

Sectonema caobangense sp. n. from Vietnam (Nematoda, Dorylaimida, Aporcelaimidae)

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Abstract: *Sectonema caobangense* sp. n. from evergreen forest soil in Vietnam is described, including scanning electron micrograph (SEM) observations and D2-D3 LSU rDNA analysis. The new species is characterized by its 3.12 to 5.80 mm long body, lip region offset by deep constriction and 21 to 23 µm broad, mural tooth 13 to 14 µm long at its ventral side, 940 to 1,112 µm long neck, pharyngeal expansion occupying 61% to 69% of total neck length, uterus a long simple tube-like structure 292 to 363 µm long or 2.7 to 2.9 times the corresponding body diameter, *pars refringens vaginae* well developed, $V = 48$ to 56, short (36–51 µm, $c = 77$ –132, $c' = 0.5$ –0.8) and rounded tail, 87 to 99 µm long spicules, and four or five irregularly spaced ventromedian supplements bearing hiatus. *Sectonema caobangense* sp. n. differs from the typical pattern of *Sectonema* in the nature of the stomatal protrusible structure, bearing a mural tooth attached to the ventral side of the stoma. Molecular data obtained and the derived evolutionary trees support a close phylogenetic relationship with other *Sectonema* species.

Key words: Bayesian inference, description, LSU ribosomal DNA, maximum likelihood, morphology, morphometrics, SEM, taxonomy.

The genus *Sectonema* Thorne, 1930 is a remarkable, free-living nematode taxon, whose representatives are characterized, among other biological features, by their comparatively large size and predator behaviour (Heyns, 1965). It includes nearly 30 species from many countries and territories, mainly dwellers of terrestrial habitats. The taxonomy of the genus is somewhat intricate as its species are often separated by small differences, for instance the morphology of lip region, the nature of the stomatal protruding structure, tail shape, etc.

Several nematological surveys have revealed that Vietnamese nematode fauna is very rich in members of the family Aporcelaimidae Heyns, 1965, including many nondescribed species (for instance, see Álvarez-Ortega et al., 2015), some of them belonging to *Sectonema* (Nguyen et al., 2016). The study of a new population containing both females and males, collected from natural areas in the northern region of Vietnam, resulted in the discovery of an unknown species of *Sectonema*, which is described in the following. For additional introductory information, see the previous contributions already mentioned.

MATERIAL AND METHODS

Nematodes: Nematodes were collected from natural areas in Vietnam, extracted from soil samples using the methods of Baermann (1917) and Flegg (1967), relaxed and killed by heat, fixed in 4% formaldehyde, processed to anhydrous glycerine following Siddiqi's (1964) technique, and mounted on permanent glass slides to allow handling and observation under light microscope (LM).

Light microscopy: Measurements of specimens were taken using a light Olympus BH-2 microscope equipped with differential interference contrast. Morphometrics included de Man's indices and usual measurements. The location of the pharyngeal gland nuclei is expressed according to Loof and Coomans (1970) and spicule terminology followed Peña-Santiago et al. (2014). Some of the best preserved specimens were photographed with a Nikon Eclipse 80i microscope and a Nikon DS digital camera. Raw photographs were edited using Adobe Photoshop CS. Drawings were made using a *camera lucida*.

Scanning electron microscopy: After their examination and identification, two specimens preserved in glycerine were selected to their observation under SEM following the protocol by Abolafia and Peña-Santiago (2005). The nematodes were hydrated in distilled water, dehydrated in a graded ethanol–acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin scanning electron microscope.

DNA extraction, PCR, and sequencing: DNA was extracted from a single individual using the proteinase K protocol. Nematode material was transferred to an 0.5-ml Eppendorf tube containing 18 µl of worm lysis buffer (50 mM KCL, 10 mM Tris pH 8.3, 2.5 mM MgCl₂, 0.45% NP 40, and 0.45% Tween 20) and 2 µl proteinase K (600 µg/ml) (Thermo Scientific). The tubes were incubated at 65°C (1 hr) and then at 95°C (15 min). PCR and sequence protocols were as described in detail by Nguyen et al. (2016).

