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Morphological and molecular characterisation of *Mulveyellus aizawlensis* sp. n. (Nematoda: Iotonchinae) from Aizawl, Mizoram, India

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Summary – Analysis of a soil sample from Aizawl district of Mizoram, India, led to the discovery of *Mulveyellus aizawlensis* sp. n. The new species is presented with female morphological data and molecular data of the D2-D3 expansion segment of 28S and 18S of rDNA, and phylogenetic analysis data based on these sequences. *Mulveyellus aizawlensis* sp. n. is morphologically characterised by a ventrally arcuate female body, presence of minute transverse striations on cuticle, a barrel-shaped buccal cavity, a dorsal tooth at anterior half of the buccal cavity, toothless subventral wall, distinct cup-shaped amphids located at the level of the dorsal tooth, a tuberculate pharyngo-intestinal junction, bean-shaped *pars refrigens vaginae*, a didelphic-amphidelphic reproductive system, a conoid ventrally-arcuate tail, and absence of caudal glands and spinneret. This study presents molecular data of the genus *Mulveyellus* for the first time and revealed a close phylogenetic relationship of this genus with *Prionchulus*.

Keywords – 18S rDNA, D2-D3 of 28S rDNA, Mononchida, *Prionchulus*, taxonomy.

The mononchs and the dorylaims were two ordinal ranks of nematodes as recognised by Jairajpuri (1969), who also proposed the Mononchida as a nematode order to include four families, namely Anatonchidae, Cobbonchidae, Iotonchidae and Mylonchulidae. Later, Iotonchinae and Hadronchinae were established as two subfamilies of Iotonchidae based on the presence and absence of subventral teeth (Jairajpuri & Khan, 1982). Under the subfamily Iotonchinae, Siddiqi (1984a) proposed four genera – *Caputonchus* Siddiqi, 1984, *Mulveyellus* Siddiqi, 1984, *Nigronchus* Siddiqi, 1984 and *Truxonchus* Siddiqi, 1984. However, Andrássy (1993) subsequently recognised 12 genera in Iotonchinae, *i.e.*, *Caputonchus*, *Hadronchoides* Jairajpuri & Rahman, 1984, *Hadronchulus* Ray & Das, 1983, *Hadronchus* Mulvey & Jensen, 1967, *Iotonchulus* Andrássy, 1993, *Iotonchus* Cobb, 1916, *Jensenonchus* Jairajpuri & Khan, 1982, *Mulveyellus*, *Nullonchus* Siddiqi, 1984b, *Parahadronchus* Mulvey, 1978, *Prionchulellus* Mulvey & Jensen, 1967 and *Prionchuloides* Mulvey, 1963, and also provided keys to their identification.

Siddiqi (1984a) proposed the genus *Mulveyellus* to accommodate species of the genera *Iotonchus* and *Mononchus* Bastian, 1865 that have continuous mouth region with adjoining body and a dorsal tooth at the anterior end of the buccal cavity. When *Mulveyellus* was proposed, six species were transferred to the genus, namely: *Mulveyellus antedontus* (Mulvey, 1961) Siddiqi, 1984 from *Iotonchus antedontus*, *Mulveyellus antedontoides* (Coetzee, 1967) Siddiqi, 1984 from *Iotonchus antidontoides*, *Mulveyellus jairi* (Lordello, 1959) Siddiqi, 1984 from *Iotonchus jairi*, *Mulveyellus longicaudatus* (Baqri, Baqri & Jairajpuri, 1978) Siddiqi, 1984 from *Iotonchus longicaudatus*, *Mulveyellus monhystera* (Cobb, 1917) Siddiqi, 1984 from *Mononchus monhys-*

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tera and *Mulveyellus vorax* (Cobb, 1917) Mulvey, 1963, Siddiqi, 1984 from *Iotonchus vorax*, and further changed their family positions from Anatonchidae to Iotonchidae. Later, Andrássy (1993) added three more species to *Mulveyellus*: *Mulveyellus shamimi* (Patil & Khan, 1982) Andrássy, 1993 from *Iotonchus shamimi*, *M. arenicola* (Altherr, 1963) Andrássy, 1993 from *Iotonchus arenicola* and *M. parazschokkei* (Allgén, 1929) Goodey, 1951, Andrássy, 1993 from *Mononchus* (*Iotonchus*) *parazschokkei*. However, he did not accept the position of *M. antedontoides*, *M. antedontus* and *M. vorax* suggested by Siddiqi (1984a) because of the presence of conspicuous subventral ribs opposite to the dorsal tooth of buccal cavity. Instead, they were transferred to the genus *Jensenonchus*. Also, because of the presence of long filiform tail and terminal spinneret, *M. longicaudatus* was transferred to a new genus, *Iotonchulus* as *I. longicaudatus* (Baqri *et al*., 1978; Siddiqi, 1984a; Andrássy, 1993). These taxonomic rearrangements left five nominal species under *Mulveyellus*, namely: *M. jairi*, *M. monhystera*, *M. shamimi*, *M. arenicola* and *M. parazschokkei*. These species are characterised by the presence of a barrel-shaped buccal cavity with dorsal tooth at anterior third to middle of buccal cavity, a toothless subventral wall, tuberculate pharyngo-intestinal junction, absence of caudal glands and spinneret and ventrally arcuate conoid tail.

