ACADEMIA JOURNAL OF BIOLOGY 2026, 48(1): 1–9
DOI: 10.15625/2615-9023/23236

REDESCRIPTION WITH NEW MOLECULAR DATA OF RARE GENUS AND SPECIES *Iotonchulus longicaudatus* Baqri, Baqri and Jairajpuri, 1978 (Mononchida, Iotonchidae) FROM VIETNAM

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Received 13 June 2025; accepted 16 March 2026

ABSTRACT

The rare genus and species *Iotonchulus longicaudatus* (Baqri, Baqri & Jairajpuri, 1978) Andrássy, 1993b was previously found only in India. This species was first recorded in Vietnam at the Cat Tien National Park (Ho Chi Minh City) and is currently recorded in several natural areas: Phia Oac (Cao Bang), Van Ban (Lao Cai) and Con Dao Island (Ho Chi Minh City). The new illustrations with re-description and new molecular data were presented.

Keywords: Taxonomy, 18S rDNA, 28S rDNA, distribution.

Citation: Vu Thi Thanh Tam, Le Thi Mai Linh, 2026. Redescription with new molecular data of rare genus and species *Iotonchulus longicaudatus* Baqri, Baqri and Jairajpuri, 1978 (Mononchida, Iotonchidae) from Vietnam. *Academia Journal of Biology*, 48(1): 1–9. https://doi.org/10.15625/2615-9023/23236

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INTRODUCTION

The genus *Iotonchulus* was proposed by Andrássy (1993) when he separated from the genus *Mulveyellus* Siddiqi, 1984 and transferred the species *Mulveyellus longicaudatus* (Baqri, Baqri & Jairajpuri, 1978) Siddiqi, 1984 to the new genus. The new genus *Iotonchulus* is distinguished from *Mulveyellus* by its shaped tail (filiform *vs* elongate conoid). Two other species from the genus *Iotonchus* Cobb, 1916, as *Iotonchus bangkokensis* Buangsuwon & Jensen, 1966, *Iotonchus longicaudatus* Baqri, Baqri & Jairajpuri, 1978 and *Iotonchus ophiocercus* Clark, 1961 were moved to the new genus *Iotonchulus*. The new genus *Iotonchulus* also differs from the genus *Iotonchus* by the position of the dorsal tooth located close to the middle of the buccal cavity. The species *Iotonchulus longicaudatus* (Baqri, Baqri & Jairajpuri, 1978) Andrássy, 1993 became the type species. Ahmad & Jairajpuri, when reviewing the order Mononchida, moved the species *Iotonchus darreni* Ahmad, Baniyamuddin & Jairajpuri, 2006, to the genus of *Iotonchulus* (Ahmad & Jairajpuri, 2010). Siddiqi (2015) erected the new genus *Supronchus*, which was different from the genus *Iotonchulus* by having a position of dorsal tooth in the posterior half of the buccal cavity and transferred two species of *Iotonchulus bangkokensis* (Buangsuwon & Jensen, 1966) Andrássy, 1993b and *Iotonchulus darreni* (Ahmad, Baniyamuddin & Jairajpuri, 2006) Andrássy, 1993 to the genus *Supronchus*. Siddiqi has also moved the species *Iotonchulus ophiocercus* (Clark, 1961) Andrássy, 1993 to the other new genus of *Megaiotonchus* Siddiqi, 2015 (Siddiqi, 2015). Therefore, until now, the genus *Iotonchulus* Andrássy, 1993 contains only one type species *I. longicaudatus* (Baqri, Baqri & Jairajpuri, 1978) Andrássy, 1993.

The species *I. longicaudatus* (Baqri, Baqri & Jairajpuri, 1978) Andrássy, 1993 was first described by Baqri, Baqri & Jairajpuri (1978) with the original population from Darjeeling, West Bengal (India). In Vietnam, *I. longicaudatus* was first reported in National Park Cat Tien, Ho Chi Minh City (Nguyen, 2000) with low qualification of the drawing and poor description. In the current paper, *I. longicaudatus* (Baqri, Baqri & Jairajpuri, 1978) Andrássy, 1993 is redescribed, distributed with the first molecular data from Vietnam.

