculture and Technology, Banswara - 327001, Rajasthan, India*

[2]*Division of Nematology, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi - 110012, India*

**Corresponding author E-mail:* vssomvanshi@iari.res.in; babuento@yahoo.co.in

ABSTRACT: *Amyna axis* (Lepidoptera: Erebidae), *Chrysodeixis* spp. (*C. acuta* and *C. eriosoma*) and *Spodoptera* spp. (*S. exigua* and *S. litura*) (Lepidoptera: Noctuidae) are important polyphagous insect-pests of leguminous crops. A mermithid (Nematoda: Mermithidae) nematode was found parasitizing the larval stages of these insects in the western Indian state of Rajasthan. Morphological and molecular analysis suggests that the nematode might be a species of the genus *Hexamermis*. The average length of post-parasitic nematode juveniles was 15 cm, and the average greatest body width was 1.5 mm. The vulva was median (V% = 48) without vulval flap. Approximately 100-125 µm long caudal appendages were present on tail. The cross-section revealed the presence of six hypodermal chords at the mid body region, and stichosome was present. The 18S rDNA sequence showed 99% similarity to mermithid nematode. A phylogenetic analysis of 18S rDNA sequences of mermithids resulted in identification of a new clade "Lineage 3" which included representatives of *Mermis* sp., *Isomermis* sp., *Limnomermis* sp., and *Pheromermis* sp. This is the first report of natural mermithid parasitism of *A. axis* and *Chrysodeixis* spp. along with its molecular characterization.

KEY WORDS: *Amyna Axis*, *Chrysodeixis*, *Hexamermis*, mermithid, soybean, *Spodoptera*

(Article chronicle: Received: 17-08-2018; Revised: 11-08-2019; Accepted: 10-09-2019)

INTRODUCTION

Lepidopteran insects pose a major threat to Indian agriculture by causing extensive damage to field and orchard crops. *Amyna axis* (Lepidoptera: Erebidae), *Chrysodeixis* spp. and *Spodoptera* spp. (Lepidoptera: Noctuidae) are economically important polyphagous insect-pests of leguminous crops, and are widely distributed throughout India (Gill *et al.*, 2015; Singh and Singh, 1991; Meena *et al.*, 2016). Mermithid nematodes (Enoplida: Mermithidae) are obligate parasites of arthropods and are known to infect insects of fifteen different orders (Nickle, 1981). Several species of mermithids under eight genera, *viz*., *Agamermis, Geomermis, Hexamermis, Limnomermis, Mermis, Ovamermis, Pentatomermis*, and *Romanomermis* have been reported from various agro-ecological zones of India (Devasahayam and Abdulla-Koya, 1994; Rahaman *et al.*, 2000). *Hexamermis* spp., are parasites of lepidopteran insects with higher level of incidence in the insects feeding on low-growing crops (Bhatnagar *et al.*, 1985). The identification of mermithid nematodes at species level is difficult because of fewer distinguishable morphological characters, environmental effects on morphology, use of inadequate number of specimens during earlier descriptions (Poinar, 1975), and the lack of information on specific host–parasite associations (St-Onge *et al.*, 2008). In addition, DNA sequence information is also not available for majority of the described mermithid species (Stubbins *et al.*, 2015). Here we report the natural mermithid infection of lepidopteran insects, *viz*., *A. axis, C. acuta*, *C. eriosoma*, *S. exigua* and *S. litura* from the western Indian state of Rajasthan. Based on the morphological and molecular observations, the nematode was found to be a species of genus *Hexamermis*. To the best of our knowledge, this is the first report of a mermithid parasitism of *A. axis* and *Chrysodeixis* spp. In addition, we also provide DNA sequence information for 18S ribosomal DNA (18S rDNA) molecular marker for the identified *Hexamermis* sp. along with an updated molecular phylogeny of the mermithid nematodes.

