

Morphological and molecular characterization of *Mylonchulus laocaiensis* sp. n. (Nematoda: Mylonchulidae) from Vietnam

Research Paper

Cite this article: Vu TTT, Le TML and Peneva V (2024). Morphological and molecular characterization of *Mylonchulus laocaiensis* sp. n. (Nematoda: Mylonchulidae) from Vietnam. *Journal of Helminthology*, **98**, e75, 1–11
<https://doi.org/10.1017/S0022149X24000671>.

Received: 02 May 2024
 Revised: 03 August 2024
 Accepted: 21 September 2024

Keywords:

morphology; *Mylonchulus*; molecular phylogeny; taxonomy; new species

Corresponding author:

T.T.T. Vu;
 Email: vtam7572@gmail.com

T.T.T. Vu^{1,2} , T.M.L. Le^{1,2} and V. Peneva³

¹Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam; ²Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam and ³Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1113 Sofia, 2, Y. Gagarin Street, Sofia, Bulgaria

Abstract

Mylonchulus laocaiensis sp. n. recorded from Vietnam, is described and illustrated, and its phylogenetic relationship within the Mylonchulidae family and Mononchida order are analysed. The new species is characterized by medium body size (L = 1.0–1.5 mm); buccal cavity goblet shaped, 26–29 × 14–16.5 μm or 1.8 (1.7–1.9) times as long as wide; posterior position of dorsal tooth apex (79%–88% from the base of buccal cavity); a small subventral tooth present on subventral wall; *pars refringens vaginae* with faint and small (2.5 × 1.7 μm) teardrop-shaped pieces, short *pars distalis vaginae*; male with short spicules (54–57 μm) with rounded head. The molecular data (18S and 28S rDNA) are provided for the new species.

Introduction

The genus *Mylonchulus* was proposed by Cobb (1916) when he divided the genus *Mononchus* Bastian, 1865 into five genera, namely: *Mononchus* Bastian, 1865, *Prionchulus* Cobb, 1916, *Mylonchulus* Cobb, 1916, *Iotonchus* Cobb, 1916, and *Anatonchus* Cobb, 1916 and the species *Mylonchulus minor* (Cobb, 1893) Cobb, 1916 became the type species. Until 1976, the genus *Mylonchulus* Cobb, 1916 contained a small number of monodelphic species, and in that year, Andrásy transferred the only opisthodelphic species *M. reversus* Cobb, 1917 to the newly erected genus *Oligonchus* Andrásy, 1976. In 1982, Jairajpuri and Khan proposed the new genus *Paramylonchulus* Jairajpuri and Khan, 1982, which was split from the genus *Mylonchulus* Cobb, 1916 based on some main characteristics such as: female genital system mono-prodelphic and the absence of teeth in subventral wall. Seven species including *M. caespitosus* Razzhivin, 1971, *M. californicus* Jairajpuri, 1970, *M. index* Cobb, 1906, *M. mashhoodi* Khan and Jairajpuri, 1979, *M. mulveyi* Jairajpuri, 1970, *M. silvaticus* Razzhivin, 1971, *M. subterraneus* Schneider, 1940 were transferred to the new genus *Paramylonchulus* Jairajpuri and Khan, 1982 with *P. index* Cobb, 1906 as the type species. Khan and Saeed (1987) proposed the new genus *Pakmylonchulus* Khan and Saeed, 1987 which differed from the genus *Mylonchulus* Cobb, 1916 by the absence of subventral teeth and the body length of less than 1 mm based on the type species of *P. amurus* Khan and Jairajpuri, 1979. Loof (1993) did not accept that the number of female genital branches (didelphic or mono-prodelphic) is basis for generic separation between *Mylonchulus* Cobb, 1916 and *Paramylonchulus* Jairajpuri and Khan, 1982 and considered the *Paramylonchulus* Jairajpuri and Khan, 1982 as a synonym of the genus *Mylonchulus* Cobb, 1916. Zullini and Peneva (2006) and Andrásy (2009) agreed with Loof (1993) and did not accept the genera *Paramylonchulus* Jairajpuri and Khan, 1982 and *Pakmylonchulus* Khan and Saeed, 1987 as valid genera only based on the characteristics of female genital system and the presence of teeth on subventral wall. These authors believed that those characteristics did not clearly differentiate these genera from *Mylonchulus*. Siddiqi (2015) proposed the new genus *Montonchus* Siddiqi, 2015 which differed from the genus *Mylonchulus* Cobb, 1916 by having two widely spaced distinct ridges across the middle of the buccal cavity. Only one species *Mylonchulus montanus* (Thorne, 1924) Goodey, 1951 was transferred to the genus *Montonchus* Siddiqi, 2015 and became the type species. Aliramaji et al. (2023) reconfirmed the validity of the genus *Paramylonchulus* based on analysis of morphological characteristics and molecular data of the species *P. iranicus* Aliramaji et al., 2023 from Iran. The main diagnostic features of the genus *Mylonchulus* are: (1) buccal cavity goblet or funnel-shaped, thickened walls; (2) dorsal tooth is very large, massive, claw-like, obliquely directed anteriorly with sharply pointed apex and situated in the anterior half of buccal cavity; (3) submedian rasp-like denticles arranged regular transverse rows; (4) non-tuberculate cardia; (5) female reproductive system amphidelphic and (6) tail of both sexes similar, short ($c' \leq 4$), varying in forms; caudal glands well developed, grouped or in tandem; spinneret opening at the tail terminus (Ahmad and Jairajpuri 2010).

Currently, 69 valid *Mylonchulus* Cobb, 1916 species have been described, with the three most recent species being *M. kermaniensis* Shokoohi et al., 2013 from Iran, *M. capsicum* Ishaque et al., 2021 from Pakistan and *M. shamimi* Handoo et al., 2021 from India. In Vietnam, 20 species of the genus *Mylonchulus* have been reported (Vu 2016).

In the present study, *Mylonchulus laocaiensis* sp. n. is described based on morphological, morphometric, and molecular data from Lao Cai Province, Vietnam.

Materials and methods

Nematode extraction, preservation and morphological studies

Soil samples were collected from a pristine forest in Bat Xat Nature Reserve and Hoang Lien National Park in Lao Cai Province, Vietnam. Nematodes were extracted from soil samples using the Baermann funnel technique (Southey, 1986).

Specimens were heat killed at 70° fixed in TAF solution (Southey, 1986) (for morphological observations) or in a DESS mixture (Yoder et al., 2006) (for molecular analyses), transferred to anhydrous glycerol (Seinhorst, 1962), and mounted on glass slides for microscopic observation. After filming and taking pictures, selected specimens were submitted for molecular studies. Measurements were performed with a Nikon digital camera on a Nikon Eclipse Ni microscope at the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST), Vietnam. Observations of morphological diagnostic features and photographs were taken with a Nikon digital camera mounted on a Nikon Eclipse Ni microscope. Illustrations were drawn using a Nikon Eclipse Ni microscope equipped with a Nikon Y-IDT drawing tube. Photographs and illustrations were edited using Adobe Photoshop CC 2018.

DNA extraction, polymerase chain reaction and sequencing

Nematode DNA was extracted from a single individual as described by Holtermann et al. (2008), and DNA extracts were stored at –20° until used as a polymerase chain reaction (PCR) template. The D2-D3 expansion segment of 28S rDNA was amplified using the forward D2A (5'-ACAAGTACCGTGGGGAAAGTTG-3') and reverse D3B (5'-TCGG AAGGAACCAGCTACTA-3') primers (Subbotin et al., 2006) and the 18S rRNA gene was amplified using primers NEM18SF: 5'-CGCGAATRGCTCATTACAACAGC-3' (forward) and NEM18SR: 5'-GGGCGGTATCTGATCGCC-3' (reverse) (Floyd et al., 2005). All PCR reactions contained 12.5 µL Hot start green PCR Master Mix (2×) (Promega, USA), 1 µL of the forward and reverse primer (10 µM each), the 3 µL DNA template and sterile Milli-Q water to 25 µL of the total volume. All PCR reactions were performed in SimpliAmp Thermal cycler (Thermo Fisher Scientific) as follows: an initial denaturation step at 95° C for 4 min, followed by 40 cycles at 95° C for 30 s, 54° C for 30 s and 72° C for 60 s with a final incubation for 5 min at 72° C. Amplicons were visualised under ultraviolet illumination after Simplisafe gel staining and gel electrophoresis. After sequencing the obtained *Mylonchulus laocaiensis* sp. n. rDNA sequence fragments were deposited in GenBank under the following accession numbers: MW544445, MW544446 (18S rDNA) and MW544448, MW544450 (D2D3 28S rDNA).