MATERIALS AND METHODS

Sampling, extraction and processing of nematodes

Soil samples were randomly collected from several natural forests in Lao Cai, Cao Bang provinces and Ho Chi Minh City (Vietnam). Nematodes were extracted from soil samples using the Baermann funnel technique (Southey, 1986). Specimens were killed at 70 ^{o}C by hot water, fixed in TAF solution (Southey, 1986), transferred to anhydrous glycerol (Seinhorst, 1959), and mounted on glass slides for microscopic observation. Measurements were performed with a Nikon digital camera on a Nikon Eclipse *Ni* microscope at the Institute of Biology, 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. Photographs were edited using Adobe Photoshop CC 2018.

DNA isolation, amplification and sequencing

Nematodes were fixed in DESS solution (Yoder et al., 2006). Genomic DNA was isolated from single individuals as described by Holterman et al. (2006) and stored at -20 ^{o}C until used as a PCR template. The D2-D3 expansion segment of 28S was amplified using the forward D2A (5'-ACAAGTACCGTGGG GAAAGTTG-3') and reverse D3B (5'-TCGG AAGGAACCAGCTACTA-3') primers (Subbotin et al., 2006), and the 18S rDNA fragment was amplified using the primers 18S (18F: 5'-TCTAGAGCTAATACATGCAC-3'/18R: 5'-TACGGAAACCTTGTTACGAC-3'). 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), 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 UV illumination after Simplisafe gel staining and gel electrophoresis.

Phylogenetic analyses

For reconstructing the phylogenetic relationships, analyses were based on 18S and 28S rDNA sequences. The newly obtained rDNA sequences were analysed using BioEdit and aligned with sequences available in GenBank using the ClustalW alignment tool implemented in the MEGA 7 version 11.0 (Kumar et al., 2016). The final 18S and 28S rDNA datasets for phylogenetic study included sequences from the present study and available sequences of members of the Dorylaimidae retrieved from GenBank. The prepared multiple alignments of 28S rDNA generated by the ClustalW algorithm were routinely manually edited in order to eliminate improper phylogenetic signals. Representative *Mononchus* sequences were used as the outgroup. The phylogenies were constructed with the MEGA 7 version 11.0. Maximum likelihood with the K2+G+I substitution model for 28S; T92+G+I substitution model for 18S data set was used. Genetic distances (number of nucleotide positions and uncorrected p-distance) were calculated in MEGA7.

RESULTS AND DISCUSSION

Order: MONONCHIDA Jairajpuri, 1969

Family: Iotonchidae Jairajpuri, 1969

Genus: *Iotonchulus* Andrássy, 1993b

***Iotonchulus longicaudatus* (Baqri, Baqri and Jairajpuri, 1978) Andrássy, 1993** (Fig. 1)

Synonym: *Iotonchus longicaudatus* Baqri, Baqri and Jairajpuri, 1978

Mulveyellus longicaudatus (Baqri, Baqri & Jairajpuri, 1978) Siddiqi, 1984

Redescription

Measurements: Nine females in good condition. Measurements in Table 1.

Female: Nematodes slender to very slender (a = 38–45) and small sized nematodes 1.1–1.3 mm long, habitus curved ventrally in the posterior half after fixation in TAF solution, tapering slightly anterior end to the base of the pharynx but sharply towards the posterior end. Maximum body width at level of vulva, 25–32 µm wide. Cuticle smooth, two layers, 1–1.5 µm thick at the base of the pharynx. Lip region round, offset with the body contour by constriction, 18–22 µm width, 2.4–2.8 times as wide as high. Lips moderately separated, prominent labial and cephalic sensilla. Amphidial fovea small, cup-shaped, its aperture oval transverse, 3–4 µm wide, located at the level of the anterior end of the buccal cavity or slightly lower. Vestibulum 6–7 µm long. Buccal cavity barrel-shaped, 1.7–1.9 times as long as wide, its walls medium cuticularized. Dorsal tooth medium size, 11–13 µm long from base to apex, forward directed, its apex pointed, situated at 49–61% of buccal cavity length from stoma base. Two foramina are present at the base of the buccal cavity, lying close to each other. The first fifth from posterior end of the buccal cavity is embedded in pharyngeal tissue.

