

Short Note

Mermithid nematode (Mermithoidea) – a parasite of lichen-eating termites in Vietnam

Boris D. Efeykin^{1,2}, Irina I. Semenyuk^{1,2}, Dinh The Dung² and Anna I. Zueva¹

¹A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia

²Joint Russian-Vietnamese Tropical Scientific and Technological Center, Hanoi, Vietnam
e-mail: bocha19@yandex.ru

Accepted for publication 10 November 2022

The mermithids (Mermithoidea) represent a group of nematodes with more than fifty genera of specialised parasites of invertebrates, mainly arthropods. Parasitic juveniles of mermithids can be found in the coelomic cavity of the host. Representatives of 15 orders of insects were reported as their hosts (Nickle *et al.*, 1972; Poinar *et al.*, 2015).

Hospitalitermes bicolor (Haviland, 1898) are lichenophagous Nasutitermitinae termites. *Hospitalitermes* is an open-foraging genus distributed from Indian to Papuan region (Amornsak *et al.*, 2003). *Hospitalitermes* termites feed in the forest canopy in large columns of mixed workers and soldiers formed by up to 500,000 individuals (Miura *et al.*, 1998). During foraging, soldiers protect workers using terpenic substances, while workers carry food balls in their mandibles (Miura *et al.*, 1998; Amornsak *et al.*, 2003).

There are very few records of mermithid nematodes parasitising termites. The earliest record appears to be that of Hegh (Hegh *et al.*, 1922) who reported an unknown mermithid attacking the European termite, *Thoracotermes brevinotus*. The work of Ruttledge *et al.* (1925) reported a mermithid parasitising *Cornitermes orthocephalus* Silvestri in Brazil. A century later, Poinar (Poinar *et al.*, 2019) recorded *A. termitivoratus* as the first case of a mermithid infecting termites in North and Central Americas.

Termites were sampled near the Thac Troi (Heaven) rapids (N11° 27.139' E107° 26.517') in the Cat Tien National Park (Vietnam).

Three out of 90 specimens of termites dissected were found infected each with a single mermithid nematode at the stage of an early parasitic larva:

1. Parasitic juvenile 42 mm long, max. diam. 115 µm, host: sample H7_st, worker, collected 16.06.2022.

2. Parasitic juvenile 37.5 mm long, max. diam. 103 µm, host: sample H7, worker, collected 16.06.2022.

3. Parasitic juvenile 390 mm long, max. diam. 106 µm, host: sample H9, worker, collected 22.06.2022.

The nematodes were fixed in 95% ethanol. Genomic DNA was extracted from each individual with the QIAmp® DNA Micro (Qiagen, USA) kit, following manufacturer's instructions. The small ribosomal subunit ribosomal RNA gene (18S rDNA) was amplified with the nematode-specific primers Nem18SF (5'-CGC GAA TRG CTC ATT ACA ACA GC-3') and Nem18SR (5'-GGG CGG TAT CTG ATC GCC-3') for the 5' region of the gene (Floyd *et al.*, 2005). The D2-D3 segment of the large ribosomal subunit ribosomal RNA gene (D2-D3 28S rDNA) was amplified with the primers D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') (Nunn, 1992). PCR reactions were performed in a total volume of 25 µl with the Encyclo PCR Kit (Evrogen, Russia). Phylogenetic reconstructions were conducted from the alignment with *Coomansus parvus* as an outgroup. Optimal evolutionary models were chosen by PartitionFinder (Lanfear *et al.*, 2012). Maximum Likelihood (ML)

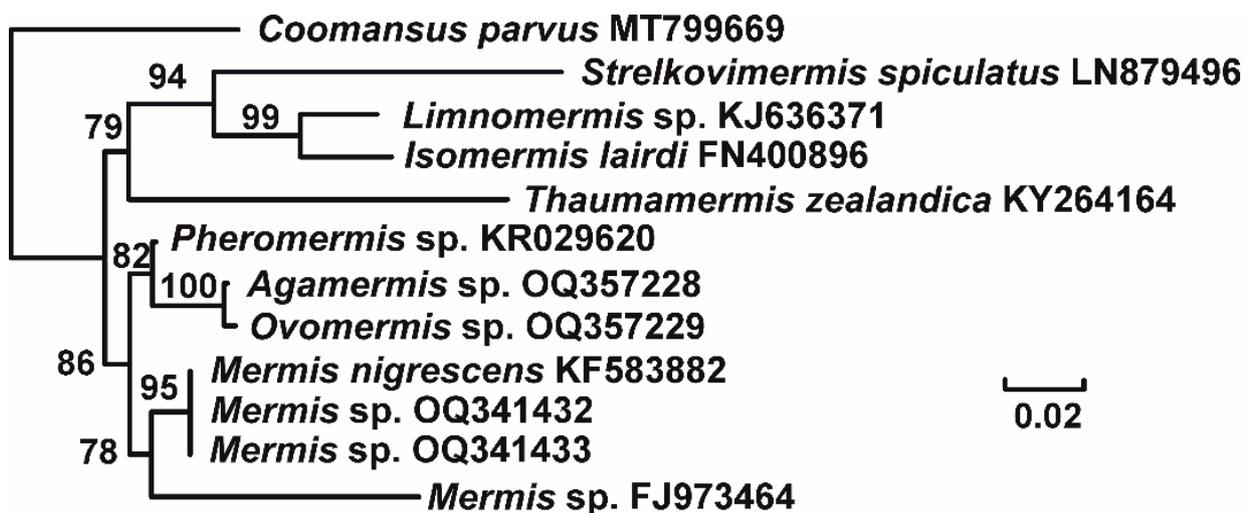


Fig. 1. ML phylogenetic tree (HKY+G+I model) based on the SSU region. Numbers near the branches denote percentage bootstrap resampling support from 1,000 replications. Bootstrap support is only shown for values exceeding 75%.

