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A new needle nematode, *Longidorus zanjanensis* sp. nov. (Nematoda: Longidoridae) from north-western Iran

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Research Paper

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Abstract

During a survey of soil nematodes in 2022, a new species of the genus *Longidorus*, described here as *Longidorus zanjanensis* sp. nov., was discovered in the rhizosphere of *Astragalus* sp. in Zanjan Province, Iran. The new needle nematode is described and illustrated based on morphological, morphometric, and molecular traits. Further, its females are characterized by having a long body ranging 5.6–7.7 mm long, lip region anteriorly flattened and almost continuous or slightly offset by a depression with body contour, *ca* 16.5–18.5 µm wide, amphidial fovea pouch-like without basal lobes, guiding ring at 35–41 µm distance from the anterior end, and an odontostyle and odontophore ranging 102–115 and 47–75 µm long, respectively. The pharyngeal bulb is 123–153 µm long, female reproductive system didelphic–amphidelphic containing sperm, vulva almost equatorial, located at 46.7–51.4% of body length, tail short, rounded to bluntly conoid, bearing two pairs of caudal pores and terminus widely rounded with distinct radial lines in hyaline region (39–50 µm long, c = 122.4–189.4, c' = 0.6–0.8). Males are common, making up to 60% of the adults, and are functional, with spicules 68.0–80.0 µm long, as well as having 8–14 ventromedian copulatory supplements. All four juvenile life developmental stages were present, with the tail of first-stage juvenile conoid shape, dorso-ventrally curved with rounded terminus. The polytomous codes delimiting the new species are: A4-B3-C3-D3-E1-F34-G12-H1-I2-J1-K6. Morphologically, the new species comes close to eight known species of the genus, namely *L. apulus*, *L. armeniacae*, *L. crassus*, *L. kheirii*, *L. soosanae*, *L. proximus*, *L. pauli,* and *L. ferrisi.* The morphological differences between the new species and the aforementioned species are discussed. Molecular phylogenetic analyses based on D2-D3 of large subunit (LSU) and internal transcribed spacer 1 (ITS1) rRNA sequences indicate that *Longidorus zanjanensis* sp. nov. is closely related to *L. hyrcanus*, *L. soosanae*, and *L. elongatus.*

Introduction

Dorylaimida Pearse, 1942 is one of the most diverse orders in terms of number of species within the phylum Nematoda (Jairajpuri & Ahmad 1992; Andrassy 2009). The family Longidoridae Thorne, 1935 (Thorne 1935), inside Dorylaimida, includes obligate plant ectoparasitic species and is one of the most economically important nematode groups in agriculture. The importance of this group of nematodes lies not only in their polyphagy and cosmopolitan distribution but also their status as vectors of plant viruses that cause significant damage to a wide range of agricultural crops (Coomans 1996; Taylor & Brown 1997; Macfarlane 2003; Decraemer & Robbins 2007; Decraemer & Geraert 2013; Archidona-Yuste *et al.* 2016a, b, 2019a). The family Longidoridae includes more than 500 species (Coomans *et al.* 2001; Decraemer & Robbins 2007), where the needle nematodes of the genus *Longidorus* Micoletzky, 1922 are one of the most diverse genera of this family. This genus includes a number of long to very long body (2–12 mm) specimens with long stylet (80–260 µm). They are a polyphagous species of many plants including various agricultural crops, and they cause damage by direct feeding on root cells as well as by transmitting nepoviruses (nepoviruses are icosahedral, with a bipartite positive stranded RNA genome, wherein each RNA encodes as a single polyprotein). The genus *Longidorus* is a diverse group with more than 177 nominal species (Gutiérrez-Gutiérrez *et al.* 2020; Clavero-Camacho *et al.* 2021). Only 11 species (6.9%) (*L. apulus* Lamberti & Bleve-Zacheo, 1977; *L. arthensis* Brown *et al.*, 1994; *L. attenuatus* Hooper, 1961; *L. caespiticola* Hooper, 1961; *L. diadecturus* Eveleigh & Allen, 1982, *L. elongatus* (de Man, 1876) Thorne & Swanger, 1936; *L. fasciatus* Roca & Lamberti, 1981; *L. leptocephalus* Hooper, 1961; *L. macrosoma* Hooper, 1961; *L. martini* Merny, 1966, and *L. profundorum* Hooper, 1965) have been reported as virus vector transmitting seven nepoviruses (*artichoke Italian latent virus*, *cherry rosette disease virus*, *tomato black ring virus*, *raspberry ringspot virus*, *Arabis mosaic virus*, *peach rosette mosaic virus*, and *mulberry ringspot virus*) (Brown *et al.* 1988; Taylor & Brown 1997; Decraemer & Robbins 2007). These nematodes spend