Pharynx cylindrical, 229–280 µm, nerve ring encircling pharynx, located at 80–96 µm or 33–38% of pharynx length. Secretory-excretory pore (SE-pore) distinct, situated posterior to the nerve ring or 35–41% of pharynx length. Pharyngo-intestinal junction tuberculate (Fig. 1B). Rectum straight, as long as one anal body width. Tail very long, filiform, 296–343 µm or 14–18 times the anal body diameter long. Three caudal glands well developed, lying in tandem and opening subventral terminally on the tail terminus (Fig. 1D). Three pairs of caudal pores located in the dorsal side of the first fifth to anterior end of the tail (Fig. 1E).

Genital system mono-prodelphic, with anterior branch well developed, 110–214 µm occupying 10–16% of total body length. Ovaries reflexed with oocytes first arranged in several rows and then in a single row, 50–100 µm length; sphincter between oviduct and ovary well developed. The uterus tube simple

and short, uterin eggs were seen in several specimens, 96–115 × 28–32 µm. Vagina length 7–11 µm occupying 25–33% corresponding body diameter. *Pars proximalis vaginae* short; *pars refringens vaginae* small 1.5–2 × 1.5 µm, sclerotized in round-shaped pieces; *pars distalis vaginae* 5–8 × 2–3 µm. Vulva transverse, slit-like, at 57.5–61% of body length. Post uterus sac shortened, occupying 50–70% of the anal body diameter long.