Phylogenetic analyses

Phylogenetic relationship analyses were based on 18S and 28S rDNA. The newly obtained rDNA sequences were analysed using the BioEdit sequences available in GenBank using the ClustalW alignment tool implemented in the MrBayes version 3.2.6 (Ronquist et al., 2012). The final 18S and 28S rDNA datasets for phylogenetic study included sequences from the current study *Mylonchulus laocaiensis* sp. n. and available sequences of Mononchida representatives from GenBank. The prepared multiple alignments of 18S and 28S rDNA generated by the ClustalW algorithm were routinely manually edited to eliminate improper phylogenetic signals. A 18S alignment of 885-bp long containing 48 sequences and an D2-D3 alignment of 800-bp long containing 45 sequences were created. Bayesian inference (BI) analysis was constructed with the program MrBayes version 3.2.6 (Ronquist et al., 2012). The analysis under the generalized time reversible and invariant sites and gamma distribution (GTR + I + G) model was initiated with a random starting tree and run with the Markov chain Monte Carlo (Larget & Simon, 1999) for 1 × 10⁶ generations. The tree was visualised and saved with FigTree 1.4.4 (Rambaut, 2018).

Results and discussion

Description

Order: MONONCHIDA Jairajpuri, 1969

Family: Mylonchulidae Jairajpuri, 1969

Genus: *Mylonchulus* Cobb, 1916

Mylonchulus laocaiensis sp. n. (Figures 1, 2 and 3).

urn:lsid:zoobank.org:pub:3826F1CD-C115-4B89-9988-840C4C667B6A

Type material:

Holotype. female, Vietnam, Lao Than mountain, 22°37'14"N; 103°37'24"E, altitude ± 1900 m, Bat Xat Nature Reserve, Lao Cai Province, 12-2021, slide *Mylonchulus laocaiensis* sp. nov. number 1.

Paratypes. The same data as holotype, 2 male paratypes number 2 and 11. 25 female paratypes on slide numbers 3–10.

Other material. Hoang Lien mountain, 2200 m, Hoang Lien National Park, Lao Cai Province, 9-2022. Twenty-one females on slide numbers 11-20. Holotype female, 15 female paratypes and one paratype males deposited in the nematode collection of the Department of Nematology, Institute of Ecology and Biological Resources of the VAST, Vietnam. Ten female paratypes and one male paratype deposited at the Vietnam National Museum of Nature of the VAST, Vietnam.

Etymology: The name of the species refers to its geographical origin from Lao Cai Province in Vietnam.

Type locality: Lao Cai Province, Vietnam.

Description:

Measurements: see Table 1.

Adult: Moderately slender to slender (a = 23–30), medium sized nematodes, 1.0–1.5 mm long, habitus curved ventrally and C-shaped after fixation in TAF solution. Body cylindrical, slightly tapering towards the anterior end but more sharply towards posterior end. Maximum body width at level of vulva. Cuticle smooth, two layers, 2.0 (1.5–2.3) µm thick at the base of the pharynx. Lip region angular, prominent set off from body contour by depression, 2.8–3.7 times as wide as high. Lips moderately separated, distinct

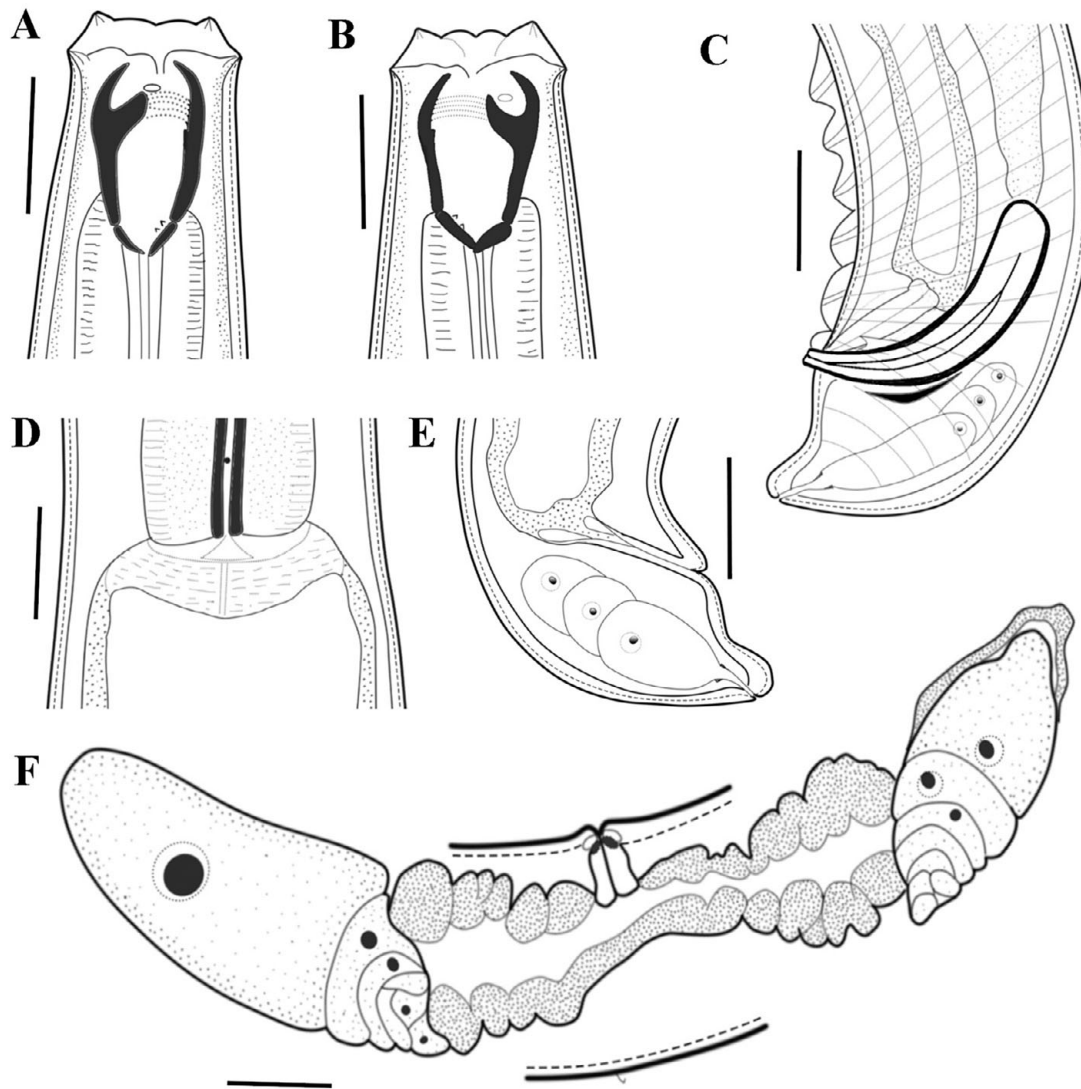


Figure 1. Holotype female and paratype male of *Mylonchulus laocaiensis* sp. n. (a): female head region; (b): male head region; (c): male tail region; (d): pharyngo-intestinal junction region; (e): female tail region; (f): female reproductive system (scale bars = 20 μ m).

with prominent labial and cephalic sensilla. Anterior sensilla are arranged in two circles: an anterior one with six inner labials and a posterior one with six outer labials and 4 cephalic papillae slightly protruding beyond the body outline. Amphidial fovea small, fusi-form shaped, its aperture oval transverse, 2.2–3 μ m wide, located 4.0–5.0 μ m from anterior end of buccal cavity or at the level of dorsal tooth apex. Vestibulum 6–7 μ m long. Buccal cavity goblet shaped, tapering at base, 1.7–1.9 times as long as wide, its walls heavily cuticularized. Dorsal tooth large, 11–12 μ m long from basis to apex, claw-like, forward directed, its apex obtusely, located at anterior fifth (80%–88%) of buccal cavity length from stoma base. A small tooth clearly present in the subventral wall. Five transverse rows of raps-like denticles on subventral walls. Two foramina present at base of buccal cavity lying close to each other. Posterior fourth of buccal cavity embedded in pharyngeal tissue.

