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Updated taxonomy and new insights into the evolutionary relationships of the genus *Sporonchulus* Cobb, 1917 (Nematoda, Mononchida) after the study of two Vietnamese species

Tam T. T. Vu[1,2], Anh D. Nguyen[1,2], Thi Mai Linh Le[1,2], Reyes Peña-Santiago[3]

1 *Institute of Ecological and Biological Resources, Vietnam Academy of Sience and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*

2 *Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*

3 *Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus "Las Lagunillas" s/n, Edificio B3, 23071- Jaén, Spain*

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Corresponding author: Tam T. T. Vu (vtam7572@gmail.com)

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Abstract

Two known species of the genus *Sporonchulus*, namely *S. ibitiensis* and *S. vagabundus*, collected from natural areas of Vietnam, are characterized, including descriptions and illustrations of both species, as well as SEM observations and molecular (18S-, 28S rDNA) analyses of *S. ibitiensis*. The identity of the two species is discussed, with detailed comparison with previously known populations. The taxonomy of the genus is updated, presenting a diagnosis, list of species, key to their identification, and a compendium of their main morphometrics. An integrative analysis, combining morphological data with a cladistic approach and the first molecular study for a representative of *Sporonchulus*, better supports a narrow relationship of this genus with Mononchidae than with Mylonchulidae members, however further research should be conducted to elucidate its phylogeny.

Key Words

Description, molecular analysis, morphology, phylogeny, 18S, 28S-rDNA

Introduction

Mononchs, the members of the nematode order Mononchida, are an interesting taxon due to their wide geographical distribution and their role as active predators. Their 47 valid genera and 432 valid species (Hodda 2022) dwell in all kind of continental, both soil and freshwater, habitats in the six continents, even Antarctica. They are comparatively large nematodes, often ranging from 1 to 3 mm long, show a very active predatory behaviour toward other nematodes, including cannibalism, and play an important role in feeding relationships of nematode assemblages. Ahmad and Jairajpuri (2010) provided an excellent monograph devoted to their morphology and taxonomy, which still is the best reference for a general overview of the group.

The genus *Sporonchulus* is a rare mononchid taxon, with only four valid species, that, however, display a Pantropical distribution, recorded in Neotropics, Africa and Indomalayan region (Ahmad and Jairajpuri 2010). Originally proposed by Cobb (1917) as a subgenus of *Mononchus* Bastian, 1865 with *M.* (*S.*) *dentatus* Cobb, 1917 as its type and only species, its rank was raised to genus level by Andrássy (1958). Taxonomy of *Sporonchulus* was studied by different authors (Mulvey 1963; Jairajpuri 1971; Andrássy 1993; Ahmad and Jairajpuri 2010) and its position in Mononchida tree was a matter of some controversy. Thus, Jairajpuri (1969) created the subfamily Sporonchulinae, within the family Mylonchulidae Jairajpuri, 1969, to include the genera *Granonchulus* Andrássy, 1958, *Judonchulus* Andrássy, 1958, *Prionchuloides* Mulvey, 1963 and *Sporonchulus* Cobb, 1917.

This idea was followed in subsequent major contributions co-authored by the same author (Jairajpuri and Khan 1982; Ahmad and Jairajpuri 2010). Nevertheless, other authors (Andrássy 1976, 1993, 2009; Zullini and Peneva 2006) did not accept Jairajpuri's scheme, did not recognise Sporonchulinae as a valid taxon, and considered all its genera as members of Mononchinae Filipjev, 1934 in Mononchidae Filipjev, 1934. These authors provided morphological evidence in favour of their proposals, but no molecular analyses were available to support them. Besides, Loof (2006) stated (p. 308) that "this genus is in need of revision".

The occurrence of *Sporonchulus* in Vietnam is not reported so far. Nevertheless, a population of *Sporonchulus ibitiensis* (Carvalho, 1951) Andrássy, 1958 was collected in the course of a general nematological survey conducted to explore the mononchid diversity of natural enclaves of the country, and fresh specimens were therefore available for sequencing. Besides, the revision of the material previously identified as *Actus conoidus* Dhanachand, Romoni & Pramodini, 2006 by Vu (2017) has revealed that it belongs to *S. vagabundus* Jairajpuri, 1971. Thus, this contribution aims to provide a morphological characterisation of the Vietnamese specimens of the mentioned above species, to carry out the first molecular study of a representative of the genus, to discuss their evolutionary relationships and to provide its updated taxonomy.

Materials and methods

Sampling, extraction and processing of nematodes

Three females of *S. vagabundus*, previously deposited at Department of Nematology, Institute of Ecology and Biological Resources (IBSR), Vietnamese Academy of Sciences and Technology (VAST), Hanoi, Vietnam, were available for study. Four females of *S. ibitiensis* obtained from soil samples were collected in a natural forest at Tram Tau town, Yen Bai Province and Du Gia Natural Reserve, Ha Giang Province (Vietnam). Nematodes were extracted using a modified Baermann funnel technique (Southey 1986), heat killed, fixed in TAF solution (Southey 1986) for morphological observations or in a DESS mixture (Yoder et al. 2006) for molecular analyses. Then, specimens were transferred to anhydrous glycerol (Seinhorst 1959, 1962), and mounted on glass slides for their observation with light microscopy. Nematodes were measured and photographed using an Eclipse 80i microscope (Nikon, Tokyo, Japan) with differential interference contrast optics, a drawing tube (camera lucida) and a DS digital camera. Line drawings were made from taken photomicrographs, after processing with Adobe Photoshop CS8. Morphometrics include Demanian indices and other measurements and ratios, some of them presented in a separate table; meanwhile, others form part of the literal description of the species. All measurements were recorded in µm, except body length in mm. After filming and taking pictures, selected specimens were submitted for molecular studies. SEM photographs were taken using SEM PRISMA E according to Abolafia (2015) at VAST. Pictures were edited using Adobe Photoshop CC2018.

DNA extraction, polymerase chain reaction (PCR) and sequencing

Nematode DNA of *S. ibitiensis* was extracted from a single individual as described by Holterman et al. (2006) and DNA extracts were stored at –20° until used as PCR template. The D2-D3 expansion segment of 28S rDNA and 18S were amplified using the forward D2A (5′–ACAAGTACCGTGGGGAAAGTTG–3′) and reverse D3B (5′–TCGG AAGGAACCAGCTACTA–3′) primers (Subbotin et al. 2006) and 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), 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 60s with a final incubation for 5 min at 72 °C. Amplicons were visualised under UV illumination after Simply safe gel staining and gel electrophoresis. Purified PCR products were sent to Apical Scientific Company for sequencing (Selangor, Malaysia). After sequencing the obtained rDNA sequences fragments were deposited in GenBank under the following accession numbers: OQ377123 (18S) and OQ377128 (28S).

Phylogenetic analyses

For exploring phylogenetic relationships, analyses were based on 18S and 28S rDNA. The newly obtained sequences were manually edited using Chromas 2.6.6 (Technelysium, Queensland, 110 Australia) and aligned with other sequences available in GenBank using ClustalW alignment tool implemented in the MEGA11 (Kumar et al. 2021). Poorly aligned regions at extremes were removed from the alignments using MEGA7. The best fit model of nucleotide substitution used for the phylogenetic analysis was statistically selected using jModelTest 2.1.10 (Darriba et al. 2012). The phylogenetic tree was generated with the Bayesian inference method using MrBayes 3.2.6 (Ronquist et al. 2012). The analysis under the generalised 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 and Simon 1999) for 1×10^6 generations. The tree was visualised and saved with FigTree 1.4.4 (Rambaut 2018).