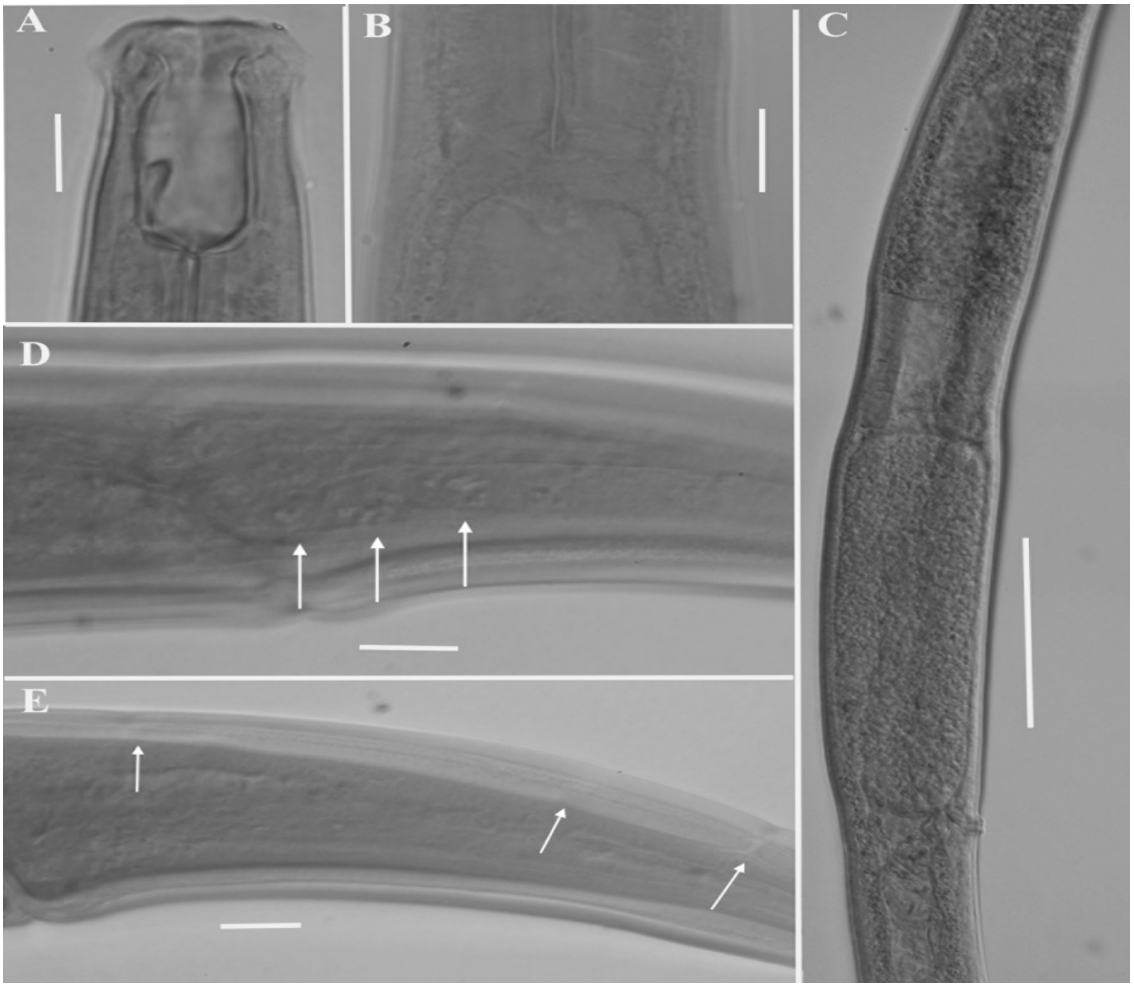

Figure 1. Iotonchulus longicaudatus (Baqri, Baqri and Jairajpuri, 1978) Andrássy, 1993b. A: Head region (dorsal tooth positon in the buccal cavity); B: Tuberculate valve in pharyngo-intestinal junction; C: Female reproductive system; D: Three caudal glands in tail region; E: Three pairs of caudal pores in dorsal side of tail (scare bars: A, B, D, E = 10 µm; C = 50 µm)

Remarks: The measurements of Vietnamese specimens corresponded well with those of the holotype and paratype specimens of the type population from Darjeeling, West Bengal, India (Baqri et al., 1978) and see Table 1.

Localities: Phia Oac National Park (22°36’29”N, 105°51’58”E, altitude at 1,710 m), Cao Bang province. Other localities are Hoang Lien-Van Ban Natural Reserve (22°02’26”N, 103°57’42”E, altitude at 1,060 m), Lao Cai province and Con Dao National Park (08°55’11”N, 106°55’58”E, altitude at 30 m), Con Dao island, Ho Chi Minh City.

Table 1. Comparison of morphometric data of *Iotonchulus longicaudatus* (Baqri, Baqri & Jairajpuri, 1978) Andrássy, 1993b (all measurements are in μm except indicated and ratios V, a, b, c, c')

Local	India			Malaysia	Vietnam			
	Darjeeling, West Bengal		Kerala	West Malaysia	Cat Tien, Ho Chi Minh City	Phia Oac, Cao Bang	Hoang Lien-Van Ban, Lao Cai	Con Dao, Ho Chi Minh city
Reference	Baqri et al., 1978		Mohandas & Prabhoo, 1979	Loof, 2006	Nguyen, 2000	Current paper		
Character	Holotype	Paratypes						
N	♀	♀♀ (n = 3)	♀♀ (n = 5)	♀♀ (n = 2)	♀ (n = 1)	♀ (n = 1)	♀♀ (n = 6)	♀♀ (n = 2)
L (mm)	1.3	1.08–1.32	1.09–1.2	1.31–1.33	1.34	1.34	1.1–1.33 (1.2 ± 0.1)	1.05; 1.1
V (%)	61	59–61	60–61	55–56	60	60.2	57.5–60 (58.8 ± 0.1)	59; 61
a	40	39–45	32–34	41–44	38	41.9	39.5–44 (42.7 ± 1.9)	38; 38
b	4.4	3.5–4.2	4.4–4.6	4.7	5.5	4.8	4.3–4.8 (4.6 ± 0.2)	4.5; 4.3
c	4.1	3.4–4.0	3.7–4	3.3–3.4	3.6	4.1	3.4–3.9 (3.7 ± 0.2)	35; 4.5
c'	13.3*	-	13.5–14.4	21	15.3	16.4	14.3–18 (16.7 ± 1.3)	15.1; 14
Buccal cavity length	24.2*	21–24	22–25	24	22.5	22.6	21.4–23.8 (22.4 ± 1)	22.6; 23.3
Buccal cavity width	15*	12–15	12–13	12	13	12	11–14 (12.9 ± 1.1)	11.4; 15
Position of dorsal tooth apex (%)	62.5*	50–66	65–68	55–57	49	49.3	48–58.6 (52.2 ± 4.2)	50.8; 61