their entire life cycle in the rhizosphere, using their needle stylet to feed on the apical root cells, inducing galls in the tips and arresting root growth (Taylor & Brown 1997; Palomares-Rius *et al.* 2017). The morphological convergence and the existence of cryptic species in this genus make the accurate identification of species considerably more difficult (De Luca *et al.* 2004; Gutiérrez-Gutiérrez *et al.* 2013; Archidona-Yuste *et al.* 2016b, 2019b). Consequently, morphological taxonomy could lead to underestimation of the diversity in the genus *Longidorus* as reported in other genera of plant-parasitic nematodes (Palomares-Rius *et al.* 2014; Archidona-Yuste *et al.* 2016a, b, c; Janssen *et al.* 2017). Therefore, accurate identification of *Longidorus* species is essential in establishing appropriate control measures and control strategies for preventing the spread of these nematodes. To date, 27 *Longidorus* species have been reported from Iran, including: *L. aetnaeus* Roca *et al.*, 1986; *L. africanus* Merny, 1966; *L. apulus*; *L. armeniacae* Bakhshi Amrei *et al.*, 2022; *L. artemisiae* Rubtsova *et al.*, 1999; *L. behshahrensis* Bakhshi Amrei *et al.*, 2020; *L. crassus* Thorne, 1974; *L. elongatus*; *L. euonymus* Mali & Hooper, 1974; *L. hyrcanus* Mobasseri *et al.*, 2023; *L. iranicus* Sturhan & Barooti, 1983; *L. kheirii* Pedram *et al.*, 2008; *L. leptocephalus*; *L. orientalis* Loof, 1982; *L. paravineacola* Ye & Robbins, 2003; *L. perangustus* Roshan-Bakhsh *et al.*, 2016; *L. persicus* Esmaeili *et al.*, 2017; *L. pisi* Edward *et al.*, 1964; *L. profundorum*; *L. protae* Lamberti & Bleve-Zacheo, 1977; *L. proximus* Sturhan & Argo, 1983; *L. sabalanicus* Asgari *et al.*, 2022; *L. soosanae* Pour Ehtesham *et al.*, 2023; *L. sturhani* Rubtsova *et al.*, 2001; *L. tabrizicus* Niknam *et al.*, 2010, and *L. vineacola* Sturhan & Weischer, 1964. In a May 2022 survey, a population of an unidentified species of *Longidorus* was recovered from the rhizosphere of *Astragalus* sp. naturally growing in the mountains of the Anguran Protected Area, west Mahneshan, Zanjan province. Molecular approaches and phylogenetic studies in combination with morphometric characters are used as a taxonomic standard for species identification and delimitation, which is known as Integrative Taxonomy (Gutiérrez-Gutiérrez *et al.* 2013; Peneva *et al.* 2013; Archidona-Yuste *et al.* 2016d). Our research aims to characterize this undescribed nematode species based on morphological characters integrated with molecular data and infer the phylogenetic relationships with the other species of genus *Longidorus*.

Materials and methods

Nematode population sampling, extraction, and morphological identification

About 100 soil samples were collected from the rhizosphere of different plants at a depth of 10–50 cm, in the Zanjan province, north-western Iran. Specimens of an unidentified *Longidorus* sp. nov. were obtained from the rhizosphere of *Astragalus* sp. in Zanjan province. Nematodes were extracted using the tray method (Whitehead & Hemming 1965), the magnesium sulphate (MgSO4) centrifugal flotation method (Coolen 1979), and a modification of Cobb's decanting and sieving method (Flegg 1967). Nematodes were handpicked under a stereomicroscope, killed by adding hot FPG (4:1:1, formaldehyde: propionic acid: glycerin) solution, transferred to anhydrous glycerine according to De Grisse (1969), and mounted on permanent glass slides to allow handling and observation. Morphometric values and photomicrographs were taken using a Dino-Eye digital eyepiece camera (Model AM7023, bundled with DinoCapture 2.0 software; AnMo Electronics Corporation, New Taipei City, Taiwan) attached to a Leitz Dialux 22 light microscope. Line drawings were first made using a drawing tube, then re-drawn and prepared for publication using CorelDRAW software version 16 (Corel Corp, Canada). Morphological comparisons were performed using the polytomous identification keys for the identification of *Longidorus* species (Chen *et al.* 1997; Loof & Chen 1999) and with the descriptions of all other characterized species up to the present. The position of pharyngeal gland nuclei was calculated according to Loof & Coomans (1972), and the juvenile developmental stages were identified according to Robbins *et al.* (1995). All measurements were recorded in micrometres (μm), except for body length in millimetres (mm) and ratios. Ratios are defined in Jairajpuri & Ahmad (1992).