Results

Descriptions of species

Sporonchulus ibitiensis **(Carvalho, 1951) Andrássy, 1958**

Mononchus ibitiensis Carvalho, 1951. Syn.
Sporonchuloides ibitiensis (Carvalho, 1951) Mohandas & Prabhoo, 1982.

Material examined. Four females from one location, in good state of preservation.

Morphometrics. See Table 1.

Description. Female. Moderately slender to slender (*a* = 28–34) nematodes of medium size, 1.09–1.37 mm long. Body cylindrical, slightly tapering towards the anterior end and more appreciably towards the posterior extremity as the tail is conical. Upon fixation, habitus strongly curved ventrad, C- to G-shaped. Cuticle smooth when observed with LM, but showing very fine transverse striation under SEM, two-layered, 1 µm thick at anterior region, 1.5 µm in midbody and 1.5–2 µm on tail. Lip region almost continuous with the adjoining body, 2.0–2.2 times as wide as high and one-half to two-thirds (53–67%) of body diameter at neck base, with totally fused lips and weakly protruding papillae; SEM observations (Fig. 1D): oral field comparatively small, with almost hexagonal oral aperture surrounded by six perioral liplets, labial papillae button-like, prominent, cephalic papillae also button-like, but visibly smaller than labial ones. Amphid fovea small, goblet-like, located at 10–11 µm from the anterior end, its aperture a short transverse slit 3–3.5 µm long, occupying up to one sixth (12–16%) of lip region diameter. Vestibulum 4.5–5.5 µm long. Buccal cavity up to twice (1.8–1.9 times) as long as wide, 1.1–1.3 times longer than lip region diameter: vertical (anterior) plates somewhat convergent at their anterior and posterior ends, their walls 1–1.5 µm thick, horizontal (posterior or basal) plates visibly oblique, with foramina, dorsal tooth apex situated at anterior third of buccal cavity (68–74% from the base), anterior subventral plates bearing each two irregular rows of small teeth with decreasing size from the base till the level of dorsal tooth. Anterior end of pharynx embracing the basal part of buccal cavity, gland nuclei obscure except S_2N = 81–85%. Nerve ring located at 92–101 µm or *ca* one-third (31–36%) of the total neck length. Pharyngo-intestinal junction non-tuberculate, 14–18 × 8.5–10 µm. Genital system diovarian, with small and equally developed branches occupying 6–13% of body length: ovaries comparatively large, 50–146 µm long, with oocytes first arranged in several rows and then in only one row; genital tract very short and poorly differentiated, oviduct 35–48 µm long or 1.0–1.2 body diameters, ***pars dilatata oviductus*** not enlarged, uterus 17–33 µm long or 0.5–0.7 body diameters, both separated by an indistinct weak sphincter; vagina 11 µm long, extending inwards to one-third (34%) of body diameter, ***pars proximalis*** 6.5 × 1.5 µm, ***pars refringens*** with two drop-shaped or somewhat trapezoidal sclerotized pieces 2.5 × 2.5 µm and a combined width of 5 µm, ***pars distalis*** 1.5 µm, vulva a transverse slit. Rectum 0.8–1.0 times the anal body diameter long. Tail conical with finely rounded tip, regularly curved ventrad, with poorly developed glands and lacking a terminal spinneret (Figs 1, 2).

Table 1. Main morphometrics of two *Sporonchulus* species found in Vietnam. Measurements in µm except L in mm.

Species	*S. ibitiensis*	*S. vagabundus*
n	4♀♀	3♀♀
Character		
L	1.26 ± 0.12 (1.09–1.37)	1.27–1.41
a	29.9 ± 2.8 (28–34)	34–39
b	4.1 ± 0.2 (3.8–4.2)	4.6–4.8
c	24.0 ± 1.5 (22–25)	23–25
c'	2.2 ± 0.0 (2.1–2.2)	2.4–2.5
V	60.4 ± 0.9 (60–62)	57–59
Lip region diameter	24.1 ± 1.9 (21–26)	20–21,
Buccal cavity length	27.3 ± 0.5 (27–28)	22–23
Buccal cavity width	14.5 ±1.7 (12–16)	11.5–12.5
Dorsal tooth apex (%)	71.6 ± 2.8 (68–74)	73–75
Neck length	293 ± 13 (284–302)	278–293
Body diameter at neck base	35.5 ± 4.9 (32–39)	34–36
midbody	42.8 ±7.3 (32–48)	33–40
anus	24.8 ± 3.3 (21–29)	22–24
Distance vulva – ant. end	761 ± 64 (674–824)	748–806
Rectum length	21.0 ± 3.8 (16–24)	20–23
Tail length	52.8 ± 6.7 (46–62)	53–60

Male. Not found.

Molecular characterization. After sequencing and editing, two sequences were obtained for phylogenetic analyses: one full length 18S rDNA with 1591 bp length (GenBank accession N° OQ377123) and one nearly 745 bp length D2D3 of LSU rRNA (28S) (GenBank accession OQ377128).

Locality and habitat. Vietnam, Yen Bai Province, Tram Tau town (coordinates 21°50'18"N, 104°44'22"E, altitude 930 m) and Ha Giang Province, Du Gia Natural Reserve, (coordinates 22°43'18"N, 105°11'38"E, altitude 780 m) where the nematodes were found in soil around the roots of forest trees.

Voucher specimens. Permanent slides are stored at the Department of Nematology, Institute of Ecology and Biological Resources, VAST, Hanoi, Vietnam.

Remarks. Present description provides new data and illustrations of *S. ibitiensis*, especially useful for comparative purposes. General morphology of Vietnamese females very well fits that of type specimens and other known populations (Mulvey 1963; Mulvey and Jensen 1967; Lordello 1970; Chaves and Geraert 1977; Mohandas and Prabhoo 1979; Jairajpuri and Khan 1982; Chaves 1990; Loof 2006; Tahseen et al. 2013; Perichi et al. 2021) of the species. Nevertheless, their morphometrics need further analysis. Table 1 shows the most relevant measurements and ratios of females herein studied; meanwhile, Table 2 includes those available from the literature. Especially relevant is the variation observed in buccal cavity length (18–34 µm), an unusually wide range in mononchid species. Actually, Vietnamese specimens display 26–28 µm long buccal cavity, totally comparable or