Pharynx cylindrical, nerve ring encircling pharynx, located at 30 (28–31)% of neck length. Secretory-excretory pore (SE-pore) indistinct, situated at 33 (31–34)% of neck length. Pharyngo-intestinal junction non-tuberculate, 19–22 \times 10–11.5 μ m. Rectum straight, shorter than anal body width, 0.8 (0.7–1) times the anal

body diameter long. Tail short 0.9–1 time the anal body diameter long; plump, ventrally bent; tail tip rounded. Three caudal glands well developed, lying in tandem and opening sub-terminally on the tail terminus with cuticularized valvular apparatus.

Female: Genital system didelphic-amphidelphic, with both branches equally developed, each branch occupying 11.5 (9.5–13.5)% of total body length. Ovaries reflexed and respectively lengths 55–70 μ m with oocytes first arranged in several rows and then in single row on alternate sides of the intestine; sphincter between oviduct and ovary not present. Oviduct 40–70 μ m long. Uterus simple, 51–60 μ m or 1.3–1.5 body diameter; uterine egg 91–103 \times 33–39 μ m. Vagina length 13 (8–15) μ m occupying 33 (28–40)% corresponding body diameter. *Pars proximalis vaginae* short; *pars refringens vaginae* small 2.0 \times 1.5 μ m, sclerotized in teardrop-shaped pieces; *pars distalis vaginae* 6–12 \times 1.5–2 μ m. Vulva transverse, slit-like, at 64%–67% of body length.

Male: Male similar to female in general morphology and body shape and size (see Table 1). Genital system diorchic, with opposed testes. Anterior testis 113–133 μ m or 11–13.6% of body length and posterior testis 96–137 μ m or 9.5%–14% of body

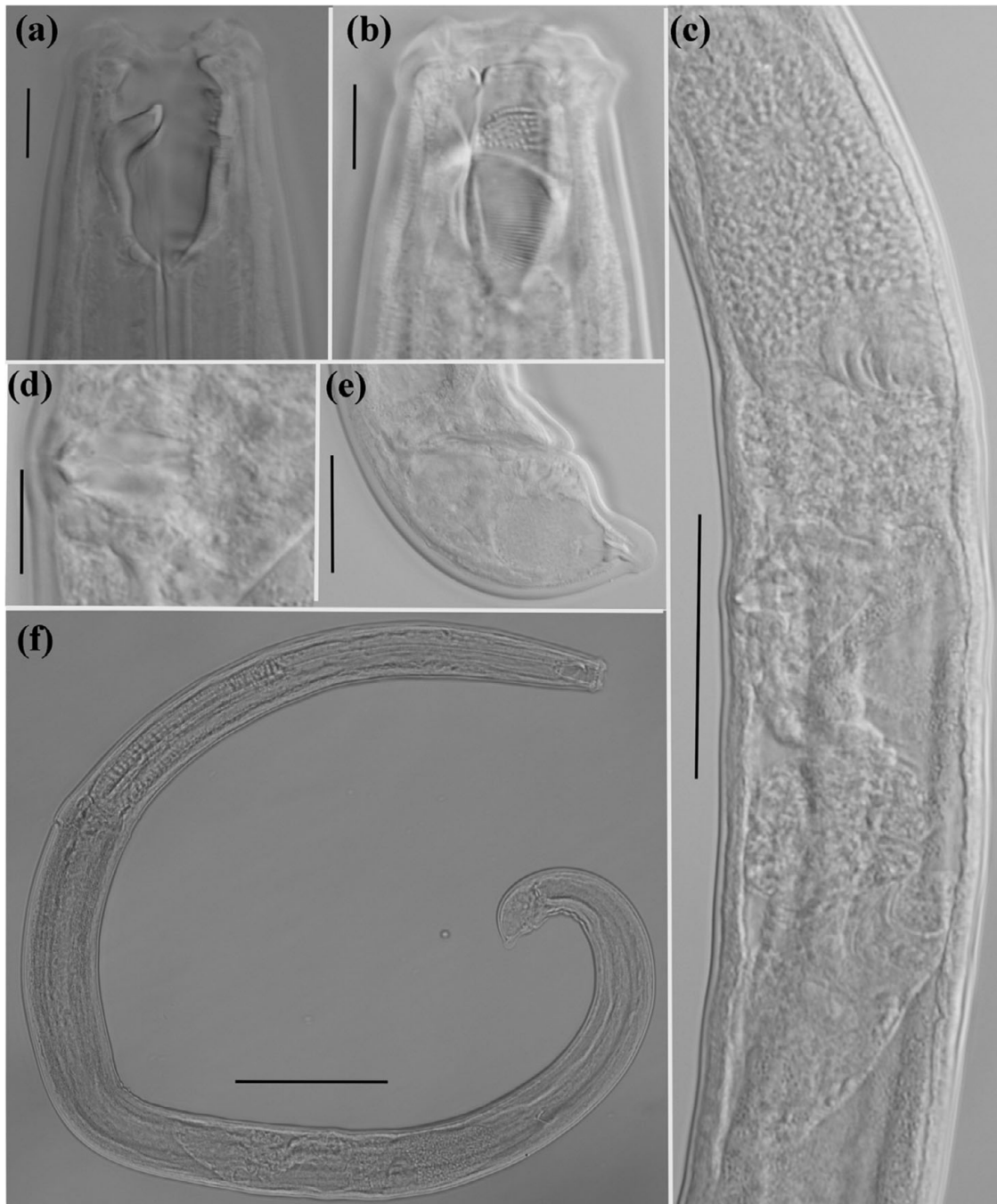


Figure 2. Holotype female of *Mylonchulus laocaiensis* sp.n. (a): head region; (b): denticular rows; (c): reproductive system; (d): vaginae region; (e): tail region; (f): entire body (scale bars: a, b, d = 10 μm ; e = 20 μm ; c = 50 μm ; f = 100 μm).

length. Spermatozoa spherical shaped, 2.5–3.5 μm in diameter. Ventromedian supplements 9–10 in number, conical shape, regularly spaced, 80–88 μm apart or occupied 8%–8.5% of body length. The anteriormost supplement situated at 89–98 μm from cloaca aperture. Spicules moderately slender 6.8–7.2 times as long as wide, 54–57 μm long, 8 μm at the maximum width and 1.6–1.8 μm at the minimum width; ventrally curved, with bifurcate terminus, 1.7 times longer than body diameter at cloacal aperture. The head of spicule round, 11 μm long and 5 μm wide. The median piece is 39.5–41 μm long and approximately 4 μm wide. The dorsal hump shallow, curvature 145°. Lateral guiding pieces absent.

Gubernaculum well developed, spatuliform in the distal end, 19–20 μm long.

Diagnosis: *Mylonchulus laocaiensis* sp. n. is characterized by moderately slender to slender body size (1.0–1.5 mm); medium of buccal cavity size (26–29 \times 14–16.5 μm); more posterior position of amphidial aperture (4–5 μm) from anterior margin of buccal cavity; 5 denticles rows and small subventral tooth present; *pars refringens vaginae* small (2.0 \times 1.5 μm), cuticularized in teardrop-shaped pieces; tail short ($c' = 0.8$ –1.0); presence of males with short spicules (54–57 μm), rounded head; accessory piece absent; 9–10 lateral supplements regularly arranged.

