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Morphological and molecular characterisation of *Neothada olearum* sp. nov. (Nematoda: Tylenchidae) from Spain

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Research Paper

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Abstract

During nematode surveys conducted to investigate the biodiversity of plant-parasitic nematodes in Mediterranean olive groves with different management strategies (organic and conventional), a nematode population of the genus *Neothada* was detected in southern Spain. Application of integrative taxonomical approaches clearly demonstrated that it is a new species described herein as *Neothada olearum* sp. nov., also representing the first report of the genus in Spain. The new species is amphimictic, characterised by a short body (563–774 µm); cuticle widely annulated (2.5–3.0 µm); total number of body annuli 214–226; 16 longitudinal ridges giving a tessellate body surface; stylet without distinct basal knobs (9.0–11.0 µm); and tail elongate-conoid, with tip bluntly rounded. The results of molecular analysis of D2-D3 28S rRNA, ITS rRNA, partial 18S rRNA, and cytochrome oxidase c subunit 1 (COI) gene sequences support for the new species status and clearly separated from *N. major* and other species within *Neothada*. Phylogenetic analyses of ribosomal and mitochondrial markers of this study suggested that *Neothada* is a monophyletic genus, clearly separated from *Thada*.

Introduction

Nematodes within the Tylenchidae family are one of the most abundant soil-inhabiting species worldwide in natural and agricultural environments, although they do not comprise economically important plant-parasites (Geraert 2008; Qing and Bert 2019). The genus *Neothada* was proposed by Khan (1973) to include small nematodes (under 1 mm) with a thick cuticle marked by transverse and longitudinal striae or groves dividing the surface into minute squares or rectangular blocks (Siddiqi 2000). *Neothada* Khan, 1973 comprises a small genus within the family Tylenchidae Thorne, 1935 distributed almost worldwide and associated with algae, mosses, lichens, and plant roots (Geraert 2008; Siddiqi 2000). Currently, *Neothada* comprises six valid species, namely: *N. tatra* (Thorne and Malek 1968) Khan, 1973 (type species), *N. cancellata* (Thorne 1941) Khan, 1973, *N. costata* (Geraert and Raski 1986) Siddiqi, 2000, *N. geraerti* (Andrássy 1982) Siddiqi, 1986, *N. hades* Heyns and van den Berg, 1996, and *N. major* Maqbool and Shahina, 1989. The systematic position of the genera *Thada* and *Neothada* has been controversial in the nematological literature. Siddiqi (2000) divided the family Tylenchidae in five subfamilies, including Thadinae Siddiqi, 1986, that contain exclusively the genera *Thada* Thorne, 1941 and *Neothada* characterised by an axial spermatheca and a short conoid to subcylindrical tail. However, Geraert (2008) based on the sensilla in the lip region, the plain vulval region (without flap or membrane), and elongated tail, often rounded or clavate included these genera under the subfamily Boleodorinae Khan, 1964 together with *Boleodorus* Thorne, 1941, *Atetylenchus* Khan, 1973, *Basiria* Siddiqi, 1959, *Neopsilenchus* Thorne and Malek, 1968, *Psilenchus* de Man, 1921, and *Ridgellus* Siddiqi, 2000.

Molecular phylogeny inferred from ribosomal RNA (rRNA) genes suggests that Tylenchidae is polyphyletic (Bert *et al.* 2008; Holterman *et al.* 2006; Kantor *et al.* 2023; Subbotin *et al.* 2006). However, currently, there is little molecular data on species of the genera *Thada* and *Neothada* which could help clarify their taxonomic and systematic position within the family Tylenchidae. These studies comprise Yaghoubi *et al.* (2015) on *N. cancellata*, Hosseinvand *et al.* (2020a, 2020b) on *Thada populus* Hosseinvand *et al.*, 2020, *N. hades*, *N. major*, and *N. cancellata*, and in these studies, using partial sequences of 18S rRNA and 28S rRNA D2-D3 expansion segments, *Neothada* spp. clustered together with *Basiria* spp., concurs with the proposal raised by Geraert (2008).

During nematode surveys conducted in the spring of 2023 to investigate the biodiversity of plant-parasitic nematodes in Mediterranean olive groves with different management strategies (organic and conventional) in Greece, Italy, Morocco, Portugal, and Spain, a nematode population of the genus *Neothada* morphologically resembling *N. major* was detected in an olive grove in southern Spain (Chiclana de Segura, province of Jaén, Spain). This finding prompted us to study this population using an integrative taxonomic approach based on morphological and molecular analyses, along with comprehensive scanning electron microscopy (SEM) studies. To our knowledge, no detection of these nematodes has been reported in Spain; consequently, the recent finding of a new population of *Neothada* sp. in olive groves in southern Spain provides an excellent chance for ribosomal and mitochondrial molecular characterisation, as well as phylogenetic studies regarded as crucial in taxonomic and systematic studies of Tylenchidae that will progress our understanding of the evolution of these nematodes.

Therefore, the main objectives of this study were: *i*) to characterise morphologically and morphometrically the new Spanish population of *Neothada* and compare with related species; *ii*) to characterise molecularly the new *Neothada* population using the D2–D3 expansion segments of 28S rRNA, ITS rRNA, partial 18S rRNA, and cytochrome oxidase c subunit 1 (COI) gene sequences; and *iii*) to study the phylogenetic relationships of the identified *Neothada* species with available sequenced species of the family Tylenchidae.

Material and methods

Nematode population and morphological characterisation

Soil samples were collected from a total of 44 olive groves (with six samples per grove) during olive flowering stage (Spring 2023), giving a total of 264 sampling sites widely distributed across the most important olive-growing regions of the Mediterranean basin (Greece, Morocco, Italy, Portugal, and Spain). Soil samples were collected within 5–30 cm soil depth, and nematodes were extracted from a 500 cm^3 subsample of soil by centrifugal flotation (Coolen 1979). Extracted specimens were heat killed, fixed in TAF, processed to glycerol by a slow evaporation method, and mounted on permanent slides (De Grisse 1969; Seinhorst 1966). The light micrographs and measurements of nematode populations, including the main diagnostic characteristics (i.e., de Man indices, body length, stylet length, lip region, tail length and shape, longitudinal ridges) were performed using a Leica DM6 compound microscope with a Leica DFC7000 T digital camera. All abbreviations used are as defined in Siddiqi (2000).

For SEM, fixed specimens were dehydrated in a gradient series of ethanol, critical-point dried, sputter-coated with gold according to the protocol by Abolafia *et al.* (2002), and observed with a Jeol IT 800 SHL Scanning Electron Microscope (5 kV; Tokio, Japan).

Molecular characterisation and phylogenetic analyses

For molecular analyses, and to avoid mistakes in case of mixed populations in the same sample, single specimens from the sample were temporarily mounted in a drop of 1M NaCl containing glass beads (to prevent nematode specimens from crushing/damaging) to ensure specimens conformed with the target population. All necessary morphological and morphometric data were recorded. This was followed by DNA extraction from single individuals as described by Archidona-Yuste *et al.* (2024). The D2–D3 expansion segments were amplified using the D2A (5'-ACAAGTACCGTGAGGGAAAG TTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') primers (De Ley *et al.* 1999). The ITS region was amplified using forward primer 18S (5′-TTGATTACGTCCCTGCCC TTT-3′) and reverse primer Vrain2r (5′-TTT CACTCGCCGTTACTAAGGGAATC-3′) (Vrain *et al.* 1992). The partial 18S rRNA was amplified using the primers 988 (5′-CTCAAAGATTAAGCCATGC-3′), 1912R (5′-TTTACGGTCAGAACTAGGG-3′), 1813F (5´- CTGCGTGA-GAGGTGAAAT -3/), and 2646R (5/- GCTACCTTGTTACGACT TTT -3/) (Holterman *et al.* 2006). Finally, the portion of the COI gene was amplified using the primers JB3 (5´- TTTTTTGGGCAT CCTGAGGTTTAT -3`) and JB5 (5'- AGCACCTAAACTTAAAA-CATAATGAAAATG -3´) (Derycke *et al.* 2005).