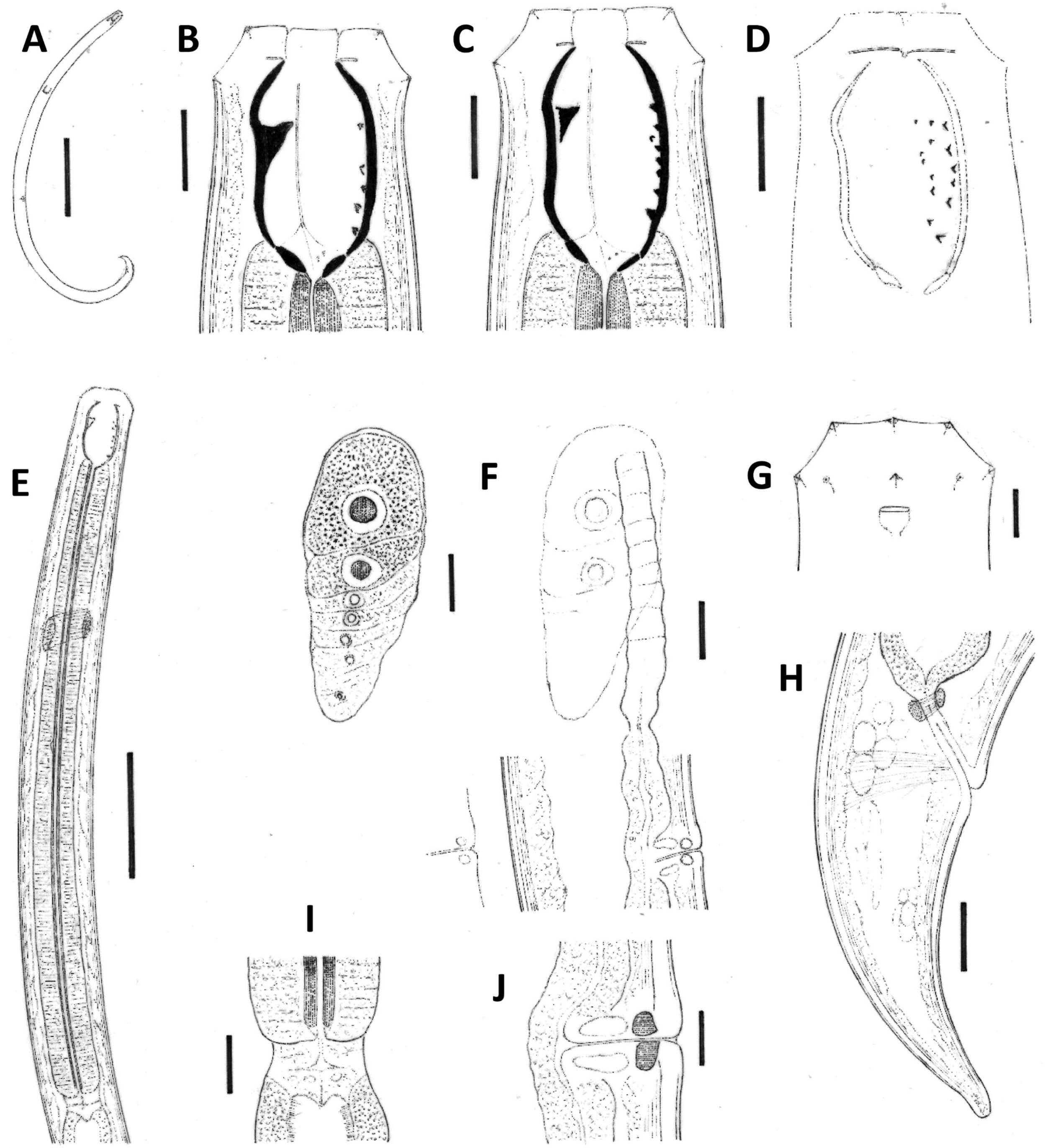

Figure 1. *Sporonchulus ibitiensis* (Carvalho, 1951) Andrássy, 1958 (Female). **A.** Entire; **B–D.** Anterior body region, lateral median (**B, C**) and lateral submedian (**D**) view; **E.** Neck region; **F.** Anterior genital branch; **G.** Anterior body region, lateral surface view; **H.** Caudal region; **I.** Pharyngo-intestinal junction; **J.** Vagina. Scale bars: 200 µm (**A**); 10 µm (**B–D, F, H, I**); 50 µm (**E**); 5 µm (**G, J**).

with appreciably overlapping ranges to those reported for Afrotropical (Mulvey and Jensen 1967; Chaves and Geraert 1977) and Indomalayan (Mulvey 1963; Mohandas and Prabhoo 1979; Jairajpuri and Khan 1982; Loof 2006) populations, but they differ from some South American specimens (Carvalho 1951; Chaves 1990), 25–33 vs 18–23 µm, indeed a remarkable difference that might be the result of a biogeographical pattern with two tentative species or subspecies. Nevertheless, a doubt persists over the true identity of several of these populations, which should be resolved before proposing any taxonomical change. Thus, South American females recorded by Carvalho (1951) and Chaves (1990) were not characterised enough for a comparative analysis. The two Brazilian females studied by Lordello (1970) showed 23 and 27.5 µm long buccal cavity. Chaves and Geraert (1977) studied three females from the former Zaire characterised by bearing a terminal subdorsal pore, which might be a remarkable distinguishing trait. Besides, two (more recent) contributions (Tahseen et al. 2013; Perichi et al. 2021) raise more uncertainties. On the one hand, Tahseen et al. (2013) studied only one Indian female with 22 µm long buccal cavity, which suggests that the species might display more variation in Indomalayan range than previously re-