MATERIALS AND METHODS

Insect sampling and nematode collection

As a part of an ongoing study to monitor the diversity of insect pests and insecticide resistance of *Spodoptera* spp. on soybean (*Glycine max*) at Agriculture Research Station, Maharana Pratap University of Agriculture and Technology, Rajasthan, India, the larvae were collected from the field for routine observation for the presence of any disease or parasitoid. The samples were collected randomly from Bagidora and Baneriya Khurd villages of Banswara and Pratapgarh districts, respectively, of Rajasthan state, India. Live nematodes were found to emerge from few of the collected insect larvae. The nematodes were preserved in DESS (Yoder *et al.*, 2006), used for further morphological observations (Kaya and Stock, 1997) and photomicrographs were obtained with Zeiss Axiocam M2m compound microscope equipped with AxioVision software.

Nematode identification

The gross morphology of the processed nematodes was studied by compound microscope. Further, the nematode specimens were cross-sectioned to observe the ultrastructural details. For molecular characterization, nematode DNA was extracted as described earlier (Subbotin *et al.*, 2000) from five individual nematode specimens obtained from each of the insect species. The 18S rDNA was PCR amplified by primers SSU18A (5'-AAAGATTAAGCCATGCATG-3') and SSU26R (5'-CATTCTTGGCAAATGCTTTCG-3') (Floyd *et al.*, 2002). The amplified products were resolved on 1% agarose gel and purified using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA). The purified products were cloned into pGEM-T Easy vector (Promega, Madison, WI, USA) and sequenced via Sanger sequencing. The raw sequences were checked for quality, aligned and consensus sequences were generated for each of the specimen. For the phylogenetic analysis, a blast search was conducted against NCBI nucleotide database, the top matching sequences were retrieved, and used for reconstruction of a Maximum Likelihood phylogenetic tree using MEGA6 software (Tamura *et al.*, 2013).

RESULTS AND DISCUSSION

The nematode infected insect larvae were recovered from both of the districts of Rajasthan, India, which are geographically approximately 120 kilometers apart from each other (Fig. 1 A). The nematode parasitism occurred only in *Amyna axis*, *Chrysodeixis* spp. and *Spodoptera* spp., while the other insect species, *viz*., *Achaea janata, Aegocera* sp., *Spilarctia obliqua* and *Aloa lactinea* were free from any nematode parasitism. The percentage of nematode parasitism in *Spodoptera* and *Chrysodeixis* was found to be 7.14% (n=140) and 9.09% (n=55), respectively, while infestation of *A. axis* was lower at 4.20% (n=47). After emergence of the nematodes, the larvae died and readily succumbed to bacterial infection and started rotting (Fig. 1 B-E). All the nematodes emerged from the anterior portion and the integument of the larval body.

The morphological observations of the post-parasitic nematode juveniles (n=5) emerged from the insect larvae revealed the presence of well-developed stichosomes – a diagnostic character of family mermithidae (Nematoda) (Kaya and Stock, 1997). Detailed observations (Fig. 2A-H) of the post-parasitic juveniles revealed the average length to be 15 cm, while the longest one was measured to be of 17 cm in length. The average greatest body width was 1.5 mm. Vulva was found almost at the mid body (V% = 48) without vulval flap. Caudal appendage was present on tail and was 100-125 µm long. Inspection of the cross-section revealed the presence of thick cuticle and well-developed musculature in the interchordal zone. Six hypodermal chords were seen at the mid body region. Analysis of the morphological characters of the nematodes isolated from the insects showed close proximity to *Hexamermis* sp. (Kamminga *et al.*, 2012; Poinar *et al.*, 1981; Poinar and Chang, 1985; Rubstov, 1976). However, because of absence of males and lack of adequate number of good quality adult nematode specimens, we were unable to identify the species of this nematode. Under low magnification, several pore-like openings were observed in the cuticle with channel-like structures underneath, running across the cuticular layers. However, detailed observations of these structures under high magnification neither revealed any cuticular opening nor presence of a channel through the cuticular strata. Instead, an elongated spindle shaped structure was observed beneath them. Similar kind of pore-like structures in the cuticle were also observed in *Romanomermis culicivorax* by Platzer and Platzer (1985), but any conclusive evidence could not be represented to designate these structures as "body pores". Instead, they were assumed as porous regions in the cuticle that may help in transmembrane transport of various substances. Several terrestrial mermithids are known to infect both immature and adult insects (Petersen, 1985) and *Hexamermis* spp. are largely reported to be associated with a plethora of lepidopteran insects under Indian condition (Bhatnagar *et al.*, 1985; Rahaman *et al.*, 2000). However, this is the first report of a *Hexamermis* like species infecting *A. axis* and *Chrysodeixis* spp. anywhere in the world.