All PCR assays were done according to the conditions described by Archidona-Yuste *et al.* (2024). Then, the amplified PCR products were purified using ExoSAP-IT (Affimetrix, USB products) and used for direct sequencing on a DNA multicapillary sequencer (Model 3130XL genetic analyser; Applied Biosystems, Foster City, CA, USA) using the BigDye Terminator Sequencing Kit V.3.1 (Applied Biosystems, Foster City, CA, USA) at the Stab Vida sequencing facilities (Caparica, Portugal). The newly obtained sequences were submitted to the GenBank database under the accession numbers indicated on the phylogenetic trees.

Phylogenetic analyses

D2–D3 expansion segments of 28S rRNA gene, ITS rRNA region, partial 18S rRNA gene, and COI mtDNA sequences of the unidentified *Neothada* species population were obtained in this study. These sequences, and other sequences from species of Tylenchidae from GenBank, were used for phylogenetic analyses. Outgroup taxa for each dataset were chosen following previously published studies (Bai *et al.* 2020; Munawar *et al.* 2021; Qing and Bert, 2019). Multiple sequence alignments of the different genes were made using the FFT-NS-2 algorithm of MAFFT V.7.450 (Katoh *et al.* 2019). The BioEdit program V.7.2.5 (Hall 1999) was used for sequence alignment visualisation and manually edited and trimmed the poorly aligned positions using a light filtering strategy (up to 20% of alignment positions), which has little impact on tree accuracy and may save computation time, as suggested by Tan *et al.* (2015). Phylogenetic analyses of the sequence datasets were based on Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The best-fit model of DNA evolution was achieved 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, and the gamma distribution shape parameters and substitution rates in the AIC were then used in MrBayes for phylogenetic analyses. The transition models with invariable sites and a gamma-shaped distribution for the D2–D3 segments of 28S rRNA gene (TIM2 + I + G), the partial 18S rRNA gene (TIM1 + I + G), and general time-reversible model with a gamma-shaped distribution (GTR + G) for the COI gene were run with four chains for 10×10^6 generations. A combined analysis of the three ribosomal genes was not undertaken because some sequences were not available for all species. The sampling for Markov chains was conducted at intervals of 100 generations. For each analysis, two runs were conducted. After discarding burn-in samples of 30% and evaluating convergence, the remaining samples were retained for more in-depth analyses. The topologies were used

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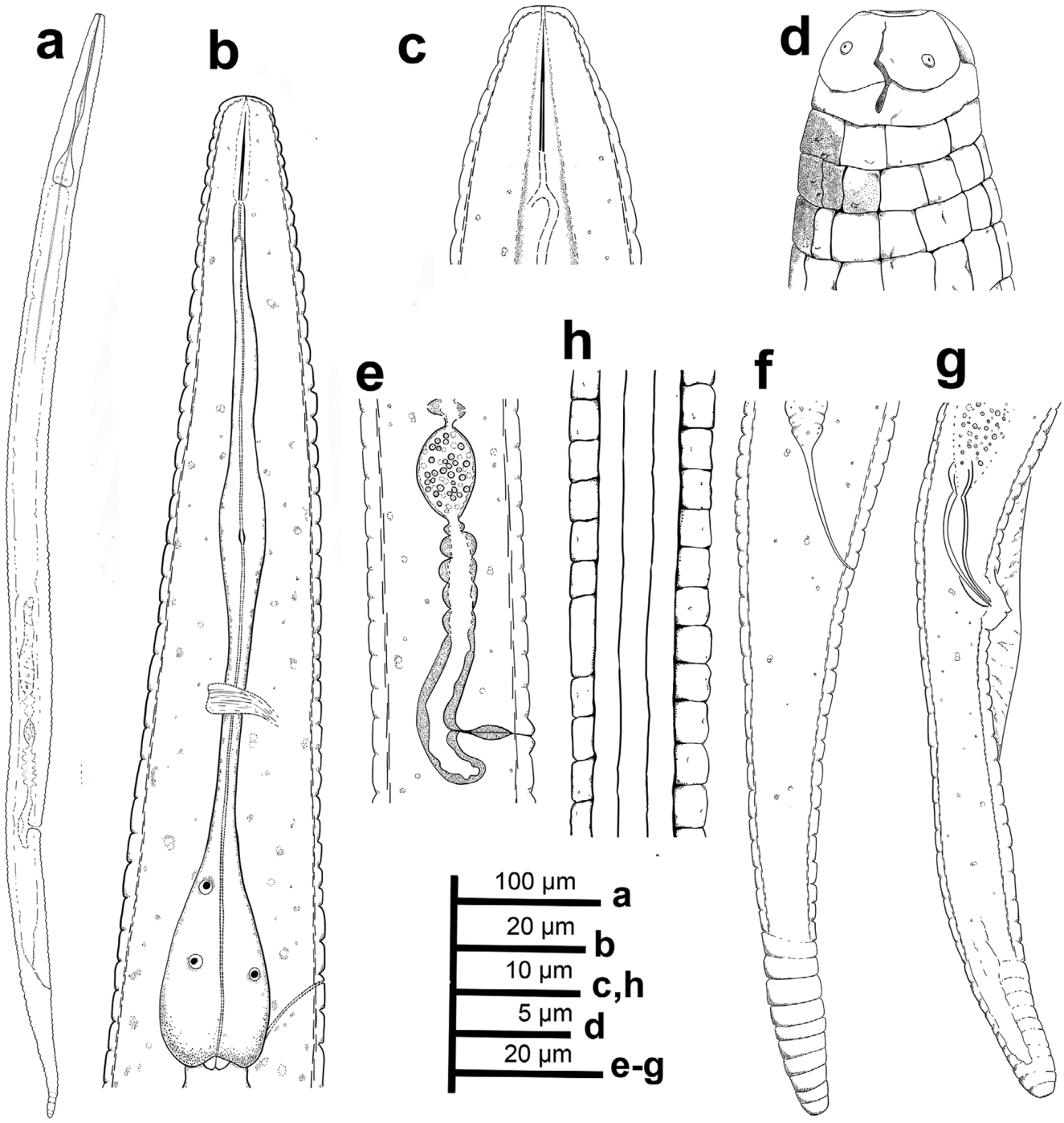

Figure 1. *Neothada olearum* sp. nov. (drawings). (a), entire female body; (b), female pharyngeal region; (c), detail of lip region showing stylet; (d), detail of lip region showing amphidial aperture and cervical papillae; (e), detail of vulval region showing spermatheca; (f), female tail; (g), male tail; (h), detail of lateral lines at mid of body.

to generate a 50% majority-rule consensus tree. For each appropriate clade, posterior probabilities (PP) were given. FigTree software version v.1.4.4 (Rambaut 2018) was used for visualising trees from all analyses.

Results

Only one soil sample, collected from the rhizosphere of cultivated olive (cv. Picual) at Chiclana de Segura, Jaén province, Spain, contained a *Neothada* species at a high population density (252 nematodes/500 cm^3 of soil). This contrasts with its very low overall frequency of occurrence across all samples (1/264 = 0.004). Detailed morphological, morphometrical, and molecular information about this species is provided below, confirming its identity as a new species of the genus *Neothada* described herein.