Molecular characterization

For the molecular phylogenetic studies, four live nematode specimens (two females and two juveniles) were selected. Each specimen was transferred to an Eppendorf tube containing 10 μl ddH2O, 8 μl lysis buffer (125 mM KCl, 25 mM Tris–Cl pH 8.3, 3.75 mM MgCl2, 2.5 mM DTT, 1.125% Tween 20, 0.025% gelatine), and 2 μl proteinase K (600 μg/ml), and crushed for 2 min with a micro-homogeniser (Subbotin *et al.* 2000). The tubes were frozen at -80 °C (15 min), then incubated at 65 °C (1 h) and at 95 °C (10 min), consecutively. After centrifugation (1 min, 16,000 × g), 4 μl of extracted DNA were added to the polymerase chain reaction (PCR) mixture in a 0.2 ml Eppendorf tube containing: 20 μl 2 × Master mix (Amplicon, Odense, Denmark), 2 μl of each primer (10 pMol/μl), and 12 μl ddH2O, to a final volume of 40 μl. The D2-D3 expansion segments of 28S rRNA were amplified using forward D2A (5′–ACAAGTACCGTGAGGGAAAGTTG–3′) and reverse D3B (5′–TCGGAAGGAACCAGCTACTA–3′) primers (Nunn 1992). The ITS1 region was amplified using forward primer 18S (5′–TTGATTACGTCCCTGCCCTTT–3′) (Vrain *et al.* 1992) and reverse primer rDNA1 5.8S (5′–ACGAGCCGAGTGATCCACCG–3′) (Cherry *et al.* 1997). PCR reactions were carried out in a DNA thermal cycler (Hybaid, Ashford, Middlesex, UK), and the amplification program was set as follows: initial denaturation at 94 °C for 10 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C (LSU), 55 °C (ITS1) for 30 s, extension at 72 °C for 1 min; and finally, the elongation step at 72 °C for 6 min. The amplified PCR products were purified using ExoSAP-IT (Affimetrix, USB products), quantified using a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA), and used for direct sequencing in both directions using the primers referred to above. The resulting products were purified and run 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 OR509844-OR509847 for D2-D3 expansion segments of 28S rRNA and OR509848-OR509851 for ITS1 region.

Phylogenetic analyses

The newly obtained sequences of *L. zanjanensis* sp. nov. (D2-D3 expansion segments of 28S rRNA, and ITS1 rRNA) and other sequences of different *Longidorus* spp. from GenBank were used for phylogenetic analyses. ITS1 rRNA did not have enough similarity with other sequences deposited in the GenBank, and for this reason, sequence similarity comparisons were only made with the

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closest phylogenetically related species. Outgroup taxa for each dataset were chosen following previously published studies (He *et al.* 2005; Holterman *et al.* 2006; Palomares-Rius *et al.* 2008; Gutiérrez-Gutiérrez *et al.* 2013; Archidona-Yuste *et al.* 2019b; Cai *et al.* 2020a, b). Multiple sequence alignments for each gene were made using the FFT-NS-2 algorithm of MAFFT V.7.450 (Katoh *et al.* 2019). Sequence alignments were visualized using BioEdit (Hall 1999) and manually edited and trimmed of poorly aligned positions using a light filtering strategy (up to 20% of alignment positions), which has little impact on tree accuracy and may save some 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 & Huelsenbeck 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, base frequency, proportion of invariable sites, and gamma distribution shape parameters and substitution rates in the AIC were then used in MrBayes for the phylogenetic analyses. The general time-reversible model with invariable sites and a gamma-shaped distribution (GTR + I + G) for the D2-D3 segments of 28S rRNA and the general time-reversible model and a gamma-shaped distribution (GTR + G) for ITS1 rRNA were run with four chains for 4 × 10^6 generations, respectively. The Markov chains were sampled at intervals of 100 generations. Two runs were conducted for each analysis. 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 1.4.4 (Rambaut 2018).

Results and Discussion

The integration of nematode morphology with the morphometric analysis and molecular data using ribosomal sequences allowed us to describe herein a new species of the genus as *L. zanjanensis* sp. nov.

Longidorus zanjanensis sp. nov.

Zoobank: urn:lsid:zoobank.org:act:7584860D-A994-495B-A5CD-5AE62DBA0AD6

Description

See Figures 1–4 and Table 1.

Female. Body ventrally bent varying from J to G shape when heat-relaxed. Cuticle appearing smooth under light microscope; its thickness varies over the body, 4–5 µm at guiding ring level, to 3–4 µm at mid-body, 7–8 µm at anterior lip of anus, and 14–23 µm at tail end (the hyaline part of tail tip), and marked by very fine superficial transverse striae mainly in tail region. Lateral chord 23–30% of corresponding body diameter. Lip region anteriorly flattened, continuous with the adjacent body (Figures 1, 2). Amphidial fovea is pouch-like without lobes at base. Stylet guiding ring located at *ca.* two times lip region diameter from anterior end. Odontostyle long and narrow, approximately 1.9 times as long as odontophore (Figures 1, 2). Nerve ring surrounding the slender portion of the pharynx posterior to the odontophore base, located at 247–280 µm from anterior end. Pharynx dorylaimoid, anterior slender part flexible, posteriorly expanding to a muscular terminal bulb occupying about 24.1 ± 1.7 (20.7–27.4)% of the total pharynx (neck region).The dorsal gland nucleus (DN) smaller, at 26.1–36.4%, and the two ventrosublateral nuclei (S1N) at about the same level and at 51.0–61.5% of the pharyngeal bulb length (location of glands nuclei according to Loof & Coomans (1972)). Cardia conoid to rounded, 5.0–7.0 µm long. Intestine with prerectum. The reproductive system didelphic–amphidelphic, with both branches almost equally developed, each branch 500–1100 µm long, with reflexed ovaries highly variable in length, anterior ovary (173–324 µm long), and posterior ovary (157–390 µm long). Oviducts slightly longer than ovaries. Uterus bipartite, quite variable in length, anterior uteri (230–276) µm long, and posterior uteri (208–286) µm long; sphincter well developed, between uterus and oviduct. Sperm commonly found in the uteri. Vagina 50–74 µm long or *ca.* 66% of corresponding body width; pars distalis 11–16 µm long, pars proximalis vaginae measuring 20–34 µm long; vulva a transverse slit. Prerectum variable in length, 3.9–11.4 times longer than anal body width and rectum simple, 0.8–0.1 times as long as tail length. Tail bearing two caudal pores, conoid, convex dorsally, and ventrally almost straight or slightly concave with rounded terminus.