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The primers used for amplification of D2-D3 region of 28S rRNA gene were D2A (5'-ACAAGTACCGTGAGG-GAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGC-TACTA-3') (Subbotin et al., 2006). The sequence obtained was submitted to the GenBank database under accession number KX018821.

Phylogenetic analyses: The newly obtained sequences were aligned with other 48 D2-D3 expansion segments

of 28S rRNA gene sequences available in GenBank using ClustalX 1.83 (Thompson et al., 1997). Outgroup taxa were chosen according to the results of previous published data (Holterman et al., 2008; Álvarez-Ortega et al., 2013). Sequence alignments were manually edited using GenDoc 2.6.002 (Nicholas et al., 1997). The sequence dataset was analysed with Bayesian inference (BI) and maximum likelihood (ML) using MrBayes

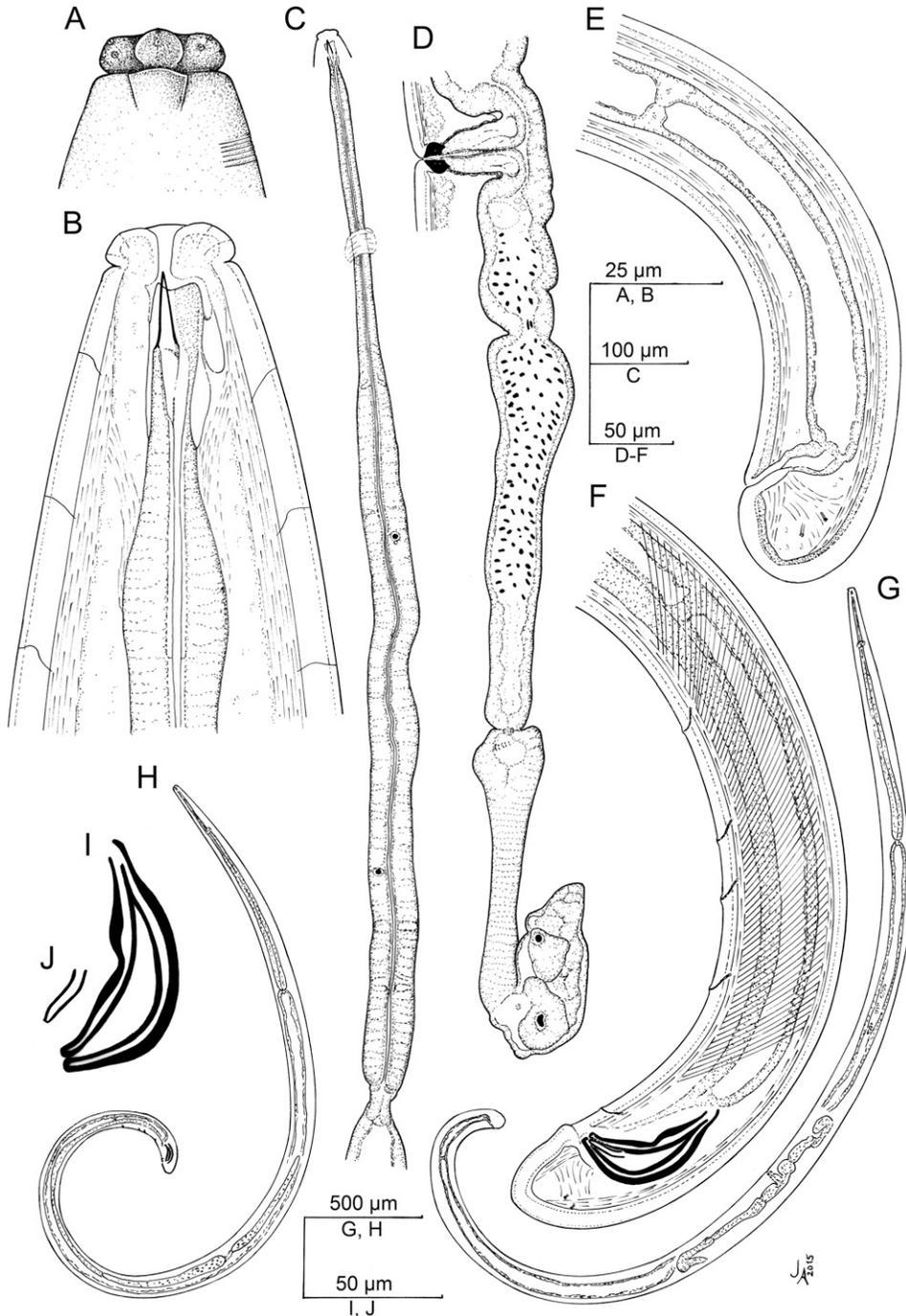


FIG. 1. *Sectonema caobangense* sp. n. (Line). A. Lip region in lateral surface view. B. Anterior region in mid-optical level, lateral view. C. Neck region. D. Female, posterior genital branch. E. Female, posterior body region. F. Male, posterior body region. G. Female, entire. H. Male, entire. I. Spicule. J. Lateral guiding piece.