Systematics

Phylum: Nematoda Rudolphi, 1808

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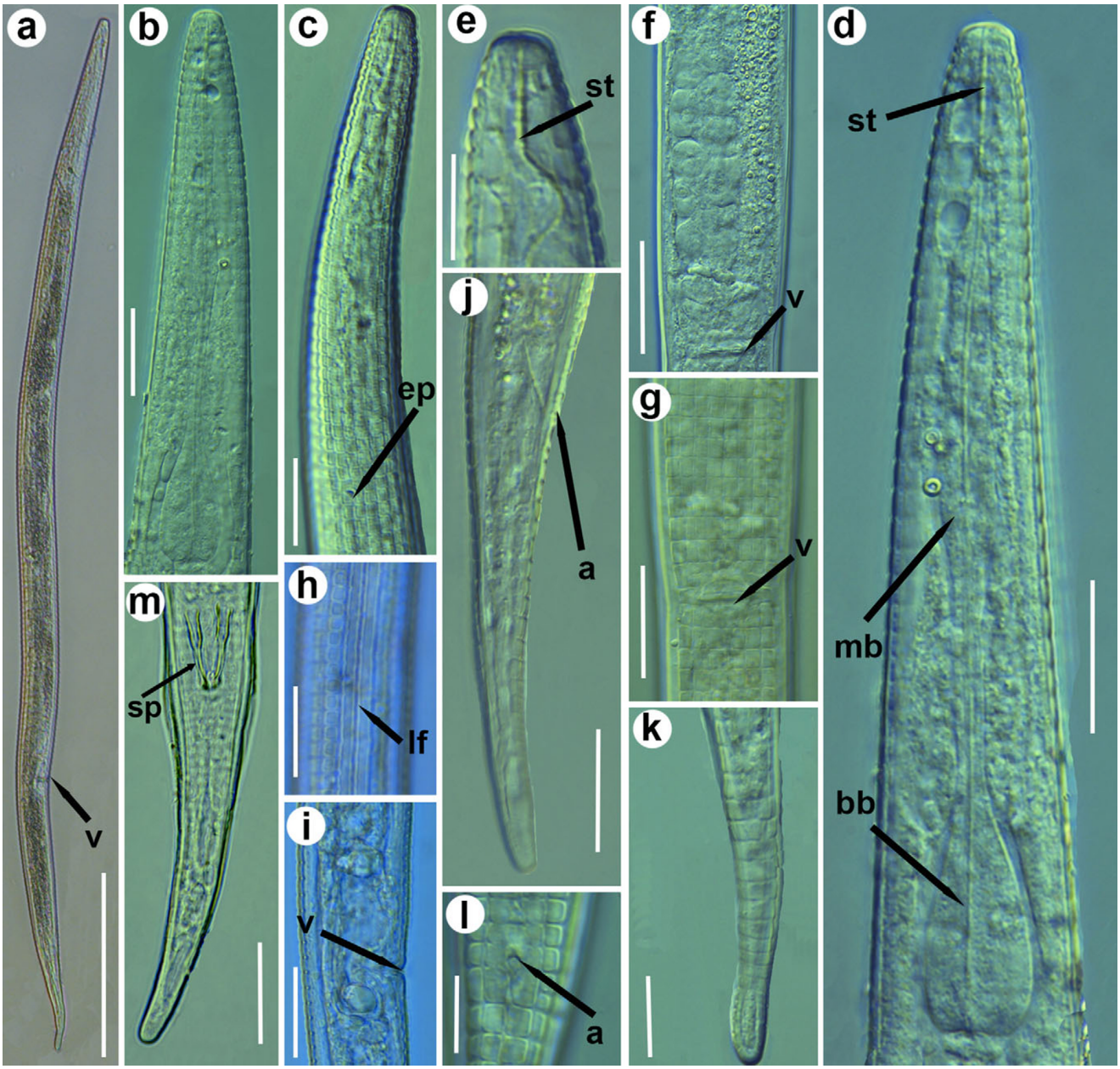

Figure 2. Light micrographs of *Neothada olearum* sp. nov. (a), entire female; (b–d), female pharyngeal region showing basal and median bulb, excretory pore, and stylet (arrowed); (e), detail of lip region showing stylet; (f–i), detail of vulval region showing longitudinal ridges, vulva, and crustaformeria; (j–l), female tail showing anus and rounded tip; (m), male tail showing a ventral view of spicules. a = anus; bb = basal bulb; ep = excretory pore; lf = lateral fields; mb = median bulb; sp = spicule; st = stylet; V = vulva. (Scale bars: a = 100 µm; b–d, f–k = 20 µm; e, l = 10 µm).

Class: Chromadorea Inglis 1983
Order: Rhabditida Chitwood 1933
Suborder: Tylenchina Chitwood 1950
Superfamily: Tylenchoidea Oerley 1880
Family: Tylenchidae Thorne, 1935
Genus: *Neothada* Khan, 1973

Neothada olearum sp. nov

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Description

Females. Body almost straight, slightly curved in posterior half after relaxed by gentle heat (Figures 1–2). Cuticle prominently annulated, 2.5–3.0 µm wide, total number of body annuli 214–226, 38–42 annuli from anterior end to posterior end of pharynx, 24–28 annuli from anus to tail tip. Lateral fields 6.5–8.0 µm wide with four smooth incisures, and 16 longitudinal ridges (14 longitudinal lines around the circumference of the body, outside the lateral fields) giving a tessellate body surface (Figures 1–3; Table 1). Lip region rounded, anteriorly flattened, continuous with body contour, with two annuli (Figures 1–3).

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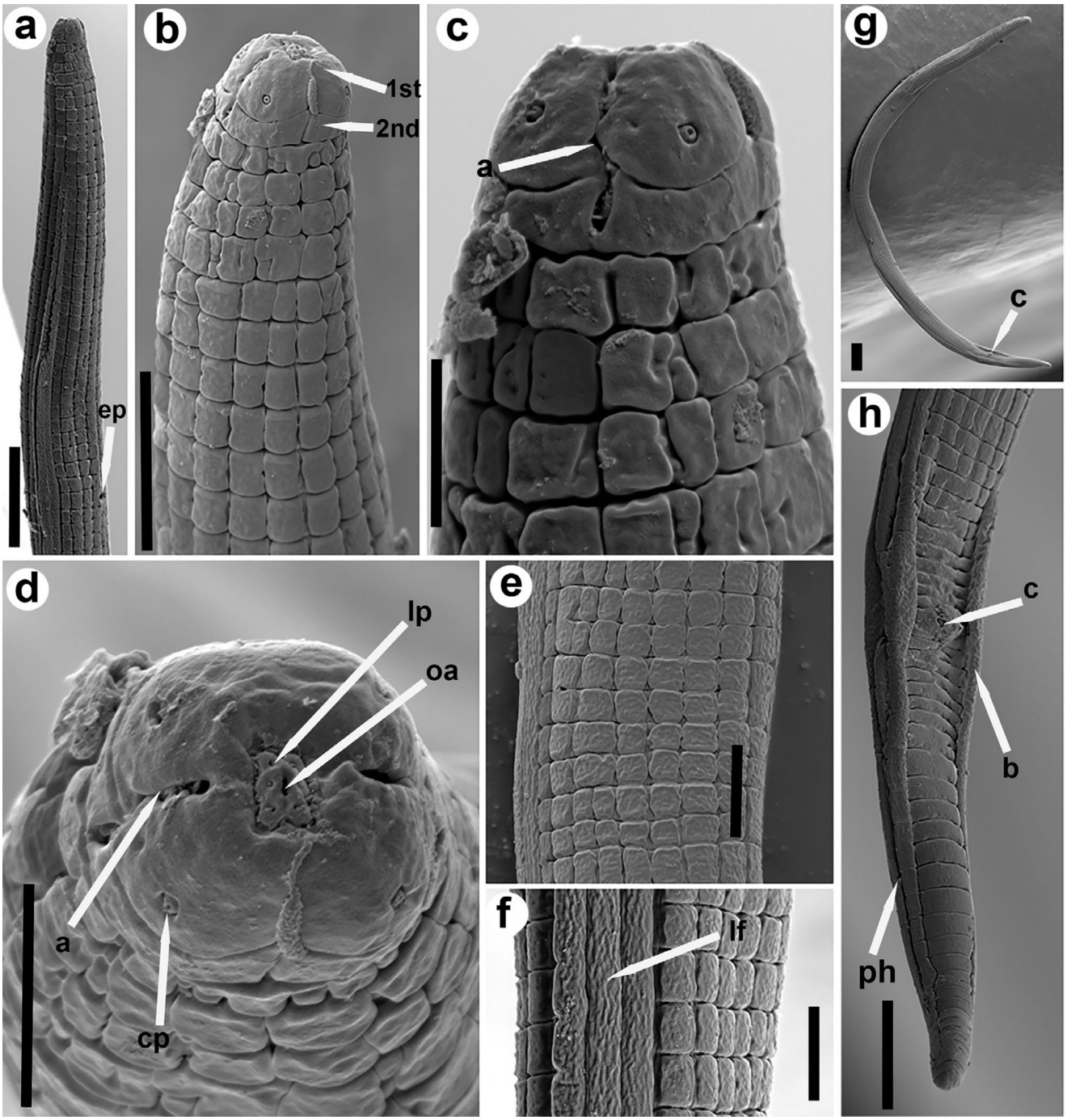

Figure 3. SEM micrographs of *Neothada olearum* sp. nov. a, b: female anterior region in lateral view showing excretory pore (ep), and detail of first (1st) and second (2nd) annuli; c: female lip region showing the amphidial aperture (a) arrowed; d: female lip region *en face* view showing the oral aperture (oa) surrounded by labial papillae (lp), cephalic papillae (cp), and amphidial aperture (a) arrowed; e, f: female mid body view showing lateral fields (lf) arrowed and longitudinal ridges; g: male whole body showing cloaca arrowed; h: male tail showing cloaca, bursa (b), and phasmid (ph) arrowed. a = amphidial aperture; b = bursa; c = cloaca; cp = cervical papillae; ep = excretory pore; lp = labial papilla; oa = oral aperture; ph = phasmid. (Scale bars: a, g, h = 20 μm; b, e, f = 10 μm; c d = 5 μm).

