Journal of Helminthology

www.cambridge.org/jhl

Two new species of the genus *Sectonema* Thorne, 1930 (Nematoda, Dorylaimida, Aporcelaimidae) from Iran, with new insights into its evolutionary relationships

M. Keramat[1], Z. Mahboubi[1], M. R. Atighi[1], E. Pourjam[1], P. Castillo[2], M. Pedram[1] and R. Peña-Santiago[3]

[1]Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran; [2]Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Avenida Menéndez Pidal s/n, 14004, Córdoba, Spain and [3]Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Spain

Research Paper

Cite this article: Keramat M, Mahboubi Z, Atighi MR, Pourjam E, Castillo P, Pedram M and Peña-Santiago R (2024). Two new species of the genus *Sectonema* Thorne, 1930 (Nematoda, Dorylaimida, Aporcelaimidae) from Iran, with new insights into its evolutionary relationships. *Journal of Helminthology*, **98**, e32, 1–12
https://doi.org/10.1017/S0022149X24000245.

Received: 02 January 2024
Revised: 19 March 2024
Accepted: 20 March 2024

Keywords:
Cladistics; description; LSU; morphology; morphometrics; phylogeny; SSU; taxonomy

Corresponding author:
R. Peña-Santiago;
Email: rpena@ujaen.es

Abstract

Two new species of the genus *Sectonema* found in northern Iran are characterized, including morphological descriptions and molecular (18S-, 28S-rDNA) analyses. *Sectonema tehranense* sp. nov. is distinguished by its 7.22 – 8.53 mm long body, lip region offset by constriction and 24 – 31 µm wide with perioral lobes and abundant setae- or cilia-like projections covering the oral field, mural tooth 15.5 – 17 µm long at its ventral side, neck 1091 – 1478 µm long, pharyngeal expansion occupying 61 – 71% of the total neck length, female genital system diovarian, uterus simple and 3.9 – 4.2 times the corresponding body diameter long, transverse vulva (V = 49 – 59), tail short and rounded (44 – 65 µm, c = 99 – 162, c' = 0.6 – 0.8), spicules 111 – 127 µm long, and 7 – 10 spaced ventromedian supplements with hiatus. *Sectonema noshahrense* sp. nov. displays a 4.07 – 4.73 mm long body, lip region offset by constriction and 23 – 25 µm wide with perioral lobes and abundant setae- or cilia-like projections covering the oral field, odontostyle 14 – 14.5 µm long, neck 722 – 822 µm long, pharyngeal expansion occupying 66 – 68% of the total neck length, female genital system diovarian, uterus simple and 2.4 – 2.7 times the corresponding body diameter long, transverse vulva (V = 54 – 55), tail convex conoid (39 – 47 µm, c = 91 – 111, c' = 0.8 – 0.9), spicules 82 µm long, and seven spaced ventromedian supplements with hiatus. Molecular analyses confirm a maximally supported (*Epacrolaimus* + *Metaporcelaimus* + *Sectonema*) clade and a tentative biogeographical pattern, with sequences of Indolamayan taxa forming a clade separated from those of Palearctic ones. Parallel or convergent evolution processes might be involved in the phylogeny of the species currently classified under *Sectonema*. This genus is certainly more heterogeneous than previously assumed.

Introduction

The genus *Sectonema* Thorne, 1930 is a diverse, widely distributed, free-living dorylaimid (order Dorylaimida) taxon. Its last published census (Álvarez-Ortega & Peña-Santiago 2019; see also Peña-Santiago 2021) consisted of 26 valid species, but, more recently, this number was increased with the inclusion of *S. reyesi* (Pedram et al. 2012), transferred from *Epacrolaimus* Andrássy, 2000 by Peña-Santiago (2023). Almost a cosmopolitan genus, it has been recorded in Afrotropical (Mauritius, South Africa), Australian (Hawaii, New Zealand, Samoa), Indomalayan (India, Vietnam), Nearctic (United States), Neotropical (Colombia, Venezuela), and Palearctic (Belgium, Bulgaria, Egypt, Georgia, Germany, Hungary, Iran, Italy, Netherlands, Romania, Russia, South Korea, Spain, Switzerland, Turkey, United Kingdom) enclaves, where it dwells soils and freshwater habitats. *Sectonema* representatives are large to very large nematodes, ranging from 2.00 to 10.45 (often 4 – 6) mm long, and active predators. The morphology, phylogeny, and taxonomy of the genus were studied by Álvarez-Ortega and Peña-Santiago (2019), who noted that its protruding stomatal structure might be either a reduced odontostyle or a mural tooth, confirmed its monophyly with molecular (28S-rDNA) analyses, identified two species groups with possible geographical projection, and maintained some discrepancies between morphological and molecular data.

Iranian nematode fauna currently consists of three *Sectonema* representatives, namely *S. reyesi*, originally described by Pedram et al. (2012), and *S. heynsi* Altherr 1968 and *S. demani* Altherr, 1965, both recorded by Abdi Gonbary et al. (2018). Nevertheless, two new populations of the genus were collected during a nematological survey conducted in northern Iran, and their study revealed that they belonged to two undescribed species. Thus, this contribution aims to characterize these unknown taxa and provide new insights into the taxonomy and evolutionary relationships of the genus.

Materials and methods

Soil sampling, nematode extraction, and morphological characterization

Twenty-five soil samples were collected from the plane trees in Tehran, Tehran province, and eucalyptus trees in Noshahr, Mazandaran province, northern Iran. The samples were placed in plastic bags, transferred to Tarbiat Modares University's nematology laboratory, and maintained at cool temperature conditions. Large body-sized nematode specimens were directly extracted from the soil samples using 20 and 60 mesh sieves (US standard mesh numbers, their openings equal to 841 and 250 µm, respectively). Nematodes of interest were handpicked under a Nikon SMZ1000 (Nikon, Tokyo, Japan) dissecting microscope, heat-killed by adding boiling 4% formalin solution and transferred to anhydrous glycerin, according to De Grisse (1969). The permanent slides were made, and the recovered specimens were studied/drawn morphologically using a Nikon E6000 (Nikon) light microscope equipped with a drawing tube.

DNA extraction, polymerase chain reaction, and sequencing

Two fresh specimens of both new species were examined on temporary slides to confirm their identity. Individual DNA samples (each from one female specimen) from each population were extracted in 50 µl TE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0) by squashing the specimens using clean slide and coverslips and the aid of a pipette tip. The DNA samples were stored at –20 °C until used as polymerase chain reaction (PCR) templates. The small subunit (SSU) rDNA of *Sectonema tehranense* sp. nov. was amplified using two below primer pairs, amplifying two overlapping fragments: forward 1096F (5′-GGTAATTCTGGAGCTAATAC-3′) and reverse 1912R (5′-TTTACGGTCAGAACTAGGG-3′) primers for the first fragment; and forward 1813F (5′-CTGCGTGAGAGGTGAAAT-3′) and reverse 2646R (5′-GCTACCTTGTTACGACTTTT-3′) primers for the second fragment (Holterman *et al.* 2006). Primers for the large subunit (LSU) D2 – D3 amplification were the forward primer 391a (5′-AGCGGAGGAAAAGAAACTAA-3′; Nadler & Hudspeth 1998) and the reverse primer 1006R (5′-GTTCGATTAGTCTT TCGCCCCT-3′; Holterman *et al.* 2008). The SSU rDNA of *Sectonema noshahrense* sp. nov. was amplified using forward 22F (5′-TCCAAGGAACAGCAGGC-3′; Blaxter *et al.* 1998) and reverse 1573 (5′-TACAAAGGGCAGGGACGTAAT-3′; Mullin *et al.* 2005), forward G18S4 (5′- GCTTGTCTCAAAGATTAAGCC-3′) and reverse 18P (5′- TGATCCWKCYGCAGGTTCAC-3′) primers (Blaxter *et al.* 1998). Primers for the LSU D2 – D3 amplification were the forward primer D2A (5′-ACAAGTACCGTGAGGGAA AGTTG-3′) and the reverse primer D3B (5′-TCGGAAGGAAC CAGCTACTA-3′; Nunn 1992).

The PCR mixture (35 mL) contained the following: 17.5 µL *Taq* DNA polymerase 2× Master Mix RED, 2-mM MgCl2 (Ampliqon, Odenese, Denmark), 9.5 µL distilled water, 1.5 µL of each primer, and 5 µL of DNA template. The thermocycling program for amplification of the ribosomal markers was as follows: denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 52°C for 40s, and extension at 72°C for 80s. The thermocycling program for amplifying the cytochrome c oxidase subunit I (COI) mtDNA was similar to the previous cycle, except the annealing temperature was set to 36°C. A final extension was performed at 72°C for 10 min for both cycles. The PCR products were sequenced using the same primers used during amplification.