The PCR amplification of 18S rDNA region yielded 930 bp fragments from all the specimens. Alignment of the sequences from all specimens revealed them to be 100% identical, suggesting that they belonged to one particular species. The representative sequence of 18S rDNA region

has been submitted to GenBank with accession number MH171724. BLAST search against NCBI nr database showed that the 18S sequence was 99% similar to a mermithid (accession no. LC114020, query coverage=96%, e-value=0). The present investigation is the first molecular characterization of a *Hexamermis* like species from India. The maximum likelihood phylogenetic analysis revealed the presence of three distinct lineages in the mermithids (Fig. 3). Lineages 1 and 2 were obtained as originally proposed by Larose and Schwander (2016) and contained unknown mermithids obtained from different *Timema* stick insect species (Larose and Schwander, 2016). Interestingly, the mermithid sp. obtained from lepidopteran insects in India grouped into "lineage 1" along with other unknown mermithids. However, use of additional mermithid 18S rDNA sequences (Bik *et al.*, 2010; Villemant *et al.*, 2015; Tripodi and Strange, 2018) resulted in generation of a new clade that we propose as "Lineage 3" in this study (Fig. 3). The new lineage 3 included representatives of *Mermis* sp., *Isomermis* sp., *Limnomermis* sp., and *Pheromermis* sp. To the best of our knowledge, this is the most comprehensive and updated molecular phylogenetic tree of mermithid nematodes based on 18S rDNA marker. However, this conclusion is limited to the fact that most sequences used for the construction of phylogenetic tree are either unidentified or undescribed mermithid sequences.

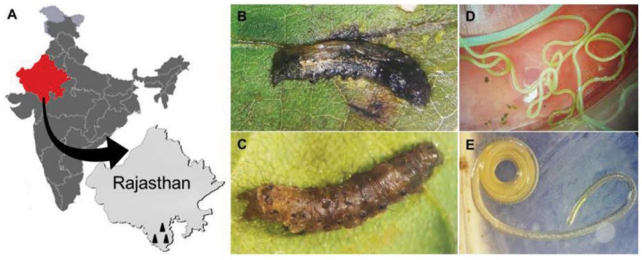

Fig. 1. Distribution of identified mermithid species in India and its parasitism [(A) The nematode was isolated from Rajasthan state of India. Dead larva of *Spodoptera litura* (B) and *Chrysodeixis acuta* (C) after emergence of nematodes (D, E)]