Mulveyellus jairi, *M. shamimi* and *M. parazschokkei* have so far been reported from India (Patil & Khan, 1982; Siddiqi, 1984a; Ahmad & Jairajpuri, 2010; Paul & Sharma, 2017). In the present study, a new population of *Mulveyellus* was recovered during a biodiversity study of mononchids in the Mizoram state of northeastern India. Based on morphological, molecular and phylogenetic data analyses, the species was found to be new to science and hence, is herein characterised as *Mulveyellus aizawlensis* sp. n.

Materials and methods

Nematode extraction and morphological study

Soil around the rhizosphere of *Melocanna* sp. (common name: Bamboo) was collected from the campus of Fernando School, Zemabawk, Mizoram, India, at a longitude of 23°43'49.8"N and latitude of 92°45'31.7"E in 2018. The sample was processed by Cobb's (1918) sieving and decanting method followed by modified Baermann's funnel technique (Thorne, 1961). The extracted nematodes were killed using warm (around 60°C) formalin alcohol (FA) (4:1) and dehydrated by glycerol-ethanol method of Seinhorst (1962). Permanent mounts of the fixed nematodes were prepared by mounting the specimens in glycerin on glass slides. The specimens were observed, measured and illustrated using a Nikon Eclipse Trinocular Research Microscope (ECLIPSE E200), equipped with a Y-IDT drawing tube and Y-TV55 camera. Drawings were made using a Y-IDT drawing tube and improved using Adobe Photoshop 2020.

Molecular characterisation

Nematode specimens previously stored in DESS solution containing 20% dimethyl sulfoxide (DMSO) and 0.25 M disodium EDTA, saturated with NaCl, pH 8.0 (Seutin *et al*., 1991; Yoder *et al*., 2006) were transferred to distilled water in a glass cavity block for washing and rehydration. The water in the glass cavity block was changed every 15 min four times. After this washing step, an individual nematode was transferred to a drop of water on a glass slide and, with the help of a metallic pin (used as a nematode picking tool), the specimen was cut into two pieces. The pieces were then transferred in a polymerase chain reaction (PCR) tube containing 20 μl of worm lysis buffer (50 mM KCl, 10 mM Tris at pH 8.3, 2.5 mM $MgCl_2$, 0.45% NP 40 (Tergitol Sigma), 0.45% Tween-20). The PCR tubes were frozen at −20°C (15 min) followed by adding 1 μl proteinase K (1.2 mg ml^{-1}), incubation of the tubes at 65°C (1 h) and 95°C (10 min) and ending by centrifuging the lysate at 14 000 *g* for 1 min (Singh *et al*., 2021). The extracted genomic DNA was used for PCR amplification of the D2-D3 expansion segments of 28S and partial 18S of ribosomal DNA.

For amplification of the D2-D3 of 28S, the primer pair D2A: 5′-ACA AGT ACC GTG AGG GAA AGT TG-3′/D3B: 5′-TCC TCG GAA GGA ACC AGC TAC TA-3′ (Nunn, 1992) was used with the thermal profile of 94°C for 4 min, 35 cycles of (94°C for 1 min, 55°C for 1.5 min and 72°C for 2 min), 72°C for 10 min and a final hold at 4°C. Amplification of the partial sequence of 18S was done using the primer pair, SSU18A: 5′-AAA GAT TAA GCC ATG CAT G-3′/SSU26R: 5′-CAT TCT TGG CAA ATG CTT TCG-3′ (Mayer *et al*., 2007) with the thermal profile of 95°C for 5 min, 35 cycles of (94°C for 1 min, 52°C for 1.5 min and 68°C for 2 min), 68°C for 10 min and a final hold at 4°C. The PCR amplicons were cleaned using exoenzymes as in Singh *et al*. (2020). After sequencing of the PCR amplicons, contigs were

prepared from the newly produced forward and backward sequences using Geneious Prime 2020.0.5 (https://www.geneious.com) and the resulting contigs were deposited in GenBank.

Phylogenetic analysis

Phylogenetic relationships of *Mulveyellus aizawlensis* sp. n. with other mononchid species were analysed based on the D2-D3 and 18S sequence alignments using the program, Geneious Prime 2020.0.5. The available D2-D3 and 18S sequences of the nematode order Mononchida were retrieved from the NCBI and aligned with the newly obtained sequences using MUSCLE alignment of Geneious Prime 2020.0.5 using default parameters, followed by trimming of the poorly aligned ends. A D2-D3 alignment of 740 bp long containing 39 sequences and an 18S alignment of 820 bp long containing 68 sequences were created. Bayesian phylogenetic analysis (MrBayes 3.2.6) was done using the GTR + I + G nucleotide substitution model for each alignment file. The analyses were run under 1×10^6 generations (four runs) and Markov chains were sampled every 100 generations and 20% of the converged runs were regarded as burn-in (Huelsenbeck & Ronquist, 2001). The Bayesian 50% majority consensus trees were inferred, and the branch supports were indicated by posterior probabilities.

Results

Morphological characterisation

***Mulveyellus aizawlensis* sp. n.**
(Figs 1, 2)

Measurements

See Table 1.