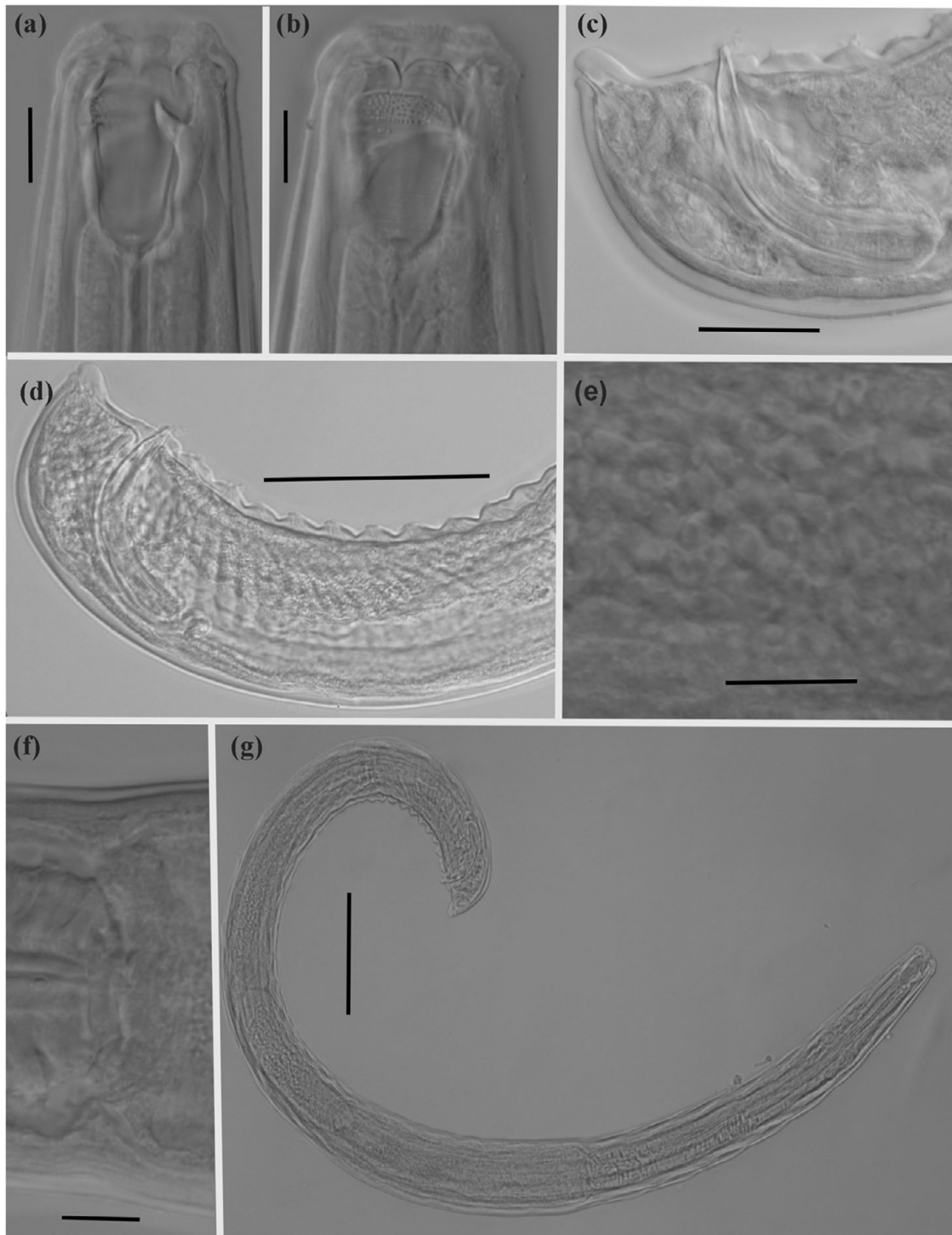


Figure 3. Paratype male of *Mylonchulus laocaiensis* sp. n. (a): head region; (b): denticular rows; (c): spicule gubernaculum and accessory piece; (d): tail region; (e): spermatozoa; entire body (scale bars: a, b, e, f = 10 μ m; c = 20 μ m; d = 50 μ m; g = 100 μ m).

In general appearance *Mylonchulus laocaiensis* sp. n. is similar to *M. oceanicus* Andr ssy, 1986, *M. doliolarus* Andr ssy, 1992, *M. ananasi* Yeates, 1992, *M. aequatorialis* Orselli and Vinciguerra, 2007, and *M. ubis* Clark, 1961 based on a group of characters: female didelphic, tail shape not broadly rounded, caudal glands well developed with spinneret present on subdorsal tail terminus (Clark, 1961; Andr ssy, 1986; Andr ssy, 1992; Yeates, 1992; Orselli and Vinciguerra, 2007) but differs from these species by the presence of a small subventral tooth and the presence of males.

Mylonchulus laocaiensis sp. n. is distinguished from *M. oceanicus* by having a more posterior amphidial aperture (at the level of dorsal tooth apex vs at the beginning of anterior buccal cavity); number of denticule rows (5 vs 6–7) and narrower buccal cavity (14–16.5 vs 16–19 μ m). *Mylonchulus laocaiensis* sp. n. differs *M. doliolarus* Andr ssy, 1992 in body size (1–1.5 μ m vs 0.6–0.7 μ m); shape of buccal cavity (goblet shaped vs more barrel shaped); anterior dorsal tooth apex (79–88% vs 76–77%); lower c' value (0.8–1 vs 1) and buccal cavity size (26–29 \times 14–16.5

Table 1. Morphometrics of *Mylonchulus laocaiensis* sp. nov. (all measurements are in μm except indicated and ratios a, b, c, c')

	Lao Cai Province			
	Bat Xat Nature Reserve			Hoang Lien National Park
	Holotype	Paratype		
	♀	♀♀ (n = 25)	♂ (n = 2)	♀♀ (n = 21)
L	1139	1096 ± 52.5 (978–1186)	982; 1013	1239 ± 125 (1040–1488)
V (%)	65	65.5 ± 1.0 (63.9–67.4)	–	65 ± 1.0 (62–67)
a	26.4	25 ± 1.9 (23–30.5)	23.5; 25.6	26.2 ± 1.4 (24–28.5)
b	3.2	3.1 ± 0.1 (3.0–3.3)	3.1; 3.1	3.2 ± 0.1 (3.1–3.3)
c	38.8	40.4 ± 3.3 (34–48)	34.4; 32.6	40.6 ± 4.0 (35–49)
c'	1.0	0.9 ± 0.1 (0.8–1.0)	0.9; 0.9	1.0 ± 0.1 (0.9–1.0)
Lip region width	23	24.3 ± 0.9 (22.5–25)	24.6; 24.8	26.2 ± 1.3 (24.5–29)
Lip region height	7.5	7.9 ± 0.8 (7–9.5)	9; 9	7.9 ± 0.7 (7–9.5)
Buccal cavity length	27.5	27.3 ± 0.7 (26–28.5)	26; 27.8	28 ± 0.7 (26.5–29)
Buccal cavity width	15.5	15.3 ± 0.6 (14–16.5)	15; 15.6	15.6 ± 0.5 (15–16.5)
Position of tooth apex (%)	82.5	81.8 ± 1.8 (79–84.6)	83.1; 80	83 ± 2.4 (78–88)
Neck length	354	350 ± 12.8 (323–379)	317; 332	383 ± 29 (335–435)
Nerve ring to anterior end	107	105 ± 5.1 (96–118)	96; 88	115 ± 8.2 (101–126)
Secretory excretory pore to anterior end	115	113 ± 5.1 (103–125)	104; 101.3	126.5 ± 10.4 (111–148)
Cuticle at the neck base	1.9	1.9 ± 0.3 (1.5–2.4)	2.1; 2.2	1.9 ± 0.2 (1.5–2.4)
Body diameter at the neck base	39.2	40 ± 2.2 (34–43)	40.3; 37	42.4 ± 2.6 (38–44)
Body diameter at the vulva	43	43.9 ± 2.7 (39.5–48)	–	47.3 ± 3.4 (41.5–50)
Body diameter at the anus	29	29 ± 1.5 (23.4–31)	33; 36	31.7 ± 2.5 (27.3–38)
Anterior branch of reproductive system	105	115 ± 18 (83–147)	–	142.5 ± 30.2 (103–181)
Posterior branch of reproductive system	107	110 ± 17.2 (78–133)	–	141.5 ± 32 (90–180)