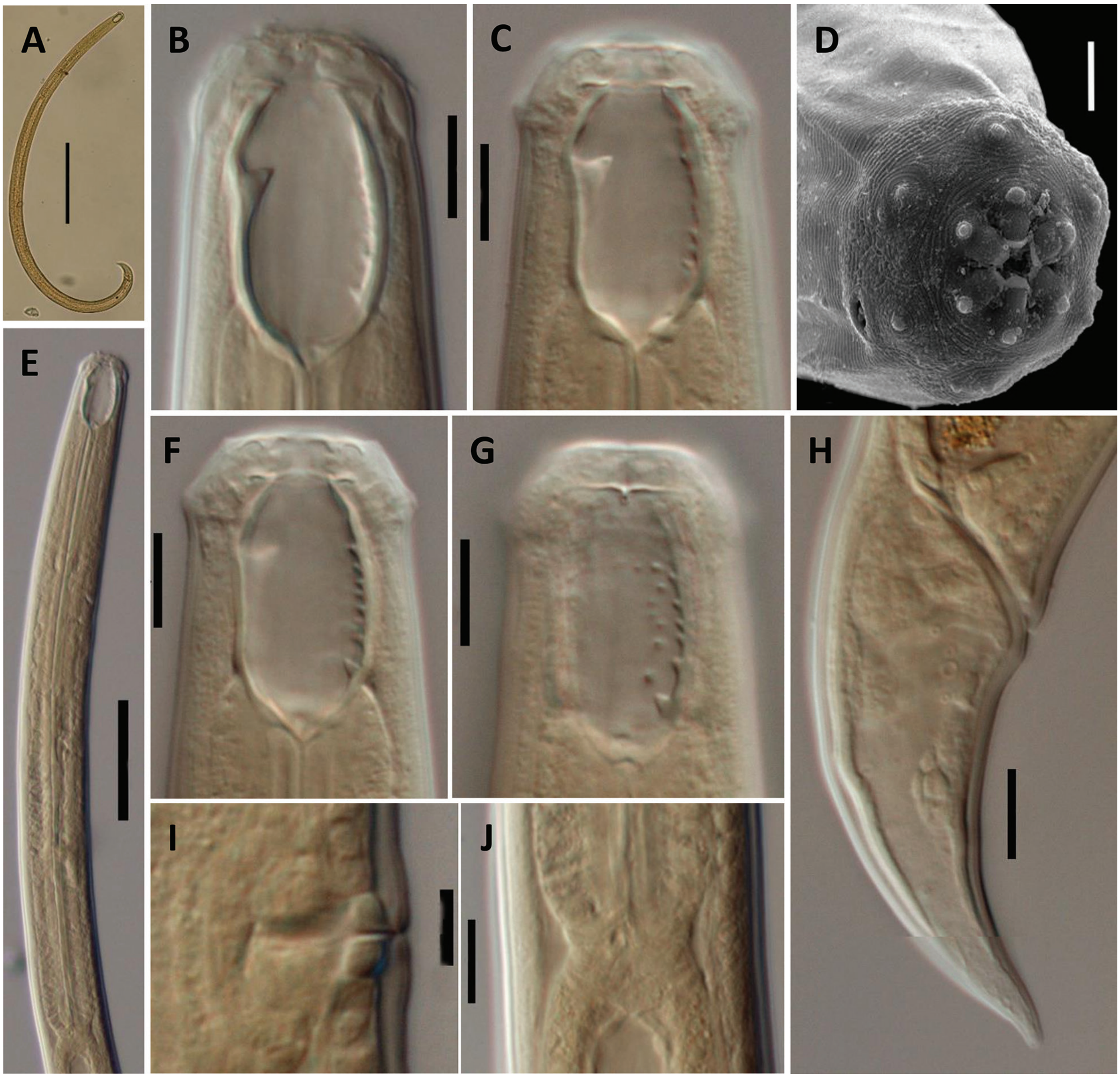

Figure 2. *Sporonchulus ibitiensis* (Carvalho, 1951) Andrássy, 1958 (Female, LM). **A.** Entire; **B, C, F, G.** Anterior body region, lateral median (**B, C**) and lateral submedian (**F, G**) view; **C.** Lip region (SEM), in face view; **E.** Neck region; **H.** Caudal region. **I.** Vagina; **J.** Pharyngo-intestinal junction. Scale bars: 200 µm (**A**); 10 µm (**B, C, F, G, H, J**); 5 µm (**D, I**); 50 µm (**E**).

ported. On the other hand, Perichi et al. (2021) described two Venezuelan populations whose buccal cavities display very wide variation in their length (27–33 and 24–34 µm) and with appreciable difference in their lip region width (26–30 and 20–27 µm, respectively).

Sporonchulus vagabundus Jairajpuri, 1971

Material examined. Three females from one location, in good state of preservation.

Morphometrics. See Table 1.

Description. Female. Moderately slender to slender (a = 28–35) of medium size, 1.27–1.41 mm long. Body cylindrical, slightly tapering towards the anterior end and more appreciably towards the posterior extremity as the tail is conical. Upon fixation, habitus strongly curved ventrad, C- to G-shaped. Cuticle smooth, two-layered, 1 µm thick at anterior region, 1–1.5 µm in midbody and 2 µm on tail. Lip region almost continuous with the adjoining body, 2.1–2.5 times as wide as high and hardly more than one-half (53%) of body diameter at neck base, with totally fused lips and weakly protruding papillae. Amphid fovea small, goblet-like, located at 8–10 µm from the anterior end, its aperture a short transverse slit 3 µm long, occupying less than one-sixth (14–15%) of lip region diameter. Vestibulum 5 µm long. Buccal cavity up to twice (1.8–1.9 times) as long as wide, hardly longer (1.1 times) than lip region diameter: vertical (anterior) plates somewhat convergent at their anterior and posterior ends, their walls 1–1.5 µm thick, horizontal (posterior or basal) plates visibly oblique, with foramina, dorsal tooth apex situated at three-fourths of buccal cavity (74–75% from the base), anterior subventral plates bearing each two irregular rows

Table 2. Compilation of main morphometrics of *Sporonchulus* species. Measurements in µm except L in mm.

Character / Species	n	L	a	b	c	V	c'	lrd	bcl	neck	abd	tail	spic	vm	country	Ref.[1]
S. coronatus	3♀♀	1.07–1.40	17–22	3.3–4.0	20–26	60–66	?	?	28	?	?	?	–	–	Brazil	1, 2
S. dentatus	♀	1.1	31*	3.7*	20*	62	2.6*	29*	33*	297*	21*	55*	–	–	Brazil	3
	♀	1.1–1.3	30–31*	4.0*	18–19*	61	2.3*	25–30	31–36	272–231*	22–26*	60–71	–		Mauritius	4
	♀	1.25	30	3.9	19	62	?	?	34	321*	?	65	–	–	Venezuela	2
?	♀♀	1.14–1.15	26–27	3.4	21–23	62–64	2.1*	?	32–34	330–335	?	49–55	–	–	Brazil	5
?	♀♀[2]	1.1–1.5	26–30	3.4–3.9	19–23	62–64	2.0–2.5	?	32–35	?	?	?	–	–	Several	6
S. Ibitiensis	♀	1.4	24	4	23*	60	2.5*	23*	18*	350*	24	60	–	–	Brazil	7
	♀	1.2	30	3.9	24	63	2.6*	?	25	308*	19*	50	–	–	Sri Lanka	2
	5♀♀	1.1–1.4	28–36	3.9–4.3	21–23	57–63	?	?	27–28	?	?	52–62	–	–	Nigeria	8
	3♀♀	1.01–1.05	23–25	3.0	17–21	57–60	2.3*	25*	26	?	20*	47*	–	–	Zaire	9
	2♀♀	0.99, 1.37	21, 26	3.4, 3.9	20, 26	71, 61	?	?	23, 27	291, 344	?	49, 53	–	–	Brazil	5
	21♀♀	1.24–1.43	20–28	3.9–4.4	23–28	59–63	1.7–2.1	22–28	28–30	?	?	47–60	–	–	India	10, 11
	4♂♂	1.18–1.28	27–30	3.8–4.0	23–24	–	1.6–1.7	22–28	30–31	?	?	52–54	30–35	11–12		
	8♀♀	0.95–1.12	25–32	3.5–4.2	17–19	58–60	2.1–2.7	19–23	21–23	210–237	?	49–64	–	–	Argentina	12
	30♀♀	1.26–1.73	24–34	3.8–5.0	20–27	58–64	1.8–3.1	20–28	26–33	299–396	21–33	53–78	–	–	Malaysia	13
	♀	1.10	27	4.0	20	61	2.0	22	22	272	27	57	–	–	India	14
	11♀♀[2]	1.0–1.5	22–33	3.4–5.2	17–28	59–63	1.4–2.3	20–30	24–34	?	24–30	36–57	–	–	Venezuela	15
	4♀♀	1.09–1.37	28–34	3.8–4.2	22–25	60–62	2.1–2.2	21–26	27–28	284–302	21–29	46–62	–	–	Vietnam	16
S. vagabundus	3♀♀[2]	1.26–1.34	28–36	4.2–4.6	22–25	58–61	2.0	19–21	21–22	280–319*	?	53–58	–	–	India	17
?	♀♀[2]	1.2–1.8	22–32	3.7–4.8	21–27	51–63	2.0–2.5	22–27	22–27	?	?	50–79	–	–	India	11
?	10♀♀[2]	0.91–1.09	20–29	3.6–4.2	19–22	59–64	1.7–2.4	20–23	24–27	232–297	22–28	45–55	–	–	India	14
	3♀♀	1.27–1.41	34–39	4.6–4.8	23–25	57–59	2.4–2.5	20–21	22–23	278–293	22–24	53–60	–	–	Vietnam	16

