

First report of *Longidorus mindanaoensis* Coomans, De Ley, Jimenez and De Ley, 2012 (Nematoda: Longidoridae) From a Mangrove Forest in Vietnam

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Longidorus spp. (needle nematodes) are migratory ectoparasitic nematodes. Besides their direct damages by direct feeding from root cells, some species could also transmit plant pathogenic viruses (Taylor and Brown, 1997). Currently, only one valid species (*Longidorus elongatus* (de Man, 1876) Micoletzky, 1927) has been reported in association with peanut (*Arachis hypogaea* L.) from Vietnam.

During this study, a population of the genus *Longidorus* was recovered from saline sea sediments in a mangrove forest in Vietnam (GPS coordinates N: 8°38'09.902"; E: 104°44'31.178"). Nematodes were extracted from soil samples using the tray method (Whitehead and Hemming, 1965). The specimens were killed, fixed in TAF, and transferred to glycerin according to Seinhorst (1959). The measurements

Abstract

Longidorus mindanaoensis was recovered from a mangrove forest in Vietnam. The recovered population is in general morphological agreement with the type population, and the characters of pharyngeal bulb, i.e. the same unique pattern of pharyngeal glands nuclei as well as the lip region morphology, amphidial fovea shape and size and position of vulva corroborated its identity. Molecular studies of the recovered population using D2–D3 expansion segments of large subunit ribosomal DNA (LSU rDNA D2–D3) revealed the D2–D3 sequence of the recovered population is 99.6% similar to the sequence of the type population. A new morphometric range for body size was recorded for the species based upon present Vietnamese population, and the present study emphasized that the diversity of *Longidorus* spp. in Vietnam could be higher than previously assumed.

Keywords

Ca Mau, Dat Mui, deepwater, marine nematode, needle nematode, Ngoc Hien, plant-parasitic nematode, saline sea sediment.

and preparing the microphotographs were performed using a Carl Zeiss Axio Lab.A1 light microscope equipped with a ZEISS Axiocam ERc5s digital camera (Nguyen et al., 2017). For molecular phylogenetic analysis, the D2–D3 expansion segments of LSU rDNA were amplified using the primers D2A and D3B (5'-ACAAGTACCGTGGGGAAAGTTG-3' and 5'-TCGGAAGGAACCAGCTACTA-3') (De Ley et al., 1999). The newly obtained sequence was compared with previously submitted sequences into the GenBank database (Altschul et al., 1997) using BLAST search. Multiple alignments were made using MUSCLE and Modeltest was used to select the best fit model in MEGA 6 (Tamura et al., 2013). MrBayes 3.2.6 (Huelsenbeck and Ronquist, 2001) in Geneious R11 (www.geneious.com) was used to infer the Bayesian