(Continued)

Table 1. (Continued)

	Lao Cai Province			
	Bat Xat Nature Reserve			Hoang Lien National Park
	Holotype	Paratype		
Egg length	–	95.4 ± 3.6 (91–103)	–	95 ± 4.1 (88–99)
Egg width	–	37 ± 1.3 (35–39)	–	39 ± 2.3 (35.7–42)
Anterior testis length	–	–	133; 114	–
Posterior testis length	–	–	137; 96	–
Vagina length	14.5	13 ± 2.6 (8–15)	–	14 ± 1.7 (12.5–16.5)
Rectum length	24	22.4 ± 1.8 (20–25)	–	25.3 ± 0.8 (23–29)
Tail length	29	27.3 ± 0.7 (25–30)	29	30.6 ± 2.9 (25–36)
Spicule length	–	–	54.5; 57	–
Spicule width	–	–	8; 8	–
gubernaculum length	–	–	19	–
Distance from cloacal opening to posteriormost supplement	–	–	9.5; 10.4	–
Distance from cloacal opening to anteriormost supplement	–	–	89; 98	–
Number of supplements	–	–	10; 9	–

–, no information.

Table 2. Genetic distances between *Mylonchulus laocaiensis* sp. n. and other *Mylonchulus* species using 18S rDNA sequence data (p-distances given in percents)

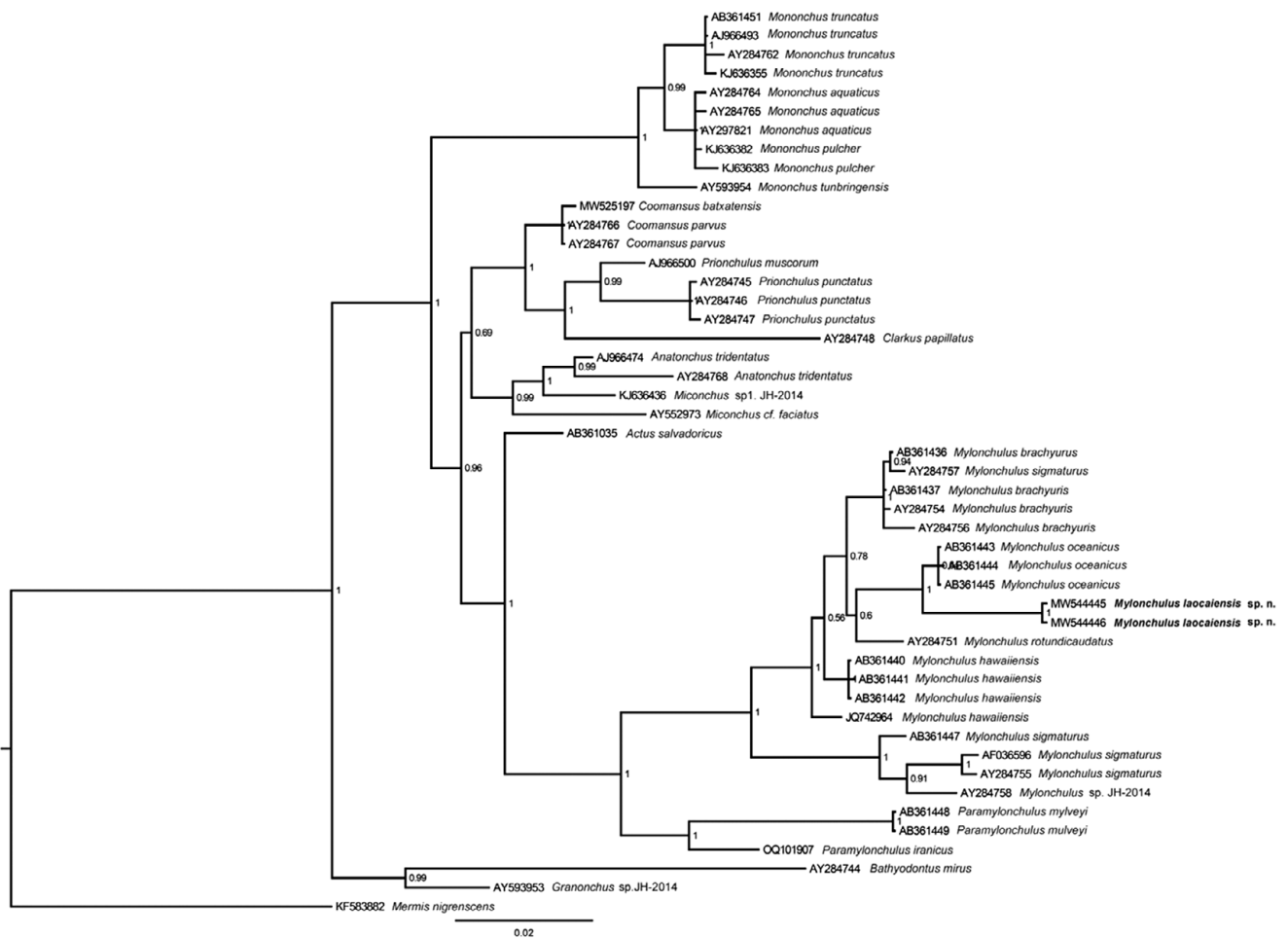
Sequence number/species	1	2	3	4	5	6	7	8	9	10	11	12
1 MW544446 <i>Mylonchulus laocaiensis</i> sp. nov.												
2 MW544445 <i>Mylonchulus laocaiensis</i> sp. nov.	0.0											
3 AB361445 <i>Mylonchulus oceanicus</i>	1.6	1.6										
4 JQ742964 <i>Mylonchulus hawaiiensis</i>	1.8	1.8	0.8									
5 AB361437 <i>Mylonchulus brachyuris</i>	2.0	2.0	1.6	0.7								
6 AB361442 <i>Mylonchulus hawaiiensis</i>	2.1	2.1	1.8	0.6	1.0							
7 AY284757 <i>Mylonchulus sigmaturus</i>	2.4	2.4	1.9	1.2	0.2	1.4						
8 AF036596 <i>Mylonchulus arenicolus</i>	4.3	4.3	4.1	2.9	4.1	3.9	3.7					
9 OQ101907 <i>Paramylonchulus iranicus</i>	4.5	4.5	4.4	3.7	4.0	4.0	4.2	4.9				
10 AB361035 <i>Actus salvadoricus</i>	5.3	5.3	5.1	4.3	5.1	4.6	5.3	5.4	3.8			
11 AB361449 <i>Paramylonchulus mulveyi</i>	6.1	6.1	5.8	5.1	5.7	5.8	5.9	7.3	3.4	5.3		
12 AJ966493 <i>Mononchus truncatus</i>	7.2	7.2	6.7	6.5	6.9	6.7	7.1	7.5	6.4	5.0	7.0	
13 AJ966500 <i>Prionchulus muscorum</i>	7.2	7.2	6.7	6.2	6.3	6.2	6.6	7.3	4.9	3.2	6.4	5.2