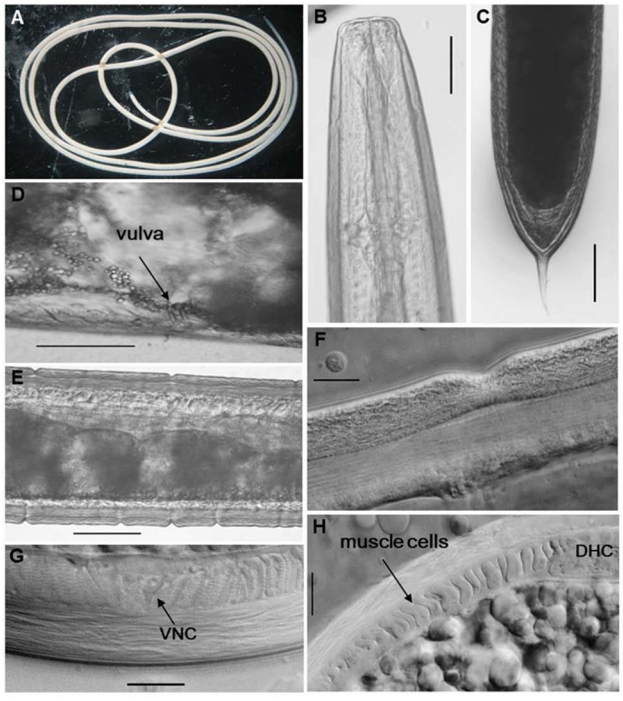

Fig. 2. Morphology of the mermithid species [(A) Adult female; (B) Anterior end of female; (C) Posterior end of female; (D) Vulva (Latero-ventral view); (E) Pore-like structures in cuticle; (F) Magnified view of cuticle showing no pore, instead a sunken area with elongated spindle shaped structure underneath; (G) Ventral nerve cord (VNC); (H) Well-developed body muscles in interchordal zone, DHC: Dorsal Hypodermal Cord (scale bar:: A: Head end to tail- 17 cm; B-E: 100 µm; F-H: 20 µm)]

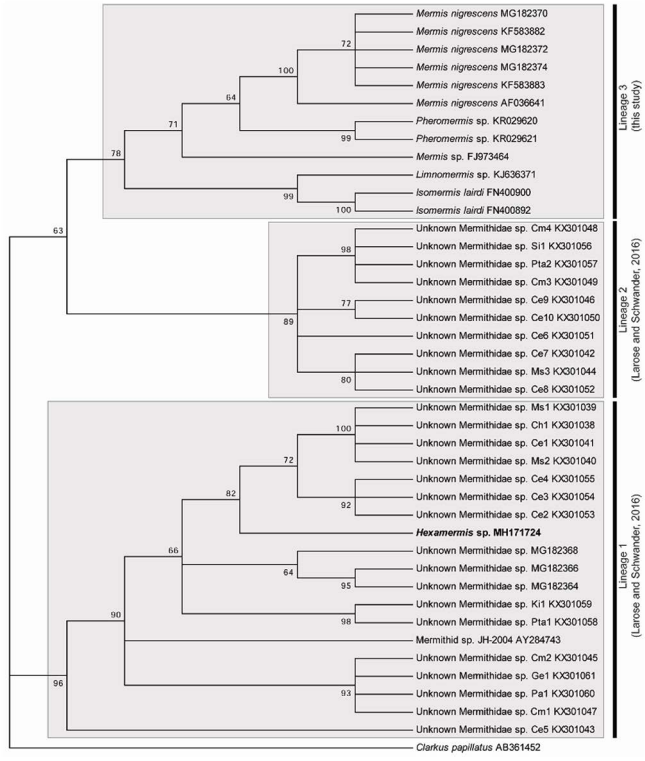

Fig. 3. Reconstruction of evolutionary relationship of identified mermithid nematode and an updated molecular phylogeny of mermithids using 18S rDNA sequences (The evolutionary history was inferred by using the Maximum Likelihood method based on Tamura 2-parameter model and bootstrapped 1000 times to get the final tree. Branches corresponding to partitions reproduced in less than 60% bootstrap replicates are collapsed and the bootstrap values are shown next to the branches. The lineages 1 and 2 were proposed by Larose and Schwander (2016), whereas lineage 3 has been proposed in this study. The nematode reported in the present study grouped into lineage 1)

The present investigation reports a natural mermithid infection of the larval stage of *A. axis*, *Chrysodeixis* spp., and *Spodoptera* spp. across two districts of Rajasthan, India. Morphological and molecular characterization (18S rDNA marker) revealed that the nematodes recovered from all the infected insect larvae showed close proximity to *Hexamermis* sp. This is the first report of mermithid nematode infection of *A. axis* and *Chrysodeixis* spp. from India. An updated 18S rDNA molecular phylogenetic analysis revealed the presence of three major lineages in mermithids, and the present mermithid species grouped into lineage 1 along with other uncharacterized mermithids. Based on our phylogenetic analysis, we propose a third lineage in the mermithids in addition to the two earlier lineages proposed by Larose and Schwander (2016).