Description

Female

Slender nematodes (a = 21-24), resemble closed 'C' shape upon fixation, 1.5-2.0 mm long. Body cylindrical, maximum width anterior to vulva or around mid-body. Cuticle with minute transverse striations, thickness varies throughout body length, maximum at anus level (3-4 μm). Lateral chord *ca* 17-21% of body width at mid-body region. Lip region continuous with adjoining body region, not offset. Labial width *ca* 3.5-5.0 times that of labial height. Labial papillae prominent, arranged in circle like a crown. Amphidial fovea cup-shaped, located at level of dorsal tooth. Amphidial canal indistinct. Stoma consisting of vestibulum and buccal cavity. Buccal cavity strong, barrel-shaped, 1.5-2.0 times as long as wide, with a dorsal tooth pointed anteriorly. Dorsal tooth positioned around anterior half or at 72-73% of buccal length from stoma base. Subventral wall toothless. Pharyngeal sleeve small, covers stoma base. Nerve ring distinct, located at 20-22% of pharynx length from neck base. Secretory-excretory pore distinct, situated at 24-27% of pharynx length from neck base. Pharyngo-intestinal junction tuberculate, situated at 22-26% of body length from anterior end. Intestine randomly granulated. Intestine tapering at vulva, occupying about half of body width at vulva. Reproductive system didelphic-amphidelphic. Genital branches symmetrical; anterior branch 230-357 μm long and posterior branch 221-330 μm long. Ovaries robust and reflexed, similar; anterior ovary 83-116 μm long and posterior ovary 73-111 μm long, not reaching oviduct-uterus junction. Oviduct large and straight. Oviduct-uterus junction without sphincter. Uterus thin and convoluted. Vagina extending over 35-41% of body diam. *Pars distalis vaginae* 4-6 μm long with concave walls. *Pars refrigens vaginae* with bean-shaped sclerotised pieces of dimensions 5-9 $\times$ 2-4 μm. *Pars proximalis vaginae* 16-19 μm long with tubular walls. Vulva with slightly protruded transverse slit, located posterior to mid-body region. Advulval papillae absent. Eggs absent. Rectum straight, 41-49 μm long, similar in length to one anal body diam. Tail conoid, 119-159 μm long, ventrally arcuate with pointed tip. Caudal glands and spinneret absent.

Male

Not found.

Type specimen

Holotype female on slide Maiz-1 and five paratype females on slides Maiz-2–Maiz-6, respectively, were deposited at the Nematode Collection of the Department of Zoology, Manipur University, Canchipur, India.

Type location and habitat

The new species was found present in a soil sample from around the roots of *Melocanna* sp. in the campus of Fernando School, Zemabawk, Aizawl district, Mizoram, India (23°43'49.8"N, 92°45'31.7"E) in September 2018.