Male. Common (about 60% of the population) and functional. Similar to females in general morphology, except for the reproductive system and posterior end more ventrally curved Male genital reproductive system diorchic. Spicules arcuate, robust, about 1.5 times longer than tail length, lateral guiding piece more or less straight. Adanal supplements paired, preceded anteriorly by a row of 8–14 irregularly spaced ventromedian supplements. Tail bluntly conoid, dorsally convex and ventrally concave, terminus widely rounded, with distinct radial lines in hyaline region. Tail length almost equivalent to cloacal body width.

Juveniles. Morphologically similar to adults in most respects except for size and development of reproductive system. All juvenile developmental stages were detected and distinguished by relative lengths of body and functional and replacement odontostyle (Figure 4). J1 characterized by a conoid tail, dorso-ventrally curved with rounded terminus, and slight depression at hyaline region level, with a c′ ratio average of 2.3, odontostyle length *ca.* 66.3 µm, and shorter distance from anterior end to stylet guiding-ring than that in adult stages. For the rest of the juvenile stages (J2, J3, J4), the replacement odontostyle were located at some distance posterior to the odontophore base and morphology of tail were similar to females (bluntly conoid with a rounded terminus, dorsally convex and ventrally almost straight or slightly concave), becoming stouter after each moult (Figure 3).

Diagnosis and relationships

Longidorus zanjanensis sp. nov. is characterised by a thick (a < 88) and long body (5.6–7.7 mm); lip region 16.5–18.5 µm wide and continuous with body contour; amphidial fovea pouch shaped, not bilobed, and extending about 1/2 part of oral aperture-guiding ring distance; relatively long odontostyle (102–115 µm); guiding ring located at 35.0–41.0 µm from anterior end; vulva located at 46.7–51.4% of body length; female tail short and bluntly conoid (39.0–50.0 µm long, c = 122.4–189.4, c' = 0.6–0.8), with two pairs of caudal pores. Males with long spicules (68–80 µm) and 8–14 ventromedian supplements. Four developmental juvenile stages were identified, with the first stage juvenile with conoid tail (c' = 2.1–2.5). According to the polytomous key by Chen *et al.* (1997), supplement by Loof & Chen

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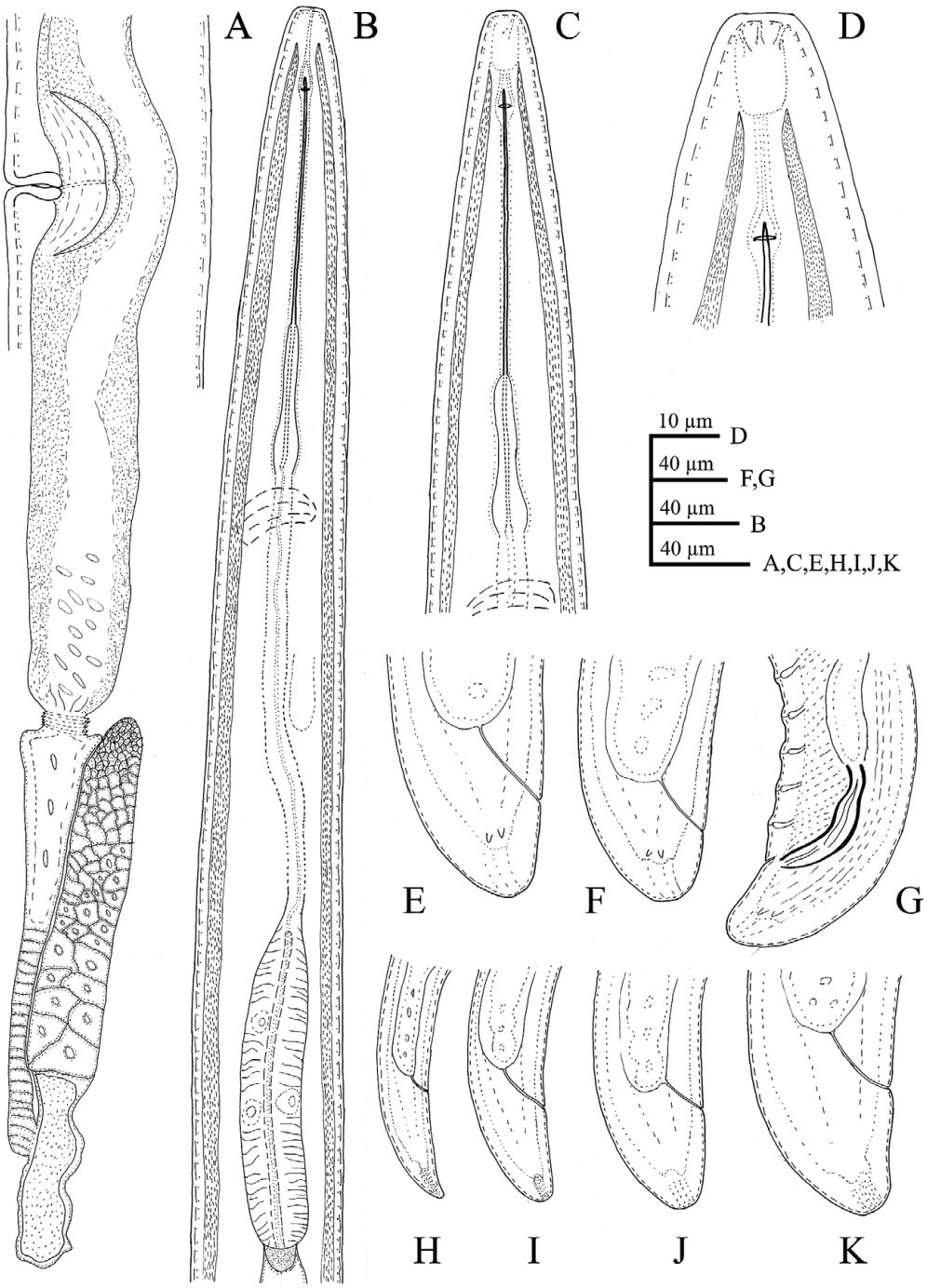