[1]References: 1 – Carvalho (1956). 2 – Mulvey (1963). 3 – Cobb (1917). 4 – Williams (1958). 5 – Lordello (1970). 6 – Ahmad and Jairajpuri (2010). 7 – Carvalho (1951). 8 – Mulvey and Jensen (1967). 9 – Chaves and Geraert (1977). 10 – Mohandas and Prabhoo (1979). 11 – Jairajpuri and Khan (1982). 12 – Chaves (1990). 13 – Loof (2006). 14 – Tahseen *et al.* (2013). 15 – Perichi *et al.* (2021). 16 – Present paper. 17 – Jairajpuri (1971).
[2]Morphometrics of specimens collected from two or more locations.
* Values calculated from literal description and/or other morphometrics.
? = Either populations whose identity raises some doubt (see text) or morphometrics not available from the corresponding reference.

of small teeth with decreasing size from the base till the level of dorsal tooth. Anterior end of pharynx embracing the basal part of buccal cavity, gland nuclei obscure. Nerve ring located at 93–101 µm or *ca* one-third (34–36%) of the total neck length. Pharyngo-intestinal junction non-tuberculate, 15 × 8–9 µm. Genital system diovarian, with small and equally developed branches occupying 6–7% of body length: ovaries comparatively large, 50–56 µm long, with oocytes first arranged in several rows and then in only one row; genital tract very short: oviduct 40–57 µm long or 1.1–1.4 body diameters, consisting of a small distal part and a well-developed ***pars dilatata***, sphincter hardly perceptible, uterus a simple tube 17–21 µm or 0.4–0.5 body diameters; vagina 11–12 µm long, extending inwards to less than one-third (29–30%) of body diameter, ***pars proximalis*** 5.5–6.5 × 1.5–2 µm, ***pars refringens*** with two trapezoidal sclerotized pieces 2.5 × 2 µm and a combined width of 4.5–5.5 µm, ***pars distalis*** 0.5–1.5 µm, vulva a transverse slit. Rectum as long as anal body diameter long. Tail conical with finely rounded tip, regularly curved ventrad, with distinct caudal glands leading to a visible ampulla with terminal spinneret 2.5 µm long (Figs 3, 4).

Male. Not found.

Locality and habitat. Vietnam, Quang Ninh Province, Bach Long Vi Island, where the nematodes were collected in soil around the roots of a natural forest.

Voucher specimens. Permanent slides are stored at the Department of Nematology, Institute of Ecology and Biological Resources, VAST, Hanoi, Vietnam.

Remarks. As mentioned in the introductory section, these specimens were originally described as *A. conoidus* by Vu (2017). Nevertheless, their general morphology and morphometrics are almost identical to those provided by Jairajpuri (1971) for the type material of *S. vagabundus*, with no appreciable difference. This species is very similar to *S. ibitiensis*, but the study of Vietnamese specimens of both species have revealed some relevant differences. Morphologically, *S. vagabundus* females show a more developed ***pars dilatata oviductus*** and bear distinct caudal glands that, most importantly, lead to appreciable terminal ampulla and spinneret (vs ampulla and spinneret absent). Morphometrically (Table 1), *S. vagabundus* shows a much shorter buccal cavity (22–23 vs 27–28 µm). Although these differences are based on the comparison of only a few specimens, they seem significant enough to separate both species.

Jairajpuri's (1971) original description and Vietnamese specimens herein studied are regarded as the basic material for the characterization of this species and for comparative purposes. Other references should be taken with more caution due to some uncertainties. Jairajpuri and Khan (1982) provided data of specimens with excessively large ranges of some relevant morphometrics (for instance, L = 1.2–1.8 mm, buccal cavity 22–27 µm), which might belong to more than one species. Tahseen et al. (2013) studied two Indian populations with smaller general size (body length 0.91–1.09 mm), and some inconsistences in their description (for instance, buccal cavity 1.4–1.6 times wider than long according to literal description, but 24–27 × 11–13 µm after the morphometrics provided in their Table 1, and buccal cavity similar-sized after their morphometrics (25–27 and 24–26 µm long), but appreciably different according to Figs 1A, 2A and their corresponding scales.