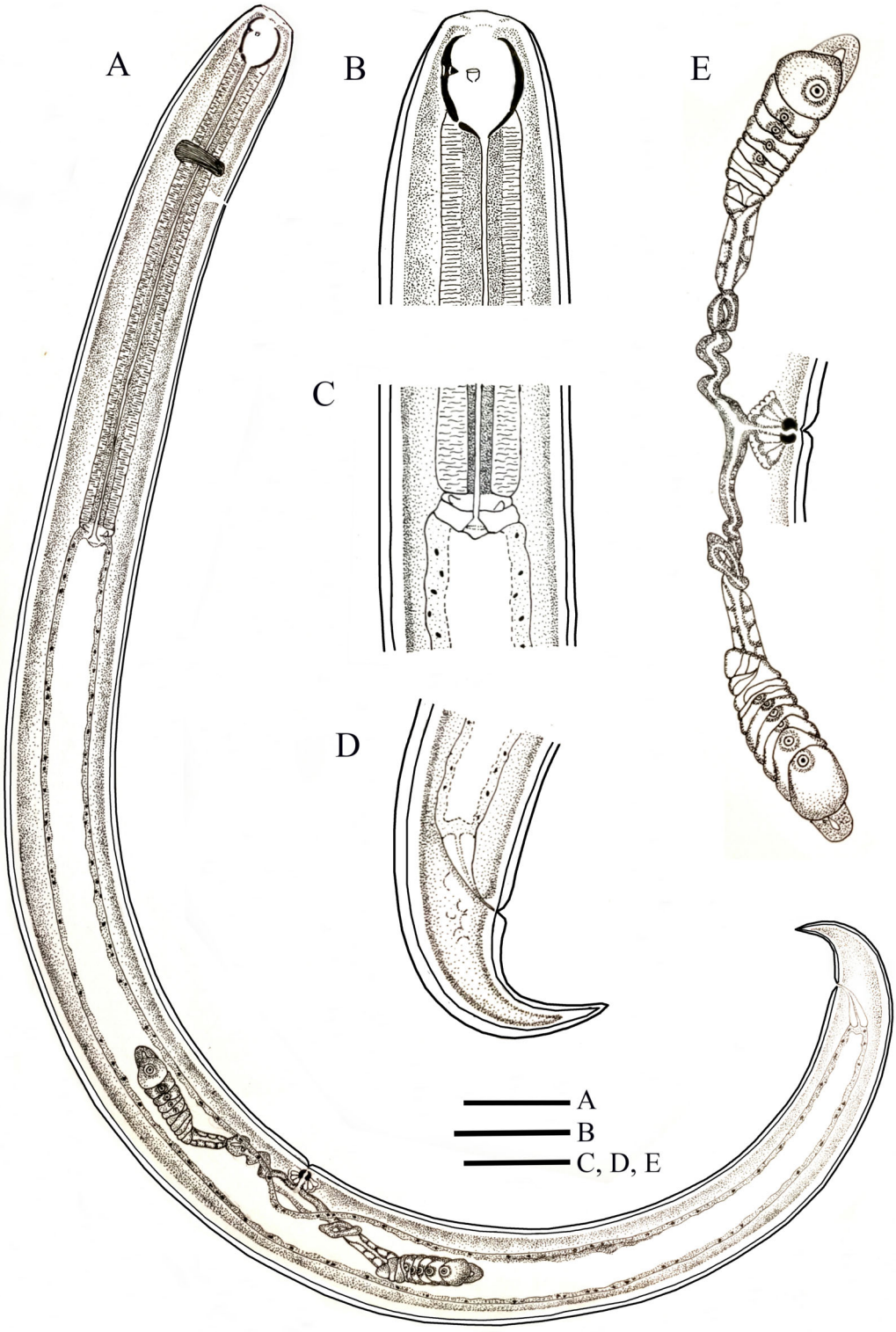

Fig. 1. Line illustration of the holotype female of *Mulveyellus aizawlensis* sp. n. A: Whole body; B: Anterior end; C: Pharyngo-intestinal junction; D: Posterior region; E: Female reproductive system. (Scale bars: A = 80 μm; B-E = 40 μm.)

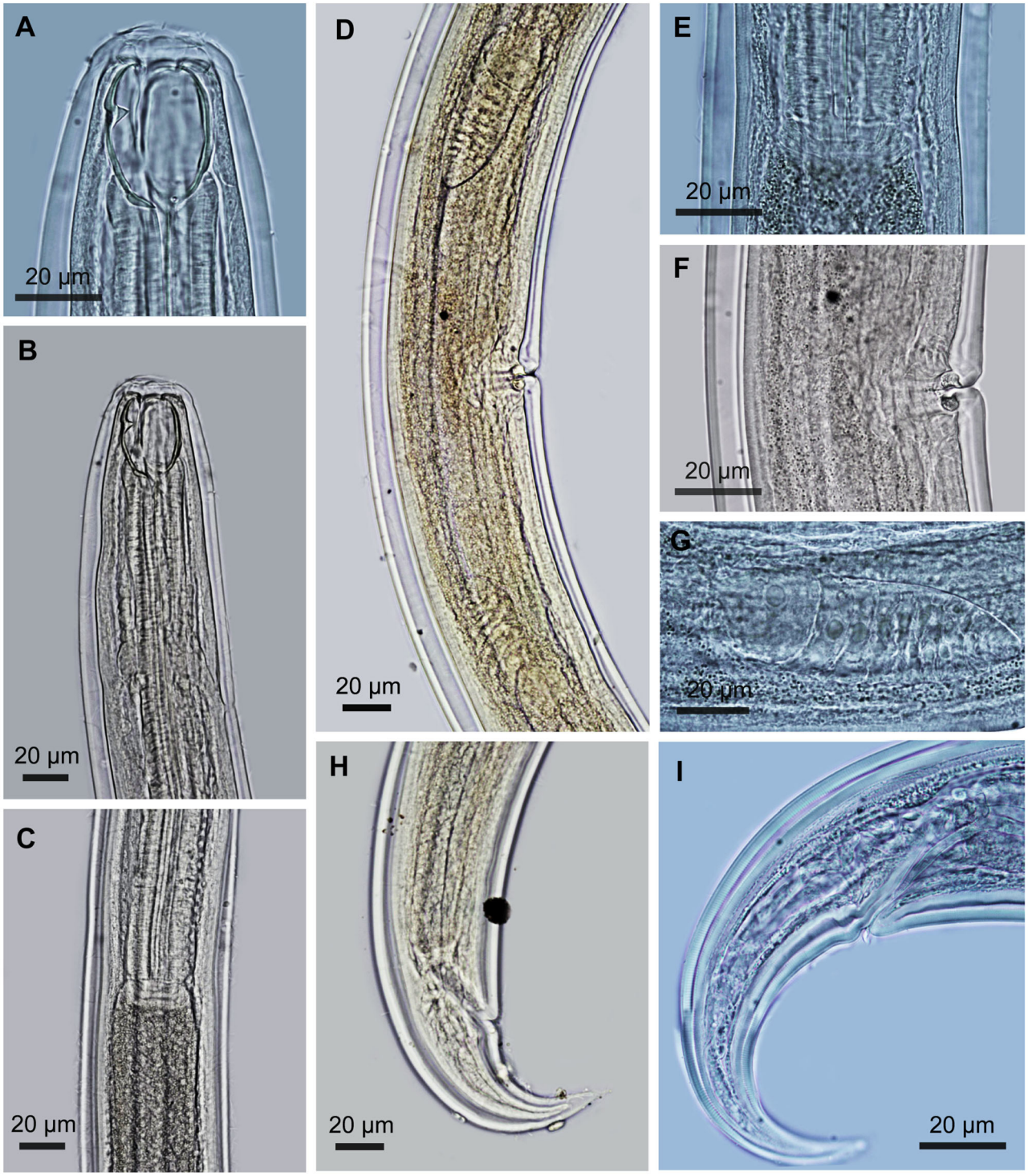

Fig. 2. Female *Mulveyellus aizawlensis* sp. n. A: Head region showing buccal cavity and dorsal tooth; B: Anterior region showing nerve ring and secretory-excretory pore; C, E: Pharyngo-intestinal junctions showing tubercles; D: Female reproductive system; F: Vulval region; G: Anterior gonad; H, I: Posterior region.