Figure 1. Line drawings of *Longidorus zanjanensis* sp. nov. A: Female reproductive system. B: Female anterior region. C: Female anterior region. D: Anterior end showing amphidial fovea. E, F: Female tail. G: Male posterior body region. H: J1 tail. I: J2 tail. J: J3 tail. K: J4 tail.

(1999), and the addition of some characters by Peneva *et al.* (2013), codes for the new species are: A4-B3-C3-D3-E1-F34-G12-H1-I2-J1-K6; and specific D2-D3 expansion segments of 28S rRNA, and ITS1 region (GenBank accession numbers: OR509844 for D2-D3 expansion segments of 28S rRNA and OR509848-OR509851 for ITS1 region).

According to the body and odontostyle length, shape of amphidial fovea, distance of guiding ring from anterior body end, lip region and tail shape, a and c' ratios, and frequency of males, the new species is close to eight known *Longidorus* species, namely *L. apulus*; *L. armeniacae*; *L. crassus*; *L. ferrisi* Robbins *et al.*, 2009; *L. kheirii*; *L. pauli* Lamberti *et al.*, 1999; *L. proximus*; and *L. soosanae.* In addition, *L. zanjanensis* sp. nov. is closely related molecularly to *L. hyrcanus*; *L. elongatus*; and also *L. soosanae.*

The new species differs from *L. apulus* by having a different amphidial fovea shape (pouch-like-shaped, not bilobed *vs.* symmetrically bilobed at base), a higher oral aperture to guiding ring distance (35.0–41.0 *vs.* 24.0–34.0 µm), lower a ratio (68.7–87.8 *vs.*

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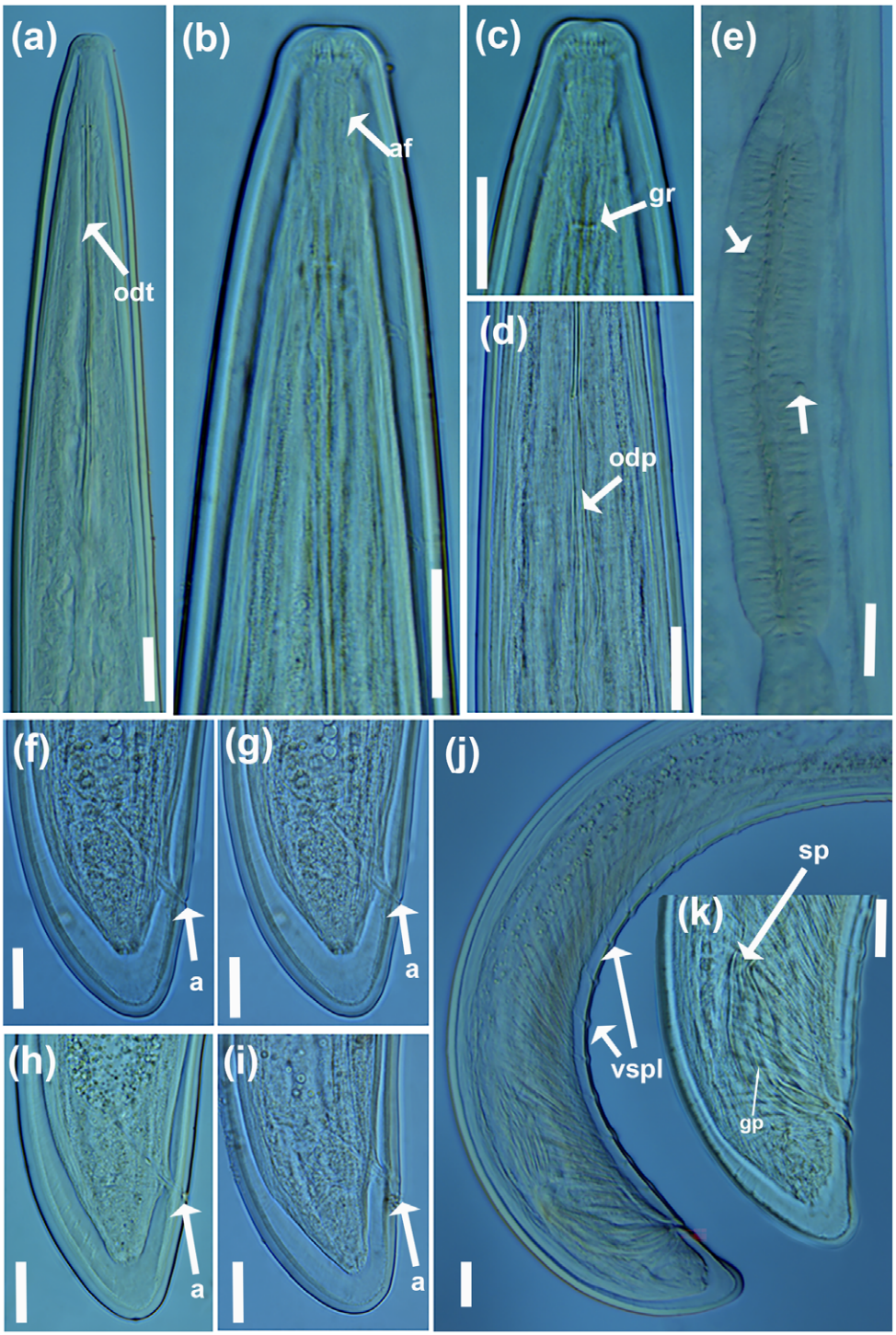