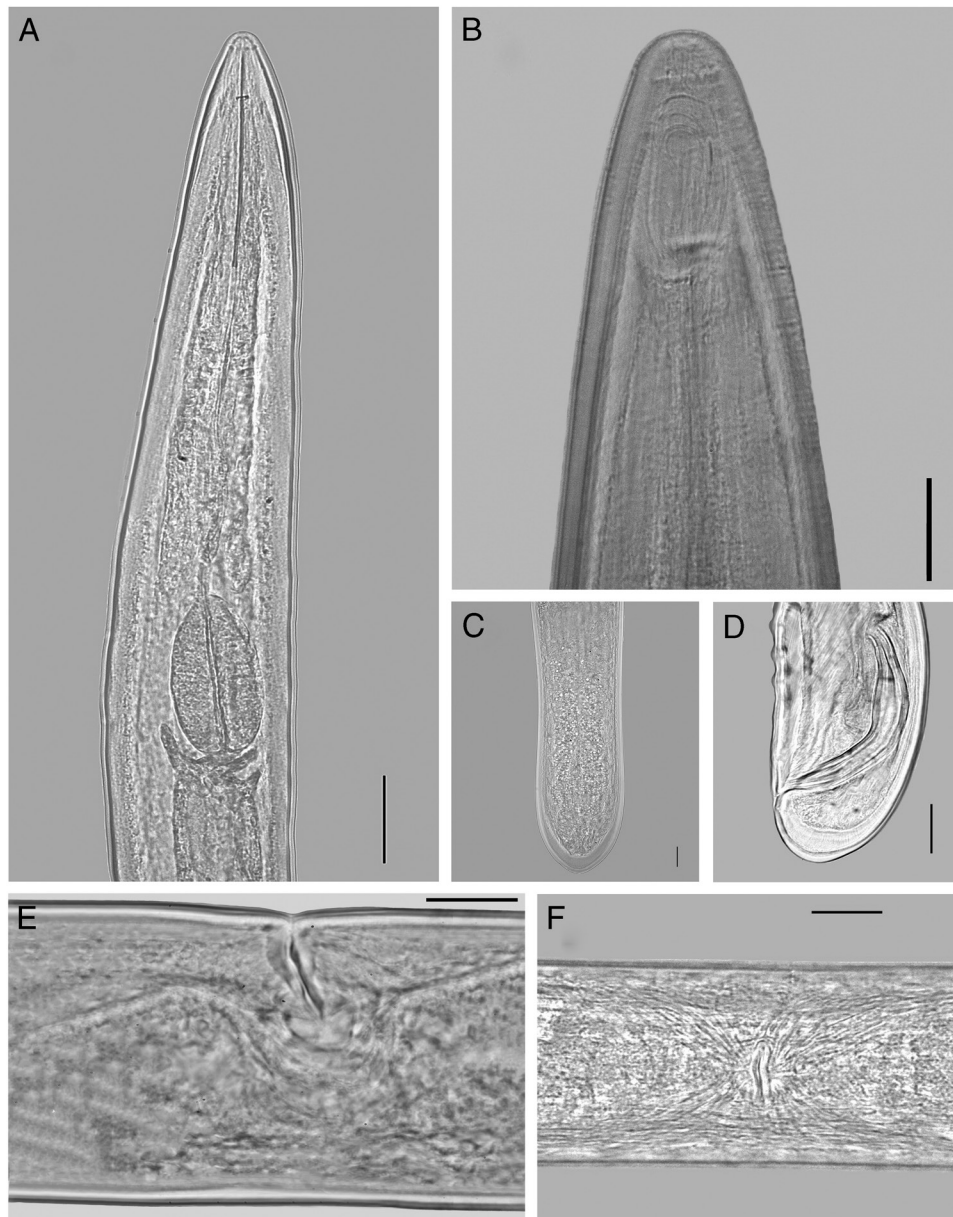


Figure 1: *Longidorus mindanaoensis* (Coomans et al., 2012) from Vietnam. (A–F) Female. (A) Esophageal region; (B) Anterior region; (C) Posterior region; (E) Vulval region; (F) Vulva region, ventral view. (D) Male posterior region. (Scale: (A) 50 μ m; (B–F) 20 μ m).

phylogenetic tree with 10^6 generations of Markov chains (4 runs, 20% burn-in) (Nguyen et al., 2019).

The morphological comparisons revealed the studied population belongs to *Longidorus mindanaoensis* (Coomans et al., 2012) that was recovered from the same habitat in the Philippines by Coomans et al. (2012). The morphological characters and measurements of the Vietnamese population of *L. mindanaoensis* (Fig. 1, Table 1) are in agreement with the original description of the species by

Coomans et al. (2012). Some unique morphological and morphometric characters like the shape of lip region and amphidial fovea, the characters of the esophageal bulb (its small size and shape as well as the arrangement of glands nuclei) and the position of vulva delimiting the species well corroborated the identity of the species. However, new morphometric data ranges were recorded for the species as follows: the females and males of the presently studied population of *L. mindanaoensis* from Vietnam are smaller

Table 1. Measurements of *Longidorus mindanaoensis* (Coomans et al., 2012) from Vietnam and the Philippines.