Phylogenetic analyses

D2 – D3 domains of the 28S rDNA and 18S rDNA sequences of the recently recovered *Sectonema* populations were obtained in this study. These sequences and other sequences belonging to species of Dorylaimida from GenBank, were used for phylogenetic analyses. Outgroup taxa for each dataset were chosen following previously published studies (Álvarez-Ortega & Peña-Santiago 2019; Álvarez-Ortega *et al.* 2021; Peña-Santiago & Castillo 2022). Multiple sequence alignments of the two ribosomal genes were made using the fast Fourier transform (FFT)-NS-2 algorithm of MAFFTversion 7.450 (Katoh *et al.* 2019). Sequence alignments were visualised using BioEdit (Hall 1999) and manually edited. Phylogenetic analyses of the sequence datasets were based on Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist & Huelsenbec 2003). The best-fit model of DNA evolution was obtained using JModelTest V.2.1.7 (Darriba *et al.* 2012) with the Akaike information criterion (AIC). The best-fit model, the base frequency, the proportion of invariable sites, the gamma distribution shape parameters, and substitution rates in the AIC were then used in MrBayes for the phylogenetic analyses. BI analyses were performed under a general time-reversible model with invariable sites and a gamma-shaped distribution (GTR + I + G) for the D2 – D3 expansion segments of the 28S rRNA and 18S rRNA genes. All Bayesian analyses were run separately per dataset with four chains for 4×10^6 generations. The Markov chains were sampled at intervals of 100 generations. After discarding burn-in samples of 30% and evaluating convergence, the remaining samples were retained for in-depth analyses. The topologies were used to generate a 50% majority-rule consensus tree. Posterior probabilities (PP) were given on appropriate clades. Trees from all analyses were visualised using FigTree software version 1.42 (Rambaut 2014). A combined analysis of the three ribosomal genes was not undertaken because some sequences were unavailable for all species.

Results

***Sectonema tehranense* sp. nov.** (Figs 1-3, morphometrics in Table 1)

Material examined: Seven females and three males from one location, in good state of preservation.

Adult: Very slender (a = 51 – 75) nematodes of very large size, 7.22 – 8.53 mm long. Body cylindrical, tapering toward both ends, but more so towards the anterior end as the tail is short and rounded. Upon fixation, the habitus curved ventrad, C- to G-shaped. Cuticle dorylaimid, 8 – 8.5 µm thick at anterior region and mid-body, and 18 µm at tail, three-layered, especially appreciable at caudal region, consisting of a smooth, thin outer layer, much thicker intermediate layer with weak radial striation, and a thinner inner layer. Body pores are only conspicuous in the cervical region, where two or three ventral and dorsal pores are perceptible. Lateral chord 15 – 27 µm wide, occupying 12 – 19% of mid-body diameter. Lip region offset by deep constriction, 2.8 – 3.4 times as wide as high, up to one-fourth (23 – 25%) of body diameter at neck base, lips separated, with perioral lobes, oral field covered of abundant setae- or cilia-like projections. Amphid fovea stirrup-shaped, its aperture 13 – 15 µm or *ca* one-half (43 – 52%) of lip region diameter. Cheilostom is short and wide, with less appreciable walls. Stomatal protruding element is a ventral, somewhat deltoid, mural tooth-like structure, its ventral side hardly

https://doi.org/10.1017/S0022149X24000245 Published online by Cambridge University Press

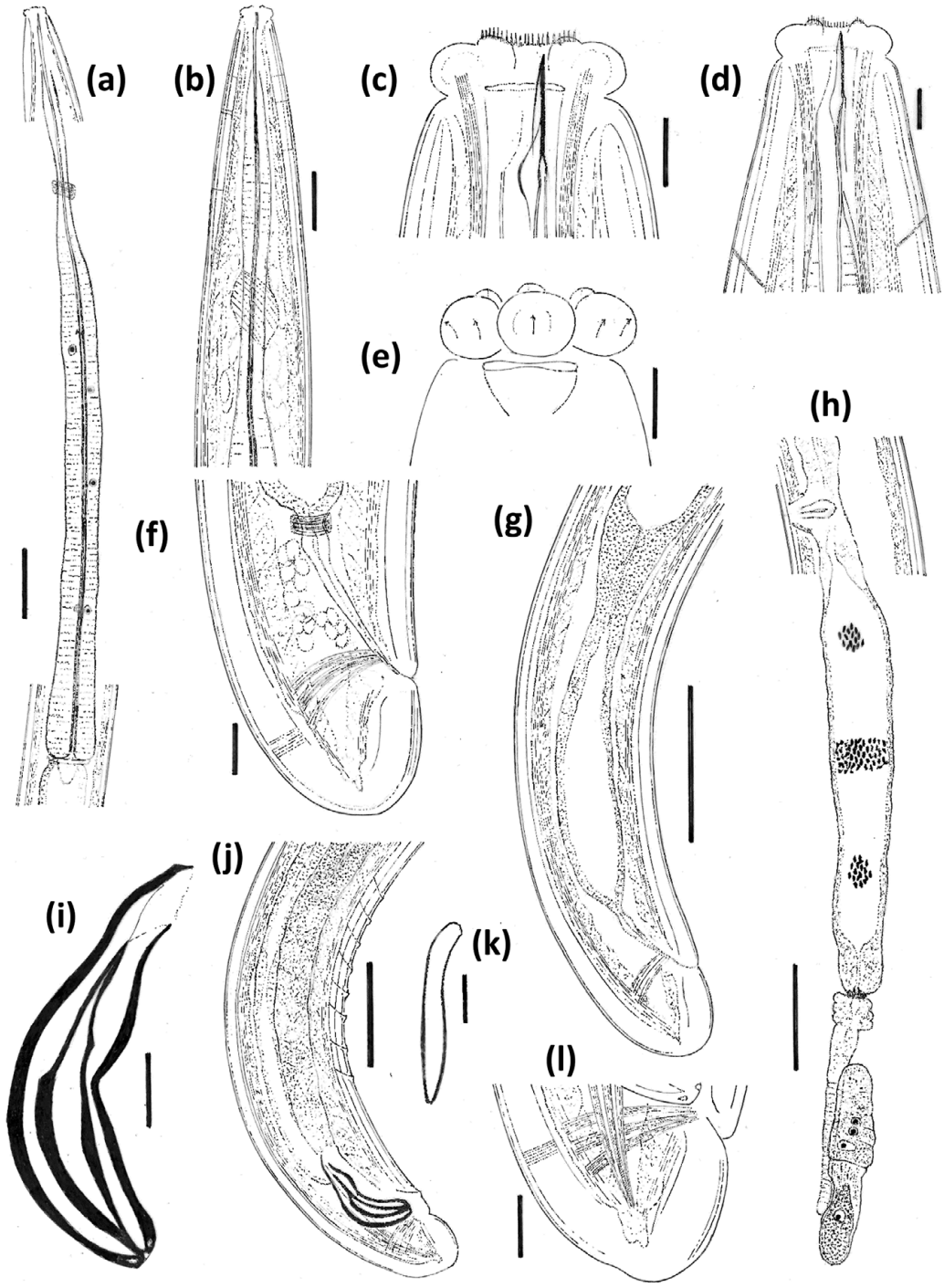

Figure 1. *Sectonema tehranense* sp. nov. (a) Neck region; (b) Anterior part of neck region; (c, d,) Anterior region, in lateral median view; (e) Anterior region, in lateral surface view; (f) Female, rectum and caudal region; (g) Female, posterior body region; (h) Female, posterior genital branch; (i) Spicule; (j) Male, posterior body region; (k) Lateral guiding piece; (l) Male, caudal region. [Scale bars: a, g, h = 100 μm; b, j = 50 μm; c-e, k, l = 10 μm; f, i = 20 μm].

longer than one-half (53 – 65%) of the lip region diameter, the dorsal one slightly longer than the ventral, 0.65 – 0.73% of lip region diameter. The guiding ring is shallow, visibly plicate, located at 8 μm or 29% of the lip region diameter from the anterior end. Odontophore is linear, lacking differentiation, and is 1.9 – 2.5 times longer than mural tooth. Pharynx entirely muscular, very gradually enlarging into the basal expansion that is 9.4 – 13.7 times as long as wide, 5.9 – 7 times the lip region diameter at neck base, and occupies *ca* two-thirds (61 – 71%) of the total neck length, gland nuclei located as follows: DO = 41 – 42, DN = 44 – 46, S_1N_1 = 50, S_1N_2 = 62 – 63, S_2N = 79 – 80. Pharyngo-intestinal junction consisting of a short, conical to conoid, 32 – 34 × 27 – 29 μm cardia, its junction to pharyngeal base surrounded by weak ring-like element.