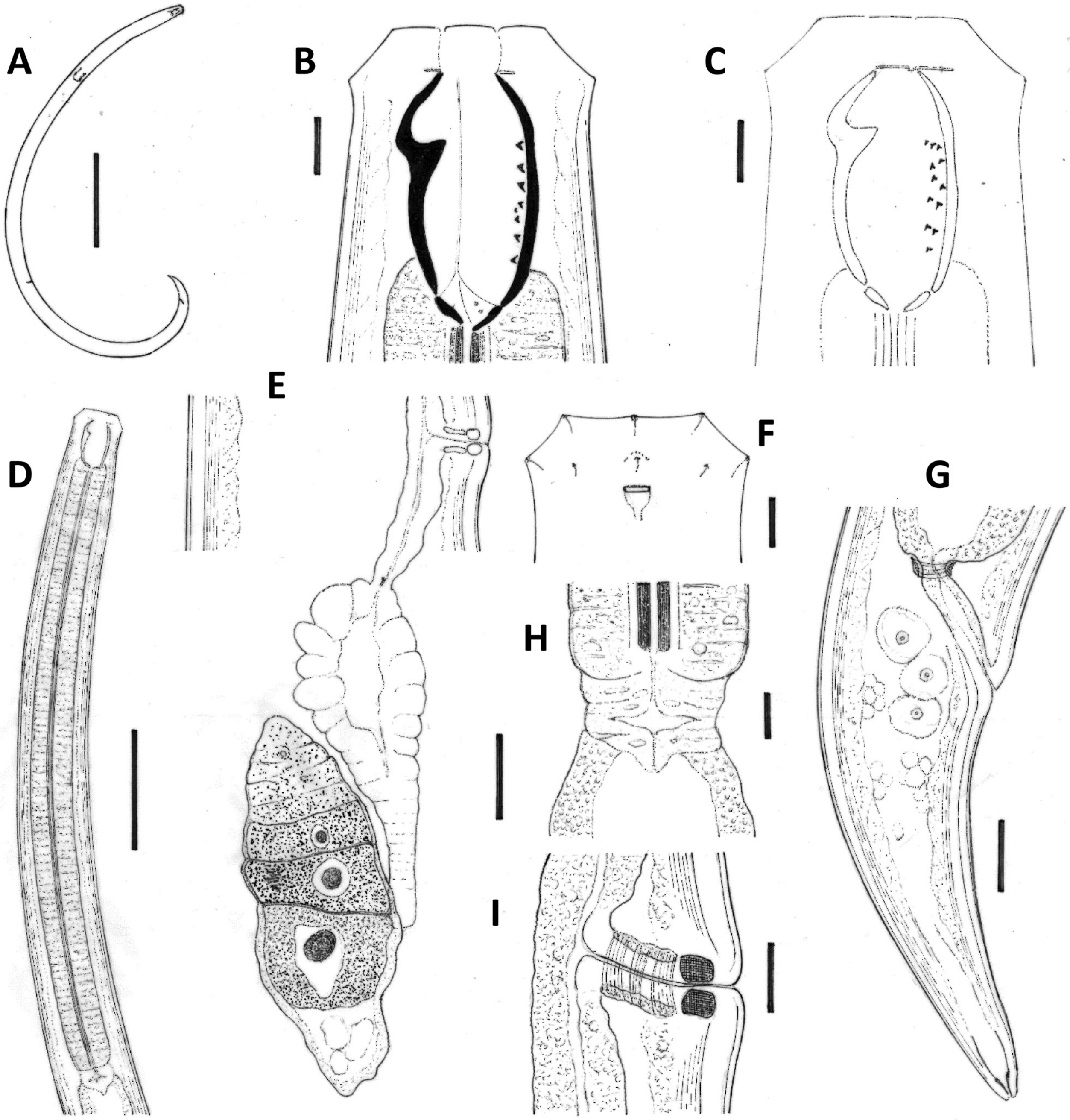

Figure 3. *Sporonchulus vagabundus* Jairajpuri, 1971 (Female). **A.** Entire; **B, C.** Anterior body region, lateral median view; **D.** Neck region; **E.** Posterior genital branch; **F.** Anterior body region, lateral surface view; **G.** Caudal region; **H.** Pharyngo-intestinal junction; **I.** Vagina. Scale bars: 200 µm (**A**); 5 µm (**B, C, F, G, H**); 50 µm (**D**); 10 µm (**E, G**).

Evolutionary relationships of *Sporonchulus* Cobb, 1917

The study of Vietnamese females of *S. ibitiensis* and *S. vagabundus* confirms at least two remarkable morphological features with evolutionary projection: the absence of tubercles at their pharyngo-intestinal junction and, most important, its buccal cavity, very similar to that found in Mononchidae members. Thus, the buccal cavity consists of more or less parallel, convergent at their both ends, vertical (anterior) plates, and visibly oblique horizontal (posterior) plates. Besides, the dorsal tooth, located at the anterior third of buccal cavity, shows an almost horizontal plane anterior margin, very slightly forward directed. This design significantly differs from that observed in Mylonchulidae, with the vertical and basal plates more distinctly converging at their posterior ends than at their anterior ends, the whole cavity becoming V-shaped, and the dorsal tooth conspicuously forwards directed, its anterior margin appearing visibly concave. The mylonchulid pattern is herein interpreted as derived (apomorphic) compared to the more primitive (plesiomorphic) mononchid (Mononchidae) pattern.

Unfortunately, the GenBank database does not include any representative of *Sporonchulus*, therefore the first sequences of both 18S and 28S rDNA are herein provid-

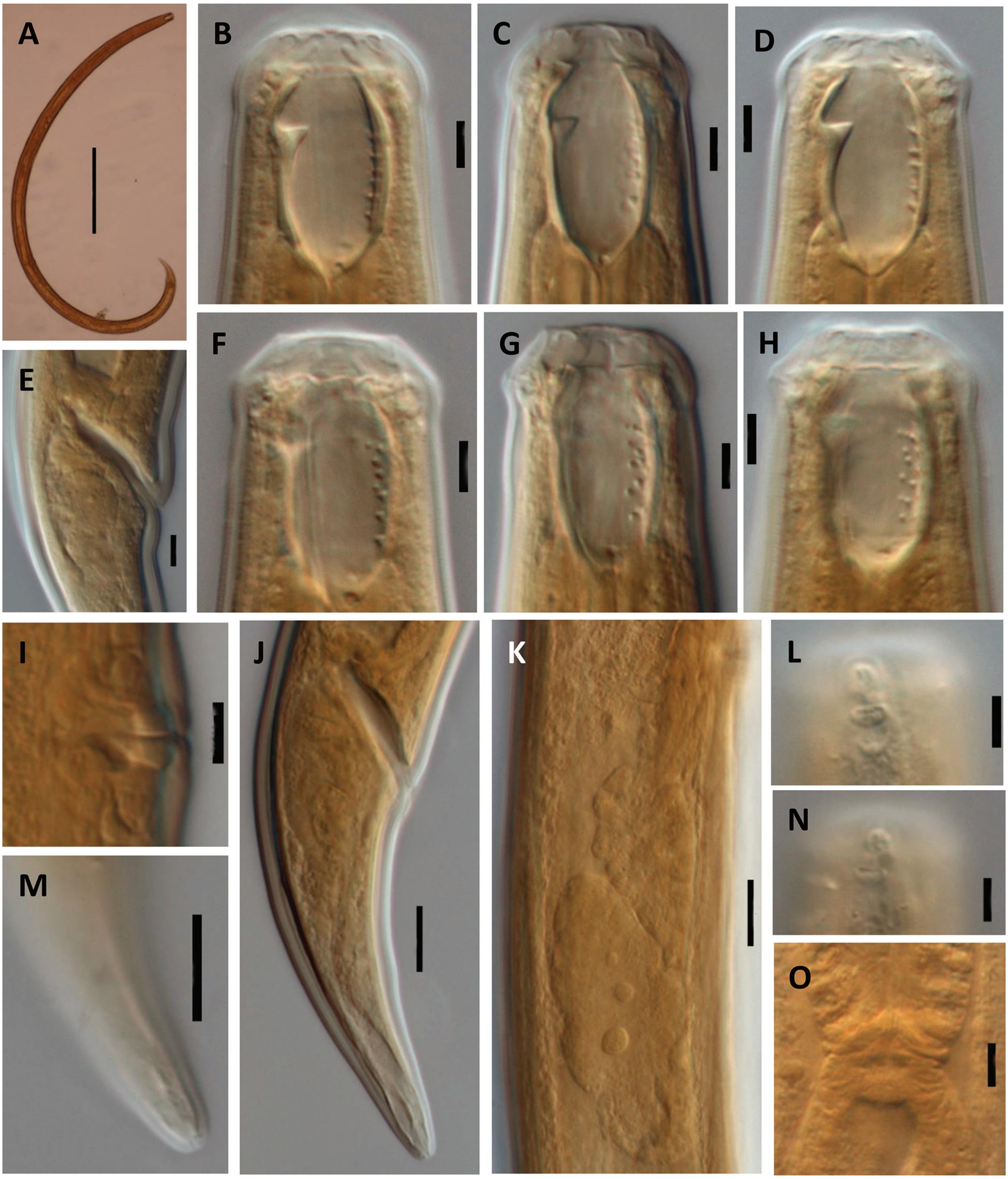

Figure 4. *Sporonchulus vagabundus* Jairajpuri, 1971 (Female, LM). **A.** Entire; **B–D.** Anterior body region, lateral median view; **E.** Caudal glands; **F–H.** Anterior body region, lateral submedian view; **I.** Vagina; **J.** Caudal region; **K.** Posterior genital branch; **L, N.** Anterior body region, lateral surface view; **M.** Detail of caudal region tip; **O.** Pharyngo-intestinal junction. Scale bars: 200 µm (**A**); 5 µm (**B–I, L, N, O**); 10 µm (**J, K, M**).