Labial framework slightly sclerotised (Figure 2). Stylet delicate without distinguishable basal knobs, anterior conical part 2.5–3.5 μm long. Dorsal pharyngeal gland opening 2.0–3.5 μm posterior to stylet base. Pharynx tylenchoid; procorpus cylindroid 34.0–40.0 μm long; median bulb fusiform, weakly muscular and without valves 6.0–7.5 μm wide x 16.0-19.0 μm long; isthmus slender, basal bulb pyriform 13.0–14.0 μm wide x 25.0-27.0 μm long (Figures 1–2). Cardias rounded, 3.0–3.5 μm long. SEM observations (Figure 3) showed a quadrangular oral plate, a first annulus wider than second (2.0–2.5 μm vs 1.0–1.5 μm wide) and showing four rounded cephalic papillae, a slit-like oral aperture surrounded by six labial papillae in *en face* view. Amphidial aperture a conspicuous longitudinal to slightly bent slit extending as far as the second annulus (Figure 3). Excretory pore slightly sclerotised, at middle of basal bulb, 33–40 annuli from anterior end. Deirid at level of excretory pore. Hemizonid not

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Table 1. Morphometrics of *Neothada olearum* sp. nov. from Chiclana de Segura, Jaén province, Spain

Character[a]	Holotype female	Paratype	
		Females	Males
n		20	4
L	643	649.4±61 (563–774)	596.3±18 (571–612)
a	25.7	27.0±3.8 (21.4–32.1)	26.5±3.5 (22.6–30.3)
b	5.8	5.8±0.4 (5.2–6.5)	5.3±0.3 (5.0–5.6)
c	8.9	8.9±0.8 (7.1–10.3)	8.0±0.4 (7.5–8.5)
c'	4.8	4.8±0.5 (3.9–5.4)	4.4±0.1 (4.3–4.5)
V/T	72.9	72.7±0.8 (71.1–74.0)	42.7±6.4 (36.0–49.2)
G1	40.7	39.6±6.5 (29.1–48.7)	–
Stylet length	10.0	10.0±0.6 (9.0–11.0)	9.9±0.3 (9.5–10.0)
Nerve ring-anterior end distance	69.0	66.0±4.1 (60.0–70.0)	64.3±1.5 (63.0–66.0)
Excretory pore anterior end distance	91.0	93.6±8.2 (84.0–114.0)	89.2±1.3 (88.0–90.5)
Tail length	72.0	73.3±3.8 (68.0–80.0)	75.1±4.4 (70.5–80.0)
Vulva-anus distance	102.0	104.4±14.5 (88.0–134.0)	-
Body width at level of:			
lip region	7.0	6.5±0.6 (6.0–7.5)	6.3±0.5 (6.0–7.0)
mid-body	25.0	24.8±5.4 (19.0–34.0)	22.1±3.7 (20.0–26.5)
anus	15.0	15.6±2.0 (13.0–19.0)	17.1±1.0 (16.0–20.0)
Spicules length	–	–	17.6±1.9 (16.0–20.0)
Gubernaculum	–	–	5.3±0.5 (5.0–6.0)

[a]Abbreviations are defined in Siddiqi (2000). All measurements are in µm and in the form: mean. ± standard deviation (range) where appropriate.

always clear; when seen, one or two body annuli anterior to excretory pore. Nerve ring surrounding anterior part of isthmus. Reproductive system monodelphic prodelphic; vulva a transverse slit in ventral view (Figure 2), without lateral flaps 11.0–12.0 µm long. Post-vulval uterine sac 0.44 times body width. Ovary single outstretched with oocytes in a single row, quadricolumella present, spermatheca axial, oval (14.0–19.0 µm x 26.0–30.0 µm) filled with rounded sperm 1.5–2.0 µm wide. Vulva anus distance 1.4 (1.2–1.7) times as long as tail. Tail elongate-conoid, tail tip bluntly rounded.

Males. Less frequent than female (1:5 ratio). Body habitus almost straight, slightly curved in posterior half after relaxed by gentle heat. Morphologically similar to female in body and tail shape, lip region, stylet, pharynx, and longitudinal ridges, except for genital system and secondary sexual features and posterior region more strongly curved ventrally. Testis single, outstretched, containing several rows of spermatogonia. Tail elongate-conoid, tail tip bluntly rounded. Spicules curved ventrally, gubernaculum simple and slightly ventrally curved.

Diagnosis and relationships

Neothada olearum sp. nov. is an amphimictic species characterised by a short body (563–774 µm); a widely annulated cuticle (2.5–3.0 µm); a total of 214–226 body annuli; 16 longitudinal ridges creating a tessellate body surface; a stylet without distinct basal knobs (9.0–11.0 µm); and an elongate-conoid tail with a bluntly rounded tip.

Morphological and morphometrically *Neothada olearum* sp. nov. is close to *N. major* from which can be separated in having 16 longitudinal ridges *vs* 20, stylet length (10.0 µm (9.0–11.0) *vs* 13.0 µm (12.0–14.4)), lower a ratio (27.0 (21.4–32.1) *vs* 37 (34–39)), and shorter spicules (17.6 µm (16.0–20.0) *vs* 21.5 µm (20.0–23.0)) (Maqbool and Shahina 1989). An Iranian population of *N. major* (Hosseinvand *et al.* 2020b) also showed morphological and morphometrical similarities with *N. olearum* sp. nov. but differs in longitudinal ridges (16 *vs* 19–20), a ratio (27.0 (21.4–32.1) *vs* 35.9 (29–44)), c' ratio (4.8 (3.9–5.4) *vs* 6.4 (5.6–7.1)), and male presence and sperm present in spermatheca *vs* absence of male and spermatheca without sperm, as well as D2–D3 expansion segments of 28S rRNA (MN970002) differing in 15 bp and six indels (Figure 4).

Neothada olearum sp. nov. can be separated from *N. tatra* by lip region (continuous with body contour *vs* set off), a longer body (649 µm (563–774) *vs* 560 µm), wider body annuli (2.5–3.0 µm *vs* 3.1–5.0 µm), and a longer tail (73 µm (68–80) *vs* 61 µm), with higher number of annuli 24–28 *vs* 18) (Thorne and Malek 1968). From *N. geraerti*, the new species can be separated by longitudinal ridges (16 *vs* 12), a longer body (649 µm (563–774) *vs* 490–570 µm), lower a ratio (27.0 (21.4–32.1) *vs* 37 (34–39)), and a longer tail (73 µm (68–80) *vs* 52–66 µm) (Andrássy 1982). From *N. cancellata*, the new species can be separated by lip region shape (rounded, anteriorly flattened *vs* conoid), a longer body (649 µm (563–774) *vs* 560–580 µm), V ratio (72.7 (71–74) *vs* 69.5), and a longer tail (73 µm (68–80) *vs* 57 µm) (Geraert 1974; Thorne 1941). *N. hades* and *N. costata* mainly differ by the absence of stylet basal knobs *vs* presence, body length (649 µm (563–774) *vs* 530–620 µm, 867 µm (788–973), respectively), longitudinal ridges (16 *vs* 14, 14, respectively), stylet length (10.0 µm (9.0–11.0) *vs* 9.0–10.5 µm, 11.2 µm (11.0–13.0), respectively), V ratio (72.7 (71–74) *vs* 70–74, 66 (65–67), respectively), and tail length (73 µm (68–80) *vs* 55–66 µm, µm 122 (114–136), respectively) (Geraert and Raski 1986; Heyns and van den Berg 1996).