Figure 2. Light micrographs of *Longidorus zanjanensis* sp. nov. (a–d) female anterior body regions showing odontostyle, odontophore, amphidial fovea, and guiding ring (arrowed); (e) detail of basal bulb showing dorsal gland and ventrosublateral nuclei (arrowed); (f-i) female tail; (j, k) male tail with details of spicules, guiding pieces of gubernaculum and ventromedian supplements (arrowed). Abbreviations: a = anus; af = amphidial fovea; gr = guiding ring; gp = guiding pieces of gubernaculum; odt = odontostyle; odp = odontophore; sp = spicule; vspl = ventromedian supplement. (Scale bars: 20 µm).

110–154), and longer spicule length (68.0–80.0 *vs.* 57.0 µm). From *L. armeniacae*, it differs by a higher oral aperture to guiding ring distance (35.0–41.0 *vs.* 29.0–35.0 µm), shorter spicule length (68.0–80.0 *vs.* 80.0–107 µm), and different tail shape in J1 (slender conical, without a digitate or subdigitate terminus *vs.* convex-conoid to conical, with a distinctly digitate terminus). It differs from *L. crassus* by having a longer body (5.6–7.7 *vs.* 5.0–6.0 mm), different amphidial fovea shape (pouch-like, not bilobed *vs.* symmetrically bilobed at base), wider lip region width (16.5–18.5 *vs.* 15 µm), higher oral aperture to guiding ring distance (35.0–41.0 *vs.* 32.5 µm), and lower a ratio (68.7–87.8 *vs.* 80.0–107 µm).

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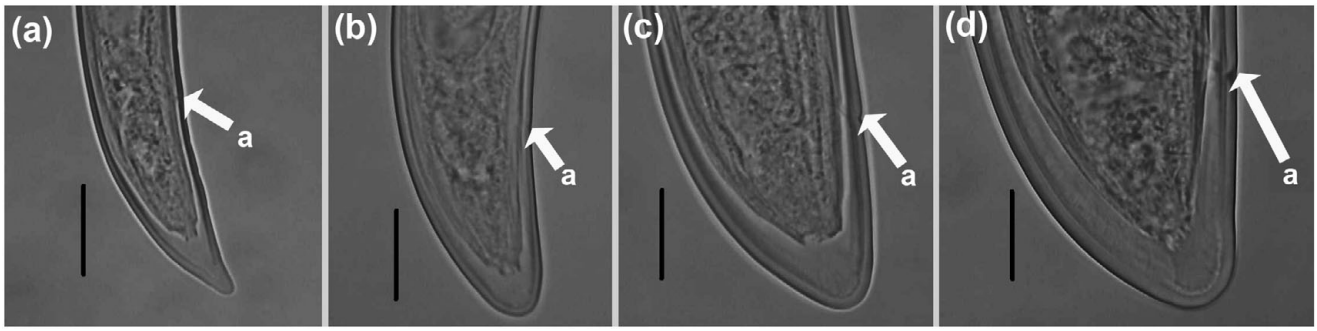

Figure 3. Light micrographs of *Longidorus zanjanensis* sp. nov. (a–d) tails of J1, J2, J3, and J4. (Scale bars: a–d = 20 µm).

