

Research Paper

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Description of a new species *Coomansus batxatensis* (Mononchida, Mononchidae) from Vietnam, with an updated key to species

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Abstract

Coomansus batxatensis sp. nov., recorded from Vietnam, is described and illustrated and its phylogenetic relationship within the Mononchida is analysed. The molecular data (18S and 28S ribosomal DNA) are provided for the new species. The new species is characterized by small body size (body length, L = 0.7–0.9 mm); buccal cavity sub-rectangular in shape, flattened at base, 21–24 × 12–13 μm or 1.9 (1.7–2.0) times as long as wide; posterior position of dorsal tooth apex (59–63% from the base of buccal cavity); *pars refringens vaginae* with faint and small (2.5 × 1.7 μm) teardrop-shaped pieces, short *pars distalis vaginae*; and males with short spicules (50–51.5 μm) with rounded head and conical blade part. The new species is close to *Coomansus parvus* but differs from it by the smaller buccal cavity size, more posterior position of the dorsal tooth apex, longer tail and presence of males. An updated identification key to *Coomansus* species and a compendium of all the species known are presented.

Introduction

The genus *Coomansus* was proposed by Jairajpuri & Khan (1977) based on the type species *Coomansus parvus* (de Man, 1880) and several species which were transferred from the genus *Clarkus* Jairajpuri, 1970. The genus *Coomansus* differs from the genus *Clarkus* Jairajpuri, 1970 in having a weak longitudinal ridge opposite to dorsal tooth, anteriorly gradually merging into the ventral wall (Jairajpuri, 1970; Ahmad & Jairajpuri, 2010). Loof & Winiszewska-Ślipińska (1993) have reviewed this genus *Coomansus* and transferred nine species of ‘*zschokkei*-group’ from the genus *Iotonchus* Cobb, 1916 and the species *Clarkus major* (Cobb, 1893) Jairajpuri, 1970 to the genus *Coomansus*. On the other side, the species *Coomansus sphagni* (Brzeski, 1960) Jairajpuri, 1970 was moved to the genus *Iotonchus*. Recently, nine species of ‘*zschokkei*-group’ were transferred from the genus *Coomansus* to the genus *Parkellus* Jairajpuri, Tahssen & Choi, 2001 based on the more posterior position of the dorsal tooth apex (Ahmad & Jairajpuri, 2010). The main feature of the genus *Coomansus* that distinguishes it from the genus *Parkellus* is the location of the dorsal tooth apex in the anterior half of the buccal cavity (Jairajpuri *et al.*, 2001). The main diagnostic features of the genus are: (1) buccal cavity barrel-shaped with one dorsal tooth situated in the anterior half of buccal cavity; (2) presence of weak longitudinal ridge on ventral wall; (3) non-tuberculate cardia; (4) didelphic–amphidelphic female reproductive system; and (5) conoid, ventrally curved tail, 2–6 anal body diameter long without spinneret at the tail terminus.

Currently, 24 *Coomansus* species have been described, with the four most recent species discovered being *Coomansus inca* Andrásy, 2011 from Peru, *Coomansus mapuche* Andrásy, 2011 from Chile, *C. papua* Andrásy, 2011 from Papua New Guinea and *Coomansus prodontus* Shah & Hussain, 2015 from Korea. In Vietnam, two species of the genus *Coomansus* – *C. parvus* (de Man, 1880) Jairajpuri & Khan, 1977 and *Coomansus venezolanus* (Loof, 1964) Jairajpuri, 1970 have been reported (Nguyen, 2007; Vu, 2016).

In the present study, *Coomansus batxatensis* sp. nov. is described based on morphological, morphometric and molecular data from the Bat Xat Nature Reserve in Lao Cai Province, Vietnam. An updated key to species based on females and a compendium of all the species known are also provided.

Materials and methods**Nematode extraction, preservation and morphological studies**

Soil samples were collected from a pristine forest in Lao Cai Province, Vietnam. Nematodes were extracted from soil samples using modified Baermann funnel technique (Southey, 1986).

They were heat killed, fixed in 4% formaldehyde (for morphological observations) or in a DESS mixture (Yoder *et al.*, 2006) (for molecular analyses), transferred to anhydrous glycerol (Seinhorst, 1959, 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 DS-Fi3 digital microscope camera (Minato, Tokyo, Japan) on a Nikon Eclipse Ni-U Upright microscope (Minato, Kyoto, Japan) at the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST), Vietnam. Observations of morphological diagnostic features and light microscope 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 (Minato, Tokyo, Japan). Photographs and illustrations were edited using Adobe Photoshop CC 2018 (www.adobe.com/productions/photoshop.html). Permanent slides are stored at the Department of Nematology, Institute of Ecology and Biological Resources, VAST, Hanoi, Vietnam.

DNA extraction, polymerase chain reaction (PCR) and sequencing

Nematode DNA was extracted from a single individual as described by Holterman *et al.* (2008) and DNA extracts were stored at -20° until used as PCR template. The D2–D3 expansion segment of 28S ribosomal DNA (rDNA) and 18S were amplified using the forward D2A (5'-ACAAGTACCGTGGGGAAAGTTG-3') and reverse D3B (5'-TCGG AAGGAACCAGCTACTA-3') primers (Subotin *et al.*, 2006) and primers 18S (18F: 5'-TCTAGAGCTAATACATGCAC-3'/18R: 5'-TACGGAAACCTTGTACGAC-3'). All PCR reactions contained 12.5 μ l Hot start green PCR Master Mix (2 \times) (Promega, Madison, Wisconsin, USA), 1 μ l of the forward and reverse primer (10 μ M each), the 3 μ l DNA template and sterile Milli-Q water to 25 μ l of the total volume. All PCR reactions were performed in SimpliAmp Thermal cycler (Thermo Fisher Scientific, Waltham, Massachusetts, USA) as follows: an initial denaturation step at 95 $^{\circ}$ C for 4 min, followed by 40 cycles at 95 $^{\circ}$ C for 30 s, 54 $^{\circ}$ C for 30 s and 72 $^{\circ}$ C for 60 s with a final incubation for 5 min at 72 $^{\circ}$ C. Amplicons were visualised under ultraviolet illumination after Simplisafe gel staining and gel electrophoresis. After sequencing, the obtained *C. batxatensis* sp. nov. rDNA sequence fragments were deposited in GenBank under the following accession numbers: MW525195–MW525197 (18S rDNA) and MW525198–MW525200 (28S rDNA).