Lip region width	20*	17–21	21–22	19–20	20	21.6	18–21 (19 ± 1)	18.7; 22
Lip region heigth	-	8–11	7–8		9	8.6	8–11.2 (9.5 ± 1.2)	8; 9
Pharynx length	-	-		281–289	243	278	229–280 (259.5± 19)	234; 256
Distance from nerve ring to the anterior end		88–98	82		90	86	80–96 (87.5 ± 6)	80; 86
Body diameter at the neck base	-	-				29.5	24–31 (28± 2.7)	29.3; –32
Body diameter at the vulva	32*	-		29–32	35	32	25–31 (28.3 ± 2.4)	28; 32
Body diameter at the anus	23*	-		19		20	18-24 (19.7 ± 2.4)	19.6; 23.7
Anterior branch of reproductive system	163*	-				204	146–214(174 ± 30)	109; 146
Post uterus sac	13*	-				14.4	15.3–17.7 (16.6 ± 1)	-; 15
Vagina length	11*	-				7.2	7–10 (9± 1.3)	11.6; 11.6
Rectum length	14.5*	14–18				20	15.7–21 (18± 2)	18; 22
Tail length	290*	283–334	290–310	396–399	370	330	302–343 (326.3± 16.7)	296; 313

Note: - no information; * calculate from original illustrations.

Sequence analysis

After sequencing and editing, two sequences were obtained for phylogenetic analyses: one 802 bp long 18S rDNA (accession PQ836286) and another 753 bp long 28S rDNA (accession PQ817663). A Blaster search showed that the 18S sequence was 100% identical to a sequence OP877774 assigned to *I. longicaudatus* (Indian population), 99.6% to sequence accession OP997536 assigned to *Iotonchus parabasidontus* Clark, 1961, 99.4% to sequence OQ170963 *Iotonchus trichurus* Cobb, 1917, 99.6% to sequence OP864955.1 *Parahadronchus siroii* Renubala & Dhanachand, 1992, respectively. For its part, the 28S sequence was 98.9% identical to OP877775 *I. longicaudatus* (Indian population), 92% to a sequence OQ170964 *I. trichurus* Cobb, 1917, 92.6% to a sequence *Iotonchus lotilabiatus* Vu, Le & Nguyen, 2021, 92.3% to sequence OP997552 *I. parabasidontus* Clark, 1961, 92.8% to sequences (OP866150 & OP866151) *Parahadronchus siroii* Renubala & Dhanachand, 1992 and 98.3% to sequence OQ170962 assigned to *Parahadronchus divendentus* Sushilkumar, Mexico & Mohilal, 2023.

Male: Not found.