Table 1. Morphometric details of *Mulveyellus aizawlensis* sp. n. females. All measurements are in μm except L in mm and are presented in the form: mean ± s.d. (range).

Parameter	Holotype	Paratype
n	1	5
L (body length)	1.65	1.82 ± 0.12 (1.65-2.02)
a (body length/maximum body width)	21.8	22.1 ± 1.19 (20.7-23.6)
b (body length/anterior end to pharyngo-intestinal junction length)	3.93	4.05 ± 0.18 (3.84-4.39)
c (length/tail length)	13.9	12.3 ± 0.92 (11.4-13.9)
c′ (tail length/body width at anus)	2.91	3.19 ± 0.24 (2.91-3.51)
G1 (anterior gonad length × 100/body length)	13.9	15.9 ± 2 (13.9-19.8)
G2 (posterior gonad length × 100/body length)	13.3	15.3 ± 1.8 (13.3-18.3)
V (anterior end to vulva distance × 100/body length)	59.2	59.8 ± 1.5 (57.9-61.1)
Body width at neck base	58.7	56.7 ± 4.0 (49.8-59.1)
Body width at vulva	81.3	85.1 ± 7.4 (74.9-92.6)
Body width at anus	40.7	46.6 ± 4.7 (40.7-52.5)
Cuticle at lip region	2.22	2.02 ± 0.13 (1.85-2.22)
Cuticle at vulva	3.97	3.31 ± 0.51 (2.52-3.97)
Cuticle at anus	4.38	3.84 ± 0.39 (3.27-4.38)
Lateral chord	16.4	20.4 ± 3.6 (16.4-24.6)
Lip width	32.4	33.7 ± 1.36 (32.4-36.2)
Lip height	6.96	8.20 ± 0.93 (6.96-9.15)
Buccal cavity length	35.9	38.6 ± 1.67 (35.9-40.8)
Buccal cavity width	24.7	24.5 ± 2.38 (21.9-28.9)
Dorsal tooth apex from stoma base	26.3	27.9 ± 1.14 (26.3-29.8)
Nerve ring from anterior end	126	138 ± 10.5 (124-151)
Anterior end to pharyngo-intestinal junction	420	451 ± 35.3 (411-499)
Anterior end to vulval opening	980	1089 ± 89 (980-1233)
Anterior gonad length	230	292 ± 43.4 (230-357)
Posterior gonad length	221	279 ± 43.4 (221-330)
Vulval opening to rectum	556	587 ± 26.7 (556-635)
Rectum	40.7	43.7 ± 2.94 (40.7-49.0)
Tail length	119	148 ± 15.2 (119-159)

ETYMOLOGY

The specific epithet *aizawlensis* is derived from the type locality district, Aizawl.

DIAGNOSIS AND RELATIONSHIPS

Mulveyellus aizawlensis sp. n. can be diagnosed by a continuous mouth region, barrel-shaped buccal cavity with a dorsal tooth at the anterior half of the cavity, amphids at the level of the dorsal tooth, lateral chord *ca* 17-21% body width, bean-shaped *pars refrigens vaginae*, a didelphic-amphidelphic reproductive system, absence of caudal glands and spinneret, and a pointed tail terminus.

Mulveyellus aizawlensis sp. n. is similar to *M. shamimi* in having a tuberculate pharyngo-intestinal junction, didelphic-amphidelphic reproductive system, conoid and ventrally arcuate tail, absence of caudal gland and spinneret. However, the new species differs from *M. shamimi* in body posture (ventrally arcuate throughout body *vs* ventrally arcuate post vulva), lip region (continuous *vs* offset), dorsal tooth position in buccal cavity (anterior half *vs* anterior third), amphids position (at the level of the dorsal tooth *vs* above the dorsal tooth), lateral chord (20 *vs* 33% of body width at mid-body), *pars refrigens vaginae* shape (bean shape *vs* droplet shape), and rectum length (similar to anal body diam. *vs* less than one anal body diam.).

Mulveyellus aizawlensis sp. n. is similar to *M. monhystera*, *M. jairi*, *M. parazschokkei* and *M. arenicola* in having a barrel-shaped buccal cavity, a tuberculate pharyngo-intestinal junction, absence of advulval papillae, conoid and ventrally arcuate tail and absence of caudal glands and spinneret. However, the new species differ from *M. monhystera* and *M. jairi* in having continuous *vs* offset lip region, dorsal tooth position at anterior half *vs* anterior

third (*M. monhystera*) and midway (*M. jairi*) of buccal cavity, amphids position at dorsal tooth level *vs* anterior to dorsal tooth (*M. monhystera*), lateral chord 20% of body width *vs* 50% of body width near mid-body (*M. monhystera*), and in the reproductive system (didelphic-amphidelphic *vs* mono-prodelphic in both *M. jairi* and *M. monhystera*).

Furthermore, *M. aizawlensis* sp. n. differs from *M. arenicola* in body length (1.6-2.0 mm *vs* 3.1 mm), dorsal tooth position in buccal cavity (anterior second *vs* posterior), tail length (short *vs* long), vulva position from anterior end (posterior to mid-body *vs* mid-body), and tail shape (ventrally arcuate *vs* curled up). Lastly, it also differs from *M. parazschokkei* in dorsal tooth position in buccal cavity (anterior half *vs* middle), tail length (short *vs* long) and tail tip (pointed *vs* rounded). A comparative account of important morphological characters of all the nominal *Mulveyellus* spp. is presented in Table 2.