Phylogenetic analyses

For phylogenetic relationships, analyses were based on 18S and 28S rDNA. The newly obtained rDNA sequences were analysed using the BioEdit sequences available in GenBank using the ClustelW alignment tool implemented in the MEGA 7 version 7.0 (Kumar *et al.*, 2016). The final 18S and 28S rDNA datasets for phylogenetic study included sequences from the current study *C. batxatensis* sp. nov. and available sequences of Mononchida representatives from GenBank. The prepared multiple alignments of 18S and 28S rDNA generated by the ClustelW algorithm were routinely manually edited in order to eliminate improper phylogenetic signals. The phylogenies were constructed with the program MEGA 7 version 7.0. Maximum

likelihood with T92+G substitution model for both 18S and 28S data sets was used.

Results and discussion

Coomansus batxatensis sp. nov.

Material examined

Holotype. Ten paratype females and one paratype male were collected at Sang Ma Sao commune. Another population including 13 females and two males was collected at Y Ty commune. All specimens were preserved in good condition.

Measurements. See table 1.

Description

Small nematodes, 0.7–0.9 mm long. Relaxed specimens arcuate, more curved ventrad at posterior end. Body tapering slightly anterior to base of pharynx but more sharply towards posterior end. Maximum body width at the level of vulva. Cuticle smooth, 2.5 (2–3) μ m thick.

Lip region rounded, 3.0 (2.7–3.3) times as wide as high, offset by a depression. Lips moderately separated and rounded. Anterior sensilla arranged in two circles: an anterior one of six inner labial papillae, posterior crown with six outer labial papillae and four cephalic papillae slightly protruding beyond the body outline. Amphid fovea funnel-like, its aperture, 3.6 (3.0–4.0) μ m wide, located 4.6 (4.0–6.0) μ m from the anterior end of buccal cavity or 4.4 (3.5–5.5) μ m to the dorsal tooth apex. Buccal cavity barrel to sub-rectangular shape, flattened at base, 1.9 (1.7–2.0) times as long as wide. Dorsal tooth medium-sized, forward directed, its apex located 60 (59–63)% of buccal cavity length from buccal cavity base. About the posterior fifth of buccal cavity embedded in pharyngeal tissues.

Nerve ring encircling the cylindrical and muscular pharynx at about 35 (32–36)% of its length. Secretory–excretory pore (SE-pore) indistinct, situated just posterior to nerve ring or 39 (38–42)% its length from anterior end. Pharyngo–intestinal junction non-tuberculate. Rectum slightly arcuate, 0.8 (0.8–1) times the anal body diameter long. Tail conical, ventrally bent, regularly tapering, with sharp tip. Caudal glands and terminal opening absent.

Female. Genital system didelphic–amphidelphic, with both branches equally developed. Ovaries on alternate sides of the intestine, well developed, with numerous oocytes, overlap the uterus–oviduct junction. Oviduct and sphincter just on the other side of the reflexed ovary. Uterus simple. Vagina length occupying about 1/3 of corresponding body diameter. *Pars proximalis vaginae* as long as wide, cylindrical, surrounded by constrictor muscles not drawn. *Pars refringens vaginae* with faint sclerotized but clear and small (2.5 \times 1.7 μ m), teardrop-shaped pieces. *Pars distalis vaginae* short. Vulva transverse in ventral view (see figures 1 & 2).

Male. Genital system diorchic, with opposed testes. Spicules are relatively slender, ventrally curved, with bifurcate terminus, 1.7–1.9 times longer than body diameter at cloacal aperture; the head of spicule round-shaped, offset by a shallow depression, conical blade part. Lateral guiding pieces with arched edges and bifurcate terminus, 12–15 μ m. This furcation is symmetrical and moderately marked. Gubernaculum is well developed, spatuliform in the terminal, 18–19.5 μ m long. Ventromedian supplements 11–12 in number, conical and regularly arranged, occupy 13.6–14.6% of the body length.

Table 1. Morphometrics of *Coomansus batxatensis* sp. nov. All measurements are in μm except where indicated.

	Bat Xat Nature Reserve, Lao Cai Province				
	Sang Ma Sao commune				
	Holotype	Paratype		Y Ty commune	
		♀	♀♀ (n = 10)	♂ (n = 1)	♀♀ (n = 13)
L	809	822.7 ± 54.4 (689–872)	799	704.1 ± 48.7 (659–798)	661; 730
Distance vulva from anterior body end	504	508.4 ± 31.1 (439–549)	–	424.6 ± 30.7 (390–467)	–
V (%)	62.3	61.8 ± 1.5 (59.5–62.5)	–	60.3 ± 0.8 (59.0–61.5)	–
a	20.6	21.3 ± 1.1 (20.0–23.3)	21.8	18.7 ± 1.3 (17.4–21.8)	18.0; 21.5
b	3.5	3.5 ± 0.2 (3.2–3.7)	3.5	3.5 ± 0.1 (3.4–3.6)	3.4; 3.5
c	9.4	10.5 ± 0.8 (9.0–11.0)	13.4	8.3 ± 1.3 (7.6–11.7)	9.3; 10.2
c'	3.6	3.3 ± 0.3 (3.0–4.0)	2.0	3.6 ± 0.3 (3.0–4.0)	2.4; 2.8
Pharynx length	228	232.8 ± 8.3 (215–242)	229	202.5 ± 11.1 (184–222)	193; 209
Nerve ring from anterior body end	82.3	82.2 ± 6.1 (70.0–86.0)	82.4	68.9 ± 5.1 (63.5–81.0)	75.0; 70.8
SE-pore from anterior body end	90.3	91.2 ± 3.9 (87.0–100.0)	96.6	84.4 ± 5.0 (78.0–95.0)	85.8; 85.5
Anterior branch of reproductive system	106	90.1 ± 11.0 (70.5–106.0)	–	79.5 ± 7.3 (67.7–88.4)	–
Posterior branch of reproductive system	87	82.5 ± 14.4 (56.5–104.0)	–	74.2 ± 7.1 (59.0–85.5)	–
Testis length	–	–	311	–	255; 321
Buccal cavity length	23.4	23.4 ± 0.6 (22.5–24.0)	23.3	22.5 ± 0.9 (21.0–24.0)	22.9; 22.7
Buccal cavity width	12.5	12.4 ± 0.4 (12.0–13.0)	12.4	12.6 ± 0.4 (12.0–13.0)	13.0; 12.1
Distance of dorsal tooth apex from the base of buccal cavity	14.0	14.3 ± 0.2 (14.0–14.5)	14.0	13.6 ± 0.7 (13.0–15.0)	13.9; 13.6
Lip region width	19.6	20.1 ± 1.0 (19.5–22.5)	21.0	19.8 ± 0.8 (19.0–21.0)	19.4; 20.8
Lip region height	6.8	6.8 ± 0.4 (6.5–7.5)	7.2	6.6 ± 0.5 (6.0–7.0)	6.6; 7.2
Body width at pharynx base	38.6	37.5 ± 2.5 (33.0–40.0)	36.7	37.7 ± 2.1 (34.5–40.5)	36.8; 34.0
Maximum body width	39.3	38.7 ± 3.5 (32.0–42.0)	–	37.7 ± 2.9 (33.5–44.0)	–
Anal body width	24.0	23.7 ± 1.3 (21.5–25.0)	29.1	24.1 ± 1.6 (21.5–27.0)	29.3; 25.7
Vagina length	14.0	13.3 ± 1.4 (10.0–14.5)	–	13.8 ± 1.0 (12.0–15.0)	–
Rectum length	19.9	19.5 ± 1.4 (17.0–22.0)	26.8	19.8 ± 1.2 (17.5–22.0)	26.3; 23.2
Tail length	86	79.0 ± 7.2 (63.0–87.0)	60	85.7 ± 10.0 (64.5–100)	71; 71

(Continued)

Table 1. (Continued.)