ed. The results of their analyses are presented in the trees shown in Figs 5, 6, respectively. A BLAST search for matches to the partial 18S rDNA sequence revealed 99% similarity to *Actus* sequences, 97–98% to *Miconchus* sp., 96.6% to *Iotonchulus*, 96% to several *Mylonchulus* sequences, etc. 28S rDNA sequence is 98% similar to that of *Actus*, 94–95% to *Coomansus*, *Parkellus* and *Prionchulus*, 93–94% to *Anatonchus*, 86–88% to *Mylonchulus*, etc. Both trees display a different main branching of Mononchina representatives. 18S tree presents *Mononchus* sequences (Mononchidae, Mononchinae) forming part of a totally supported (100%) clade that is the sister group of the re-

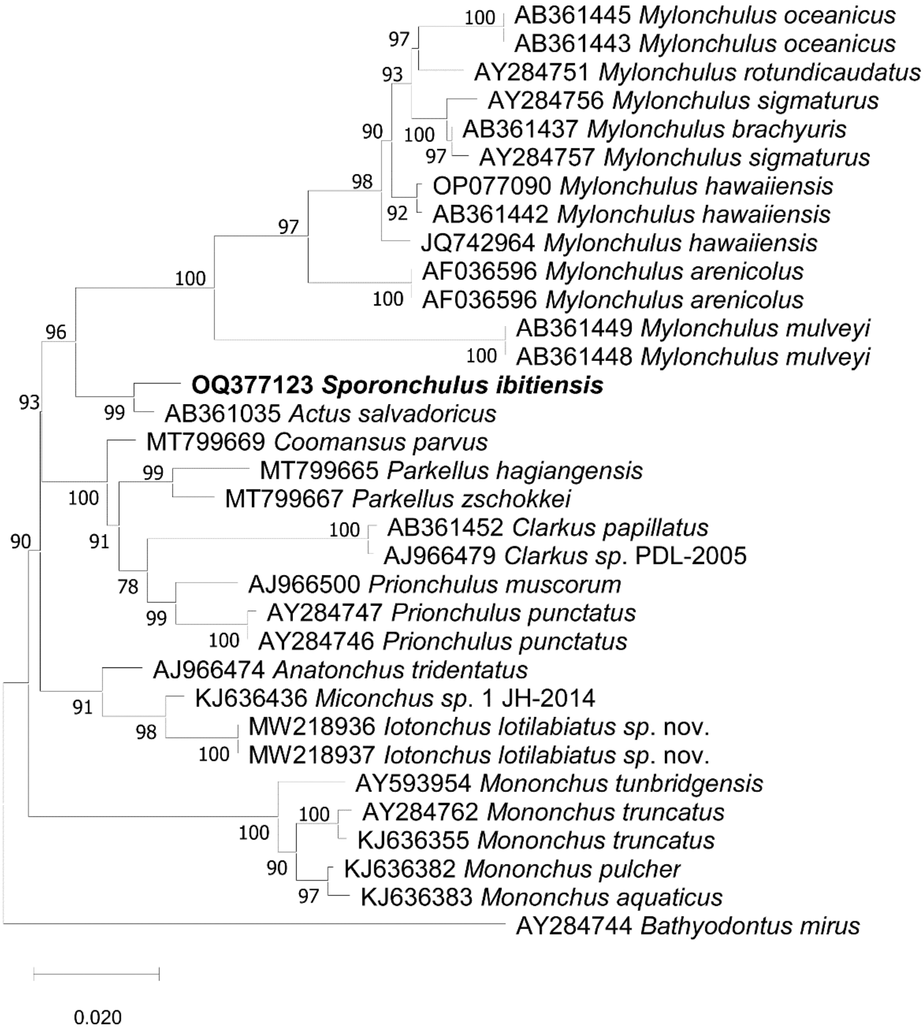

Figure 5. Bayesian Inference tree from the newly sequenced *Sporonchulus ibitiensis* (Carvalho, 1951) Andrássy, 1958 based on sequences of the 18S rDNA region. Bayesian posterior probabilities (%) are given for each clade. Scale bar shows the number of substitutions per site.

maining members of the suborder, all of them components of another, highly supported (98%) clade. Conversely, 28S tree presents *Mylonchulus* sequences (Mylonchulidae, Mylonchulinae) forming a maximally supported clade, which is the sister group of the remaining taxa included in another, maximally supported clade. It is remarkable that neither of these two trees confirms the traditional (morphological) division of Mononchina into two superfamilies, namely Anatonchoidea and Mononchoidea, a system accepted for several decades (Jairajpuri 1969; Jairajpuri and Khan 1982; Andrássy, 1976, 1993, 2009), which did not obtain confirmation when the first molecular analyses, focused on SSU rDNA (Holterman et al. 2006, 2008; Olia et al. 2008; van Megen et al. 2009), were available. Regarding the position of *Sporonchulus* sequences in Mononchida tree, both trees significantly differ in their topology. Thus, 18S tree shows *Sporonchulus* sequence as part of a highly supported (99%) clade also including *Actus salvadoricus*, this clade being the sister group of a maximally supported (100%) *Mylonchulus* clade, both clades grouped in a less supported major (96%) clade. These results argued in favour of the monophyly of Sporonchulinae and of its inclusion in the family Mylonchulidae. Conversely, in LSU tree the *Sporonchulus* sequence is included in a weakly supported (85%) large clade together with *Mononchus* sequences, which is the sister group of a maximally supported clade including all the sequences belonging to Anatonchoidea genera (*Anatonchus*, *Iotonchus*, *Miconchus*, *Parahadronchus*).

As mentioned in the introductory section, the position of *Sporonchulus* and Sporonchulinae was a matter of some controversy. Several authors (Andrássy 1976, 1993, 2009; Zullini and Peneva 2006) claimed they belonged to Mononchidae. This hypothesis is herein morphologically supported. Conversely, molecular analyses do not agree about the position of *Sporonchulus* sequences (see above), but 28S tree provides better resolution in the main branching of Mononchina, with *S. ibitiensis* sequence forming part of the large clade which is the sister group of *Mononchus* clade, thus supporting the results derived from morphological analysis. Although a further study should be conducted to clarify the subject, the belonging of *Sporonchulus* and Sporonchulinae to Mononchidae is herein tentatively accepted.