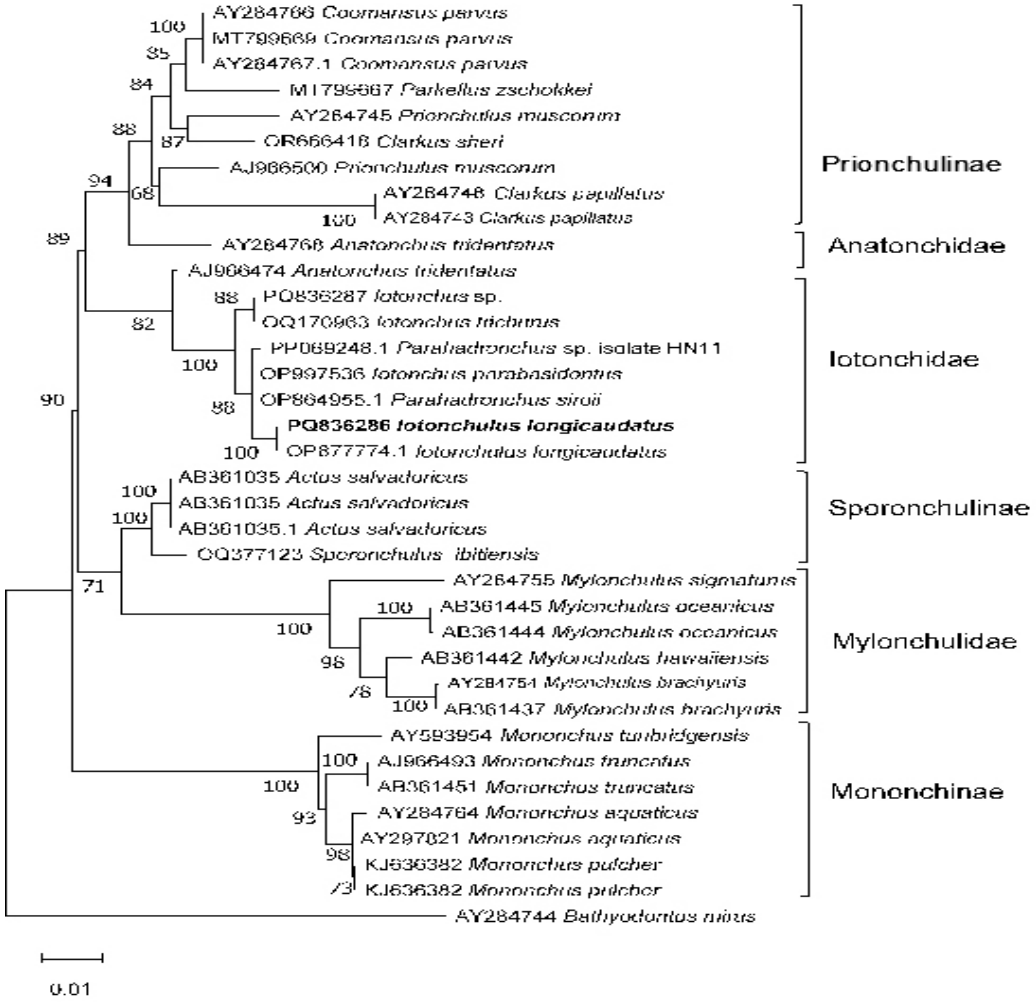

Figure 2. Phylogenetic relationships of *Iotonchulus longicaudatus* from the analysis of the 18S rDNA sequences under ML (K2+G+I model). Numbers to the left of the branches are bootstrap values for 1000 replications