3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and MEGA 6 (Tamura et al., 2013), respectively. The best-fit model of DNA evolution for BI was obtained using the program MrModeltest 2.3 (Nylander, 2004) with the Akaike Information Criterion in conjunction with PAUP* 4b10 (Swofford, 2003). Bayesian inference analysis under the GTR + G + I model was initiated with a random starting tree and run with the four Metropolis-coupled Markov chain Monte

Carlo for 10^6 generations. Maximum likelihood analysis was implemented under the best-fitting evolutionary model (GTR + I + G), obtained using the program MEGA 6, and 1,000 bootstrap replications. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities are given on appropriate clades. The trees were visualized with the program FigTree v1.4.0 and drawn with Adobe Acrobat XI Pro 11.0.1.



FIG. 2. *Sectonema caobangense* sp. n. (Female, LM). A, E. Lip region in lateral, surface view. B–D. Anterior region in lateral, median view. F. Pharyngeal expansion. G. Pharyngo-intestinal junction. H, J. Vagina. I. Vagina and uterine egg. K, N, O. Caudal region. L. Posterior body region. M. Posterior uterus, containing sperm cells inside. (Scale bars: A–C, E, H, J, N, O = 10 μ m; D, G, I, K = 20 μ m; F = 100 μ m; L, M = 50 μ m).