Molecular characterisation

Two identical sequences of the D2-D3 expansion segment of 28S rDNA (OP237017; 783 bp and OP237018; 980 bp) and a partial sequence of 18S of rDNA (OP237020; 877 bp) were generated for this species. These sequences also represent the first molecular data of the genus. Based on the NCBI BLAST sequence similarity searches, the D2-D3 sequence was found closest to an unidentified *Prionchulus* sp. (KY750804; query coverage of 93% and percent identity of 96.61%) and the 18S sequence was closest to *Prionchulus muscorum* (Dujardin, 1845) Wu & Hoeppli, 1929 (AJ966500; query cover of 100%; percent identity of 97.83%).

Phylogenetic analysis

Based on the D2-D3 tree, the new species formed a clade with the above unidentified *Prionchulus* sp. (KY750804) with maximum support, which together formed an unresolved sub-clade (also including an *Anatonchus* sp. sequence) within a larger *Prionchulus* Cobb, 1916 clade (PP = 1), comprising mostly of unidentified *Prionchulus* spp. (Fig. 3). In the 18S tree, the new species also formed a clade with an unknown *Prionchulus* sp. (MW218002) (PP = 0.59) and together formed a sister clade (PP = 0.99) to *P. punctatus* Cobb, 1917 and *P. muscorum* (Fig. 4).

Discussion

The superfamily Anatonchoidea was divided into the families Iotonchidae and Anatonchidae by Jairajpuri (1969). The former is diagnosed by the presence of a large buccal cavity, anteriorly directed dorsal tooth that may be situated either at anterior or posterior half of buccal cavity, unarmed subventral walls or provided with a smooth or serrated longitudinal ribs, whereas the latter is diagnosed by the presence of broad buccal cavity, retrorse and equal sized dorsal tooth and subventral teeth located in anterior to posterior half of buccal cavity. These two families can be separated based on apices of dorsal tooth (anterior *vs* retrorse) and ventral teeth (smaller than dorsal tooth, if present *vs* equal in size) (Jairajpuri, 1969). The genus *Mulveyellus* of Iotonchidae has some morphological similarities with *Anatonchus* of Anatonchidae such as having smooth cuticle, a large buccal cavity and a tuberculate pharyngo-intestinal junction (Jairajpuri, 1969). However, they differ in the buccal cavity shape (barrel *vs* oblong or spheroid), dorsal tooth apice (anteriad *vs* retrorse), dorsal tooth position in buccal cavity (anterior third to somewhat posterior to middle *vs* mid-region), ventral teeth (absent *vs* present), advulval papillae (absent *vs* present), tail (conoid *vs* elongate to filiform), and caudal glands and spinneret (absent *vs* present).

Mulveyellus appears to be a rare predatory nematode genus with four species reported so far from the Indian subcontinent. With the discovery of *M. aizawlensis* sp. n. from northeast India, the total number of nominal species under *Mulveyellus* now stands at six. Some of the morphological characters that were found very useful in separating *M. aizawlensis* sp. n. from the remaining five species include the lip region, amphid position, reproductive system, *pars refrigens vaginae* shape and tail terminus, in combination with the general morphometrical data (see given comparisons).

On the other hand, the phylogenetic analyses based on the alignments of the D2-D3 and 18S sequences with the available mononchid sequences revealed a close relationship of *Mulveyellus* with *Prionchulus*, but, surprisingly, these two genera belong to different mononchid families (*i.e.*, *Mulveyellus* in Iotonchidae and *Prionchulus* in Mononchidae Filipjev, 1934) and exhibit distinct morphological dissimilarities. Some major morphological differences between the two genera include the presence of tubercles in pharyngo-intestinal junction, absence of any ridges and denticulation in the buccal cavity of *Mulveyellus* (except for two ventro-sublateral teeth

Table 2. Comparative account of *Mulveyellus aizawlensis* sp. n. with five *Mulveyellus* species. Lengths are given in mm; dimensions and widths are in μm.

Parameters	*M. aizawlensis* sp. n.	*M. shamimi* (Patil & Khan, 1982) Andrássy, 1993	*M. jairi* (Lordello, 1959) Siddiqi, 1984	*M. monhystera* (Cobb, 1917) Siddiqi, 1984	*M. parazschokkei* (Allgén, 1929) Andrássy, 1993	*M. arenicola* (Altherr, 1963) Andrássy, 1993
Length	1.65-2.02	1.72	0.8-1.6	0.95-1.17	1.1-2.8	3.1
a	20.7-23.6	24	23-30	21-25	23-27	41
b	3.84-4.39	3.6	3.4-4.3	3.8-4.0	3.4-4.6	5.4
c	11.4-13.9	14	12-16	14-19	8-15	13
c′	2.91-3.51	3.15	2.5-3.0	2.0-3.0	3.5-4.0	5.0–6.0
V	57.9-61.1	64	71-76	79-81	57-65	52
Rectum length	40.7-49.0	43	–	23-28	–	–
Dorsal tooth apex from stoma base length	72-73%	70%	Midway of buccal cavity	Anterior two-thirds	Midway of buccal cavity	–
Buccal cavity length	35.9-40.8	42	35-40	23-28	–	–
Buccal cavity width	22.0-28.9	20	21-25	13-15	–	–
Tail length	119-159	120	75-115	53-65	–	–
Lip region width	32.4-36.2	34	–	21-30	–	–
Lip height	6.96-9.15	10	–	–	–	–
Mouth region	Continuous	Set off	Set off	Set off	–	–
Amphid position	At level of dorsal tooth	Above dorsal tooth level	–	Anterior to dorsal tooth	–	–
Lateral chord	20% at mid-body	33% at mid-body	–	50% at mid-body	–	–
Female reproductive system	Amphidelphic	Amphidelphic	Prodelphic	Prodelphic	Amphidelphic	–
Tail terminus	Pointed	Pointed acutely	Blunt tip	Rounded terminus	Rounded terminus	–