	Bat Xat Nature Reserve, Lao Cai Province				
	Sang Ma Sao commune				
	Holotype	Paratype		Y Ty commune	
		♀	♀♀ (n = 10)	♂ (n = 1)	♀♀ (n = 13)
Position of nerve-ring at pharynx length (%)	36.1	35.3 ± 1.9 (32.0–36.0)	36.0	34.0 ± 1.8 (30.5–35.0)	38.8; 33.9
Position of SE-pore at pharynx length (%)	39.6	39.2 ± 1.4 (38.0–42.0)	42.2	41.7 ± 1.5 (39.5–44.0)	44.4; 40.9
Position of dorsal tooth apex (%) from the base of buccal cavity	60.2	60.4 ± 1.8 (59.0–63.0)	60.2	60.0 ± 2.0 (59.0–63.0)	61.0; 60.1
Buccal cavity length / buccal cavity width	1.9	1.9 ± 0.1 (1.7–2.0)	1.9	1.8 ± 0.1 (1.7–1.9)	1.8; 1.9
Body width at pharynx base / lip region width	2.0	1.9 ± 0.1 (1.7–2.0)	1.7	1.9 ± 0.1 (1.8–2.1)	1.9; 1.6
G1 (%)	13.1	10.9 ± 1.0 (9.8–13.1)	–	11.3 ± 1.3 (9.8–13.1)	–
G2 (%)	10.8	9.9 ± 1.3 (7.9–10.9)	–	10.5 ± 0.8 (8.7–11.6)	–
Testis (%)	–	–	38.9	–	38.5; 43.9
Rectum / anal body width	0.8	0.8 ± 0.1 (0.8–1.0)	0.9	0.8 ± 0.1 (0.7–1.0)	0.9; 0.9
Spicule length	–	–	51.5	–	50.8; 49.9
Spicule width	–	–	9.5	–	9.6; 7.3
gubernaculum length	–	–	17.9	–	19.4; 19.0
Accessory piece length	–	–	15.0	–	14.6; 12.1
Distance from cloacal opening to first supplement	–	–	14.0	–	11.5; 9.9
Distance from cloacal opening to anteriormost supplement	–	–	113.9	–	96.8; 99.5
Number of supplements	–	–	12	–	12; 11

L = body length, V = distance from vulva to the anterior end of body (mk) × 100/body length (mk), a = body length (mk)/maximum body diameter (mk), b = body length (mk)/pharynx length (mk), c = body length (mk)/tail length (mk), c' = tail length (mk)/anal body diameter (mk), G1 = anterior genital branch length (mk) × 100/body length (mk), G2 = posterior genital branch length (mk) × 100/body length (mk) (mk = micrometer).

The anteriormost supplement is situated at 97–114 µm from cloacal aperture. The distance between the last and anteriormost supplement is 85–100 µm. Male tail with two pairs of small subventral papillae (see figures 2 & 3).

Type habitat and locality. The type population was collected in soil around the roots of forest trees at the Ky Quan San mountain (22°30'22"N, 103°36'52"E, altitude 2150 m) in Sang Ma Sao commune, Bat Xat Nature Reserve, Lao Cai Province, Vietnam. The other population was collected in soil around the roots of forest trees at the Nhieu Co San mountain (22°35'45"N, 103°37'09"E, altitude 2320 m) in Y Ty commune, Bat Xat Nature Reserve, Lao Cai Province, Vietnam.

Type materials. Holotype female and three paratype females from Sang Ma Sao population on slide *C. batxatensis* sp. nov. no. 1; seven other paratype females and one paratype male from Sang Ma Sao population on slides *C. batxatensis* sp. nov. nos 2–4. Thirteen females and two males from Y Ty population on slides *C. batxatensis* sp. nov. nos 5–10. All slides have been

deposited in the nematode collection of the Department of Nematology, Institute of Ecology and Biological Resources of VAST, Vietnam.

Etymology. The name of the species refers to its geographical origin from Bat Xat Nature Reserve, Lao Cai Province in Vietnam.

Diagnosis

Coomansus batxatensis sp. nov. is characterized by small adult body size (0.7–0.9 mm), small size of buccal cavity (21–24 × 12–13 µm), rather posterior position of dorsal tooth apex (12.5–15.0) µm from posterior margin of buccal cavity or 59–63% of buccal cavity length, *pars refringens vaginae* with faint and small (2.5 × 1.7 µm) teardrop-shaped pieces, short *pars distalis vaginae*, the presence of males with short spicules (50–51.5 µm), rounded head and conical blade.

In general appearance, *C. batxatensis* sp. nov. is similar to *C. parvus* (de Man, 1880) Jairajpuri & Khan, 1977, *Coomansus*

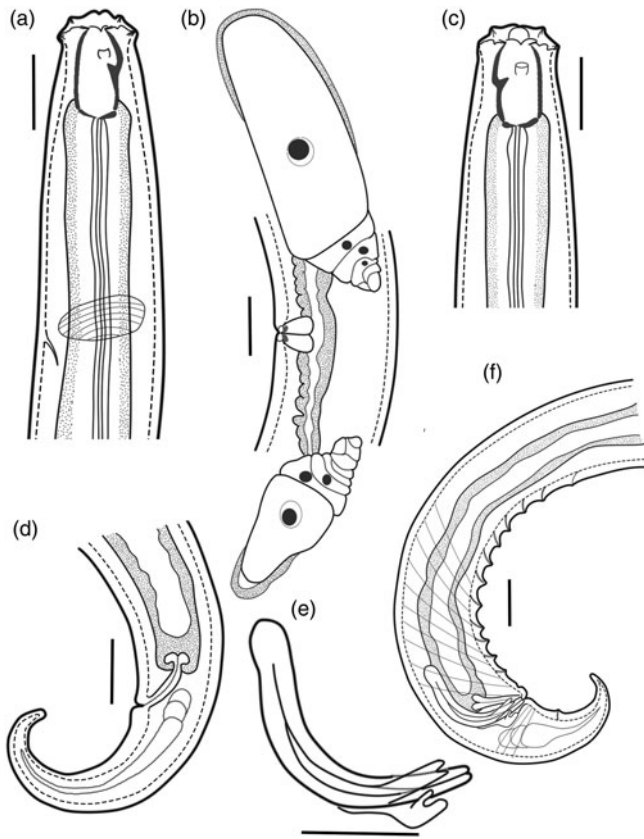


Fig. 1. Holotype female and paratype male of *Coomansus batxatensis* sp. nov.: (a) female head region; (b) female reproductive system; (c) male head region; (d) female tail; (e) spicule, gubernaculum and accessory piece; (f) male posterior region. Scale bars: 20 µm.