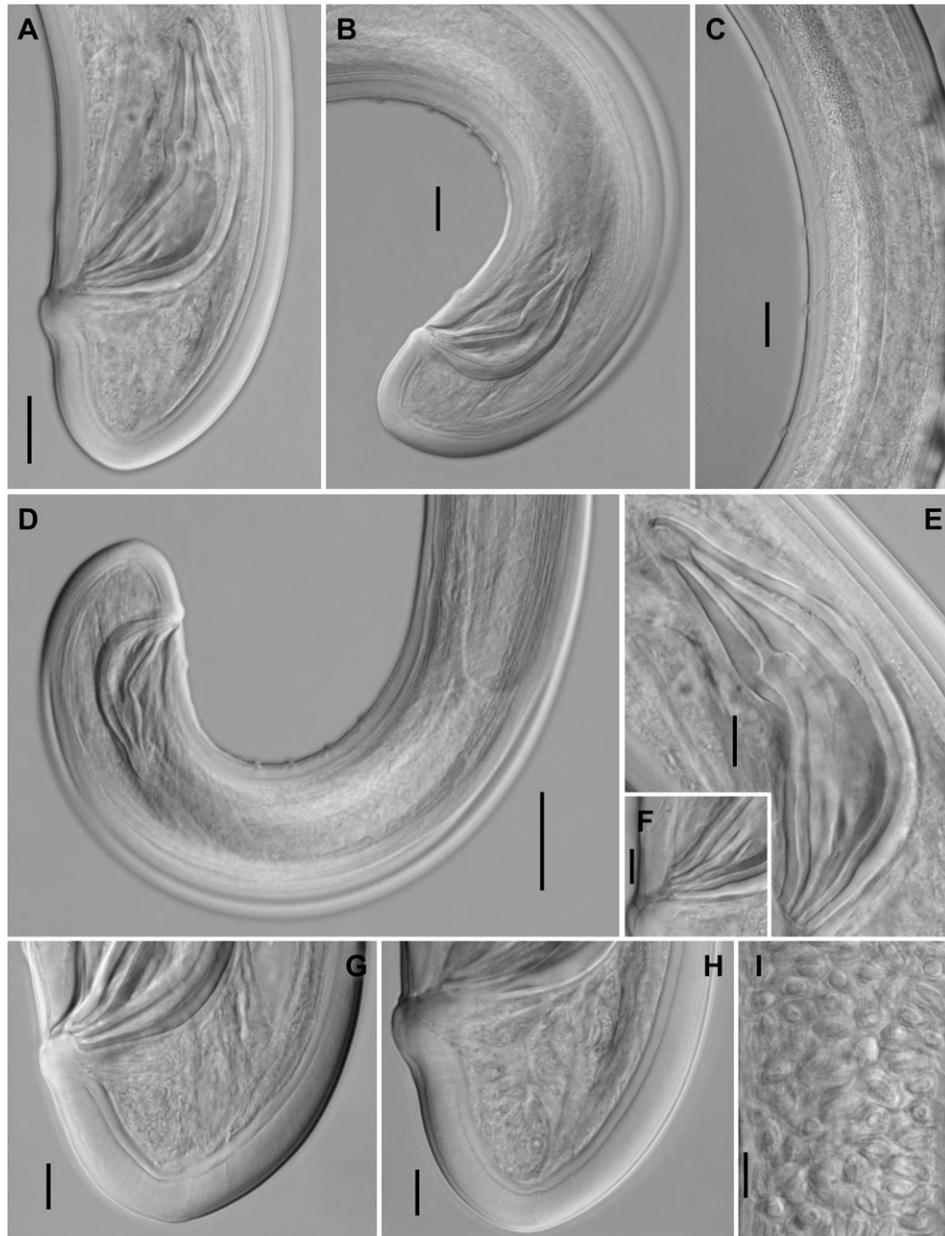


FIG. 3. *Sectonema caobangense* sp. n. (Male, LM). A. Spicule and caudal region. B, D. Posterior body region. C. Ventromedian supplements arrangement. E. Spicule. F. Lateral guiding piece. G, H. Caudal region. I. Sperm cells. (Scale bars: A–C = 20 μ m; D = 50 μ m; E–I = 10 μ m).

RESULTS

Sectonema caobangense sp. n.

urn:lsid:zoobank.org:act:6C8D8A69-DF2B-481E-905A-913649522473

(Figs. 1–4; Table 1)

Material examined: Six females and six males from Vietnam, in variable state of preservation.

Description

Adult: Moderately slender to slender nematodes of big size, 4.02 to 5.80 mm long in females, 3.12 to 4.21 mm long in males. Body cylindrical, distinctly tapering toward the anterior end, less so toward the posterior end as the caudal region is rounded. Habitus regularly curved ventrad after

fixation, C- or G-shaped, more curved at posterior body region in males, and occasionally spiral-like. Cuticle 4.0 to 4.5 μ m thick at anterior region, 5 to 7 μ m in mid-body and 8.5 to 12.0 μ m on tail; three-layered, especially distinguishable at caudal region, where it consists of thinner outer layer bearing fine transverse striation through the entire body, thicker intermediate layer with radial striation, and thin inner layer. Lateral chord 10 to 18 μ m wide at mid-body, occupying one-tenth to one-sixth (9–16%) of mid-body diameter. Two ventral and two dorsal body pores are usually present at level of odontophore. Lip region offset by deep constriction, 3.0 to 3.4 times as wide as high and less than one-fourth (19–23%) of body diameter at neck base; lips (under SEM) low, mostly amalgamated, each with four or five concentric striations; labial papillae, both inner and