	<i>Longidorus mindanaoensis</i> (Vietnamese population)		<i>Longidorus mindanaoensis</i> (Coomans et al., 2012)	
	Female	Male	Female	Male
<i>n</i>	8	5	32	22
<i>L</i>	4.82 ± 0.45 (3.95–5.26)	4.44 ± 0.18 (4.27–4.63)	6.59 ± 0.48 (5.44–7.37)	6.14 ± 0.23 (5.60–6.45)
<i>a</i>	59 ± 10.5 (46–70)	61 ± 14.2 (48–85)	87 ± 5.2 (75–97)	93 ± 4.3 (84–102)
<i>b</i>	10.7 ± 0.9 (9.3–11.8)	10.5 ± 0.5 (10.0–11.2)	13.2 ± 1.15 (11–16.8)	12.8 ± 0.91 (11–14.7)
<i>c</i>	204 ± 40 (143–250)	182 ± 4.3 (177–188)	241 ± 31 (176–296)	197 ± 19 (167–234)
<i>c'</i>	0.5 ± 0.1 (0.4–0.6)	0.6 ± 0.0 (0.5–0.6)	0.58 ± 0.04 (0.49–0.67)	0.68 ± 0.05 (0.58–0.79)
<i>V</i>	36 ± 1.8 (32–38)	–	36 ± 1.6 (33–39)	–
Odontostyle	126 ± 3.5 (122–133)	126 ± 5.8 (116–131)	134 ± 5.4 (117–142)	134 ± 5.9 (120–145)
Odontophore	90 ± 10.4 (75–104)	89 ± 8.1 (76–97)	70 ± 5.7 (61–80)	68 ± 7.3 (48–83)
Anterior end to nerve ring	217 ± 15.0 (192–236)	205 ± 10.1 (197–222)	217 ± 9 (195–233)	216 ± 8 (195–240)
Pharynx	453 ± 30 (419–511)	424 ± 30 (380–455)	493 ± 35 (400–584)	480 ± 32 (413–540)
Max. body diam. (MBD)	84 ± 12.5 (70–106)	75 ± 13.9 (54–92)	76 ± 5.6 (66–86)	66 ± 3.1 (60–72)
Body diam. at anus/cloaca (ABD)	50 ± 5.6 (44–58)	42 ± 1.6 (40–44)	48 ± 2.2 (44–52)	46 ± 2 (42–50)
Tail length	24 ± 5.0 (21–36)	24 ± 1.0 (23–26)	28 ± 2.5 (22–33)	31 ± 3.1 (27–38)
Anterior genital tract	862 ± 139 (656–1,045)	–	1,009 ± 174 (653–1,259)	–
Posterior genital tract	964 ± 140 (754–1,121)	–	1,053 ± 168 (733–1,324)	–
Spicule length	–	90 ± 4.8 (84–97)	–	87 ± 5 (73–97)
Spicule width	–	13.3 ± 1.1 (11.8–14.5)	–	–
Prerectum	284 ± 28 (266–348)	359 ± 30 (309–389)	372 ± 112 (174–740)	532 ± 107.5 (340–714)

Note: All measurements are in µm (except for *L* in mm) and in the form: mean ± s.d. (range).

than the type population (Table 1), and therefore, related indices, including *a*, *b* are also relatively smaller compared with the data given for the type population. The present observation is in accordance with the results of Coomans et al. (2012) and Archidona-Yuste et al. (2016) showing the body length of *Longidorus* spp. could be highly variable (Coomans et al., 2012; Archidona-Yuste et al., 2016).

The D2–D3 sequence of our population of *L. mindanaoensis* (accession number: MN071244) from Vietnam was 805 bp long. It had a 99.6% identity to

the same sequence of the type population and three different nucleotides were detected between both populations. This variation could be explained by intraspecies variation in this genomic region and is recently observed for several other longidorids and trichodorids (Pedram et al., 2017; Fouladvand et al., 2019). It had a 69.3 to 80.7% identity with sequences available in the GenBank database (154–265 different nucleotides). In the inferred Bayesian phylogenetic tree, the newly generated sequence and the original sequence of the type population formed a basal