μm vs $16\text{--}17 \times 14\text{--}10 \mu\text{m}$). *Mylonchulus laocaiensis* sp. n. differs from *M. ubis* by having a longer tail ($c = 22\text{--}26$ vs $c = 34\text{--}49$ and $c' = 1.6$ vs $c' = 0.8\text{--}1$); larger buccal cavity ($26\text{--}29 \times 14\text{--}16.5 \mu\text{m}$ vs $16 \times 14\text{--}9 \mu\text{m}$) and position of anterior amphidial aperture (at level of dorsal tooth apex vs posterior to dorsal tooth apex). *Mylonchulus laocaiensis* sp. n. can be distinguished from *M. aequatorialis* by

having a higher c value ($34\text{--}49$ vs $24\text{--}32$) but lower c' value ($0.8\text{--}1$ vs $1.2\text{--}1.4$); buccal cavity size ($26\text{--}29 \times 14\text{--}16.5 \mu\text{m}$ vs $20\text{--}21 \times 8\text{--}10 \mu\text{m}$) and number of raps-like denticles rows (5 vs 6). From *M. ananasi* it differs by having a more posterior position of vulva ($V = 62\text{--}68\%$ vs $V = 56\text{--}58\%$); higher c value ($34\text{--}49$ vs $24\text{--}27$) but lower c' ratio ($0.8\text{--}1$ vs $1.4\text{--}1.7$); more anterior position of dorsal

Table 3. Genetic distances between *Mylonchulus laocaiensis* sp. n. and other Mononchidae species using 28S rDNA sequence data (p-distances given in percents)

Sequence number/species	1	2	3	4	5	6	7	8
1 MW544448 <i>Mylonchulus laocaiensis</i> sp. nov.								
2 MW544450 <i>Mylonchulus laocaiensis</i> sp. nov.	0.2							
3 ON927922 <i>Mylonchulus sigmaturus</i>	10.0	10.0						
4 OQ132796 <i>Paramylonchulus index</i>	24.1	24.4	18.8					
5 MF325197 <i>Clarkus papillatus</i>	24.2	24.2	18.3	13.2				
6 MF325333 <i>Prionchulus punctatus</i>	26.8	26.8	19.1	13.7	3.5			
7 MF125526 <i>Mononchus maduei</i>	29.6	29.6	27.2	25.2	12.6	13.0		
8 MF125524 <i>Mononchus aquaticus</i>	29.6	29.6	27.2	25.2	12.6	13.0	0.0	
9 MF125530 <i>Mononchus truncatus</i>	31.5	31.5	29.2	25.1	12.6	11.8	4.7	4.7

**Figure 4.** Bayesian Inference tree from the newly sequenced *Mylonchulus laocaiensis* sp. n. based on sequences of the 18S rDNA region. Bayesian posterior probabilities (%) are given for each clade. Scale bar shows the number of substitutions per site.

tooth apex (79–88% vs 75%) and larger size of buccal cavity (26–29 × 14–16.5 vs 22–24 × 13–14) μm.

Mylonchulus laocaiensis sp. n. is also similar to *M. williamsi* Loof, 2006, *M. contractus* Jairajpuri, 1970, *M. brachyuris* (Büschli, 1873) Cobb, 1917, *M. capsicum* Ishaque et al., 2021 and *M. shamimi* Handoo et al., 2021 based on group of characters: female didelphic, tail conoid shape, caudal glands well developed with spinneret present on subdorsal tail terminus and having a small subventral

tooth (Loof, 2006; Jairajpuri, 1970; Cobb, 1917; Ishaque et al., 2021; Handoo et al., 2021).

It differs from *M. williamsi* and *M. contractus* by having a larger body size (1–1.5 μm vs 0.9–1 μm and 0.6–0.7 μm); vulva position more posterior ($V = 62\text{--}68\%$ vs $V = 55\text{--}58\%$ and $57\text{--}63\%$) and shorter tail ($c' = 0.8$ vs 1.6–1.9 and 1–1.6). *Mylonchulus laocaiensis* sp. n. can be distinguished from *M. brachyuris*, *M. capsicum* and *M. shamimi* by having larger body size (1–1.5 μm vs 0.6–1 μm, 0.9–

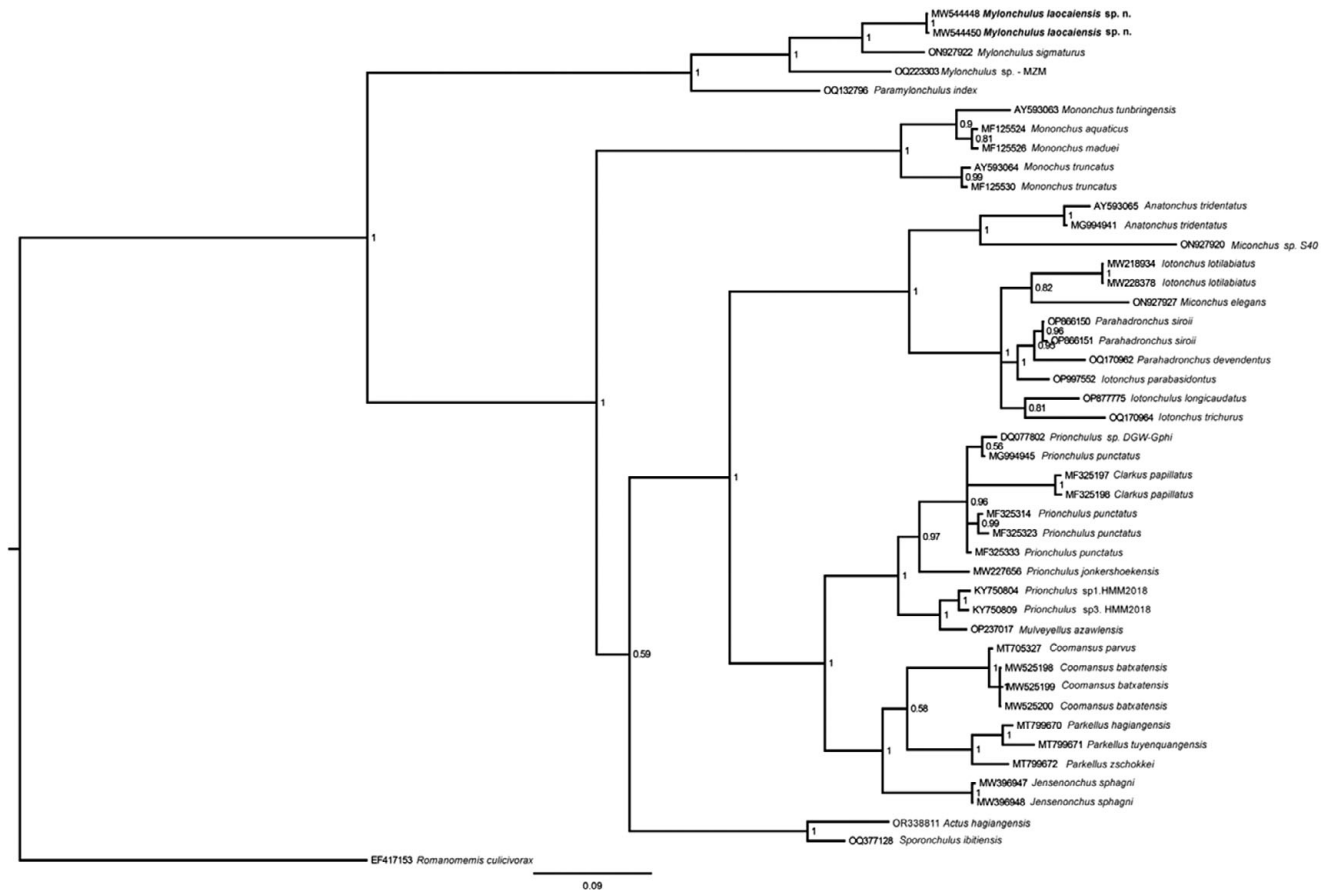


Figure 5. Bayesian Inference tree from the newly sequenced *Mylonchulus laocaiensis* sp. n. based on sequences of the 28S rDNA region. Bayesian posterior probabilities (%) are given for each clade. Scale bar shows the number of substitutions per site.