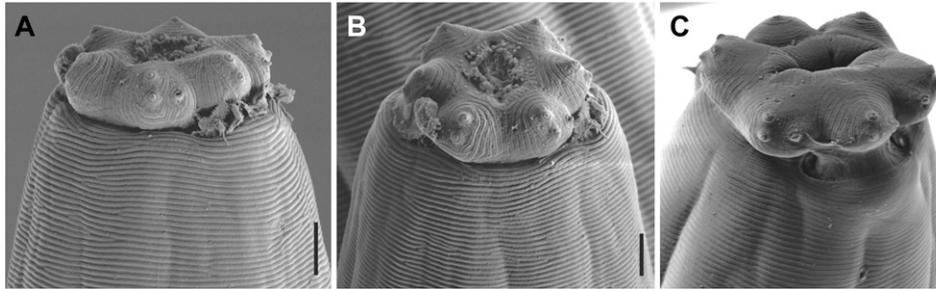


FIG. 4. *Sectonema caobangense* sp. n. (SEM). A. Juvenile, lip region in sublateral view. B. Juvenile, lip region in ventral view. C. Male, lip region in lateral view. (Scale bars: A–C = 3 μ m).

outer, button-like, surrounded by a small ring-like incisure each, and bearing a pore at its center; cephalic papillae not surrounded by any ring-like incisures and with a short, transverse, slit-like opening; oral field visibly hexagonal, delimited by short radial incisures; oral aperture a large dorsoventral slit, the lip region hence showing bilateral symmetry. Amphideal fovea cup-shaped, its opening occupying 9 to 12 μ m or two-fifths to three-fifths (43–59%) of lip region diameter. Cheilostome nearly cylindrical, lacking any differentiation. Mural tooth attached sub-ventrally, 3.4 to 3.7 times longer than its maximum width, 0.6 times as long as lip region diameter and 0.24% to 0.43% of body length; dorsal side always distinctly longer than the ventral one and with more or less sigmoid in contour. Guiding ring simple, somewhat plicate, at 0.5 to 0.6 lip region diameters from anterior end. Odontophore linear, rod-like, 3.3 to 3.7 times the mural tooth length at its ventral side. Anterior region of pharynx enlarging very gradually; basal expansion

12.6 to 16.2 times as long as wide, 5.5 to 7.0 times as long as body diameter, and occupying 61% to 69% of total neck length; gland nuclei (obscure in most specimens) located as follows: DN = 49 ($n = 1$) and S₂N = 79–81 ($n = 3$). Nerve ring situated at 200 to 229 μ m from anterior end or 19% to 21% of total neck length. Cardia rounded conoid, 15–20 \times 17–18 μ m; a ring-like structure is present surrounding its junction to pharyngeal base.

Female: Genital system didelphic-amphidelphic, with both branches almost equally and well developed, the anterior 698, 743 μ m ($n = 2$) long or 15% of body length bearing one uterine egg inside and the posterior 545 to 630 μ m long or 11% to 14% of body length (709 μ m long or 14% of body length bearing one uterine egg inside). Ovaries variably sized, usually not surpassing the sphincter level, the anterior 111 to 442 μ m, the posterior 115 to 435 μ m long; oocytes arranged first in two or more rows, then in a single row. Oviduct 274 to 224 μ m

TABLE 1. Morphometrics of *Sectonema caobangense* sp. n. Measurements in μ m (except L, in mm), and in the form: average \pm standard deviation (range).