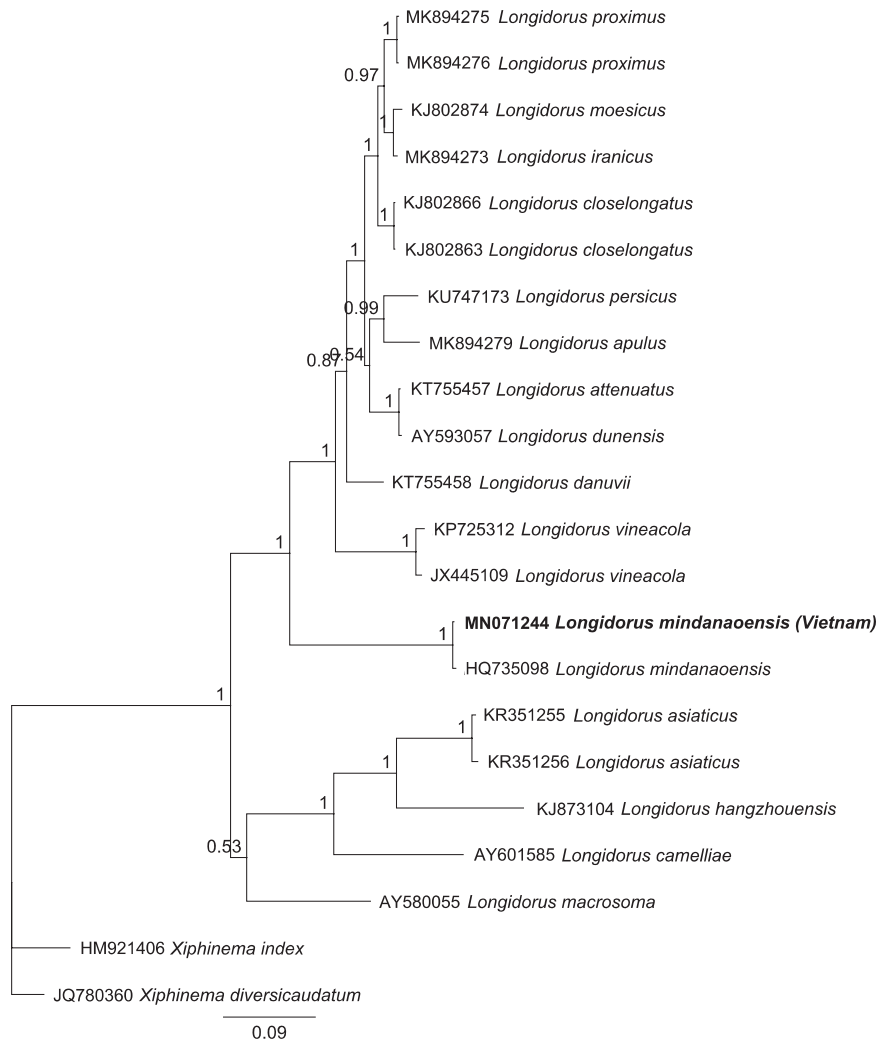


Figure 2: Bayesian phylogenetic tree generated using D2–D3 expansion segments of *Longidorus mindanaoensis* (Coomans et al., 2012) from Vietnam using *Xiphinema index* (Thorne and Allen, 1950) and *Xiphinema diversicaudatum* (Micoletzky, 1927) (Thorne, 1939) as outgroup taxa under the GTR+G evolutionary model.

clade to some other selected sequences of the genus (Fig. 2). This genomic fragment has already used in molecular phylogenetic analyses of longidorids and has successfully delimited several species belonging to cryptic complexes (Pedram et al., 2012; Gutiérrez-Gutiérrez et al., 2013; Pedram et al., 2017; Zhao et al., 2017; Fouladvand et al., 2019).

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References

- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25:3389–402.
- Archidona-Yuste, A., Navas-Cortés, J. A., Cantalapiedra-Navarrete, C., Palomares-Rius, J. E. and Castillo, P. 2016. Unravelling the biodiversity and molecular phylogeny of needle nematodes of the genus *Longidorus* (Nematoda: Longidoridae) in olive and a description of six new species. *PLoS One* 11: e0147689.