Female: Genital system diovarian, with both branches equally developed, the anterior 747 – 965 μm long or 10 – 12% of body length, the posterior 729 – 912 μm or 10 – 12% of body length. Ovaries are variably large, often reaching and surpassing the

https://doi.org/10.1017/S0022149X24000245 Published online by Cambridge University Press

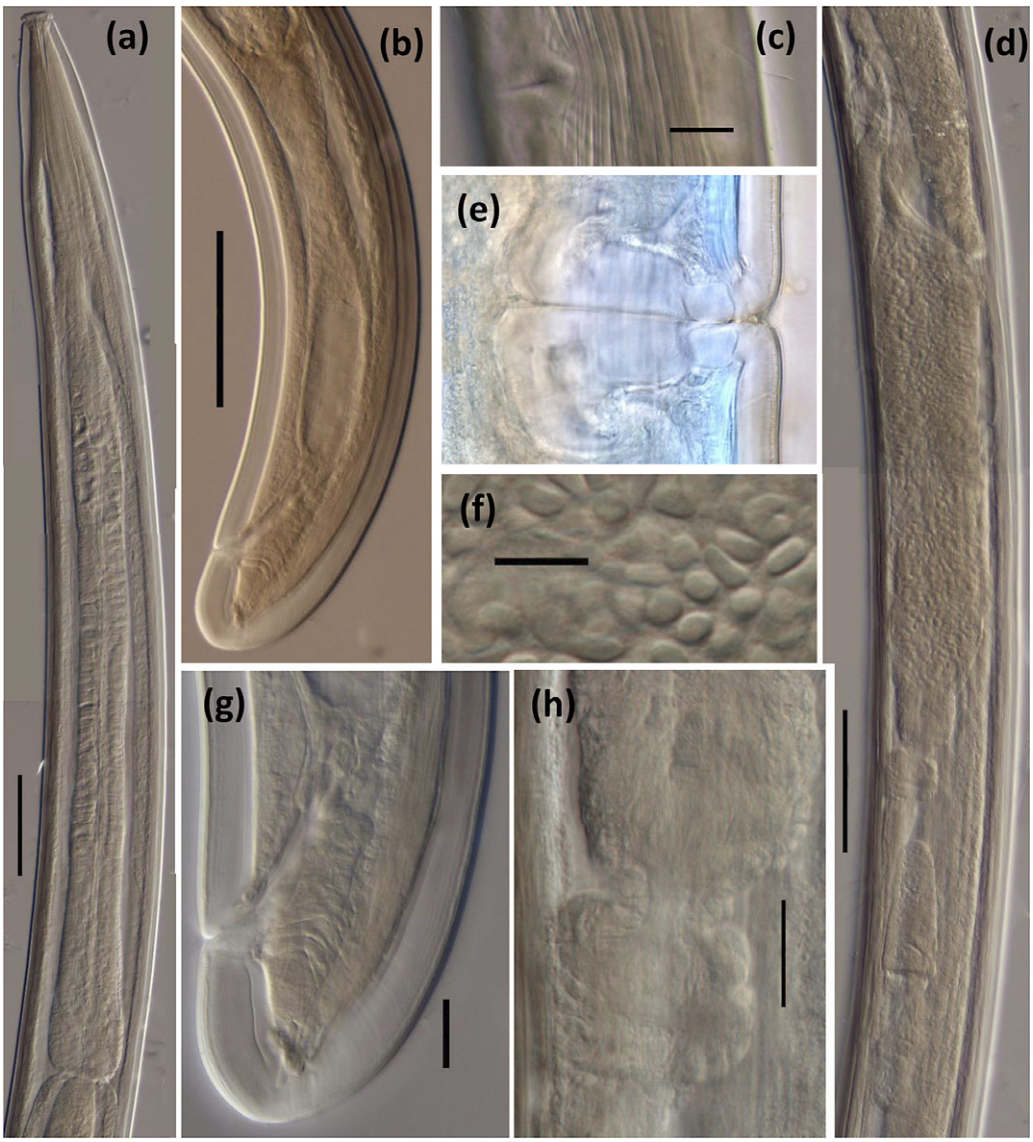

Figure 2. *Sectonema tehranense* sp. nov. (female, LM). (a) Neck region; (b) Posterior body region; (c, e) Vagina, in ventral and lateral view, respectively; (d) Posterior genital branch; (f) Sperm cells inside the uterus; (g) Posterior body region; (h) Oviduct-uterus junction. [Scale bars: a, b, d = 100 μm; c, g, h = 20 μm; e, f = 10 μm.].

oviduct-uterus junction, the anterior 184 – 347, the posterior 194 – 275 μm long, with oocytes first arranged in two or more rows and then in only one row. Oviduct joining subterminally the ovary, 172 – 360 μm long or 1.4 – 1.7 body diameters, consisting of a distal slender portion made of prismatic cells and a moderately developed proximal *pars dilatata* with visible lumen inside. A strong narrowing, surrounded by a distinct muscle sphincter, separated the oviduct and uterus. The uterus is a simple tube-like structure, 370 – 507 μm long or 3.9 – 4.2 times the corresponding body diameter. Vagina extending inward 75 – 76 μm, reaching 55 – 61% of body diameter: *pars proximalis* 48 – 52 × 40 – 48 μm, with somewhat sigmoid walls surrounded by weak circular musculature, *pars refringens* consisting of (lateral view) two close together, almost trapezoidal sclerotized pieces 13.5 – 15.5 × 10 – 12.5 μm, and with a combined width of 24 – 27 μm, and *pars distalis* 9 – 10 μm long. The vulva is a transverse slit. Prerectum 3.5 – 5.4, rectum 0.8 – 1.1 anal body diameters long. The caudal region is short and rounded, almost hemispheroid, but hardly straight ventrally; caudal pores are two lateral and subdorsal pairs.

Male: Prerectum 5.2, cloaca 1 – 1.2 times longer than body diameter at the level of cloacal aperture. Genital system is diorchic, with opposite testes. Sperm cells (once inside the uterus) are spindle-shaped to somewhat ovoid, 8 × 2.5 μm. In addition to the ad-cloacal pair, situated at 21 – 25 μm from the cloacal aperture, there is a series of 7 – 10 spaced (21 – 28 μm apart) ventromedian supplements, the last of them located at 128 – 138 μm from the ad-cloacal pair, with a distinct hiatus. Spicules dorylaimid, 4.4 times longer than wide, 1.7 times the body diameter at cloacal aperture: head 23 – 24 μm long or 19% of total spicule length, with its dorsal side appreciably longer than the ventral one; median piece occupying almost half (54%) of spicule maximum width; posterior end 28 – 29 μm wide; ventral hump and hollow conspicuous, the former situated at 46 – 52 μm or 43% of spicule anterior end; curvature 130°. Lateral guiding piece 26 – 32 μm long, 8 times as long as wide,

https://doi.org/10.1017/S0022149X24000245 Published online by Cambridge University Press