Etymology

The species epithet is the Latin term *olearum* = belonging to or corresponding to olives, as type material was found in an olive grove.

Type host and locality

The new species was recovered from the rhizosphere of olive (*Olea europaea* subsp. *europaea* L.) at Chiclana de Segura, Jaén province, Spain (coordinates 38°29'04.69" N, -3°05'15.43" W, 620 m a.s.l.).

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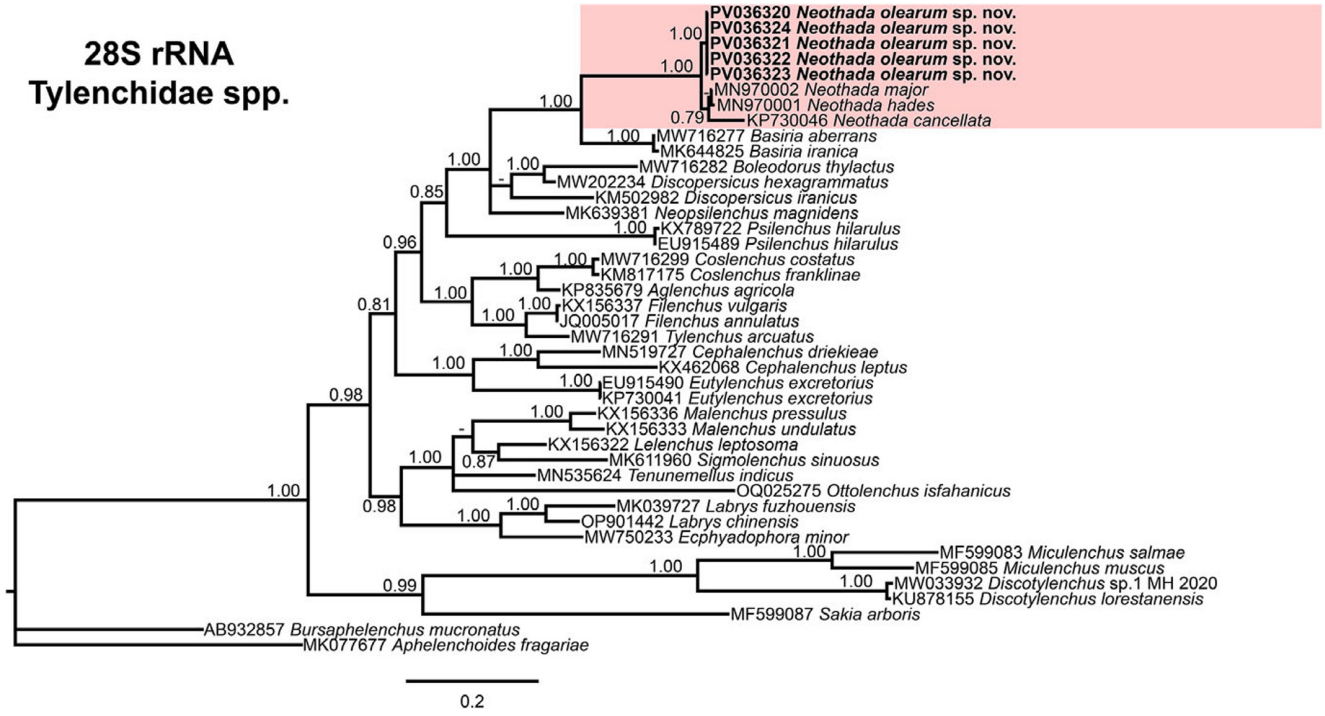

Figure 4. Phylogenetic relationships of *Neothada olearum* sp. nov. with species of Tylenchidae. Bayesian 50% majority rule consensus tree as inferred from D2 and D3 expansion segments of 28S rRNA sequence alignment under the TIM2 + I+ G model (−lnL = 11212.8251; AIC = 22605.650240; freqA = 0.1728; freqC = 0.2285; freqG = 0.3370; freqT = 0.2618; R(a) = 1.0546; R(b) = 2.8012; R(c) = 1.0546; R(d) = 1.0000; R(e) = 5.0928; R(f) = 1.0000; Pinva = 0.0138; and Shape = 0.6600). Posterior probabilities more than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in bold, and coloured box indicate clade association of the new species. Scale bar = expected changes per site.

Type material

Holotype female and 17 female and four male paratypes deposited at Institute for Sustainable Agriculture (IAS) of Spanish National Research Council (CSIC), Córdoba, Spain (Slide numbers ER51-02-ER51-08); and three females at the USDA Nematode Collection (T-8128p).

Molecular characterisation

Neothada olearum sp. nov. was molecularly characterised by the sequences of three ribosomal genes (D2–D3 expansion segments of 28S rRNA, ITS rRNA, and partial 18S rRNA), and the partial mitochondrial gene COI. The amplification of these regions yielded single fragments of approximately 900, 1100, 1800, and 400 bp, respectively, based on gel electrophoresis. Five D2–D3 of 28S rRNA sequences from 712 to 729 bp (PV036320–PV036324), five ITS rRNA sequences from 1126 bp (PV036325–PV036329), five 18S rRNA sequences from 1708 to 1719 bp (PV036330–PV036334), and four COI sequences of 248 to 266 bp (PV068612–PV068615) were generated for this new species without intraspecific sequence variations. D2–D3 expansion segments of 28S rRNA of *N. olearum* sp. nov. (PV036320–PV036324) was 98.0% similar to *N. major* (MN970002) from Iran, differing by 15 bp and six indels (Hosseinvand *et al.* 2020b); 95.8% similar to *N. cancellata* (KP730046) from Iran, differing by 28 bp and five indels (Yaghoubi *et al.* 2015); 96.8% similar to *N. hades* (MN970001) from Iran, differing by 19 bp and five indels (Hosseinvand *et al.* 2020b); and 80.6% similar to *Basiria aberrans* (MW716277) from China, differing by 146 bp and 48 indels (unpublished). ITS of *N. olearum* sp. nov. (PV036325–PV036329) were the first sequences of this marker for *Thada* or *Neothada* genera in NCBI and no significant similarity was found in the BLAST search. In any case, our ITS sequences of *N. olearum* sp. nov. (PV036325–PV036329) showed an 89.1% similarity with *Basiria aberrans*-PP575000 (differing by 25 bp and 14 indels, but coverage = 20% only), and an 88.4% similarity with *Sigmolenchus sinuosus*-MK611962 (differing by 21 bp and zero indels, but coverage = 16% only). The partial 18S of *N. olearum* sp. nov. (PV036330–PV036334) was 95.1–94.5% similar to several *Basiria* species such as *B. aberrans* (KJ869353) from Iran, *B. gracilis* (EU130839) from California, USA, and *B. graminophila* (KJ869317) from Iran, differing by 85–95 bp and 18–31 indels (Helder *et al.* 2014; Subbotin *et al.* 2008). Also, 18S of *N. olearum* sp. nov. (PV036330–PV036334) was 92.1–92.5% similar to *Boleodorus thylactus* (KJ869348) from Iran and *B. volutus* (FJ969117) from The Netherlands, differing by 129–137 bp and 32–42 indels (Helder *et al.* 2014). It also differs from *Thada populus* (MN557353) from Iran by 139 bp and 27 gaps (91.4% similarity) (Hosseinvand *et al.* 2020a). Finally, COI of *N. olearum* sp. nov. (PV068612–PV068615) were the first sequences of this marker for the genus *Neothada.* COI of *N. olearum* sp. nov. was 83.6–84.1% similar to *B. aberrans* (MN577605, MN577606) from China, differing by 40–42 bp (Bai *et al.* 2020). It differs from *Aglenchus geraerti* (MN577603) or *Coslenchus rafiqi* (MN577602) from China by 47 bp and (79.9% similarity) (Bai *et al.* 2020); from *B. thylactus* (MN577607, MN577608) from China in 52 bp and two indels (78.4%) (Bai *et al.* 2020); and from *Lelenchus leptosoma*1 and 2 (MN577615, MN577616, MN577595, MN577596) from China in 58–70 bp (71.0–73.6%) (Bai *et al.* 2020).