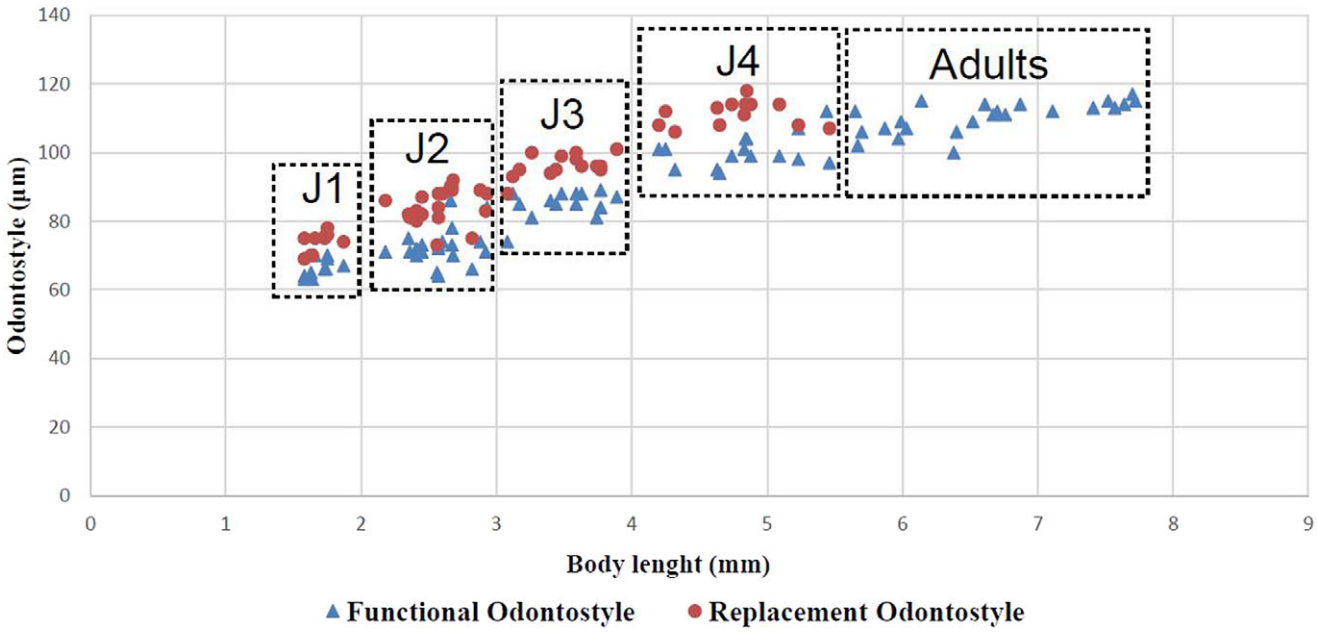

Figure 4. Relationship between body length and functional and replacement odontostyle length in all developmental juvenile life stages and mature adults of *Longidorus zanjanensis* sp. nov.

From *L. ferrisi*, it differs by a longer body (5.6–7.7 *vs.* 4.3–5.9 mm), different amphidial fovea shape (pouch-like, not bilobed *vs.* symmetrically bilobed at base), and longer spicule length (68.0–80.0 *vs.* 53.0–63 µm). From *L. kheirii*, it differs by having a smaller body (5.6–7.7 *vs.* 6.7–9.0 mm), smaller odontostyle and odontophore (102–115 *vs.* 113–130 µm and 47.0–75.0 *vs.* 69.0–97.5), narrower lip region width (16.5–18.5 *vs.* 19.5–23.0 µm), smaller tail (39.0–50.0 *vs.* 47.0–72.0 µm), and smaller spicule length (68.0–80.0 *vs.* 85.0 µm). From *L. pauli*, it differs by a smaller body [average 6.7 (5.6–7.7) *vs.* average 7.6 (6.5–8.6 mm)], different amphidial fovea shape (pouch-like, not bilobed *vs.* asymmetrically bilobed at base), wider lip region width (16.5–18.5 *vs.* 13.9–16.8 µm), lower a ratio (68.7–87.8 *vs.* 120.3–143.5), higher oral aperture to guiding ring distance (35.0–41.0 *vs.* 27.2–35.8 µm), longer spicule length (68.0–80.0 *vs.* 61.0–69 µm), and a lower number of ventromedian supplements in the male tail (8–14 *vs.* 12–16). From *L. proximus*, it differs by a lip region shape (continuous *vs.* expanded, high, separated from the rest of body by a depression), lower a and c ratio (68.7–87.8 *vs.*104–138 and 122.4–189.4 *vs.* 165–249), and position of pharyngeal gland nuclei (normal *vs.* more posterior). It differs from *L. soosanae* by having an anterior body region shape (uniformly narrowing towards anterior end *vs.* bottle-shaped), longer odontostyle (102–115 *vs.* 92.0–103 µm), lower a ratio (68.7–87.8 *vs.* 79–114), longer tail (39–50 *vs.* 33–42 µm), and longer spicule length (68.0–80.0 *vs.* 50.0–64 µm). From *L. hyrcanus*, it differs by a longer body (5.6–7.7 *vs.* 5.0–5.8 mm), different shaped lip region (anteriorly flattened, continuous with the adjacent body *vs.* rounded, continuous with body contour), different amphidial fovea shape (not bilobed *vs.* asymmetrically bilobed at base), wider lip region width (16.5–18.5 *vs.* 11.5–14.0 µm), longer spicule length (68.0–80.0 *vs.* 55.0–68 µm), different tail shape in J1 (slender conical, without a digitate or subdigitate terminus *vs.* slender, with broadly rounded tail end), and shorter tail of J1 (43.0–50.0 *vs.* 24.0 µm). Finally, from *L. elongatus*, the new species differs mainly by having a longer body (5.6–7.7 *vs.* 4.5–6.4 mm), different amphidial fovea shape (pouch-like, not bilobed *vs.* asymmetrically bilobed at base), longer odontostyle (102–115 *vs.* 81.0–102 µm), and higher oral aperture to guiding ring distance (35.0–41.0 *vs.* 29.0–36.0 µm).