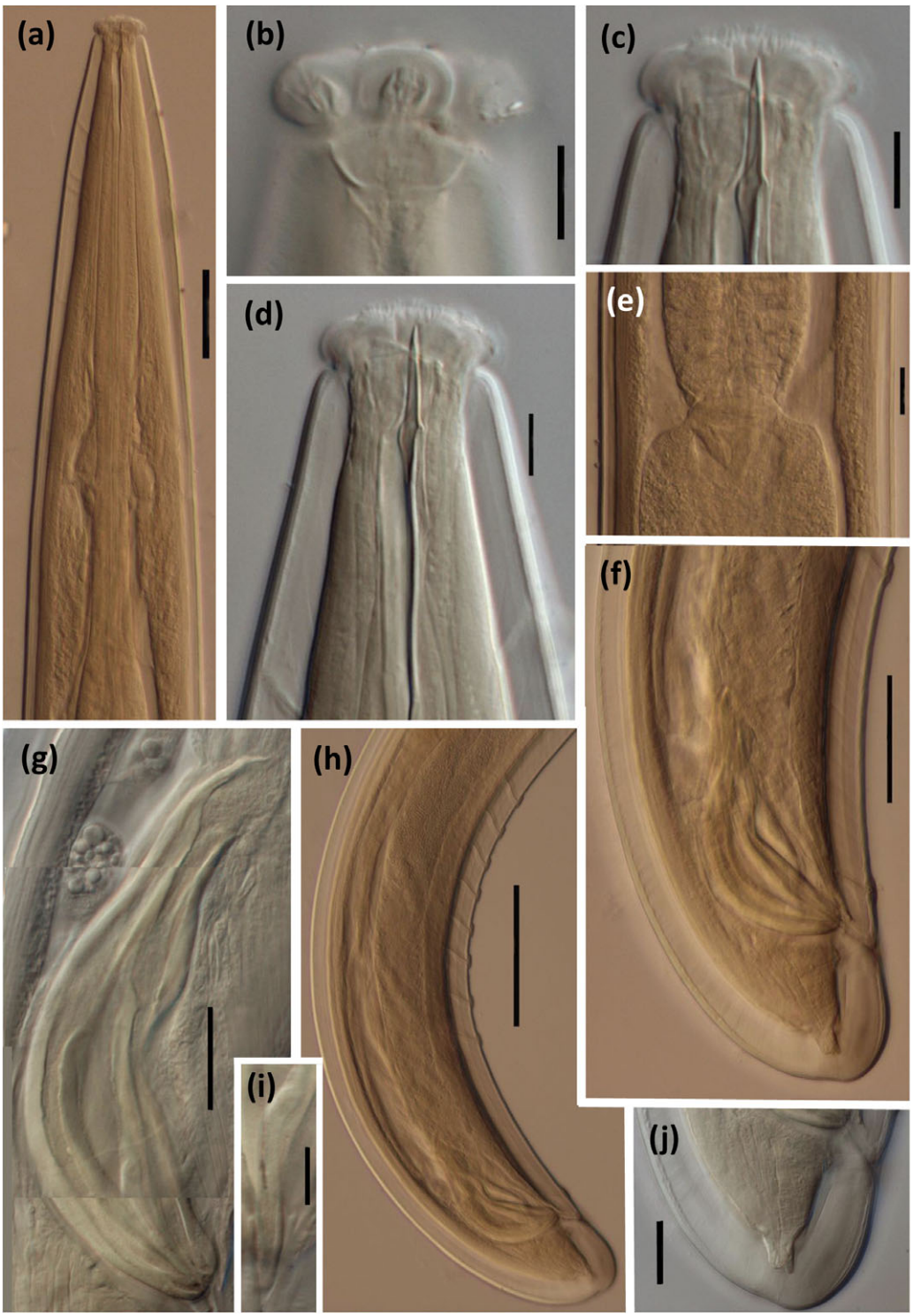

Figure 3. *Sectonema tehranense* sp. nov. (male, LM). (a) Anterior part of neck region; (b) Anterior body region, lateral surface view; (c, d) Anterior body region, lateral median view; (e) Pharyngo-intestinal junction; (f, h) Posterior body region; (g) Spicule; (i) Lateral guiding piece; (j) Caudal region. [Scale bars: a, f, h = 50 μm; b–d, i = 10 μm; e, g, j = 20 μm.].

tapering at its posterior end. The caudal region is similar to that of females.

Molecular characterization

One sequence of D2 – D3 expansion segments of 28S rRNA gene with 637 bp was obtained of *Sectonema tehranense* sp. nov. (PP035456). Its analysis and comparison with sequences from NCBI including *Sectonema heynsi, Sectonema barbatoides* Heyns, 1965, *Epacrolaimus declinatoaculeatus* (Kreis 1924) Andrássy, 2000, *Sectonema septentrionale* Peña-Santiago & Álvarez-Ortega, 2015, and *Sectonema caobangense* Álvarez-Ortega, Nguyen, Abolafia, Bonkowski & Peña-Santiago, 2016 (MH727509, AY593030, ON814781, MH915544, KX018821) showed an identity of 98.8%,

https://doi.org/10.1017/S0022149X24000245 Published online by Cambridge University Press

Table 1. Main morphometrics of two new species of the genus *Sectonema* Thorne, 1930 from Iran Measurements in µm except L in mm, and, when possible, in the form: average ± SD (range)

Species	*S. tehranense* sp. n.			*S. noshahrense* sp. n.		
Province	Tehran			Noshahr		
Habitat	Plane tree			Eucalyptus tree		
	Holotype	Paratypes	Paratypes	Holotype	Paratypes	Paratype
Character n	♀	6♀♀	3♂♂	♀	3♀♀	♂
L	7.22	7.56 ± 0.35 (7.25 – 8.53)	7.25 – 8.53	4.31	4.07 – 4.31	4.73
a	58	66.8 ± 9.8 (56 – 75)	51 – 75	52	41 – 53	63
b	6.3	6.1 ± 0.3 (5.8 – 6.4)	5.8 – 6.4	5.6	5.6	5.7
c	131	124 ± 18 (99 – 153)	100 – 162	91	91 – 109	111
V	49	53.1 ± 3.7 (49 – 59)	–	54	54 – 55	–
c’	0.7	0.7 ± 0.1 (0.6 – 0.8)	0.7 – 0.8	0.9	0.8 – 0.9	0.9
Lip region diameter	24	27.6 ± 2.2 (24 – 31)	28	23	23 – 25	24
Odontostyle/mural tooth length	17.5	19.6 ± 1.1 (17.5 – 21)	15.5 – 17	14.5	14 – 14.5	14
Odontophore length	40	40.3 ± 1.1 (37 – 42)	42 – 44	30	31 – 32	29
Neck length	1155	1274 ± 81 (1091 – 1337)	1129 – 1478	775	722 – 775	822
Pharyngeal expansion length	762	845 ± 80 (713 – 952)	602 – 850	517	480 – 518	560
Body diameter at neck base	136	127 ± 9 (116 – 138)	116 – 126	100	87 – 100	78
mid–body	121	132 ± (121 – 138)	136 – 145	121	102 – 121	75
anus	67	76.9 ± 8.3 (67 – 89)	71 – 78	51	44 – 51	41
Distante vulva – anterior end	3485	4004 ± 385 (3485 – 4442)	–	2337	–	–
Prerectum length	232	357 ± 62 (232 – 388)	280 – 366	205	172 – 205	180
Rectum/cloaca length	74	75.3 ± 3.8 (71 – 78)	75 – 88	43	35 – 50	43
Tail length	48	57.9 ± 7.2 (48 – 65)	44 – 58	41	39 – 41	47
Spicules length	–	–	111 – 127	–	–	82
Ventromedian supplements	–	–	7 – 10	–	–	7

96.4%, 94.7%, 94.2%, and 92.1%, respectively, differing in 5 bp and 1 indel, 23 bp and 1 indel, 23 bp and 5 indels, 26 bp and 7 indels, and 36 bp and 10 indels, respectively.

Similarly, one sequence of partial 18S rRNA gene with 1236 bp was obtained (PP035454). It showed a high identity with others from NCBI, including *Epacrolaimus declinatoaculeatus* (ON764423, 99.8% identity, differing in 2 bp and 1 indel), *Sectonema barbatoides* (AY284814, 99.7%, 4 bp, 1 indel), and *Sectonema* sp. JH-2004 (AY284812, 99.5%, 6 bp, 1 indel).

Diagnosis

The new species is characterized by its 7.22 – 8.53 mm long body, lip region offset by constriction and 24 – 31 µm wide with perioral lobes and abundant setae- or cilia-like projections covering the oral field, mural tooth 15.5 – 17 µm long at its ventral side and 17.5 – 20.5 µm long at its dorsal side, neck 1091 – 1478 µm long, pharyngeal expansion occupying 61 – 71% of the total neck length, female genital system diovarian, uterus simple and 370 – 507 µm long or 3.9 – 4.2 times the corresponding body diameter, transverse vulva (V = 49 – 59), tail short and rounded (44 – 65 µm, c = 99 – 162, c' = 0.6 – 0.8), spicules 111 – 127 µm long, and 7 – 10 spaced ventromedian supplements with hiatus.

Separation from its relatives

In being very large in size (more than 5 mm long), having a mural tooth as protruding stomatal structure, and abundant setae- or cilia-like projections covering the oral field, the new species is very similar to *S. heynsi* and *S. reyesi*. Nevertheless, it can be distinguished from *S. heynsi*, only known to occur in freshwater habitats of Germany (see redescription by Peña-Santiago & Álvarez-Ortega 2014), in its larger general size (body 7.22 – 8.53 *vs* 6.45 mm long), shorter mural tooth (15.5 – 17 *vs* 20 µm at its ventral side), much longer uterus (370 – 507 *vs* 263 µm), and male present (*vs* absent). From *S. reyesi*, another Iranian taxon (see redescription by Peña-Santiago 2023), in its shorter mural tooth (15.5 – 17 *vs* 19.5 – 21 µm at its ventral side), odontophore (37 – 44 *vs* 51 – 56 µm), uterus (370 – 507 *vs* 669 – 690 µm) and spicules (111 – 127 *vs* 138 µm).

https://doi.org/10.1017/S0022149X24000245 Published online by Cambridge University Press

Type locality and habitat

Iran, Tehran province, coordinates 35°47'55.7"N and 51°23'22.0"E, where the new species was found in the rhizosphere of a plane tree (*Platanus orientalis* L.).

Type material

Female holotype, four female paratypes and two male paratypes were deposited with WaNeCo collection, Wageningen, The Netherlands (http://www.waneco.eu/). One female paratype and one male paratype at nematode collection of the University of Jaén, Spain.

Etymology

The species name refers to Tehran, the Iranian province where it was collected.