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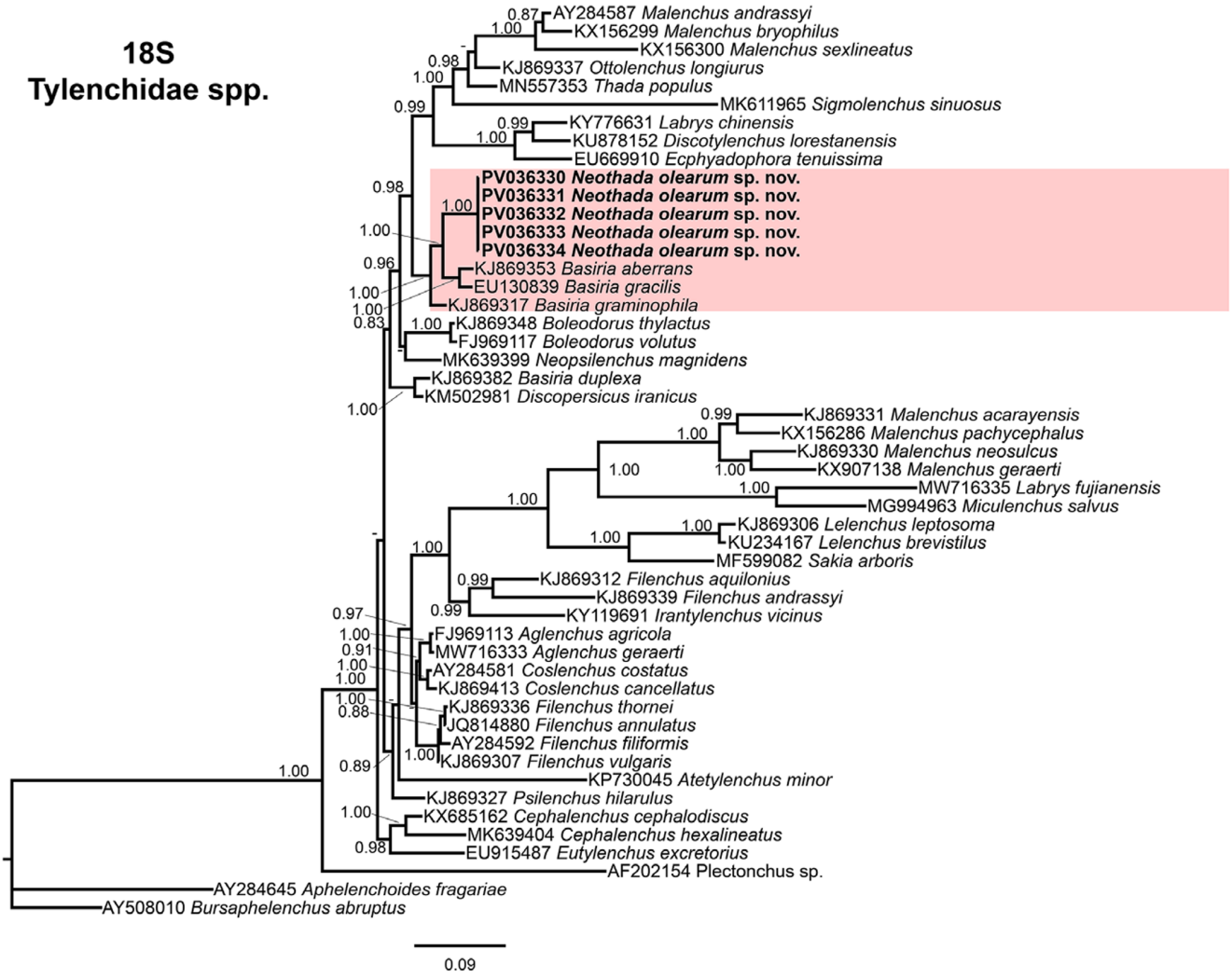

Figure 5. Phylogenetic relationships of *Neothada olearum* sp. nov. with species of Tylenchidae. Bayesian 50% majority rule consensus tree as inferred from 18S rRNA sequence alignment under the TIM1 + I+ G model (−lnL = 17362.9696; AIC = 34937.939200; freqA = 0.2417; freqC = 0.2246; freqG = 0.2879; freqT = 0.2458; R(a) = 1.0000; R(b) = 2.5654; R(c) = 1.1081; R(d) = 1.1081; R(e) = 4.9202; R(f) = 1.0000; Pinva = 0.1660; and Shape = 0.4570). Posterior probabilities more than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in bold, and coloured box indicate clade association of the new species. Scale bar = expected changes per site.

Phylogenetic relationships of Neothada olearum sp. nov. with other Tylenchidae spp.

Phylogenetic relationships among *Neothada olearum* sp. nov. with other Tylenchidae spp., as inferred from analyses of D2–D3 expansion segments of 28S rRNA, the partial 18S rRNA, and the partial COI mtDNA gene sequences using BI, are shown in Figures 4, 5, and 6, respectively. The BLAST search of the newly generated ITS sequences of *N. olearum* sp. nov. showed a low coverage (less than 35%) with sequences of Tylenchidae spp. and as a result, no further phylogenetic analysis was performed using ITS sequences. The phylogenetic trees generated with the ribosomal and mitochondrial DNA markers included 42, 50, and 37 sequences with 738, 1690, and 441 characters in length, respectively (Figures 4–6). The D2–D3 expansion segments of 28S rRNA tree of Tylenchidae spp. showed a well-supported subclade (PP = 1.00), including *N. olearum* sp. nov. (PV036320–PV036324) together with a well-supported subclade (PP = 1.00), including three *Neothada* species (*N. major*-MN970002, *N. hades*-MN970001, and *N. cancellata*-KP730046). This clade clustered with a separate well-supported subclade (PP = 1.00) including *B. aberrans*-MW716277 and *Basiria iranica*-MK644825 (Figure 4).

The 50% majority rule consensus 18S rRNA gene BI tree showed also a well-supported subclade (PP = 1.00) with *N. olearum* sp. nov. (PV036330–PV036334) together with a well-supported subclade (PP = 1.00), including *B. aberrans*-KJ869353 and *Basiria gracilis*-EU130839, while *Basiria graminophila*-KJ869317 and *Basiria duplexa*-KJ869382 are clearly separated from this subclade (Figure 5). Interestingly, the only available 18S sequence for *Thada populus* (MN557353) clustered within a distinct, well-supported subclade (PP = 1.00) with other Tylenchidae genera such as *Malenchus*, *Ottolenchus*, and *Sigmolenchus* (Figure 5).

Finally, the 50% majority rule consensus of the partial COI gene BI tree showed also a well-supported subclade (PP = 1.00) with *N. olearum* sp. nov. (PV068612–PV068615) together with a well-supported subclade (PP = 1.00), including *B. aberrans*-MN577605-MN577606 (Figure. 6).

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Figure 6. Phylogenetic relationships of *Neothada olearum* sp. nov. with species of Tylenchidae. Bayesian 50% majority-rule consensus trees as inferred from cytochrome c oxidase subunit I (COI) mtDNA gene sequence alignments under the GTR + G model (−lnL = 4051.7660; AIC = 8265.532060; freqA = 0.2622; freqC = 0.0899; freqG = 0.1832; freqT = 0.4647; R(a) = 1.2981; R(b) = 10.6042; R(c) = 4.1144; R(d) = 4.1433; R(e) = 6.3708; R(f) = 1.0000; Pinva = 0.3800; and Shape = 0.0000). Posterior probabilities more than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in bold, and coloured box indicate clade association of the new species. Scale bar = expected changes per site.