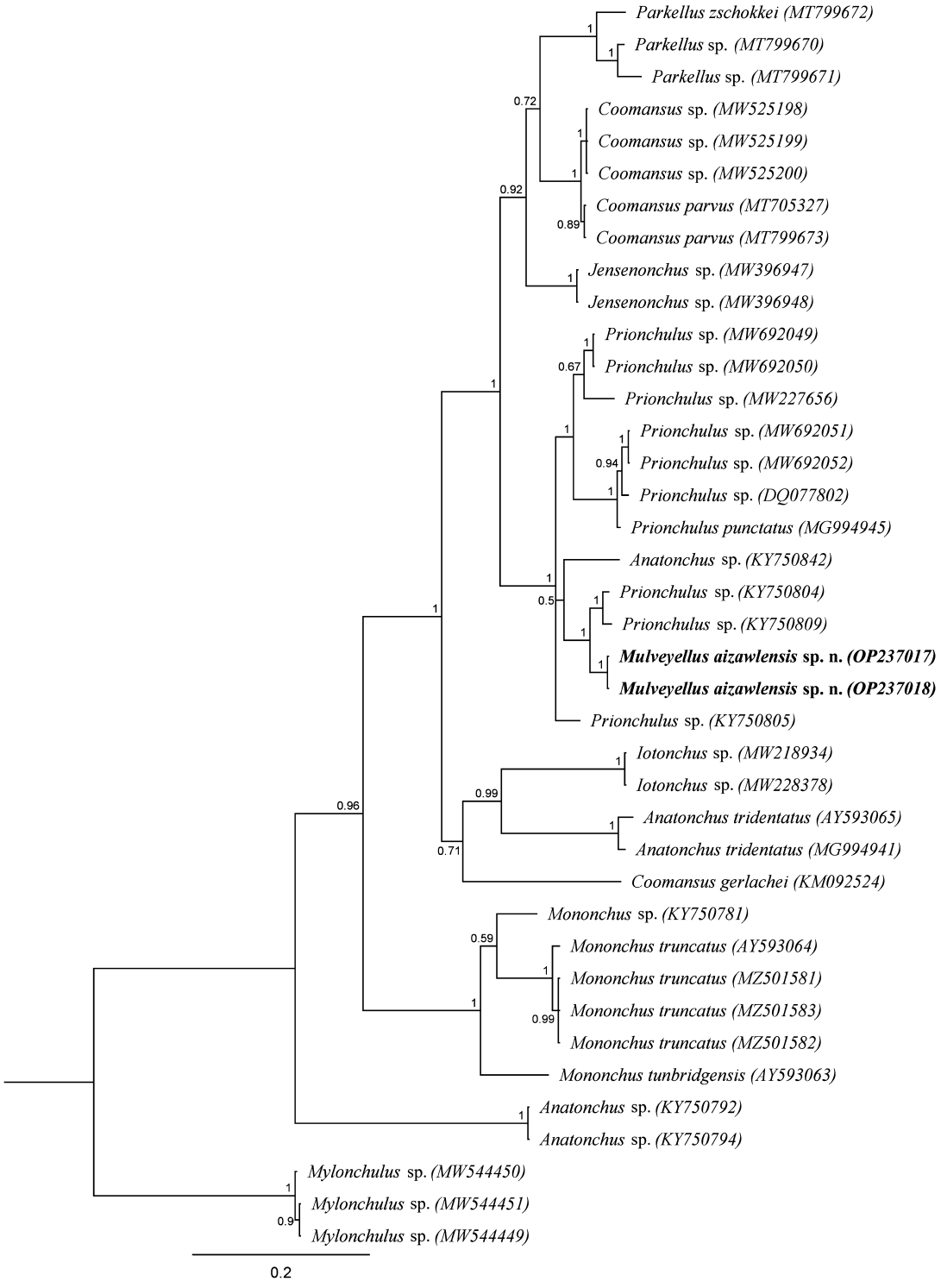

Fig. 3. A 50% majority rule Bayesian phylogenetic tree of Mononchida including *Mulveyellus aizawlensis* sp. n. from India, based on the D2-D3 expansion segments of 28S rDNA sequences under the GTR + I + G model. The new species is indicated in boldface. Posterior probabilities of above 0.50 are given next to clades.