arvensis (Eroshenko, 1972) Jairajpuri & Khan, 1977, *Coomansus ouinnensis* Yeates, 1992, *Coomansus obliquoris* (Eroshenko, 1972) Jairajpuri & Khan, 1977, *Coomansus indicus* Jairajpuri & Khan, 1982 and *C. ulsani* Choi, Khan & Lee, 1999 based on a small body size less than 1.6 mm (Coetzee, 1968; Eroshenko, 1972; Jairajpuri & Khan, 1977, 1982; Popovici, 1990; Yeates, 1992; Choi *et al.*, 1999; Zullini *et al.*, 2002) but it differs all of them by having presence of males.

Coomansus batxatensis sp. nov. is distinguished from *C. parvus* by having more posterior dorsal tooth apex (59–63% vs. 62–73%); lower *c* value (7.6–11.7 vs. 10–19) but higher *c'* value (3–4 vs. 2–3). From *C. arvensis* it differs by having more posterior dorsal tooth apex (59–63% vs. 74–85%); lower *c* value (7.6–11.7 vs. 14–16) but higher *c'* value (3–4 vs. 2–2.5). *Coomansus batxatensis* sp. nov. is different from *C. ouinnensis* by having body stouter (*a* = 17.4–23.3 vs. 22–29), vulva more anterior (*V* = 59–62.5% vs. 62–64%), more posterior dorsal tooth apex (59–63% vs. 73–78%) and smaller size of buccal cavity (21–24 × 12–13 vs. 25–28 × 13–15) µm.

The new species *C. batxatensis* sp. nov. is very close to *C. obliquoris* in having a very short body and in the shape and size of buccal cavity. It is distinguished from *C. obliquoris* by having a slenderer body (*a* = 17.4–23.3 vs. 14), lower *c* value (7.6–11.7 vs. 11–14), more anterior dorsal tooth apex (59–63% vs. 56–58%), weakly sclerotized walls of buccal cavity vs. strongly sclerotized and in the shaped of pieces of *pars refringens vaginae* (teardrop vs. round). From *C. indicus* it differs by having shorter body length (0.7–0.9 vs. 1.1–1.4) mm, vulva position more anteriorly

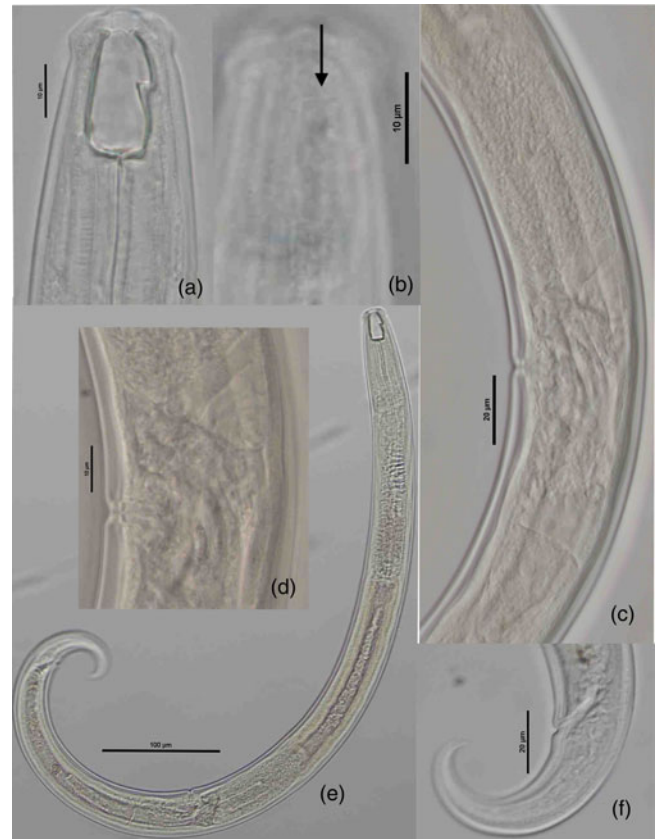


Fig. 2. Holotype female *Coomansus batxatensis* sp. nov.: (a) holotype female head region; (b) amphidial aperture; (c) reproductive system; (d) vulval region; (e) entire body; (f) tail.

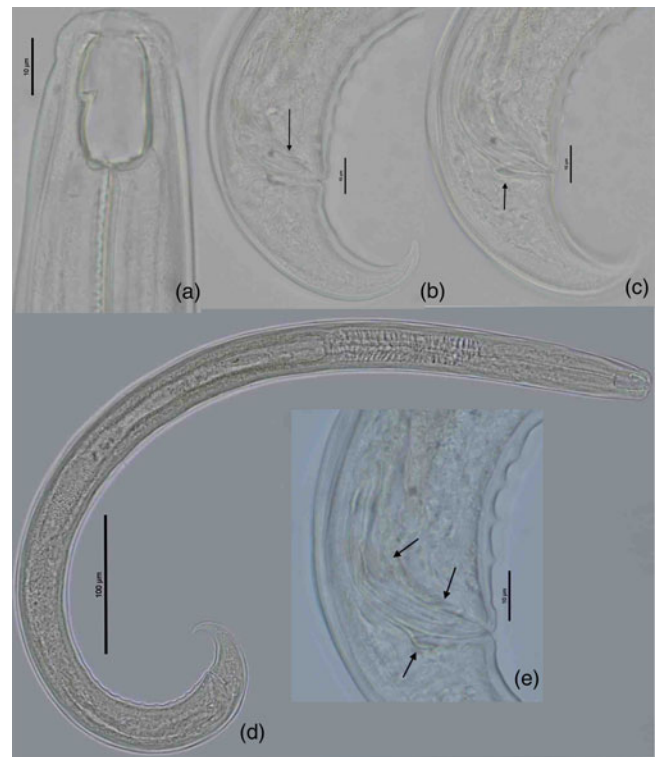


Fig. 3. Paratype male *Coomansus batxatensis* sp. nov.: (a) paratype male head region; (b) posterior region with accessory piece; (c) posterior region with spicule and gubernaculum; (d) entire body; (e) spicule, gubernaculum and accessory piece.

Table 2. Compendium of species belonging to the genus *Coomansus* Jairajpuri & Khan, 1977.