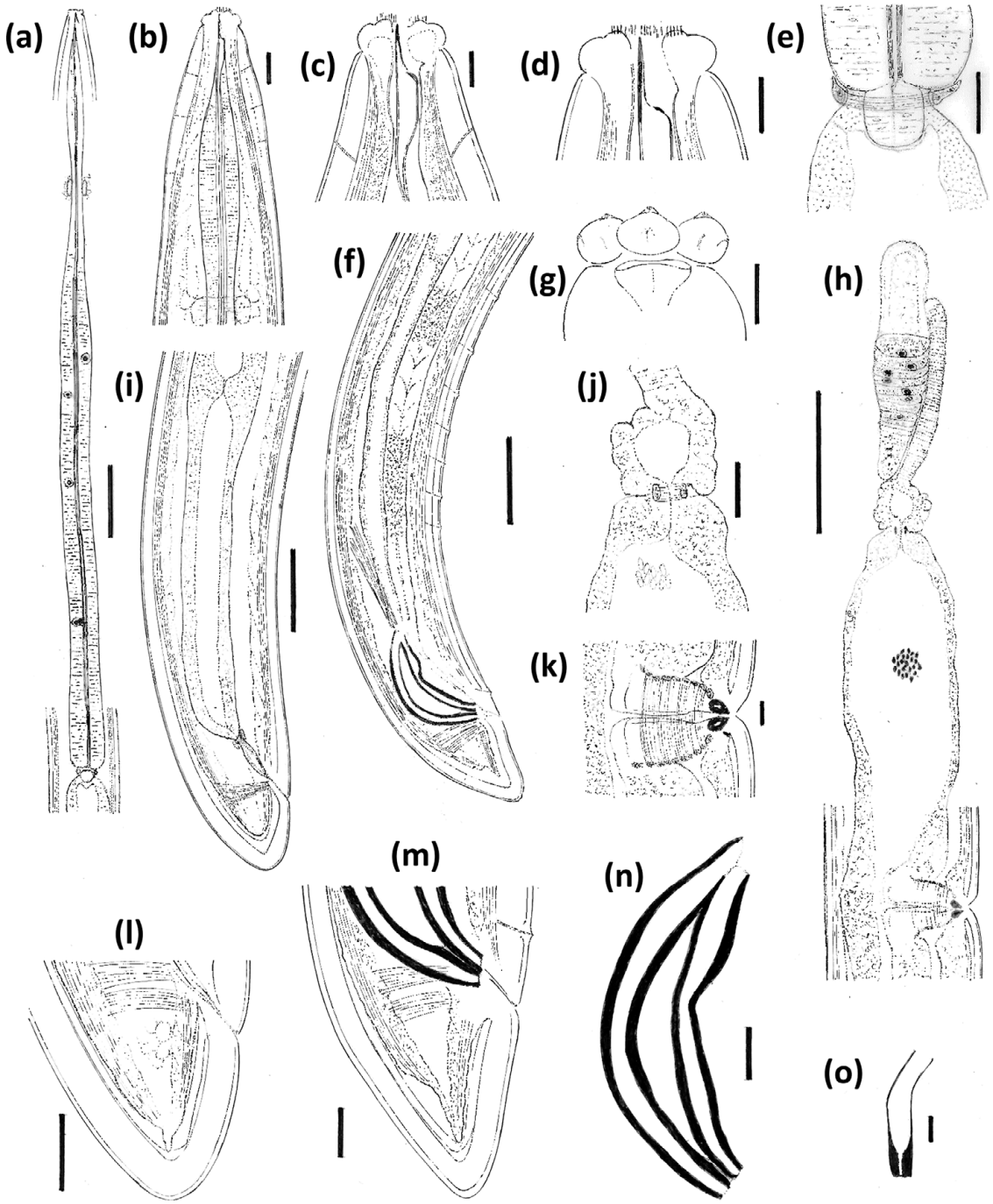

Figure 4. *Sectonema noshahrense* sp. nov. (a) Neck region; (b–d) Anterior body region, lateral median view; (e) Pharyngo-intestinal junction; (f) Male, posterior body region; (g) Anterior body region, lateral surface view; (h) Female, anterior genital branch; (i) Female, posterior body region; (j) Oviduc-uterus junction; (k) Vagina; (l) Female, caudal region; (m) Male, caudal region; (n) Spicule; (o) Lateral guiding piece. [Scale bars: a, f, h, i = 50 μm; b, j, l = 20 μm; c-e, g, k, m, n = 10 μm; o = 4 μm.].

https://doi.org/10.1017/S0022149X24000245 Published online by Cambridge University Press

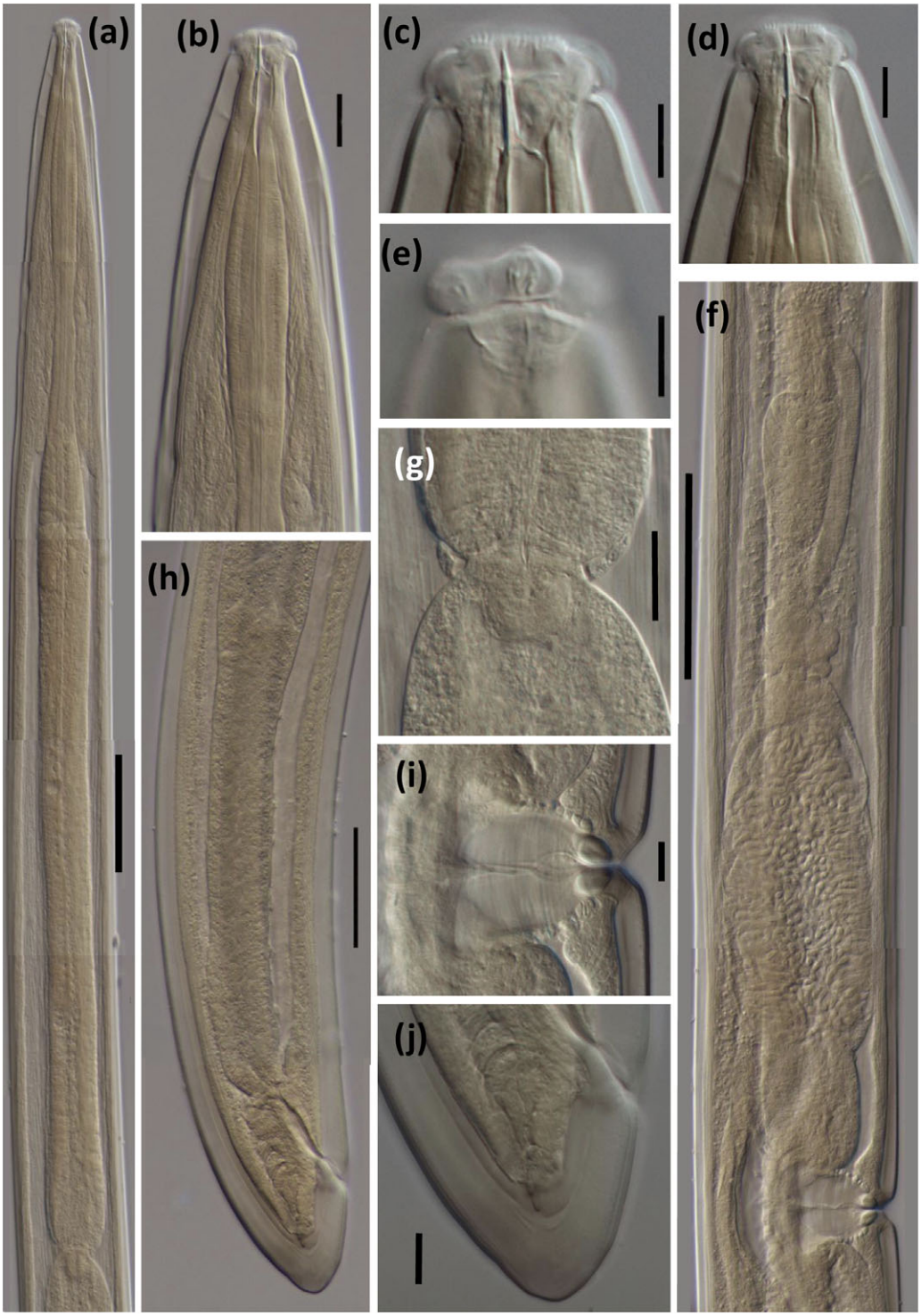

Figure 5. *Sectonema noshahrense* sp. nov. (female, LM). (a) Neck region; (b–d) Anterior body region, lateral median view; (e) Anterior body region, lateral surface view; (f) Female, anterior genital branch; (g) Pharyngo-intestinal junction; (h) Posterior body region; (i) Vagina; (j) Caudal region. [Scale bars: a, h = 50 μm; b, g = 20 μm; c-e = 10 μm; f = 100 μm; i, j = 10 μm.].