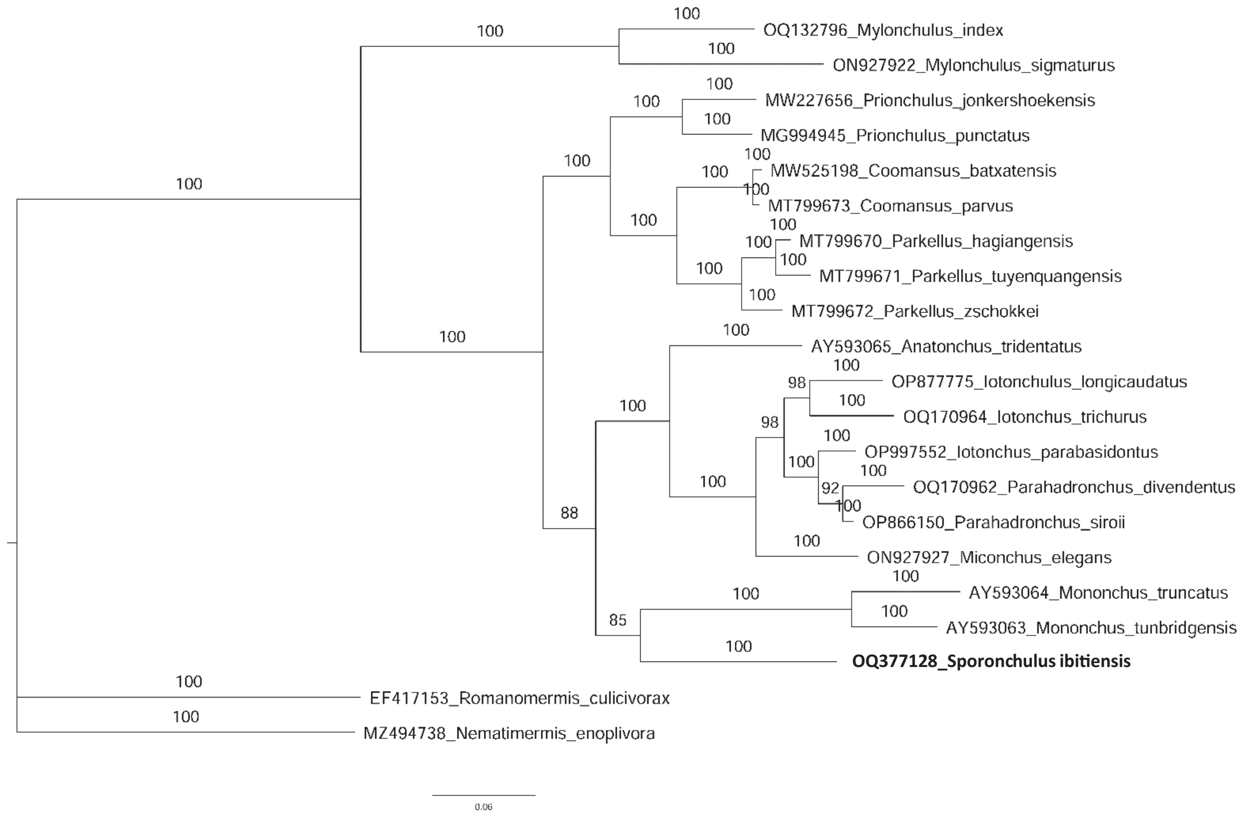

Figure 6. Bayesian Inference tree from the newly sequenced *Sporonchulus ibitiensis* (Carvalho, 1951) Andrássy, 1958 based on sequences of the 28S rDNA region. Bayesian posterior probabilities (%) are given for each clade. Scale bar shows the number of substitutions per site.

Taxonomy of *Sporonchulus* Cobb, 1917

Sporonchulus Cobb, 1917

= *Sporonchuloides* Mohandas & Prabhoo, 1982, syn. by Andrássy (1993).

Historical outline. Cobb (1917) proposed the subgenus *Sporonchulus* under the genus (*Mononchus*) to accommodate three new species, namely *M.* (*S.*) *decurrens*, *M.* (*S.*) *dentatus* (type) and *M.* (*S.*) *recessus*, and characterized it by the presence of irregularly arranged denticles opposed to the dorsal tooth. Andrássy (1958) raised the rank of *Sporonchulus* to generic level, regarded *M. ibitiensis* Carvalho, 1951 and *M. coronatus* Carvalho, 1956 as members of *Sporonchulus*, and transferred *M.* (*S.*) *decurrens* to *Granonchulus*, and *M.* (*S.*) *recessus* to *Judonchulus*. Since then, the taxonomy of the genus has been a matter of some controversy. Goodey (1963) follows Andrássy's ideas. Mulvey (1963) listed five species, recovering *S. recessus* and describing a new species, *S. minutus*. Jairajpuri (1971) added a sixth species, *S. vagabundus*, and noted that the genus consisted of two groups of species, but he did not propose any nomenclatorial change. Jairajpuri and Khan (1982) included only four species (*coronatus*, *dentatus*, *ibitiensis* and *vagabundus*), a scheme accepted in subsequent contributions (Andrássy 1993, 2009; Ahmad and Jairajapuri 2010).

Mohandas and Prabhoo (1982) created the genus *Sporonchuloides* to transfer two *Sporonchulus* species, namely *ibitiensis* (type) and *coronatus*, with "at least two (of their denticles) arranged in longitudinal ribs", meanwhile retained under *Sporonchulus* three species: *dentatus*, *recessus* and *vagabundus*. Moreover, *Sporonchuloides* was classified under Mononchidae instead of under Mylonchulidae. Nevertheless, Andrássy (1993) discussed the identity of this genus and concluded that the denticles of *S. ibitiensis* were "not arranged along ribs", and regarded *Spronchuloides* as identical and a junior synonym of *Sporonchulus*.

Diagnosis. Mononchidae. Sporonchulinae. Small- to medium-sized nematodes, 0.95–1.78 mm long. Cuticle two-layered. Lip region almost continuous with the adjoining body, with fused lips. Buccal cavity with slightly convergent vertical plates and visibly oblique transverse plates, dorsal tooth situated at the anterior third of the cavity, each vertical subventral plate bearing two irregular rows of small teeth, ca 24 in total. Pharyngo-intestinal junction lacking tubercles. Female genital system diovarian or mono-opistho-ovarian, with poorly differentiated genital tract, distinct ***pars refringens vaginae*** and a transverse vulva. Tail conical, regularly curved ventrad, with variably developed caudal glands and terminal spinneret. Males very rare, with dorylaimid spicule and 11–12 shortly spaced ventromedian supplements without hiatus.

Separation from its relatives. Within Sporonchulinae, and by having dorsal tooth located at the anterior third of buccal cavity, *Sporonchulus* resembles the genera *Granonchulus* and *Actus* Baqri & Jairajpuri, 1974. It can be distinguished from *Granonchulus* in the absence (vs presence) of a transverse row of denticles on the vertical subventral plates at level of the dorsal tooth, and small teeth arranged in four (two on each plate) irregular longitudinal rows (vs teeth scattered, not distinctly arranged in longitudinal rows) on the vertical subventral plates. From *Actus* in bearing higher number of subventral teeth (ca 24 vs ca 10) arranged into four (vs two) irregular longitudinal rows, two rows per subventral plate (vs only one row per plate).

Type species:

S. dentatus **Cobb, 1917**
= *Mononchus* (*Sporonchulus*) *dentatus* Cobb, 1917

Other species:

S. coronatus **(Carvalho, 1956) Andrássy, 1958**
= *Mononchus coronatus* Carvalho, 1956
= *Sporonchuloides coronatus* (Carvalho, 1956) Mohandas & Prabhoo, 1982

S. ibitiensis **(Carvalho, 1951) Andrássy, 1958**
= *Mononchus ibitiensis* Carvalho, 1951
= *Sporonchuloides ibitiensis* (Carvalho, 1951) Mohandas & Prabhoo, 1982

S. vagabundus **Jairajpuri, 1971**

Key to species

1 Female genital system mono-opistho-ovarian .... *Sporonchulus coronatus*
– Female genital system diovarian 2
2 *Pars dilatata oviductus* well-developed. Caudal glands conspicuous, with terminal aperture at tail end .... *S. vagabundus*
– *Pars dilatata oviductus* hardly appreciable. Caudal glands and their aperture inconspicuous 3
3 Larger buccal cavity, 32–35 μm long .... *S. dentatus*
– Smaller buccal cavity, up to 30 μm long, only exceptionally more .... *S. ibitiensis*

Table 2 compiles the main morphometrics of known populations of *Sporonchulus* species for comparative purposes.

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