Discussion

The small size of Tylenchidae nematodes as well as their morphological simplicity and similarity make species identification very difficult, and integrative taxonomical approaches (morphological, including LM and SEM, and molecular) are crucial for an accurate identification (Qing and Bert 2019). The main objective of this study was to identify and describe, morphologically and molecularly, a new population of *Neothada* detected in an olive grove in Chiclana de Segura, Jaén province, southern Spain, as well as clarify the phylogenetic relationships within the family Tylenchidae using ribosomal and mitochondrial markers. All the provided results from a morphological and molecular point of view confirmed that the unknown *Neothada* population is a new valid species of the genus herein described as *N. olearum* sp. nov. The present study enhances the knowledge of the biodiversity of Tylenchidae genera occurring in Spain. Our SEM results provide clear evidence that lip region structure is consistent with previous studies on *N. costata*, *N. geraerti*, and *N. hades* (Geraert and Raski 1986; Heyns & van den Berg 1996), maintaining the general structure of a quadrangular oral plate with fine oral aperture surrounded by six labial papillae, and amphidial apertures are not confined to the oral plate but continue on the lateral side as longitudinal slits, which fits with lip pattern type II-a by Qing and Bert (2018). The present results also supported the hypothesis that the number of longitudinal striae, which seems to be constant within each species, is an important diagnostic character, since our new species is quite close to an Iranian population of *N. major* (morphological and molecularly characterised) from which *N. olearum* sp. nov. differs mainly in the number of longitudinal ridges (16 *vs* 19–20) and other characters and ratio (Hosseinvand *et al.* 2020b).

Phylogenetic analyses of ribosomal and mitochondrial markers of this study confirmed that the family Tylenchidae is a heterogeneous group, which agrees with previous studies (Munawar *et al.* 2021; Qing and Bert, 2018, 2019; Qing *et al.* 2017, 2018). Also, phylogenetic analyses are in concordance by grouping *N. olearum* sp. nov. with species of the genus *Basiria*, which are also consistent with previous studies and confirm its classical taxonomic position (Bai *et al.* 2020; Hosseinvand *et al.* 2020b; Munawar *et al.* 2021; Qing *et al.* 2017, 2018; Qing and Bert, 2018, 2019; Yaghoubi *et al.* 2015). Although to date, only a few *Neothada* species have been molecularly characterised, including *N. cancellata*, *N. hades*, *N. major*, and *N. olearum* sp. nov., based on 28S rRNA, the present results suggest that *Neothada* is a monophyletic genus within Tylenchidae; nevertheless further studies on other species and other gene markers are needed to confirm this hypothesis. Data on 18S rRNA gene phylogeny confirm that the genera *Thada* and *Neothada* are well-separated in two different clades, supporting that the longitudinal ridges differentiating both genera is a good trait to delimitate them as proposed by Khan (1973) and accepted by many other nematologists (Geraert 2008; Heyns and van den Berg 1996; Siddiqi 2000). Although the COI gene has only been explored for a limited number of Tylenchidae species, this molecular marker is one of the most important standard barcoding genes that has been used for many free-living and plant-parasitic nematodes (Kantor *et al.* 2023; Palomares-Rius *et al.* 2017; Powers 2004). Our results on COI are congruent with the phylogenetic relationships showed by Bai *et al.* (2020). We also concur with previous statements that the higher mutation rate of this marker offers a better separation of closely related species and is mainly valuable for the identification of potential cryptic species (Palomares-Rius *et al.* 2014). In addition, phylogeny of ribosomal and mitochondrial markers of *N. olearum* sp. nov. also confirmed that longitudinal

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ridges have originated several times independently within Tylenchidae (e.g., *Coslenchus*, *Neothada*, *Eutylenchus*) as previously proposed by Qing and Bert (2019).

In summary, the present study confirms the usefulness of applying integrative taxonomy in Tylenchidae to decipher the real diversity of these nematodes, which may be much higher, given that molecular diversity (rRNA and mtDNA markers) can remarkably exceed the low morphological diversity and that the majority of Tylenchidae species still remain completely undocumented (Qing and Bert 2019). In addition, on the basis of the present results and as suggested by Heyns and van den Berg (1996), some of the few documented reports of *N. cancellata sensu lato* need to be confirmed by integrative taxonomy.

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Ethical standard. The result of this work has not been published previously and is not under consideration elsewhere.

Ethical approval. The conducted research is not either related to human or animals use.

References

Abolafia, J, Liébanas, G and Peña-Santiago R (2002) Nematodes of the order Rhabditida from Andalucia Oriental, Spain. The subgenus *Pseudacrobeles* Steiner, 1938, with description of a new species. *Journal of Nematode Morphology and Systematics* **4**, 137–154.

Andrássy I (1982) Further twenty-five nematoda species new to the fauna of Hungary. *Allattani Közlemények* **69**, 139–146.

Archidona-Yuste, A, Clavero-Camacho, I, Ruiz-Cuenca, AN, Cantalapiedra-Navarrete, C, Liébanas, G, Castillo, P and Palomares-Rius, JE (2024) The more we search, the more we find: discovering and expanding the biodiversity in the ring nematode genus *Xenocriconemella* De Grisse and Loof, 1965 (Nematoda: Criconematidae). *Zoological Letters* **10**, 8. DOI: 10.1186/s40851-024-00230-3

Bai, M, Qing, X, Qiao, K, Ning, X, Xiao, S, Cheng X and Liu, G (2020) Mitochondrial COI gene is valid to delimitate Tylenchidae (Nematoda: Tylenchomorpha) species. *Journal of Nematology* **52**, 1–12. DOI: 10.21307/jofnem-2020-038

Bert, W, Leliaert, F, Vierstraete, AR, Vanfleteren, JR and Borgonie, G (2008) Molecular phylogeny of the Tylenchina and evolution of the female gonoduct (Nematoda: Rhabditida). *Molecular Phylogenetics and Evolution* **48**, 728–744. DOI: 10.1016/j.ympev.2008.04.011

Chitwood BG (1933) A revised classification of the Nematoda. *Journal of Parasitology* **20**, 131.

Chitwood BG (1950) General structure of nematodes. In Chitwood, BG and Chitwood MB (eds), *An Introduction to Nematology. Section 1, Anatomy*. Baltimore, MD: Monumental Printing Co., 7–27.

Coolen WA (1979) Methods for extraction of *Meloidogyne* spp. and other nematodes from roots and soil. In Lamberti, F and Taylor CE (eds), *Root-knot Nematodes* (Meloidogyne *species). Systematics, Biology and Control*. New York: Academic Press, 317–329.

Darriba, D, Taboada, GL, Doallo, R and Posada, D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772. DOI: 10.1038/nmeth.2109

De Grisse, AT (1969) Redescription ou modifications de quelques techniques utilisées dans l'étude de nématodes phytoparasitaires. *Mededelingen Rijksfaculteit Landbouwwetenschappen Gent* **34**, 315–359.

De Ley, P, Félix, MA, Frisse, LM, Nadler, SA, Sternberg, PW and Thomas, WK (1999) Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology* **1**, 591–612. DOI: 10.1163/156854199508559

de Man, JG (1921) Nouvelles recherches sur les nématodes libres terricoles de la Hollande. *Capita Zoologica* **1**, 3–62.

Derycke, S, Remerie, T, Vierstraete, A, Backeljau, T, Vanfleteren, J, Vincx, M and Moens, T (2005) Mitochondrial DNA variation and cryptic speciation within the free-living marine nematode *Pellioditis marina*. *Marine Ecology Progress Series* **300**, 91–103. DOI: 10.3354/meps300091

Geraert, E (1974) The morphology and the systematic position of the genus *Thada* (Nematoda: Tylenchida). *Mededelingen Fakulteit Landbouwwetenschappen Gent* **39**, 701–712.

Geraert, E (2008) *The Tylenchidae of the word: identification of the family Tylenchidae (Nematoda)*. Ghent, Belgium: Academia Press, 540.

Geraert, E, Raski, DJ (1986) Three new species of *Basirienchus* g. n. from Southern Chile compared with *Campbellenchus, Neothada* and *Basiria* (Nematoda: Tylenchidae). *Nematologica* **31**, 266–288.

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.