Sectonema noshahrense sp. nov. (Figs 4 & 5, morphometrics in Table 1)

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

Adult: Very slender (a = 41 – 63) nematodes of large size, 4.07 – 4.73 mm long. The body is cylindrical, tapering toward both ends, but more toward the anterior end as the tail is convex conoid. Upon fixation, habitus curved ventrad, C- to G-shaped. Cuticle dorylaimid, 9 μm thick at the anterior region, 8 μm in mid-body, and 14 μm at the tail, three-layered, especially appreciable at the caudal region, consisting of a smooth, thin outer layer, much thicker intermediate layer with weak radial striation, and a thinner inner layer. Body pores are only conspicuous in the cervical region, where two or three ventral and dorsal pores are present. Lateral chord

https://doi.org/10.1017/S0022149X24000245 Published online by Cambridge University Press

23 µm wide, occupying *ca* one-fourth (23%) of mid-body diameter. Lip region offset by deep constriction, 2.5 – 2.6 times as wide as high, *ca* one-fourth (25 – 27%) of body diameter at neck base, lips separated, with perioral lobes, oral field covered of abundant setae- or cilia-like projections. Amphid fovea stirrup-shaped, its aperture 16 – 17 µm or *ca* one-half (56%) of lip region diameter. Cheilostom is short and wide, with less appreciable walls. Stomatal protruding element a reduced odontostyle, 3.2 times as long as wide (4.5 µm at its base), hardly longer than one-half (58 – 59%) of lip region diameter, and 0.35% of body length. Guiding ring shallow. Odontophore is linear, lacking differentiation, and is 2.1 times longer than odontostyle. Pharynx is entirely muscular, very gradually enlarging into the basal expansion that is 9.6 – 9.9 times as long as wide, 5.1 times the body diameter at the neck base, and occupies *ca* two-thirds (66 – 68%) of the total neck length, gland nuclei located as follows: DO = 43, DN = 46, S_1N_1 = 51, S_1N_2 = 62, S_2N = 81. Pharyngo-intestinal junction consists of a short, rounded, 19 × 19 µm cardia, its junction to the pharyngeal base surrounded by a weak ring-like element.

Female: Genital system diovarian, with both branches equally developed, the anterior 425 – 438 µm long or 10 – 11% of body length, the posterior 362 – 462 µm or 9 – 11% of body length. Ovaries are comparatively small, not reaching the oviduct-uterus junction, the anterior 129 – 177, the posterior 127 – 188 µm long, with oocytes first arranged in two or more rows and then in only one row. Oviduct joining subterminally the ovary, 165 – 222 µm long or 1.3 – 1.8 body diameters, consisting of a distal slender portion made of prismatic cells and a moderately developed proximal *pars dilatata* with visible lumen inside. A strong narrowing, surrounded by a distinct muscle sphincter, separates oviduct and uterus. Uterus a simple tube-like structure, 212 – 275 µm long or 2.4 – 2.7 times the corresponding body diameter. Vagina extending inward 52 µm, reaching 51% of body diameter: *pars proximalis* 35 × 30 µm, with somewhat sigmoid walls surrounded by weak circular musculature, *pars refringens* consisting of (in lateral view) two close together, drop-shaped sclerotized pieces 9.5 × 6.5 µm, and with a combined width of 15.5 µm, and *pars distalis* 8 µm long. Vulva a transverse slit. Prerectum 3.5 – 4, rectum 0.8 – 1 times the body diameter at the level of cloacal aperture. Caudal region convex conoid; inner core reaching up to 70% of tail length, with cuticle visibly thickened at tip; caudal pores two pairs, one lateral, another subdorsal.

Male: Genital system diorchic, with opposite testes. In addition to the ad-cloacal pair of supplements, located at 18.5 µm from the cloacal aperture, there is a series of seven irregularly spaced supplements, the posteriormost of which lying out the range of spicules, at 230 µm from the ad-cloacal pair. Spicules are robust and massive, especially in their posterior half, the head occupying 14% of the total length, the median piece 10 – 15 µm wide. Lateral guiding pieces 21 µm long. Tail similar to that of females.

Molecular characterization

One sequence of D2 – D3 expansion segments of 28S rDNA with 739 bp was obtained for *Sectonema noshahrense* sp. nov. (PP035457). It showed an identity of 95.9% with the sequence of *Sectonema tehranense* sp. nov., differing in 17 bp and 2 indels. These sequences were analysed and compared with sequences from NCBI, including *Sectonema heynsi, Sectonema barbatoides*, *Epacrolaimus declinatoaculeatus*, *Sectonema septentrionale*, and *Sectonema caobangense* (MH727509, AY593030, ON814781, MH915544, KX018821), showing an identity of 96.5%, 97.8%, 95%, 94.6%, and 92.4%, differing in 26 bp and 7 indels, 16 bp and 4 indels, 37 bp and 13 indels, 40 bp and 12 indels, and 57 bp and 18 indels, respectively.

Similarly, one sequence of partial 18S rDNA with 1253 bp was obtained (PP035455). It showed an identity of 99.8% with that of *S. tehranense* sp. nov., differing in 2 bp and 1 indel. It showed also a high identity with others from NCBI, including *Epacrolaimus declinatoaculeatus* (ON764423, 99.8% similarity, differing in 2 bp and 1 indel), *Sectonema barbatoides* (AY284814, 99.7%, 4 bp, 1 indel), and *Sectonema* sp. JH-2004 (AY284812, 99.5%, 6 bp, 1 indel).

Diagnosis

The new species is characterized by its 4.07 – 4.73 mm long body, lip region offset by constriction and 23 – 25 µm wide with perioral lobes and abundant setae- or cilia-like projections covering the oral field, odontostyle 14 – 14.5 µm long, neck 722 – 822 µm long, pharyngeal expansion occupying 66-68% of the total neck length, female genital system diovarian, uterus simple and 212 – 275 µm long or 2.4 – 2.7 times the corresponding body diameter, transverse vulva (V = 54 – 55), tail convex conoid (39 – 47 µm, c = 91 – 111, c' = 0.8 – 0.9), spicules 82 µm long, and seven spaced ventromedian supplements with hiatus.

Separation from its relatives

In having a large size (less than 5 mm long), a reduced odontostyle comparatively short as protruding stomatal structure, and abundant setae- or cilia-like projections covering the oral field, the new species is very similar to *S. barbatum* Heyns, 1965, at present only known to occur in the United States (Heyns 1965). Nonetheless, both species differ in the length of their reduced odontostyle (14 – 14.5 µm or up to three-fifths of lip region diameter *vs* 16 µm or three-fourths of lip region diameter in *S. barbatum*) and their neck (722 – 822 µm, b = 5.6 – 5.7 *vs* 589 µm, b = 6.6, in females), and their caudal region (c' = 0.8 – 0.9 *vs* c' = 1, with regular inner core *vs* inner core with a terminal projection almost reaching the tail tip). The record of *S. barbatum* in Switzerland (Loof & Coomans 1970) is questionable as the neck of the only female studied was 970 µm, significantly different from that of type specimen, *ca* 589 µm.

Type locality and habitat

Iran, Mazandaran province, city of Noshahr, coordinates 36° 34'45.9"N 51°48'49.2"E, where the new species was collected from the rhizosphere of eucalyptus trees (*Eucalyptus camaldulensis* L.)

Type material

Female holotype, two female paratypes and one male paratype deposited with with WaNeCo collection, Wageningen, The Netherlands (http://www.waneco.eu/). One female paratype at nematode collection of the University of Jaén, Spain.

Etymology

The specific epithet refers to Noshahr, the city where the new species was collected.

https://doi.org/10.1017/S0022149X24000245 Published online by Cambridge University Press

Evolutionary relationships of *Sectonema*

Derived from morphological observations

The two Iranian species herein described belong to a group of large- to very large-sized (more than 4 mm long) *Sectonema* representatives. They perfectly fit the general morphology of the genus (Álvarez-Ortega & Peña-Santiago 2019), including the two basic types of protrusible stomatal structure, either a reduced odontostyle (RO, *S. noshahrense* sp. nov.) or a mural tooth (MT, *S. tehranense* sp. nov.). The drastic reduction of the size of the stomatal protruding structure (and probably the corresponding increase of its aperture) is a remarkable apomorphic trait of the *Sectonema* morphological pattern, probably an autapomorphy within Dorylaimina. It is also its most recognizable feature to separate it from its closest relatives, for instance, *Aporcelaimus* Thorne & Swanger, 1936, *Metaporcelaimus* Lordello, 1965 and *Epacrolaimus* Andrássy, 2000 (see below), as these genera bear a typical odontostyle with both dorsal and ventral arms well perceptible and variably large aperture. Heyns (1965) illustrated the evolutionary process of reducing the size of odontostyle in aporcelaims (Aporcelaimidae Heyns 1965), assuming that *Sectonema* species derived from an ancestor that bore typical odontostyle. On this matter, two issues deserve attention. On the one hand, if this process occurred only once or happened in several taxa and/or with different intensity throughout the evolutionary history of the dorylaims/aporcelaims. On the other hand, if the two types of reduced stomatal structure (reduced odontostyle and mural tooth) represent two different (alternative) results of the reducing process, or they are two successive stages of the process.