Species	<i>n</i>	<i>L</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>c'</i>	<i>V</i>	B.C.L	B.C.W	Spicule	Suppl.	%DT	Country	References
<i>arvensis</i>	? ♀♀	1.1	17–24	3.5	14–16	2.0–2.5	61–62	26 ^a	13 ^a	–	–	85	Russia-Far East	Eroshenko, (1972)
<i>batxatensis</i> sp. nov.	24♀♀	0.7–0.9	17–23	3.2–3.7	8–12	3.0–4.0	59–62.5	21–24	12–13	–	–	59–63	Vietnam	Current paper
	3♂♂	0.7–0.8	18–22	3.4–3.5	9–13.5	2.0–2.8	–	22.5–23	12–13	50–51.5	11–12	60–61		
<i>compbelli</i>	? ♀♀	1.9–3.0	–	–	10–14	–	52–57	–	–	–	–	69–72	New Zealand	Mulvey (1967)
	? ♂♂	2.1–2.9	–	–	17–21	–	–	–	–	98–113	10–11	–		
	1♀	2.0	29	4.0	17	3.3 ^a	61	45	31	–	–	–	Nigeria	Mulvey & Jensen (1967)
<i>composticola</i>	74♀♀	1.8–2.7	25–42	3.7–5.3	7.2–11	6.3 ^a	50	35	20	–	–	83–94	New Zealand	Clark (1960)
	3♂♂	3.0–3.5	35–39	4.5–4.8	18.5–21.5	2.4 ^a	–	–	–	58	12	–		
<i>conoidus</i>	1♀	2	40.4	4.1	17.6	3.2	61	42	21	–	–	65.4	India	Mohilal & Dhanachand (1997)
<i>fletcherensis</i>	5♀♀	3.4–3.6	31–37	3.8–4.1	22–25	2.7 ^a	57–60	70–75	39–43	–	–	68–71	Canada	Mulvey (1978)
	6♂♂	3.0–3.5	33–40	3.3–3.9	23–29	2.0 ^a	–	70–76	39–45	115–130	18–20	–		
<i>gerlachei</i>	1♀	3.7	–	–	13.3	–	50	–	–	–	–	–	Canada	Mulvey (1967)
	1♂	3.2	–	–	22.2	–	–	–	–	140	12	–		
	4♀♀	2.8–4.1	28–44	4.9–5.6	15–19	3.5–4.0	51.5–56	47.5–51	25.5–29	–	–	85–87	New Zealand	Jiménez-Guirado et al. (1998)
	4♂♂	3.2–3.7	37–39	4.7–5.1	20–22	1.8–2.1	–	49–50.5	22.5–27.5	147–165	9–11	82–88		
20♀♀	2.6–4.4	27–38	4.8–5.7	13–20	3.0–3.8	49–55	43–50	24–26	–	–	84–87	Argentina	Chaves (1990)	
<i>inca</i>	4♀♀	2.0–2.4	34–40	3.5–3.8	6.3–8.5	6.3–8.2	59–62	50–53	26–30	–	–	64–68	Peru	Andrassy (2011)
<i>indicus</i>	10♀♀	1.1–1.4	18–24	3.0–3.3	15–19	2.0–2.6	70–72	35–40	19–23	–	–	52–55	India	Jairajpuri & Khan (1977)
<i>intestinus</i>	7♀♀	2.0–2.5	22–26	4.0–5.4	12–14	3.0–3.6	55–61	40–45	18–20	–	–	81–84	Argentina	Vinciguerra & La Rosa, (1990)
	10♂♂	2.1–2.9	21–27	4.1–5.2	15–19	1.6–2.2	–	39–45	17–21	128–150	8–11	80–82		
<i>kastrolli</i>	141♀♀	3.7–5.3	42–68	3.8–4.5	33–47	2.0	63–71	56–73	26–32	–	–	68–70	Germany	Mulvey (1967)
	60 ♂♂	3.5–5.9	39–50	3.5–4.4	39–59	–	–	–	–	110–120	11–15	–		
<i>magellanicus</i>	8♀♀	1.8–2.0	26–30	4.7–5.3	12.6–14.9	2.6–3.5	51–57	33–36	18–20.5	–	–	76–79.6	Chile	Jiménez-Guirado et al. (1998)
	2♂♂	1.8; 2.0	29.6; 30	5.0; 5.0	21.4; 18	1.5; 1.9	–	32.5; 33	20; 22	98; 97	11; 12	–		
<i>major</i>	?♀♀	3.4	34.5	5.3	20	3.3	55	45	29	–	–	68–70	Australia	Loof & Winiszewska-Ślipińska (1993) (after Cobb, 1916)
	1♂	3.4	–	–	20	–	–	–	–	140	12	–		
	3♀♀	3.4–3.8	29–34	4.0	26–27	2.2–2.3	60–66	52–62	35–39	–	–	70		
	3♂♂	2.4–3.6	25–36	3.8–4.1	20–26	–	–	–	–	89–91	10–12	–	Germany	Loof & Winiszewska-Ślipińska (1993) (after Meyl, 1955)
	5♀♀	3.5–4	39–46	4.0–4.5	28–35	1.8–2.6	60–64	62–70	28–32	–	–	70–73	European	Loof & Winiszewska-Ślipińska (1993)