Population	Character	<i>n</i>	Cao Bang Natural Reserve		
			Holotype	Paratypes	
			♀	5♀	6♂
L		4.96	4.88 \pm 0.67 (4.02–5.80)	3.64 \pm 0.47 (3.12–4.21)	
a		42	42.1 \pm 6.6 (32–50)	34.1 \pm 5.1 (29–42)	
b		4.5	4.5, 5.4 ($n = 2$)	4.0 \pm 0.3 (3.7–4.2)	
<i>c</i>		113	111 \pm 14 (98–132)	84.1 \pm 5.5 (77–93)	
<i>c'</i>		0.6	0.6 \pm 0.0 (0.5–0.6)	0.7 \pm 0.1 (0.6–0.8)	
V		55	51.8 \pm 2.8 (48–56)	54.7 \pm 3.0 (51–60)	
Lip region diameter		23	22.4 \pm 0.9 (21–23)	22.4 \pm 0.4 (22–23)	
Odontostyle length at ventral side		14	13.6 \pm 0.3 (13–14)	13.4 \pm 0.3 (13–14)	
Odontostyle length at dorsal side		16	16.4 \pm 0.8 (16–17)	16.3 \pm 0.4 (16–17)	
Odontophore length		50	47.1 \pm 1.7 (45–49)	47.0 \pm 1.4 (45–49)	
Guiding ring from anterior end		12	11.9 \pm 1.0 (11–13)	11.1 \pm 0.6 (11–12)	
Neck length		1,100	1,075, 1,112 ($n = 2$)	983 \pm 38 (940–1011)	
Pharyngeal expansion length		745	652, 766 ($n = 2$)	649 \pm 15 (632–662)	
Diameter at neck base		117	107 \pm 11 (91–118)	102 \pm 5 (95–109)	
At midbody		117	117 \pm 11 (101–128)	107 \pm 5 (99–113)	
At anus		76	74.8 \pm 4.0 (71–81)	66.2 \pm 3.5 (60–71)	
Prerectum length		253	202 \pm 50 (150–270)	282 \pm 47 (250–363)	
Rectum length		51	59.5 \pm 9.2 (49–66)	68.6 \pm 3.4 (66–74)	
Tail length		44	43.9 \pm 2.3 (40–46)	43.3 \pm 4.9 (36–51)	
Spicule length		–	–	94.5 \pm 4.5 (87–99)	
Ventromedian supplements		–	–	4.7 \pm 1.0 (4–6)	