Helder, J, Mooijman, P, van den Elsen, S, van Megen, H, Vervoort, M, Quist, C, Bert, W, Karegar, A, Karssen, G and Decraemer, W (2014) Biological and systematic implications of phylogenetic analyses of ~2,800 full length small subunit ribosomal DNA sequences. *Journal of Nematology* **46**, 173–174.

Heyns, J, van den Berg, E (1996) *Neothada hades* n. sp. from South Africa, with notes on the genus and a key to the species (Nematoda: Tylenchidae). *South African Journal of Zoology* **31**, 165–169.

Holterman, M, Van Der Wurff, A, van **Den 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 Phylogenetics and Evolution* **23**, 1792–1800. DOI: 10.1093/molbev/msl044

Hosseinvand, M, Eskandari, A, Ghaderi, R, and Karegar, A (2020a) Morphological and molecular data of two species of the rare genera *Thada* Thorne, 1941 and *Tenunemellus* Siddiqi, 1986 (Nematoda: Tylenchidae) from Iran. *Journal of Helminthology* **94**, e149, 1–10. https://doi.org/10.1017/S0022149X20000279

Hosseinvand, M, Eskandari, A, Ghaderi, R (2020b) Morphological and molecular characterization of two species of *Neothada* Khan, 1973 (Nematoda: Tylenchidae) from Iran, with notes on *N. cancellata*. *Journal of Nematology* **52**, 1–10. DOI: 10.21307/jofnem-2020-131

Inglis, WG (1983) An outline classification of the phylum Nematoda. *Australian Journal of Zoology* **31**, 243–255. DOI: 10.1071/ZO9830243

Kantor, MR, Handoo, ZA, Subbotin, SA, Mowery, JD, Hult, MN, Rogers, S and Skantar, AM (2023) Molecular and morphological characterization of *Tylenchus zeae* n. Sp. (Nematoda: Tylenchida) from corn (*Zea Mays*) in South Carolina. *Journal of Nematology* **55**, 1–13. DOI: 10.2478/jofnem-2023-0003

Katoh, K, Rozewicki, J and Yamada, KD (2019) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Brief Bioinformatics* **20**, 1160–1166. DOI: 10.1093/bib/bbx108

Khan, E (1964) *Boleodorus mirus* n. sp. (Tylenchida: Boleodorinae n. sub.fam.) from Kufri, Simla (H.P.) India, with a key to the species of the genus *Boleodorus* Thorne, 1941. *Zoologischer Anzeiger* **173**, 336–341.

Khan, SH (1973) On the proposal for *Neothada* n. gen. (Nematoda: Nothotylenchinae). *43rd Annual Session, Abstracts, Section Biology Sciences, Proceedings Natural Academy of Sciences, India*, 17–18.

Maqbool, MA and Shahina, E (1989) Nematodes of northern areas in Pakistan. Description of *Neothada major* n. sp. and *Pratylenchoides maqsoodi* n. sp. (Nematoda: Tylenchina). *Revue de Nématologie* **12**, 211–216.

Munawar, M, **Yevtushenko, DP and Castillo, P** (2021) Overview of the genus *Boleodorus* and first reports of *Boleodorus thylactus* and *B. volutus* from Southern Alberta, Canada. *Animals* **11**, 1760. DOI: 10.3390/ani11061760

Palomares-Rius, JE, **Cantalapiedra-Navarrete, C and Castillo P** (2014) Cryptic species in plant-parasitic nematodes. *Nematology* **16**, 1105–1118. DOI: 10.1163/15685411-00002831

Palomares-Rius, JE, **Cantalapiedra-Navarrete, C**, **Archidona-Yuste, A**, **Subbotin, SA and Castillo, P** (2017) The utility of mtDNA and rDNA for barcoding and phylogeny of plant-parasitic nematodes from Longidoridae (Nematoda, Enoplea). *Scientific Reports* **7**, 10905. DOI: 10.1038/s41598-017-11085-4

Powers, T (2004) Nematode molecular diagnostics: from bands to barcodes. *Annual Review of Phytopathology* **42**, 367–383. DOI: 10.1146/annurev.phyto.42.040803.140348

Qing, X and Bert, W (2018) 3D printing in zoological systematics: integrative taxonomy of *Labrys chinensis* gen. nov., sp. nov. (Nematoda: Tylenchomorpha). *Journal of Zoological Systematics and Evolutionary Research* **56**, 35–47. DOI: 10.1111/jzs.12191

Qing, X and Bert, W (2019) Family Tylenchidae (Nematoda): an overview and perspectives. *Organisms Diversity & Evolution* **19**, 391–408. DOI: 10.1007/s13127-019-00404-4

Qing, X, **Decraemer, W**, **Claeys, M and Bert, W** (2017) Molecular phylogeny of *Malenchus* and *Filenchus* (Nematoda: Tylenchidae). *Zoologica Scripta* **46**, 625–636. DOI: 10.1111/zsc.12236

Qing, X, **Pereira, TJ**, **Slos, D**, **Couvreur, M and Bert, W** (2018) A new species of *Malenchus* (Nematoda: Tylenchomorpha) with an update phylogeny of the Tylenchidae. *Nematology* **20**, 815–836. DOI: 10.1163/15685411-00003177

Rambaut, A (2018) FigTree v1.4.4, A Graphical Viewer of Phylogenetic Trees. Available at http://tree.bio.ed.ac.uk/software/figtree/ (accessed 25 January 2025).

Ronquist, F and Huelsenbeck, JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574. DOI: 10.1093/bioinformatics/btg180

Rudolphi, CA (1808) *Entozoorum sive vermium Intesstinalium Historia Naturalis.* 1. Amsterdam, 525.

Seinhorst, JW (1966) Killing nematodes for taxonomic study with hot F.A. 4:1. *Nematologica* **12**, 178. DOI: 10.1163/187529266X00239

Siddiqi, MR (1959) *Basiria graminophila* n. g., n. sp. (Nematoda: Tylenchinae) found associated with grass roots in Aligarh, India. *Nematologica* **4**, 217–222.

Siddiqi, MR (1986) *Tylenchida: Parasites of Plants and Insects*. CAB, Farnham Royal, Slough, UK, 645.

Siddiqi, MR (2000) *Tylenchida: parasites of plants and insects*. CABI, Wallingford, UK, 833.

Subbotin, SA, **Sturhan, D**, **Chizhov, VN**, **Vovlas, N and Baldwin, JG** (2006) Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. *Nematology* **8**, 455–474. DOI: 10.1163/156854106778493420

Subbotin, SA, **Ragsdale, EJ**, **Mullens, T**, **Roberts, PA**, **Mundo-Ocampo, M and Baldwin, JG** (2008) A phylogenetic framework for root lesion nematodes of the genus *Pratylenchus* (Nematoda): evidence from 18S and D2-D3 expansion segments of 28S ribosomal RNA genes and morphological characters. *Molecular Phylogenetics and Evolution* **48**, 491–505. DOI: 10.1016/j.ympev.2008.04.028

Tan, G, **Muffato, M**, **Ledergerber, C**, **Herrero, J**, **Goldman, N**, **Gil, M and Dessimoz, C** (2015) Current methods for automated filtering of multiple sequence alignments frequently worsen single-gene phylogenetic inference. *Systematic Biology* **64**, 778–791. DOI: 10.1093/sysbio/syv033

Thorne, G (1935) Notes on free-living and plant-parasitic nematodes. II. *Proceedings Helminthological Society of Washington* **2**, 96–98.

Thorne, G (1941) Some nematodes of the family Tylenchidae which do not possess a valvular median oesophageal bulb. *Great Basin Naturalist* **2**, 37–85.

Thorne, G and Malek, RB (1968) Nematodes of the Northern Great Plains. Part I. Tylenchida (Nemata: Secernentea). *Technical Bulletin South Dakota Agricultural Experimental Station* **31**, 1–111.

Vrain, TC, **Wakarchuk, DA**, **Levesque, AC and Hamilton, RI** (1992) Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology* **15**, 563–573.

Yaghoubi, A, **Pourjam, E**, **Atighi, MR and Pedram, M** (2015) Description of *Atetylenchus minor* n. sp. (Tylenchina: Tylenchidae) and data on two other species of the family. *Nematology* **17**, 981–994. DOI: 10.1163/15685411-00002918

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