ACKNOWLEDGEMENTS

We thank Dr. P. R. Shashank, Scientist, Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India, for identification of the insects. We also thank Dr. Sergei A. Subbotin for his critical remarks that helped in the improvement of the manuscript. The authors are grateful to Rashtriya Krishi Vikas Yojana (RKVY), Government of India, for funding this work. The In-House funding support from the ICAR-Indian Agricultural Research Institute is also acknowledged.

REFERENCES

Bhatnagar V, Pawar CS, Jadhav DR, Davies JC. 1985. Mermithid nematodes as parasites of *Heliothis* spp. and other crop pests in Andhra Pradesh, India. *Proc Indian Acad Sci Animal Sci*. **94**: 509–515. https://doi.org/10.1007/BF03186360

Bik HM, Lambshead PJD, Thomas WK, Lunt DH. 2010. Moving towards a complete molecular framework of the Nematoda: a focus on the Enoplida and early-branching clades. *BMC Evol Biol*. **10**: 353. https://doi.org/10.1186/1471-2148-10-353 PMid:21073704 PMCid:PMC2995457

Devasahayam S, Abdulla-Koya KM. 1994. Seasonal incidence of *Hexamermis* sp. (Dor., Mermithidae) parasitising larvae of top shoot borer *Cydia hemidoxa* Meyr. (Lep., Tortricidae) on black pepper. *J Appl Entomol*. **117**: 31–34. https://doi.org/10.1111/j.1439-0418.1994.tb00704.x

Floyd R, Abebe E, Papert A, Blaxter M. 2002. Molecular barcodes for soil nematode identification. *Molecular Ecol*. **11**: 839–850. https://doi.org/10.1046/j.1365-294X.2002.01485.x PMid:11972769

Gill AK, Arora R, Jindal V. 2015. Beet armyworm *Spodoptera exigua* (Hubner): A newly emerging pest of Egyptian clover in Punjab. *Range Manag Agroforestry* **36**: 170–174.

Kamminga KL, Davis JA, Stock SP, Richter AR. 2012. First report of a mermithid nematode infecting *Piezodorus guildinii* and *Acrosternum hilare* (Hemiptera: Pentatomidae) in the United States. *Fla Entomol*. **95**: 214–217. https://doi.org/10.1653/024.095.0137

Kaya HK, Stock SP. 1997. Techniques in insect nematology, pp. 281-324. In: Lacey L (Ed.). *Manual of Techniques in Insect Pathology*. London, UK. https://doi.org/10.1016/B978-012432555-5/50016-6

Larose C, Schwander T. 2016. Nematode endoparasites do not codiversify with their stick insect hosts. *Ecol Evol*. **6**: 5446–5458. https://doi.org/10.1002/ece3.2264 PMid:27551395 PMCid:PMC4984516

Meena AK, Nagar R, Swaminathan R. 2016. Occurrence of genus *Amyna* (Noctuidae: Bagisarinae) among the semilooper complex infesting soybean at Udaipur. *J Entomol Zool Stud*. **5**: 949–953.