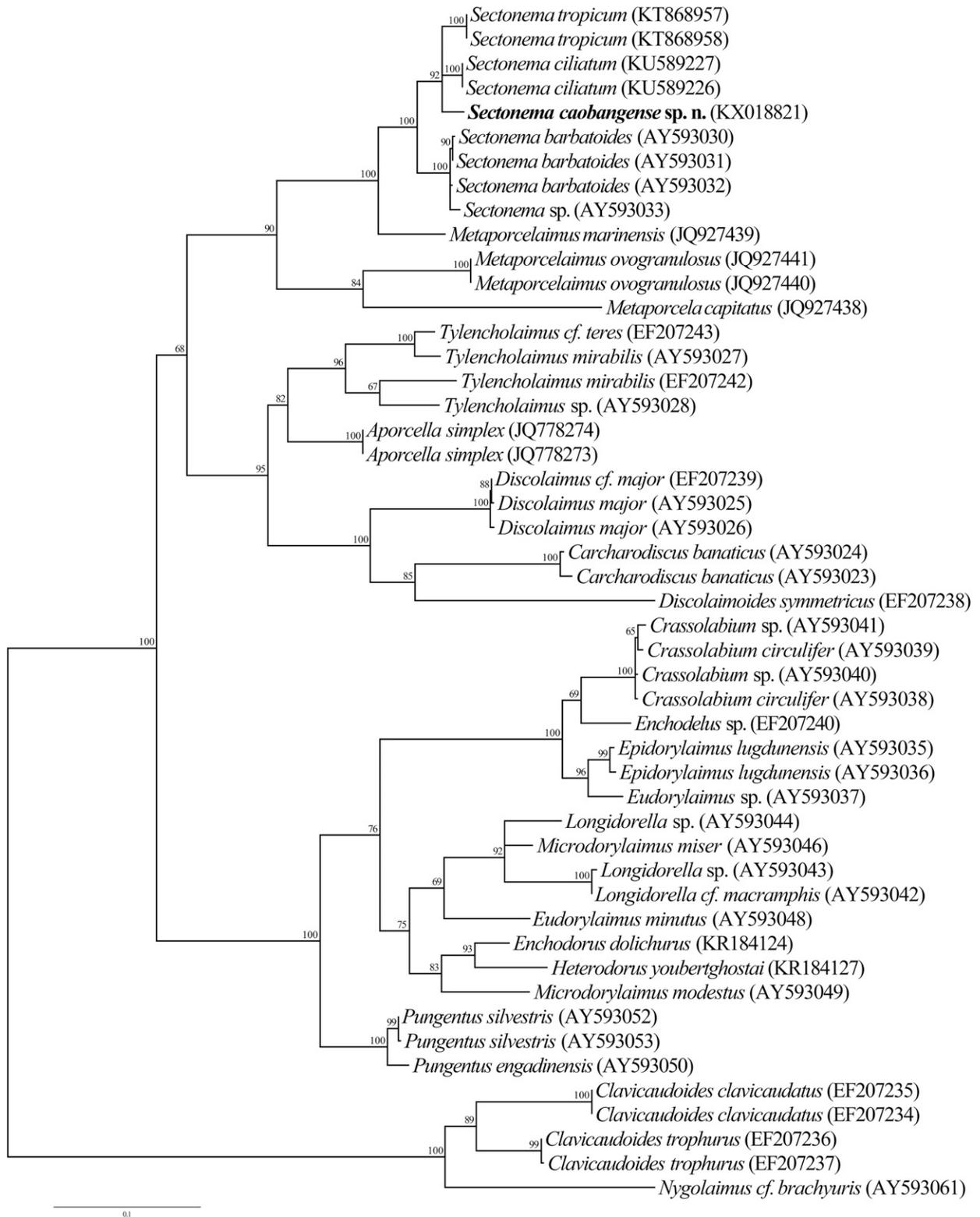


FIG. 6. Maximum likelihood tree as inferred from D2-D3 expansion segments of 28S rRNA gene sequence alignments under the GTR + I + G model. Bootstrap values are given for appropriate clades. Newly obtained sequences are indicated by bold letters.

bearing an uterine egg inside), usually containing abundant sperm cells inside. Uterine eggs ovoid, 145, 160 ($n = 2$) \times 74, 84 ($n = 2$) μm , 1.7, 2.2 ($n = 2$) times as long as wide. Vagina extending inward 56 to 67 μm or

one-half to three-fifths (46–57%) of body diameter: *pars proximalis* 37 to 51 \times 25 to 34 μm , with somewhat sigmoid walls and surrounded by weak musculature; *pars refringens* in lateral view consisting of two

triangular to drop-shaped pieces measuring 10 to 13 × 8 to 9 μm and with a combined width of 17 to 22 μm; *pars distalis* 3.5 to 7.0 μm long. Vulva a transverse slit. Prerectum 2.0 to 3.7, rectum 0.7 to 0.9 anal body diameters long. Tail short and rounded. Caudal pores two pairs, one lateral, another sub-dorsal.

Male: Genital system diorchic, with opposite testes. In addition to the ad-cloacal pair, situated at 18 to 20 μm from cloacal aperture, there is a series of four or five irregularly spaced, 13 to 56 μm apart, ventromedian supplements, the posteriormost of which lying out the range of spicules and situated at 54 to 103 μm from the ad-cloacal pair. Spicules robust and massive, especially in its posterior half, 3.6 to 4.2 times its maximum width, 1.3 to 1.6 times the body diameter at level of the cloacal aperture: dorsal contour regularly convex, ventral contour very weakly concave, with distinct hump and hollow; curvature 125 to 133°; head occupying 11% to 20% of spicule total length, its dorsal contour conspicuously curved at its anterior end and longer than the ventral one, which is short and straight; median piece 5.3 to 6.3 times longer than wide, occupying 53% to 60% of spicule maximum width, reaching the posterior tip; posterior end 7 to 9 μm wide. Lateral guiding pieces 24 to 29 μm long, 5.4 to 6.0 times longer than wide. Prerectum 3.8 to 5.5, cloaca 1.0 to 1.2 the corresponding body widths long. Tail similar to that of female. Caudal pores two pairs, one sub-lateral, another sub-dorsal.