	7♂♂	3.1–3.5	41–46	3.9–4.5	2128	1.8–2.3	–	57–65	24–31	99–108	11–14	62–71	European	Loof & Winiszewska-Słipińska (1993)
<i>mapuche</i>	6♀♀	2.3–2.4	26–31	4.6–4.9	10.5–12.7	4.4–5.2	49–54	36–39	19–21	–	–	50–53	Chile	Andrassy (2011)
<i>meridionalis</i>	7♀♀	2.2–2.7	28–38	4.2–4.8	15.5–20.5	2.8–3.5	54–57	39–45	18.5–20.5	–	–	83.5–95	New Zealand	Jiménez-Guirado <i>et al.</i> (1998)
	4♂♂	2.1–2.7	30–35	4.2–4.8	22.8–24.4	1.8–1.9	–	40.5–45	18.5–21.5	93.5–111	12–13	–		
<i>mesadenus</i>	1♀	3.4	40	4.5	12.4	4.2 ^a	54	50	25	–	–	84–94	New Zealand	Clark (1960)
	1♂	3.3	40	4.3	20.5	2.2 ^a	–	–	–	125	–	–		
	7♀♀ 11♂♂	3.0–3.8 2.7–3.7	34–44.5 28–41	4.2–4.9 4.0–4.7	12–16 17–23.5	3.6–5.6 1.9–2.8	52.5–56 –	44–54 47.5–53	23–29.5 23.5–27	– 119–142	– 9–11	85–91.5 85–93		
<i>obliquoris</i>	11♀♀ 1♂	2.9–4.1 3.5	35–46 38.2	4.3–4.9 4.7	13–17 22.6	3.8–4.9 2.2	53–57 –	48.5–55 53	23–28 23	– 129.5	– 11	88.5–93 92	New Zealand	Jiménez-Guirado <i>et al.</i> (1998)
	2♀♀	0.7	14	3.2–3.4	11–14	2.0	60–66	21–22	13	–	–	56–58		
<i>ouinnensis</i>	9♀♀	1.0–1.1	22–29	3.3–3.9	10–12	3.0–3.5	62–65	25–28	13–15	–	–	73–78	New Caledonia	Yeates (1992)
<i>papua</i>	7♀♀	3.1–3.6	24–32	4.2–4.9	11.3–13.5	3.8–5.0	52–54	49–53	26–28	–	–	76–78	Papua New Guinea	Andrássy (2011)
<i>parvus</i>	? ♀♀	0.7–1.2	17–26	2.9–4.5	10–19	2.0–3.0	58–67	24–29	12–16	–	–	63–72	Cosmopolitan	Loof, (1964), Ahmad & Jairajpuri (2010)
<i>pretoriensis</i>	6♀♀	1.6–1.8	26–34	4	15–18	2.8 ^a	60–66	34 ^a	19.4 ^a	–	–	61	South Africa	Coetzee (1968)
	? ♀♀	1.6–1.8	26–30	3.8–3.9	16–19	2.5–3.0	66–69	32–33	14–16	–	–	56–62	India	Jairajpuri & Khan (1982)
<i>prodontus</i>	? ♀♂	1.5–1.8	–	–	–	2.5–2.7	62–64	30–31	16–17	65	14	66–70	India	Shah & Hussain (2015)
<i>spelendius</i>	? ♀♀	2.6–3.3	26–30	5.0–6.0	14–17	3.0–3.7	47–58	42–44	22–23	–	–	56–60	Ecuador	Andrássy (1993)
<i>ulsani</i>	3♀♀	1.2–1.5	23.5–26	3.4–3.8	13.6–14.8	2.7–3.2	65–68	36–39	21–23	–	–	58–64	India	Choi <i>et al.</i> (1999)
<i>venezolanus</i>	1♀	2.2	21	4.2	18	–	60	42	18	–	–	67	Venezuela	Loof (1964)
	1♀	2.6	34	4.5	19	3	61	38	16	–	–	80	India	Jairajpuri & Khan (1982)
	5 ♀♀ 1♂	1.9–2.0 1.8	27–31 32	4.1–4.4 4.1	20–29 21	1.7–2.7 2.1	60–64 –	40–41 40	19–20 19.4	– 74	– 74	70–74 73	Vietnam	Vu (2016)

Measurements in μm excepted L in mm.

L = body length, σ = body length (mk)/maximum body diameter (mk), b = body length (mk)/pharynx length (mk), c = body length (mk)/tail length (mk), c' = tail length (mk)/anal body diameter (mk), V = distance from vulva to the anterior end of body (mk) \times 100/body length (mk), B.C.L = buccal cavity length, B.C.W = buccal cavity width, Spicule = length of spicule, Suppl. = ventromedian supplements, DT: dorsal tooth position length from the base of buccal cavity (mk) \times 100/buccal cavity length (mk), mk = micrometer.

^aCalculated from original illustrations.

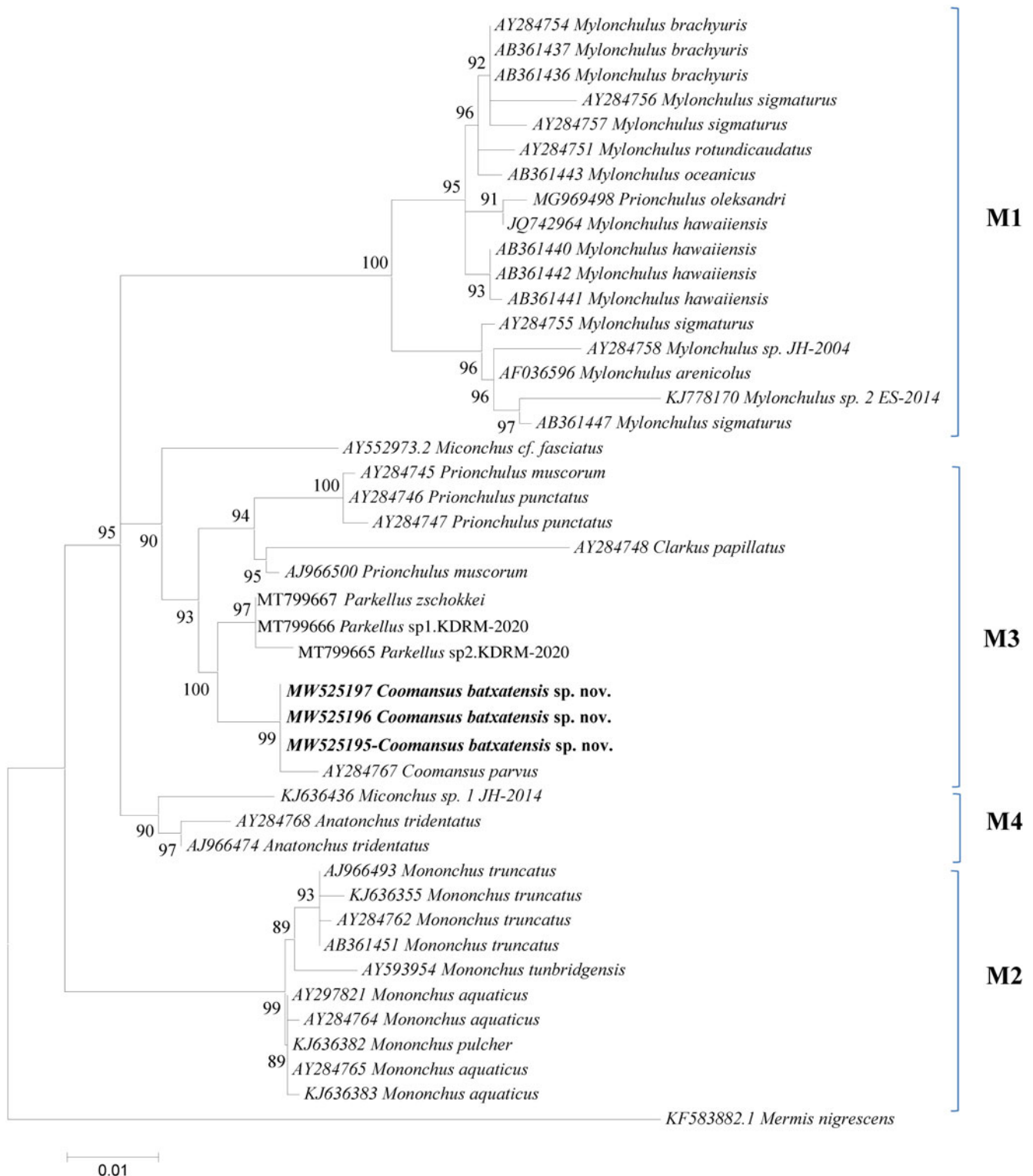


Fig. 4. 18S rDNA-based Bayesian phylogeny of the Mononchida. The new *Coomansus batxatensis* species is indicated in bold. Numbers near nodes indicate posterior probabilities. The scale bar indicates the expected number of substitutions per site.