Nickle W. 1981. Mermithid parasites of agricultural pest insects. *J Nematol*. **13**: 262–266. PMid:19300760 PMCid:PMC2618092

Petersen JJ. 1985. Nematodes as biological control agents: Part I. Mermithidae. *Adv Parasitol.* **24**: 307–344. https://doi.org/10.1016/S0065-308X(08)60565-5

Platzer A, Platzer EG. 1985. Permeability of the body wall of *Romanomermis culicivorax* to Lanthanum. *J Nematol*. **17**: 261–269. PMid:19294092 PMCid:PMC2618473

Poinar GO Jr. 1975. Entomogenous nematodes: A Manual and Host List of Insect-nematode Associations. Brill, Leiden. 317 pp. PMid:19308185 PMCid:PMC2620133

Poinar GO Jr., Mondet B, Gouteux JP, Laveissiere C. 1981. *Hexamermis glossinae* sp. nov. (Nematoda: Mermithidae), a parasite of tse-tse flies in West Africa. *Can J Zool.* **59**: 858–861. https://doi.org/10.1139/z81-122

Poinar GO Jr., Chang P. 1985. *Hexamermis cathetospiculae* n. sp. (Mermithidae: Nematoda), a parasite of the rice stemborer, *Tryporyza incertulus* (Wlk.) (Pyralidae: Lepidoptera) in Malaysia. *J Nematol*. **17**: 360–363. PMid:19294107 PMCid:PMC2618465

Rahaman PF, Sharma SB, Wightman JA. 2000. A review of insect-parasitic nematodes research in India: 1927–1997. *Int J Pest Manage*. **46**: 19–28. https://doi.org/10.1080/096708700227543

Rubstov IA. 1976. Mermithids (Nematoda, Mermithidae) – parasites of insects from Mongolia. pp. 596–614. In: *Insects of Mongolia*. Part 4. (Academy of Sciences of the USSR, Ed.) . Nauka, Leningrad.

Singh OP, Singh KJ. 1991. Economic threshold level for green semilooper, *Chrysodeixis acuta* (Walker) on soybean. *Trop Pest Manage*. **37**: 399–402. https://doi.org/10.1080/09670879109371624

St-Onge M, Larue B, Charpentier G. 2008. A molecular revision of the taxonomic status of mermithid parasites of black flies from Quebec (Canada). *J Invertebr Pathol*. **98**: 299–306. https://doi.org/10.1016/j.jip.2008.04.001 PMid:18511068

Stubbins FL, Agudelo P, Reay-Jones FP, Greene JK. 2015. First report of a mermithid nematode infecting the invasive *Megacopta cribraria* (Hemiptera: Plataspidae) in the United States. *J Invertebr Pathol*. **127**: 35–37. https://doi.org/10.1016/j.jip.2015.02.008 PMid:25731127

Subbotin SA, Waeyenberge L, Moens M. 2000. Identification of cyst-forming nematodes of the genus *Heterodera* (Nematoda: Heteroderidae) based on the ribosomal DNA-RFLPs. *Nematology* **2**: 153–164. https://doi.org/10.1163/156854100509042

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol*. **30**: 2725–2729. https://doi.org/10.1093/molbev/mst197 PMid:24132122 PMCid:PMC3840312

Tripodi AD, Strange JP. 2018. Rarely reported, widely distributed, and unexpectedly diverse: molecular characterization of mermithid nematodes (Nematoda: Mermithidae) infecting bumble bees (Hymenoptera: Apidae: Bombus) in the USA. *Parasitology* **145**: 1558-1563. https://doi.org/10.1017/S0031182018000410

Villemant C, Zuccon D, Rome Q, Muller F, Poinar Jr. GO, Justine J. 2015. Can parasites halt the invader? Mermithid nematodes parasitizing the yellow-legged Asian hornet in France. *Peer J.* **3**: e947. https://doi.org/10.7717/peerj.947 PMid:26038716 PMCid:PMC4451032

Yoder M, De Ley IT, King IW, Mundo-Ocampo M, Mann J, Blaxter M, Poiras L, De Ley P. 2006. DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology* **8**: 367–376. https://doi.org/10.1163/156854106778493448