Molecular data: One D2-D3 28S rRNA gene sequence in length of 801 bp was obtained. The evolutionary relationships of the new species with several representatives of the order Dorylaimida are presented in Figs. 5 and 6.

Diagnosis: The new species is characterized by its 3.12 to 5.80 mm long body, lip region offset by deep constriction and 21 to 23 μm broad, mural tooth 13 to 14 μm at its ventral side and 15.5 to 17.0 μm at its dorsal side, neck 940 to 1,112 μm long, pharyngeal expansion 632 to 766 μm long or occupying 61% to 69% of total neck length, uterus a simple tube-like structure 292 to 363 μm long or 2.7 to 2.9 times the corresponding body diameter, *pars refringens vaginae* well developed, $V = 48$ to 56, female tail short and rounded (40–46 μm, $c = 98$ –132, $c' = 0.5$ –0.6), male tail similar to that of female (36–51 μm, $c = 77$ –93, $c' = 0.6$ –0.8), spicules 87 to 99 μm long, and four or five irregularly spaced ventromedian supplements bearing hiatus.

Relationships: In the morphology of its stomatal protruding structure (mural tooth-like), the new species resembles, *S. anisonchium* Sidiqqi, 1995, *S. basilgoodeyi* Heyns, 1965 and *S. transsilvanicum* Popovici, 1978. It differs from *S. anisonchium* in its longer mural tooth (13–14 vs. 10–10.5 μm at its ventral side) having its dorsal side slightly (vs. distinctly) longer than the ventral one, rounded tail (vs. convex-conoid with broadly rounded terminus), comparatively longer male tail

($c = 77$ –93 vs. $c = 105$ –125), longer lateral guiding piece (24–29 vs. 13–14 μm), and lower number of ventromedian supplements (four or five vs. seven or eight). From *S. basilgoodeyi* in its longer mural tooth (13–14 vs. 12 μm at its ventral side), longer neck (940–1112 vs. 874–924 μm), more anterior vulva ($V = 48$ –56 vs. $V = 58$), uterus a simple tube-like structure (vs. tripartite) and comparatively shorter (2.7–2.9 vs. about 3.4 times the corresponding body diameter), shorter spicules (87–99 vs. 110 μm), and lesser ventromedian supplements (four or five vs. eight). And from *S. transsilvanicum* in its narrower lip region (20–23 vs. 25–26 μm broad), shorter uterus (292–363 μm long or 2.7–2.9 times the corresponding body diameter vs. about 385 μm long or 3.5 times the corresponding body diameter), and shorter spicules (87–99 vs. 100–114 μm).

Molecular analyses and the derived trees (Figs. 5, 6) show that the new species forms a well-supported clade with other *Sectonema* species, especially with *S. ciliatum* Álvarez-Ortega, Nguyen, Abolafia, Vu, Bonkowski, and Peña-Santiago, 2016 and *S. tropicum* Nguyen, Abolafia, Bonkowski, Peña-Santiago and Álvarez-Ortega, 2016, two species that, however, significantly differ from *S. caobangense* sp. n. in relevant morphological features such as the nature of the stomatal protruding structure (mural tooth-like vs. a reduced odontostyle). Thus, further studies (more taxa and/or genes) should be conducted to clarify the taxonomy of the genus.

Type locality and habitat: Northern Vietnam, Cao Bang Province, Cao Bang Natural Reserve (GPS coordinates: 22° 34' 07" N and 105° 52' 34" E), in a tropical evergreen forest soil with *Dipterocarpus* sp. and *Cinnamomum* sp. as its dominant plant species.

Type material: Female holotype and four female and four male paratypes deposited in the nematode collection of the University of Jaén, Spain. One female and one male paratypes deposited in the nematode collection of the Institute of Ecology and Biological Resources, Hanoi, Vietnam.

Etymology: The specific epithet refers to geographical origin of the species.

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