($V = 59\text{--}63\%$ vs. $70\text{--}72\%$), lower c value ($7.6\text{--}11.7$ vs. $15\text{--}19$) but higher c' ratio ($3\text{--}4$ vs. $2\text{--}2.6$), more anterior position of dorsal tooth apex ($59\text{--}63\%$ vs. $52\text{--}55\%$) and smaller size of buccal cavity ($21\text{--}24 \times 12\text{--}13$ vs. $35\text{--}40 \times 19\text{--}23$) μm .

The new species *C. batxatensis* sp. nov. can be distinguished from *C. ulsani* by having vulva more anterior

($V = 59\text{--}63\%$ vs. $65\text{--}68\%$), body stouter ($a = 17.4\text{--}23.3$ vs. $23.5\text{--}26$), lower c value ($7.6\text{--}11.7$ vs. $13.6\text{--}14.8$) and smaller size of buccal cavity ($21\text{--}24 \times 12\text{--}13$ vs. $36\text{--}39 \times 21\text{--}22$) μm .

Table 2 presents a compendium of *Coomansus* species morphometrics for comparative purposes.

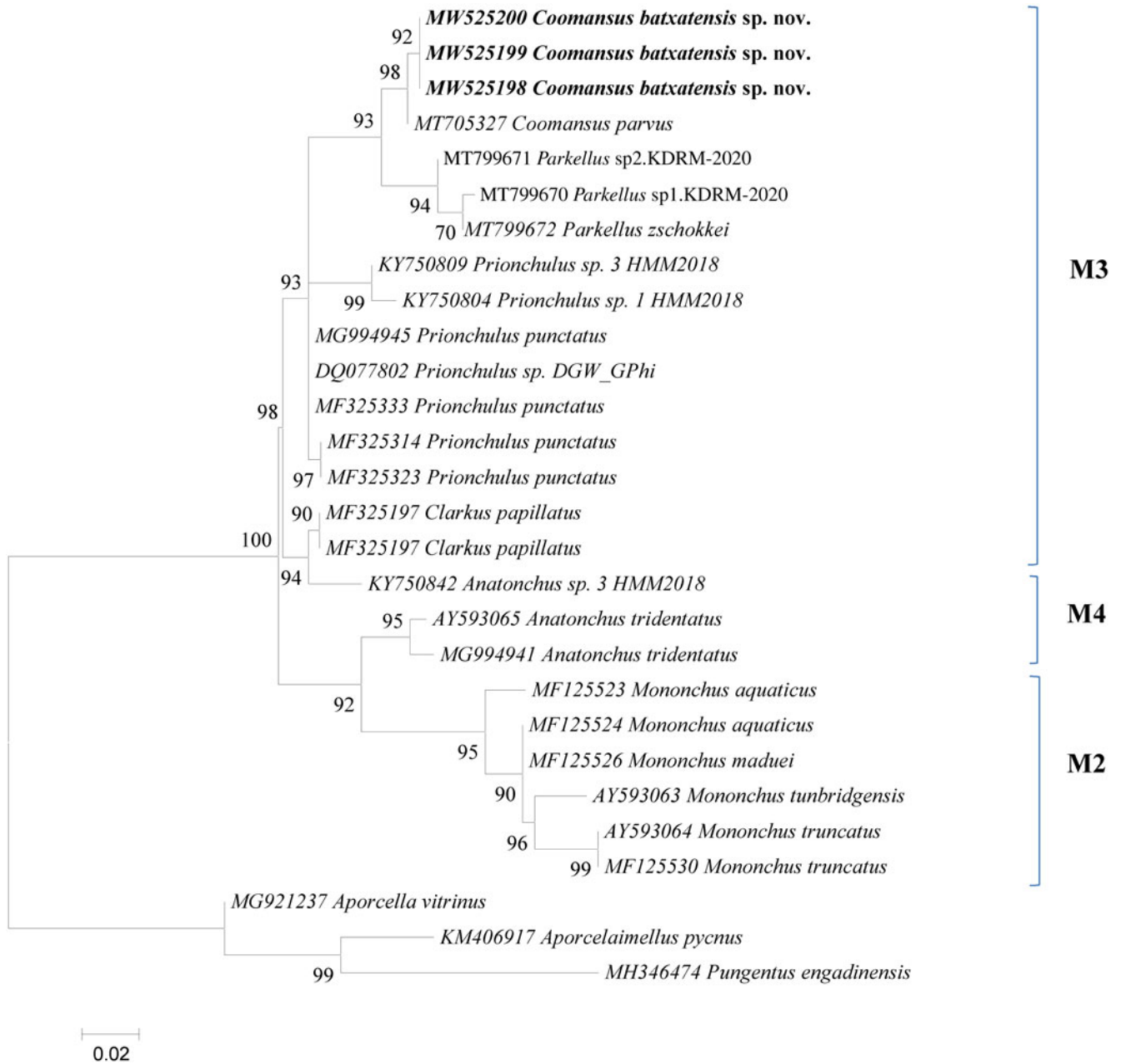


Fig. 5. D2–D3 region of 28S rDNA-based Bayesian phylogeny of the Mononchida. The new *Coomansus batxatensis* species is indicated in bold. Numbers near nodes indicate posterior probabilities. The scale bar indicates the expected number of substitutions per site..