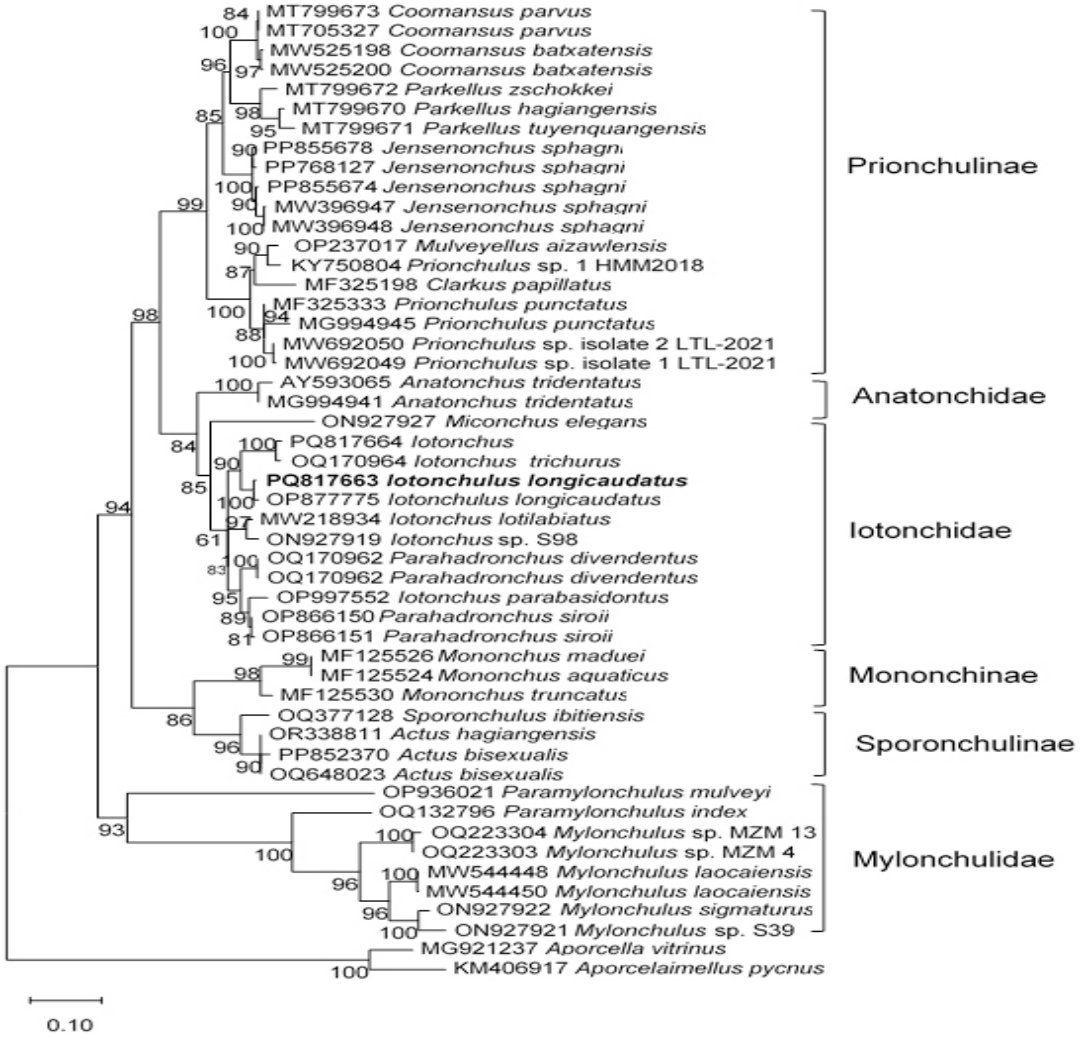

Figure 3. Phylogenetic relationships of *Iotonchulus longicaudatus* from the analysis of the 28S rDNA sequences under ML (K2+G+I model). Numbers to the left of the branches are bootstrap values for 1000 replications

rDNA phylogenetic relationships among Mononchida

The results derived from the analyses of these 18S and D2-D3 regions of 28S sequences are presented in the phylogeny trees of Figures 2 and 3, respectively. In both phylogenetic trees, the species I. longicaudatus clustered and positioned within the family Iotonchidae clade, encompassing representatives of species belonging to genera Iotonchus and Parahadronchus. This clade is sistered to the species of the family Anatonchidae (including genera Anatonchus and Miconchus) (Figs. 2 & 3). The same results were recorded previously (Holterman et al., 2008; Vu et al., 2021; Vu et al., 2025). This positioning was confirmed by phylogenetic analyses based on both the 18S and 28S rDNA data.

Acknowledgements: This result is a part of the research project "Biodiversity of predatory free-living nematodes in the order Iotonchidae at Cao Bang province", grant number NCVCC09.02/25-25, funded by the Vietnam Academy of Science and Technology.

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