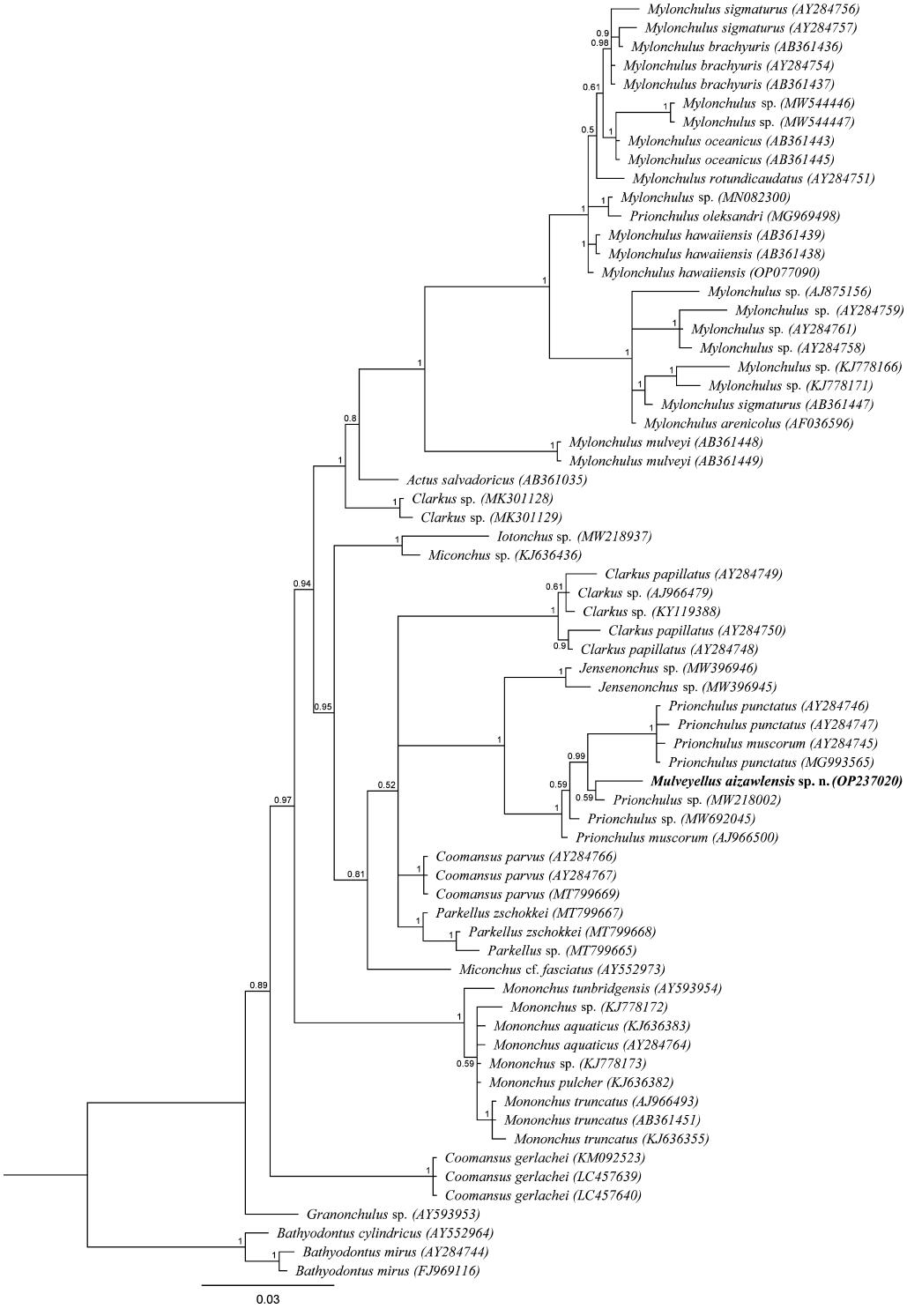

Fig. 4. A 50% majority rule Bayesian phylogenetic tree of Mononchida including *Mulveyellus aizawlensis* sp. n. from India, based on the 18S rDNA sequences under the GTR + I + G model. The new species is indicated in boldface. Posterior probabilities of above 0.50 are given next to clades.

in *M. shamimi*) *vs* non-tuberculated pharyngo-intestinal junction and presence of two longitudinal, denticulate ridges on subventral wall of buccal cavity, appearing almost like the teeth of a saw in *Prionchulus*. Nevertheless, both show similarities in other morphological characters such as body length, a barrel-shaped buccal cavity, a dorsal tooth situated within the anterior half of buccal cavity, a short and ventrally arcuate conoid tail, reduced caudal glands and no tail terminal opening. Also, interestingly, several members of Anatonchidae, Iotonchidae and Mononchidae appear to cluster together (*e.g.*, a maximally supported clade formed by *Parkellus* Jairajpuri, Tahseen & Choi, 2001, *Coomansus* Jairajpuri & Khan, 1977, *Jensenonchus*, *Prionchulus*, *Anatonchus* and *Mulveyellus* in the 28S tree and *Jensenonchus*, *Prionchulus* and *Mulveyellus* in the 18S tree) in the phylogenetic trees, indicating that their familial and generic morphological characters do not always correspond to their molecular data as was also observed by Ahmad & Jairajpuri (2010). Unfortunately, most of the sequences included in the analyses obtained from GenBank are not associated with morphological data and therefore, their genera or species information could not be verified. More molecular data linked to morphological data of mononchid species are required to draw better phylogenetic relationships among the families and genera.

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