Derived from molecular analyses

The results of molecular analyses of 28S and 18S sequences are presented in the trees of Figures 6 and 7, respectively. Previous findings (Álvarez-Ortega & Peña-Santiago 2019; Peña-Santiago & Castillo 2022) are confirmed in both cases. On the one hand, 18S tree shows that *Sectonema* and *Epacrolaimus* form a maximally (100%) supported clade, but the internal relationships are less satisfactorily resolved than in those provided by Peña-Santiago and Castillo (2022). On the other hand, 28S tree shows better resolution and provides additional information of interest. Thus, a (*Sectonema* + *Epacrolaimus* + *Metaporcelaimus*) clade is maximally (100%) supported, but internal resolution is imperfect. *Sectonema* sequences are split into three highly/maximally supported subclades. One of them, constituted by sequences of three Indomalayan (Vietnamese) species, appears as the sister group of

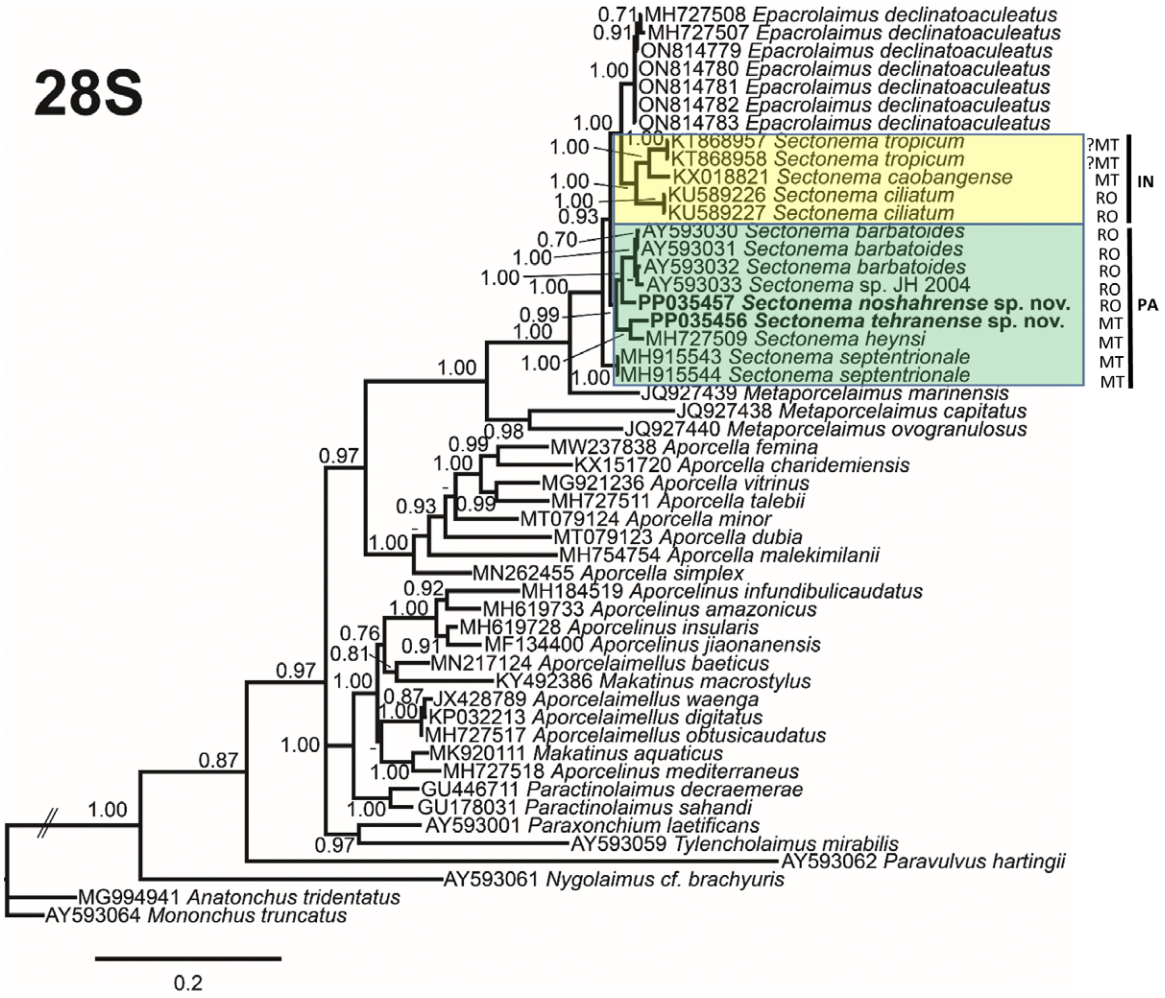

Figure 6. Phylogenetic relationships of two new species of the genus *Sectonema* Thorne, 1930 with species of Dorylaimida. Bayesian 50% majority rule consensus tree inferred from D2 – D3 expansion segments of 28S rRNA sequence alignment under the GTR + I + G model. Posterior probabilities >0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in bold, and the coloured box indicate the clade association of the studied species. Scale bar = expected changes per site.

https://doi.org/10.1017/S0022149X24000245 Published online by Cambridge University Press

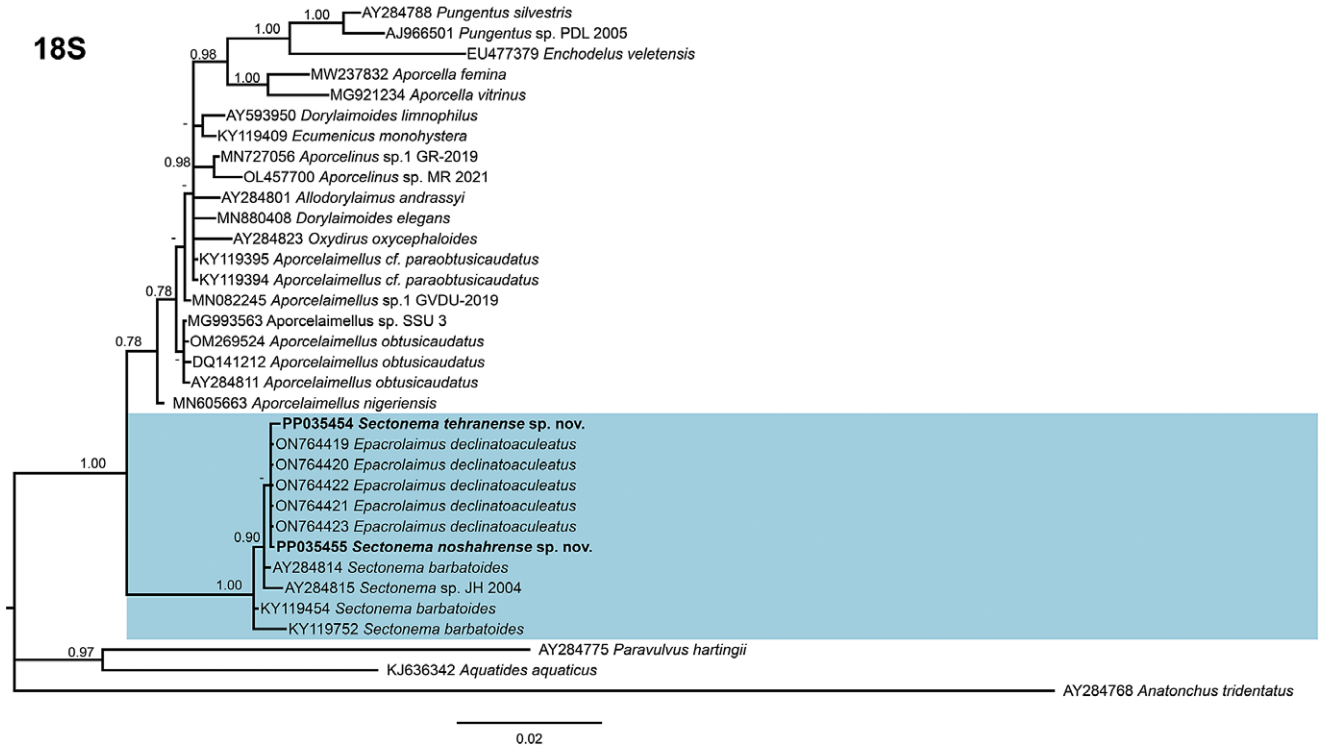

Figure 7. Phylogenetic relationships of two new species of the genus *Sectonema* Thorne, 1930 with species of Dorylaimida. Bayesian 50% majority rule consensus tree inferred from 18S rRNA gene sequence alignment GTR + I+ G model. Posterior probabilities >0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in bold, and the coloured box indicate the clade association of the studied species. Scale bar = expected changes per site. IN = Indomalayan (Vietnamese) sequences, MT = mural tooth, PA = Palearctic sequences, RO = reduced odontostyle.