analysis was performed using IQ-TREE web server (Trifinopoulos *et al.*, 2016) with 10,000 ultrafast bootstrap replicates (Hoang *et al.*, 2018). The trees were visualised using TREEVIEW (Page, 1996) and FIGTREE v.1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). Sequences for each rDNA region obtained from all three sampled nematodes were found identical.

An analysis of the 18S rDNA sequence of the mermithid studied has revealed the high level of similarity with the sequence of *Mermis nigrescens* (Dujardin, 1842) (KF583882) from an earwig in New Zealand (Presswell *et al.*, 2013) and with the sequence of another parasitic juvenile of the Mermithidae family (OQ341432) from a coelomic cavity of a tropical millipede *Orthomorpha coarctata* collected at the same locality in Viet Nam (Cat Tien National park). While juvenile morphology of Mermithidae is usually scarce in distinguishing characters, the parasitic juveniles of millipedes featured visibly swollen anterior ends with a prominent neck constriction of the body beneath the cuticle. Such a feature was recorded for *Mermis* spp. The juveniles parasitic in termites were much smaller and represented an early parasitic stage lacking any visible modifications of the anterior end. The partial LSU rDNA sequence obtained in the study did not clarify the taxonomic position of the examined Mermithidae as this locus was not investigated up to now for other representatives of the genus *Mermis* (Dujardin, 1842). However, the LSU rDNA sequence obtained was similar to that of the Mermithidae gen. sp. from

a diplopod collected at the same locality, with difference in 6 bp per 852 bp long alignment. The non-insect arthropod hosts, as seems, serve as hosts for representatives of the family Mermithidae in tropical and subtropical habitats. The finding of related mermithids both in diplopod and insect hosts can be considered as an indication on low specificity in the choice of hosts in tropical mermithids.

ACKNOWLEDGEMENTS

This study was supported by Russian Science Foundation grant [22-14-00363].

REFERENCES

- AMORNSAK, W., SARNTHOY, O. & KIRTIBUTR, N. 2003. New records of two Nasutitermitinae termites (Isoptera: Termitidae) from hill evergreen forest on Khao Kitchakut National Park in Thailand. *Agriculture and Natural Resources* 37: 421-428.
- HEGH, E. 1922. *Les Termites; Partie Générale*. Belgium, Industrie Fincan. 358 pp.
- HOANG, D.T., CHERNOMOR, O., VON HAESELER, A., MINH, B.Q. & VINH, L.S. 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 35: 518-531. DOI: 10.1093/molbev/msx281
- LANFEAR, R., CALCOTT, B., HO, S.Y. & GUINDON, S. 2012. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695-1701. DOI: 10.1093/molbev/mss020

- MIURA, T. & MATSUMOTO, T. 1998. Foraging organization of the open-air processional lichenfeeding termite *Hospitalitermes* (Isoptera, Termitidae) in Borneo. *Insectes Sociaux* 45: 17-32.
- NICKLE, W.R. 1972. A Contribution to our Knowledge of the Mermithidae (Nematoda). *Journal of Nematology* 4: 113-146.
- NUNN, G.B. 1992. *Nematode molecular evolution: an investigation of evolutionary patterns among nematodes based upon DNA sequences*. Ph.D. thesis, University of Nottingham, Nottingham, UK, 187 pp.
- PAGE, R.D. 1996. TreeView: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12: 357-358. DOI: 10.1093/bioinformatics/12.4.357
- POINAR JR., G.O. 2015. Phylum Nemata. In: *Thorp and Covich's Freshwater Invertebrates* (J.H. Thorp & D.C. Rogers Eds). pp. 273-302. Cambridge, UK, Academic Press. DOI: 10.1016/B978-0-12-385026-3.00014-0
- POINAR JR., G.O., MYER, A. & FORSCHLER, B.T. 2019. Mermithid nematode parasites of eastern subterranean termites in North America. *Nematology* 22: 235-237. DOI: 10.1163/15685411-00003298
- RUTTLEDGE, W. 1925. Note on the occurrence of a mermithid worm in the body of a termite, *Cornitermes orthocephalus* Silvestri. *Parasitology* 17: 187-188. DOI: 10.1017/S0031182000004546
- TRIFINOPOULOS, J., NGUYEN, L.T., HAESLER, V.A. & MINH, B.Q. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* 44: 232-235. DOI: 10.1093/nar/gkw256
URL: <http://tree.bio.ed.ac.uk/software/figtree/> (accessed: October 5, 2022).