1 μm); in number of transverse rows of denticles (5 vs 6–7); lower c' value (0.8–1 vs 1–1.7) and larger buccal cavity (26–29 \times 14–6.5 vs 18–25 \times 9–15 or 20–22 \times 10–12 or 26 \times 13).

Mylonchulus laocaiensis sp. n. is also similar to *M. hawaiiensis* (Cassidy, 1931) Goodey, 1951 and *M. lacustris* (Cobb, 1915) Andr ssy, 1958 based on group of characters: female didelphic, body length, vulva position, tail conoid shape; having a subventral tooth and presence of males (Goodey, 1951; Andr ssy, 1958). *Mylonchulus laocaiensis* sp. n. differs *M. lacustris* by shorter tail ($c' = 0.8$ vs 1.7–2.8); spinneret position (subdorsal tail terminus vs central tail terminus) and spicule length (54–57 μm vs 44 μm). *Mylonchulus laocaiensis* sp. n. can be distinguished from *M. hawaiiensis* by number of transverse rows of denticles (5 vs 6); more posterior position of dorsal tooth apex (79–88% vs 69–79%); lower c' value (0.8–1 vs 1.3–2); spinneret position (subdorsal tail terminus vs central tail terminus) and spicule length (54–57 μm vs 40–44 μm).

Sequence analysis

Molecular sequences of two individuals of *Mylonchulus laocaiensis* sp. n. were analysed in this study. After sequencing and editing, two sequences were obtained: two 885–932 bp nearly full length of SSU rRNA (18S), were deposited at the GenBank accession number MW544445, MW544446 (sequences were 100% identical) and two 804–824 bp length D2D3 expansion of rRNA (28S), were deposited at the GenBank accession number MW544448, and MW544450 (sequences were 99.8% similar) (Tables 2 and 3).

The interspecific nucleotide variation within the acquired 18S rDNA sequences was: *M. laocaiensis* sp. n. vs *M. oceanicus* (accession number AB361445) = 1.6%; vs *M. hawaiiensis* (accession number JQ742964, AB361441–AB3614453) = 1.8–2.1%; vs *M. brachyuris* (accession number AB361437) = 2.0%; vs *M. sigmaturus* (AY284756) = 2.4% and vs *M. arenicola* (accession number AF036596) = 4.1% (Table 2). Furthermore, *Mylonchulus laocaiensis* sp. n. differs from *Paramylonchulus mulveyi* (accession number AB361448) = 6.1%; vs *P. iranicus* (accession number OQ101907) = 4.5% (Table 2).

Based on the 28S rDNA sequences, the dissimilarity of nucleotides between *Mylonchulus laocaiensis* sp. n. vs *M. sigmaturus* (accession number ON927922) = 10% and vs *Paramylonchulus index* (accession number OQ132796) = 24.4% (Table 3).

rDNA phylogenetic relationships among Mononchina

The results derived from the analyses of these 18S and D2–D3 regions of 28S sequences are presented in the molecular trees of Figures 4 and 5, respectively. In the phylogenetic tree of 18S, the new species *Mylonchulus laocaiensis* sp. n. clustered with the other species of the genus presented, *M. oceanicus* (AB361443–AB361445), *M. hawaiiensis* (AB361440–AB361442, JQ742964), *M. rotundicaudatus* (AY284751), *M. sigmaturus* (AB361447, AY284755–AY284757, ON927922), *M. brachyuris* (AB361436, AB361437, AY284754), *M. arenicolus* (AF036596) and positioned as a clade of *Mylonchulus* genus. The new species *Mylonchulus laocaiensis* sp. nov. is sister to *M. oceanicus* but they are clearly

separated, with high genetic distance (Figure 4). *Mylonchulus* species were grouped together with genera *Paramylonchulus* (*Paramylonchulus mulveyi* (AB361448, AB361449), *P. iranicus* (OQ101907)) and presented as subfamily Mylonchulinae. The subfamily Mylonchulinae is sistered with subfamily Sporonchulinae (*Sporonchulus* (*S. ibitiensis* (accession number OQ377123)) and *Actus* (*A. salvadoricus* accession number AB361035)). This phylogenetic tree to those reported by Ollia et al. (2008), Aliramaji et al. (2023), Singh et al. (2023), Vu et al. (2024). Conversely, 28S tree presents Mylonchulinae sequences (*Mylonchulus laocaiensis* sp. n. (accession number MW544448-544450), *M. sigmaturus* (accession number ON927922), *Mylonchulus* sp_MZM (accession number OQ223303) and *Mylonchulus* (*Paramylonchulus*) *index* (accession number OQ132796)) forming a 100% supported clade Mylonchulidae, which is the sister group of the remaining taxa included in another (Figure 5). Both species of the genus *Mylonchulus* (*Mylonchulus laocaiensis* sp. n. and *M. sigmaturus*) differ from *Mylonchulus* (*Paramylonchulus*) *index* by 18.8%–24.4% base pairs (Table 3) which supports well the validity of the genus *Paramylonchulus* (following Aliramaji et al., 2023). The second major clade therefore consists of four subclades, namely (((Mononchinae + ((Sporonchulinae + (Anatonchidae + Prionchulinae))), but the resolution is not totally satisfactory as the clade ((Sporonchulinae + (Anatonchidae + Prionchulinae)) presents low support. The same results were reported by Singh et al. (2023) and Vu et al. (2024). The analysis of D2-D3 regions of 28S sequences are always more believable than 18S because they have mutations on these regions. Neither of the trees confirms the systematic division of Mononchina suborder into two superfamilies: Mononchoidea and Anatonchoidea, a system that have been accepted until now (Jairajpuri, 1969; Andrassy, 1976, 2009). Interestingly, is that not only species of family Mylonchulidae (genera *Mylonchulus* and *Paramylonchulus*), but also some other genera such as *Mulveyellus* and *Jensenonchus* (family Anatonchidae) did not place in the family based on the 28S tree (Singh et al., 2023; Vu et al., 2024). The authors believe that the shape of buccal cavity is an important characteristic for the division of the suborder Mononchina rather than the non-tuberculate or tuberculate pharyngo-intestinal junction. Thus, further molecular research should be conducted to elucidate this matter.

Acknowledgement. This study is a part of research project “Studies on selected nematode taxa of the orders Mononchida and Dorylaimida from Vietnam and Bulgaria”, grant number QTBG01.04/23-24 funded by the Vietnam Academy of Science and Technology (VAST).

Author contribution. Vu T.T.T. and Peneva V. analysed morphometric data, morphological characteristic, gave a line-drawing and photos, prepared and submitted manuscript. Le T.M.L. analysed DNA data. All authors are in agreement about the content of the manuscript.

Data availability statement. Holotype, 15 female paratypes and one male paratype have been deposited at the Department of Nematology, Institute of Ecology and Biological Resources, Vietnam. Ten female paratypes and one male paratype deposited at the VNMN of the Vietnam Academy of Science and Technology (VAST), Vietnam. The DNA sequence have been deposited in the GenBank database.

Competing interest. Authors declare that they have no competing interests.

Ethics approval consent to participate. Not applicable.

Consent for publication. Not applicable.