Key to species

Currently, 25 species of the genus *Coomansus* Jairajpuri & Khan, 1977 have been recorded. The following key is based on characteristics of females:

- 1 Small nematode, L < 1.6 mm 2
 - Large nematode, L ≥ 1.6 mm 8
- 2 V = 70–72 *indicus* Jairajpuri & Khan, 1977
 - V < 70 3
- 3 Dorsal tooth apex from the base of buccal cavity at >75% 4
 - Dorsal tooth apex from the base of buccal cavity at <75% 5

- 4 Tail 3–3.5 anal body diameter long; buccal cavity = 25–28 × 13–15 μm; ♂: unknown
 *ouinnensis* (Yeates, 1992) Andrassy, 1993
 – Tail 2–2.5 anal body diameter long; buccal cavity = 23–24.5 × 12–13 μm; ♂: unknown
 *arvensis* (Eroshenko, 1972) Jairajpuri & Khan, 1977
- 5 Plump body; $a = 14$; ♂: unknown *obliquoris* (Eroshenko, 1972) Jairajpuri & Khan, 1977
 – Body slenderer; $a > 17$ 6
- 6 Buccal cavity longer than 30 μm; ♂: unknown *ulsani* Choi, Khan & Lee, 1999
 – Buccal cavity length less than 30 μm 7
- 7 Tail 3–4 anal body diameter long; small size of buccal cavity: 21–24 × 12–13 μm, $c = 7.6$ –11.7
 *batxatensis* sp. nov.
 – Tail 2–3 anal body diameter long; larger size of buccal cavity: 24–29 × 14–16 μm, $c = 10$ –19; ♂: unknown
 *parvus* (de Man, 1880) Jairajpuri & Khan, 1977
- 8 Tail 6–8.5 anal body diameter long 9
 – Tail < 6 anal body diameter long 10
- 9 Larger size of buccal cavity = 50–53 × 26–30 μm; ♂: unknown *inca* Andrassy, 2011
 – Small size of buccal cavity = 32–38 × 20 μm *composticola* (Clark, 1960c) Jairajpuri & Khan, 1977
- 10 Dorsal tooth apex from the base of buccal cavity at > 80% 11
 – Dorsal tooth apex from the base of buccal cavity at ≤ 80% 14
- 11 Tail > 4 anal body diameter long *mesadeus* (Clark, 1960c) Jairajpuri & Khan, 1977
 – Tail ≤ 4 anal body diameter long 12
- 12 $a < 28$ *intestinus* (Vinciguerra & La Rosa, 1990) Andrassy, 1993
 – Body slenderer; $a ≥ 28$ 13
- 13 Body length: $L > 2.8$ mm *gerlachei* (de Man, 1904) Jairajpuri & Khan, 1977
 – Body length: $L < 2.8$ mm *meridionalis* Jiménez-Guirado, Wouts & Bell, 1998
- 14 Dorsal tooth apex from the base of buccal cavity at ≥ 66% 15
 – Dorsal tooth apex from the base of buccal cavity at < 66% 22
- 15 Body length ≤ 3 mm 16
 – Body length > 3 mm 19
- 16 $V ≥ 60$ 17
 – $V < 60$ 18
- 17 Body length : $L > 1.8$ mm; buccal cavity size = 38–42 × 16–20 μm *venezolanus* (Loof, 1964) Jairajpuri & Khan, 1977
 – Body length: $L ≤ 1.8$ mm; smaller size of buccal cavity = 30–31 × 16–17 μm *prodontus* Shah & Hussain, 2015
- 18 Larger size of buccal cavity = 45 × 31 μm; dorsal tooth apex from the base of buccal cavity at < 75%
 *compbelli* (Allgén, 1929) Jairajpuri, 1970a
 – Smaller size of buccal cavity = 33–36 × 18–22 μm; dorsal tooth apex from the base of buccal cavity at > 75%
 *magellanicus* Jiménez-Guirado, Wouts & Bell, 1998
- 19 Tail length = 3.8–8.0 anal body diameter long; ♂: unknown *papua* Andrassy, 1993
 – Tail length shorter; $c' < 3.5$ 20
- 20 $c' ≤ 2$ anal body diameter long *kastrolli* (Altherr, 1958) Jairajpuri, 1970
 – $c' > 2$ anal body diameter long 21
- 21 Large buccal cavity size = 70–75 × 39–43 μm *fletcherrensis* Mulvey, 1978
 – Smaller size of buccal cavity = 45–70 × 29–39 μm *major* (Cobb, 1893) Loof & Winiszewska-Ślipińska, 1993
- 22 $V < 60$; body length: $L > 2$ mm 23
 – $V < 60$; body length: $L ≤ 2$ mm 24
- 23 Body length: $L ≥ 2.6$ mm; $c > 14$; $c' = 3.0$ –3.7; buccal cavity size = 42–44 × 22–23 μm; ♂: unknown
 *splendius* Andrassy, 1993
 – Body length: $L < 2.6$ mm; $c' = 4.4$ –5.2; buccal cavity size = 36–39 × 19–21 μm; ♂: unknown
 *mapuche* Andrassy, 2011
- 24 $a = 26$ –34; $c' = 2.5$ –3.0; buccal cavity size = 32–34 × 14–19 μm; ♂: unknown
 *pretoriensis* (Coetzee, 1968) Jairajpuri & Khan, 1977
 – Body slenderer, $a = 40$; $c' = 3.2$; larger size of buccal cavity = 42 × 21 μm; ♂: unknown
 *conoidus* Mohilal & Dhanachand, 1997

Phylogenetic analysis

Molecular sequences of three individuals of *C. batxatensis* sp. nov. were analysed in this study. After sequencing and editing, six sequences were obtained: three 847–919 bp nearly full length of SSU rRNA (small subunit ribosomal ribonucleic acid) (18S), GenBank accession numbers MW525195, MW525196, MW525197 and three 765–798 bp length D2D3 of LSU rRNA (large subunit ribosomal ribonucleic acid) (28S), GenBank accession numbers MW525198, MW525199 and MW525200.

The interspecific nucleotide variation within the acquired 18S rDNA sequences was: *C. parvus* (accession no. AY284767) vs. *C. batxatensis* sp. nov. = 0.01. There was no intraspecific variation within the acquired 28S rDNA sequences. The interspecific variation was: *C. parvus* (accession no. MT705327) vs. *C. batxatensis* sp. nov. = 0.02.

rDNA phylogenetic relationships among Mononchida

The results derived from the analyses of the 18S and D2–D3 region of 28S sequences are presented in the molecular trees of [figs 4](#) and [5](#), respectively. In both phylogenetic trees the new species *C. batxatensis* sp. nov. clustered with the only other species of the genus presented, *C. parvus*, both species forming a sister group to the genus *Parkellus* in agreement with [Jairajpuri et al. \(2001\)](#) and [Ahmad & Jairajpuri \(2010\)](#).

The new species *C. batxatensis* sp. nov. and *C. parvus*, clustered together in a group, were positioned within the M3 clade (following the nomenclature of [Holterman et al., 2008](#); [Olia et al., 2008](#)), encompassing representatives of genera *Clarkus*, *Parkellus* and *Prionchulus* of the Mononchidae family ([figs 4](#) and [5](#)). This positioning was confirmed by phylogenetic analyses based on both the 18S and D2–D3 region of 28S rDNA data.

In conclusion, the validity of *C. batxatensis* sp. nov. is supported by its morphological and molecular characterization. Based on 18S rDNA and D2–D3 extension region of 28S rDNA sequences, the genus *Coomansus* appears as a sister group of *Parkellus*, supporting the validity of the latter genus.

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Conflicts of interest. None.

Ethical standards. The author asserts that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of animals.

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