- Coomans, A., De Ley, I. T., Jimenez, L. A. and De Ley, P. 2012. Morphological, molecular characterisation and phylogenetic position of *Longidorus mindanaoensis* n. sp. (Nematoda: Longidoridae) from a Philippine Avicennia mangrove habitat. *Nematology* 14:285–307.
- De Ley, P., Felix, M. A., Frisse, L., Nadler, S., Sternberg, P. and Thomas, W. K. 1999. Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology* 1:591–612.
- De Man, J. G., 1876. Onderzoekingen over vrij in de aarde levende Nematoden. *Tijdschrift der Nederlandsche Dierkundige Vereeniging Ver.* 2:78–196.
- Fouladvand, Z. M., Pourjam, E., Castillo, P. and Pedram, M. 2019. Genetic diversity, and description of a new dagger nematode, *Xiphinema afratakhtehnsis* sp. nov., (Dorylaimida: Longidoridae) in natural forests of southeastern Gorgan, northern Iran. *PLoS ONE* 14:e0214147.
- Gutiérrez-Gutiérrez, C., Cantalapiedra-Navarrete, C., Montes-Borrego, M., Palomares-Rius, J. E. and Castillo, P. 2013. Molecular phylogeny of the nematode genus *Longidorus* (Nematoda: Longidoridae) with description of three new species. *Zoological Journal of the Linnean Society* 167:473–500.
- Huelsenbeck, J. P. and Ronquist, F. 2001. MR-BAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–5.
- Micoletzky, H. 1927. Neue und seltene freilebende Nematoden aus dem Wolgagebiet (Kama). *Zoologischer Anzeiger* 73:112–23.
- Nguyen, H. T., P., T. Q., Couvreur, M., Singh, P. R., Decraemer, W. and Bert, W. 2019. Description of *Rotylenchus rhomboides* n. sp. and a Belgian population of *Rotylenchus buxophilus* (Tylenchomorpha: Hoplolaimidae). *Journal of Nematology* 51:1–20.
- Nguyen, T. D., Le, T. M. L., Nguyen, H. T., Nguyen, T. a. D., Liébanas, G. and Trinh, Q. P. 2017. Morphological and molecular characteristics of *Pratylenchus haiduongensis* sp. n., a new species of root-lesion nematode associated with carrot in Vietnam. *Journal of Nematology* 49:276–85.
- Pedram, M., Pourjam, E., Namjou, S., R., A. M., Cantalapiedra-Navarrete, C., Liébanas, G., Palomares-Rius, J. E. and Castillo, P. 2012. Molecular and morphological characterisation of *Paralongidorus iranica* n. sp. and *Paralongidorus bikanerensis* (Lal & Mathur, 1987) Siddiqi, Baujard & Mounport, 1993 (Nematoda: Longidoridae) from Iran. *Nematology* 14:427–43.
- Pedram, M., Roshan-Bakhsh, A., Pourjam, E., Atighi, M. R., Decraemer, W. and Bagheri, A. 2017. Additional data on Iranian trichodorids (Triplonchida: Trichodoridae) and first record of a rare species, *Trichodorus variabilis* Roca, 1998. *Nematology* 19: 121–9.
- Seinhorst, J. W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 4:67–9.
- Tamura, K., Stecher, G., Peterson, D., Filipowski, A. and Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution* 30:2725–9.
- Taylor, C. A. and Brown, D. J. F. 1997. *Nematode vectors of plant viruses*, CABI, Wallingford, UK.
- Thorne, G. and Allen, M. W. 1950. *Paratylenchus hamatus* n. sp. and *Xiphinema index* n. sp., two nematodes associated with fig roots, with a note on *Paratylenchus anceps*. *Proceedings of the Helminthological Society of Washington* 17:27–35.
- Thorne, G. 1939. A monograph of the nematodes of the superfamily Dorylaimoidea. *Capita Zool* 8:1–261.
- Whitehead, A. G. and Hemming, J. R. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annales of Applied Biology* 55:25–38.
- Zhao, L., Ye, W., Maria, M., Pedram, M. and Gu, J. 2017. *Xiphinema japonicum* n. sp. (Nematoda: Longidorinae) from the rhizosphere of Japanese podocarpus macrophyllus (Thunb.), a cryptic species related to *Xiphinema bakeri* Williams, 1961. *Journal of Nematology* 49:404–17.