References

- Ahmad W and Jairajpuri MS (2010) Mononchida: the predaceous nematodes. Brill Leiden-Boston, 298p.
- Aliramaji F, Taheri A and Shokoohi E (2023) Description of *Paramylonchulus iranicus* sp. n. (Nematoda: Mononchida) from Iran. *Journal of Helminthology*, 97: e53 (1–10). DOI: <https://doi.org/10.1017/S0022149X23000366>
- Andrassy I (1958) Über das System der Mononchiden (Mononchidae Chitwood, 1937). *Annales Historico-Naturalis Musei Nationalis Hungarici* 50: 151–171.
- Andrassy I (1976) *Evolution as a basis for the systematization of nematodes*. London, UK, Pitman Publishing, 288 pp.
- Andrassy I (1986) Fifteen new nematode species from the southern hemisphere. *Acta Zoologica Hungarica* 32:1–33.
- Andrassy I (1992) A taxonomic survey of the family Mylonchulidae (Nematoda). *Opuscula Zoologica Budapest* 25:11–35. https://opuscula.elte.hu/PDF/Opuscula25_1992/Andrassy,%20I_25_2.pdf
- Andrassy I (2009) Free-living nematodes of Hungary. III. *Pedozoologica Hungarica* N°5. *Hungarian Natural History Museum*. Budapest, Hungary, 608 pp.
- Clark WC (1961) The Mononchidae (Enoplida, nematode) of New Zealand. IV. The genus *Mylonchulus* (Cobb, 1916) Penak, 1953. *Nematologica* 6: 1–6.
- Cobb NA (1916) Notes on new genera and species of nematodes. Four subdivisions of *Mononchus*. *Journal of Parasitology* 2:195–196.
- Cobb NA (1917) The mononchs (*Mononchus* Bastian, 1865). A genus of free-living predatory nematodes. Contributions to a science of Nematology VI. *Soil Science* 3:431–484. <https://doi.org/10.1097/00010694-191705000-00004>.
- Floyd RM, Rogers AD, Lamshead PJD and Smith CR (2005) Nematode-specific PCR primers for the 18S small subunit rRNA gene. *Molecular Ecology Notes* 5(3), 611–612. DOI:10.1111/j.1471-8286.2005.01009.x
- Goodey T (1951) *Soil and freshwater nematodes*. London, UK, Methuen, 390 pp.
- Handoo ZA, Kantor MR and Khan E (2021) Description of ten new species including two new genera of nematodes (Nematoda) associated with economically important crops of Kashmir Valley, Jammu and Kashmir. *Pakistan Journal of Nematology* 39(1):24–40. DOI | <https://doi.org/10.17582/journal.pjn/2021/39.1.24.40>.
- Holterman M, Rybarczyk K, Evan den Elsen S, van Megen H, Mooyman P, Peña Santiago R, Bongers T and Helder J (2008) A ribosomal DNA-based framework for the detection and quantification of stress-sensitive nematode families in terrestrial habitats. *Molecular Ecology Resources* 8:23–34. DOI: 10.1111/j.1471-8286.2007.01963.x.
- Ishaque U, Iqbal EY, Kazi N, Dawar S and Raza S (2021) Description of *Mylonchulus capsicum* n. sp., with observation on *M. polonicus* and *M. maritimus* (Mononchida: Mylonchulidae) from Pakistan. *Pakistan Journal of Phytopathology* 33(2):275–282. DOI: 10.33866/phytopathol.033.02.0664.
- Jairajpuri MS (1970) Studies on Mononchida of India. II. The genera *Mononchus*, *Clarkus* n.gen. and *Prionchulus* (Family Mononchidae Chitwood, 1937). *Nematologica* 16:213–221. <https://doi.org/10.1163/187529270X00225>.
- Jairajpuri MS (1969) Studies on Mononchida of India. I. The genera *Hadronchus*, *Iotonchus* and *Miconchus* and a revised classification of Mononchida, a new order. *Nematologica* 15(4):557–581. <https://doi.org/10.1163/187529269X00894>
- Khan HA and Saeed M (1987) *Pakmylonchulus*, new genus (Mononchida: Mylonchulidae) with notes on *Mylonchulus nainitalensis* Jairajpuri, 1970 and *M. sigmaturus* (Cobb, 1917) Altherr, 1953 from Pakistan. *Pakistan Journal of Zoology* 19: 313–320.
- Khan WU and Jairajpuri MS (1979) Studies on Mononchida of India XIII. The genus *Mylonchulus* (Cobb, 1916) Altherr, 1953 with descriptions of three new species. *Nematologica*. 25:406–418.
- Larget B and Simon DL (1999) Markov Chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* 16, 750–759.
- Loof PAA (1993) Status of *Mylonchulus cavenis* W. Schneider, 1940 (Nematoda: Mononchida). *Nematologica* 39:177–182. https://brill.com/view/journals/nema/39/1-4/article-p177_15.xml.

- Loof PAA** (2006) Mononchina (Dorylaimida) from Western Malaysia. *Nematology* **8**(2):287–310. https://brill.com/view/journals/nemy/8/2/article-p287_12.xml.
- Olia M, Ahmad W, Araki M, Minaka N, Oba H and Okada H** (2008) *Actus salvadoricus* Baqri & Jairajpuri (Mononchida, Mylonchulidae) from Japan with comment on the phylogenetic position of the genus *Actus* based on 18S rDNA sequences. *Japanese Journal of Nematology* **38**:57–69. <https://agris.fao.org/agris-search/search.do?recordID=JP2009002243>.
- Orselli L and Viciguerra MT** (2007) Three new and rare species of Mononchida (Nematoda) from Ecuador. *Journal of Nematode Morphology and Systematics* **9**(2):137–146. <https://revistaselectronicas.ujaen.es/index.php/jnms/article/view/166>.
- Rambaut A** (2018) Figtree, a graphical viewer of phylogenetic trees. Available at: <https://github.com/rambaut/figtree/releases/tag/v1.4.4>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA and Huelsenbeck JP** (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **6**: 539–542. DOI:10.1093/sysbio/sys029.
- Seinhorst JW** (1962) On the killing, fixation and transferring to glycerine of nematodes. *Nematologica* **8**:29–32. DOI: 10.1163/187529262X00981
- Siddiqi MR** (2015) On the identity of *Mononchus digiturus* Cobb, 1893, *Mononchus minor* Cobb, 1893, *Mononchus trionchus* Thorne, 1924, *Mononchus montanus* Thorne, 1924 and *Mononchus amphigonius* Thorne, 1924. *International Journal of Nematology* **25**(1):77.
- Shokoohi E, Mehrabi-Nasab A, Mirzaei M and Peneva V** (2013) Study of mononchids from Iran, with description of *Mylonchulus kermaniensis* sp. n. (nematoda: Mononchida). *Zootaxa*. **3599**(6):519–534.
- Singh SS, Singh PR, Singh SM, Singh MOK and Meitei NM** (2023) Morphological and molecular characterisation of *Mulveyellus aizawlensis* sp. n. (Nematoda: Iotonchinae) from Aizawl, Mizoram, India. *Nematology* **25**:717–728. DOI:10.1163/15685411-bja10250.
- Southey JF** (1986) *Laboratory methods for work with plant and soil nematodes*. London: Her Majesty's Stationery Office.
- Subbotin SA, Sturhan D, Chizhov VN, Vovlas N and Baldwin JG** (2006) Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rDNA gene sequences. *Nematology* **8**: 455–474.
- Vu TTT** (2016) New data of two *Mylonchulus* species (Mononchida: Mylonchulidae) and an updated key to species from Vietnam. *Journal of Biology* **38**(3): 287–292. DOI: <https://doi.org/10.15625/0866-7160/v38n3.8023>
- Vu TTT, Nguyen AD and Peña-Santiago R** (2024) Updated taxonomy and new insights into the evolutionary relationships of the genus *Sporonchulus* Cobb, 1917 (Nematoda, Mononchida) after the study of two Vietnamese species. *Zoosystematics and Evolution* **100**: 155–166. <https://doi.org/10.3897/zse.100.118675>
- Yeates G** (1992) Nematodes from New Caledonia. 1. Introduction and Mononchoidea. *Fundamental and Applied Nematology* **15**(2):101–126.
- Yoder M, De Ley IT, King I, Mundo-Ocampo M, Mann J, Blaxter M, Poiras L and 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>
- Zullini A and Peneva V** (2006) Order Mononchida, 468–496. In Abebe E, Andrassy I, Traunspurger W (Eds) *Freshwater nematodes: Ecology and taxonomy*. CABI publishing, 752 p.