Epacrolaimus sequences, as previously observed by Peña-Santiago and Castillo (2022). Still, this relationship is now better supported (100 *vs* 75%). The two remaining subclades include sequences of Palearctic specimens/species, divided into a group with two sequences of *S. septentrionale* from peninsular Spain, and another larger group with non-Iberian European sequences and the two Iranian species herein studied. Regarding *Metaporcelaimus* sequences, the *M. marinensis* sequence appears closer to *Sectonema* sequences than to the other two sequences of the same genus (*cf.* Álvarez-Ortega & Peña-Santiago 2019).

Integrative approach and concluding remarks

The genus *Sectonema* is a typical case of nematode taxon in which morphological and molecular data do not match. It is easily recognizable and distinguishable from its most relatives by the peculiar structure of its protruding stomatal structure, either a reduced odontostyle or a mural tooth (see above). Available molecular analyses show that a (*Epacrolaimus* + *Metaporcelaimus* + *Sectonema*) clade is maximally supported and that *Sectonema* sequences are split into three subclades, one of them being the sister group of *Epacrolaimus* clade. The study of the two Iranian species herein described reveals two interesting results. First, the confirmation of a tentative biogeographical pattern, previously observed (Álvarez-Ortega & Peña-Santiago 2019), with sequences of Indolamayan taxa forming a clade separated from those of the Palearctic ones and the sister group of *Epacrolaimus* sequences. Second, the process resulting in the reduction of odontostyle probably occurred at least twice throughout the evolutionary history of the genus, as it is observed in both biogeographical groups because these groups include sequences/species (Fig. 7) bearing reduced odontostyle and mural tooth. This means that processes of parallel or convergent evolution might be involved in the phylogeny of the species currently classified under *Sectonema*, that this genus certainly is more heterogeneous than previously assumed, and that further research is required to elucidate its taxonomy and systematics.

Financial support. University of Jaén, Spain, through the research program 'PAIUJA 2023/2024: EI_RNM2_2023'.

Competing interest. The authors have no competing interests.

Ethical standard. The authors assert 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 laboratory animals.

References

Abdi Gonbary A, Naghavi A and Niknam G (2018) Morphological and molecular identification of *Sectonema heynsi*, *Sectonema demani* and *Epacrolaimus declinatoaculeatus* from East Azarbaijan province, Iran. 23rd Iranian Plant Protection Congress. 27–30 Aug. 2018, Gorgan, Iran.

Altherr E (1965) La faune des sables sumergés des rives du Rhin près de Krefeld. Nématodes. *Gewässer und Abwässer* **39/40**, 80–101.

Altherr E (1968) Nématodes de la nappe phréatique du réseau fluvial de la Saale (Thuringe) et psammniques du Lac Stechlin (Brandebourg du nord). *Limnologica* **6**, 247–320.

Álvarez-Ortega S, Nguyen TAD, Abolafia J, Bonkowski M and Peña-Santiago R (2016) *Sectonema caobangense* sp. n. from Vietnam (Nematoda, Dorylaimida, Aporcelaimidae). *Journal of Nematology* **48**, 95–103.

Álvarez-Ortega S and Peña-Santiago R (2019) Morphology, phylogeny and taxonomy of the genus *Sectonema* (Nematoda, Aporcelaimidae). *Zoologica Scripta* **48**, 535–544.

https://doi.org/10.1017/S0022149X24000245 Published online by Cambridge University Press

Álvarez-Ortega S, **Subbotin SA and Inserra RN** (2021) Morphological and molecular characterization of *Xiphinemella esseri* Chitwood, 1957 (Dorylaimida: Leptonchidae) from Florida, with the first molecular study of the genus. *Journal of Nematology* **53**, e2021−32.

Andrássy I (2000) Four large-sized species of the family Aporcelaimidae (Nematoda, Dorylaimida) with proposal of a new genus, *Epacrolaimus* gen. n. *Opuscula Zoologica Budapestinensis* **32**, 3−26.

Blaxter ML, **De Ley P**, **Garey JR**, **Liu LX**, **Scheldeman P**, **Vierstraete A, … and Thomas WK** (1998) A molecular evolutionary framework for the phylum Nematoda. *Nature* **392**(**6671**), 71−75.

Darriba D, **Taboada GL**, **Doallo R, and Posada D** (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772.

De Grisse AT (1969) Redescription ou modifications de quelques technique utilis [a] es dan l'etude des nematodes phytoparasitaires. *Mededelingen Rijksfaculteit Landbouwwetenschappen Gent* **34**, 351–369.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95−98.

Heyns J (1965). On the morphology and taxonomy of the Aporcelaimidae, a new family of dorylaimid nematodes. *Entomology Memoirs Department of Agriculture Technical Services Republic of South Africa* **10**, 1−51.

Holterman M, **Rybarczyk K**, **van den Elsen S**, **van Megen H**, **Mooyman P**, **Peña-Santiago R**, **Bongers T**, **Bakker J and Helder J** (2008) A ribosomal DNA-based framework for the detection and quantification of stress-sensitive nematode families in terrestrial habitats. *Molecular Ecology Resources* **8**, 23−34.

Holterman M, **Vander Wurff A**, **Vanden Elsen S**, **Van Megen H**, **Bongers T**, **Holovachov O**, **Bakker J and Helder J** (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology and Evolution* **23**, 1792–800.

Katoh K, **Rozewicki J and Yamada KD** (2019) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Brief Bioinformatics* **20**, 1160−1166.

Kreis HA (1924). Die Seen im Aela- und Tinzenhorngebiet II. Nematodes. *Jahresbericht der Naturforschenden Gesellschaft Graubündens* n. F. **63**, 23–68.

Loof PAA and Coomans A (1970) On the development and location of the oesophageal gland nuclei in Dorylaimina. *Proceedings of the IX International Nematology Symposium* (Warsaw, Poland, **1967**): 79–161.

Lordello LGE (1965) *Contribuiçao para o conhocimento dos nematóides brasileiros da familia Dorylaimidae.* Thesis Escola Superior Agricultura "Luiz de Queiroz, Univ. S. Paulo **9**, 1−68.

Mullin PG, **Harris TS, and Powers TO** (2005) Phylogenetic relationships of Nygolaimina and Dorylaimina (Nematoda: Dorylaimida) inferred from small subunit ribosomal DNA sequences. *Nematology* **7**, 59−79.

Nadler SA, **Hudspeth DSS** (1998) Ribosomal DNA and phylogeny of the Ascaridoidea (Nemata: Secernentea): implications for morphological evolution and classification. *Molecular Phylogenetics and Evolution* **10**, 221–236.

Nunn GB (1992) *Nematode molecular evolution* (Doctoral dissertation, University of Nottingham).

Pedram M, **Pourjam E and Vinciguerra MT** (2012) Description of a new species of the rare genus *Epacrolaimus* Andrássy, 2000 (Dorylaimida, Aporcelaimidae) and new data on male of *Paraxonchium laetificans* (Andrássy, 1956) Altherr & Loof, 1969 (Dorylaimida, Paraxonchiidae) from Iran. *Zootaxa* **3327**, 53–61.

Peña-Santiago R (2021) *Dorylaimida Mundi (Nematoda) – Checklist of genera and species, with their records. Monographic papers on Nematology nº 7.* Editorial Universidad de Jaén. Jaén, Spain. 957 pp.

Peña-Santiago R (2023) On identity of *Epacrolaimus reyesi* Pedram, Pourjam & Vinciguera, 2012 (Dorylaimida, Aporcelaimidae). *Nematology* **25**, 115–119.

Peña-Santiago R and Álvarez-Ortega S (2014) Re-description of three new species of the genus *Sectonema* Thorne, 1930 (Nematoda; Dorylaimida: Aporcelaimidae) originally studied by E. *Altherr*. Zootaxa **3881**, 63–74.

Peña-Santiago R and Álvarez-Ortega S (2015) Description of *Sectonema septentrionale* sp. n. (Nematoda: Dorylaimida: Aporcelaimidae) from Northern Iberian Peninsula. *Zootaxa* **3947**, 573–580.

Peña-Santiago R and Castillo P (2022) On the identity of the genus *Epacrolaimus* Andrássy, 2000 (Nematoda, Dorylaimida), with new insights into its phylogeny. *Journal of Helminthology* **96**, e72.

Rambaut A (2014) FigTree v1.4.2, A Graphical Viewer of Phylogenetic Trees.

Ronquist F and Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572−1574.

Thorne G (1930). Predaceous nemas of the genus *Nygolaimus* and a new genus *Sectonema*. *Journal of Agricultural Research* **41**, 445–466.

Thorne G and Swanger HH (1936) A monograph of the nematode genera *Dorylaimus* Dujardin, *Aporcelaimus* n. gen., *Dorylaimoides* n. gen., and *Pungentus* n. gen. *Capita Zoologica* **6**, 1−223.

https://doi.org/10.1017/S0022149X24000245 Published online by Cambridge University Press