Etymology

The specific epithet refers to the province of Zanjan, north-western Iran where the new species was collected.

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Table 1. Morphometrics of *Longidorus zanjanensis* sp. nov. from Zanjan, Iran. All measurements are in μm (except L, in mm) and in the form: mean ± standard deviation (range)

Character	Holotype	Paratypes					
	Female	Female	Male	Juvenile			
				J1	J2	J3	J4
*n**	–	10	15	10	21	13	13
L	6.8	6.7 ± 0.7	6.4 ± 0.7	1.6 ± 0.8	2.6 ± 0.2	3.5 ± 0.2	4.7 ± 0.3
		(5.6–7.7)	(5.2–7.7)	(1.5–1.9)	(2.1–3.0)	(3.1–3.8)	(4.2–5.4)
a	68.7	75.0 ± 5.4	81.4 ± 9.3	84.2 ± 4.5	84.3 ± 6.4	87.3 ± 4.6	91.9 ± 13.3
		(68.7–87.8)	(74.0–97.4)	(78.0–92.5)	(75.4–104.7)	(77.4–92.1)	(69.8–113.5)
b	13.0	11.5 ± 1.3	11.7 ± 1.0	5.5 ± 0.6	7.4 ± 1.0	8.3 ± 0.7	9.7 ± 1.2
		(9.5–13.0)	(10.7–13.1)	(4.8–7.1)	(5.5–9.2)	(7.1–9.9)	(7.8–12.3)
c	146.2	151.5 ± 18.3	135.3 ± 12.2	36.5 ± 2.6	59.5 ± 7.5	75.7 ± 7.4	103.4 ± 12.2
		(122.4–189.4)	(125.6–149.3)	(32.3–39.0)	(48.6–79.1)	(61.5–87.3)	(86.4–124.5)
c’	0.7	0.7 ± 0.05	0.8 ± 0.06	2.3 ± 0.1	1.4 ± 0.1	1.1 ± 0.1	0.9 ± 0.1
		(0.6–0.8)	(0.8–1.0)	(2.1–2.5)	(1.0–1.6)	(1.0–1.4)	(0.7–1.0)
V or T	46.7	49.5 ± 1.6	49.0 ± 3.6	–	–	–	–
		(46.7–51.4)	(43.7–53.8)				
Lip region diam.	18	17.3 ± 0.7	17.3 ± 0.7	9.5 ± 0.3	11.4 ± 0.5	12.9 ± 0.9	15.4 ± 0.8
		(16.5–18.5)	(16.0–19.0)	(9.0–10.0)	(10.5–13.0)	(11.0–14.5)	(14.0–17.0)
Odontostyle length	114	110.3 ± 4.2	110.2 ± 4.6	66.3 ± 2.6	72.7 ± 5.2	85.7 ± 2.6	99.0 ± 3.1
		(102–115)	(100–117)	(63–70)	(64–86)	(81–89)	(94–104)
Odontophore length	70	59.2 ± 8.9	47 ± 9.2	16.8 ± 2.8	44.0 ± 7.2	53 ± 4.3	55.6 ± 6.8
		(47–75)	(38–65)	(13–20)	(25–55)	(45–60)	(45–65)
Replacement odontostyle length	–	–	–	73.8 ± 3.0	84.7 ± 4.9	96.7 ± 2.5	111.3 ± 3.6
				(69–78)	(73–92)	(93–101)	(106–118)
Total stylet length	184	169.5 ± 11.1	147 ± 27.1	83.1 ± 3.9	116.7 ± 97	138.7 ± 4.3	154.6 ± 8.1
		(156–189)	(106–182)	(76–90)	(100–141)	(133–148)	(143–169)
Oral aperture-guiding ring distance	35	37.9 ± 1.6	38.3 ± 1.6	19.7 ± 0.9	23.6 ± 1.5	26.4 ± 1.8	32.0 ± 1.4
		(35–41)	(36–41)	(18–21)	(21–28)	(22–30)	(30–35)
Pharynx length	528	580.3 ± 24.6	537.3 ± 48.2	307.8 ± 32.9	356.3 ± 39.2	425.5 ± 25.6	494.3 ± 61.8
		(528–612)	(401–586)	(246–347)	(283–413)	(380–463)	(392–622)
Pharynx bulb length	145	139.8 ± 8.7	124.2 ± 19.9	71.3 ± 6.6	87 ± 10.2	105.4 ± 5.6	118.2 ± 12.2
		(123–153)	(95–140)	(55–77)	(71–109)	(95–116)	(101–135)
Pharynx bulb diam.	32	30.4 ± 2.7	30.0 ± 3.6	15.8 ± 1.5	20.2 ± 1.9	24.1 ± 1.6	26.0 ± 3.8
		(27–35)	(27–34)	(14–18)	(15–23)	(21–27)	(15–29)
Body diam. at pharynx base	79	70.4 ± 8.0	71.3 ± 7.7	26.4 ± 1.5	37.2 ± 2.7	46.7 ± 4.5	56.5 ± 7.1
		(60–82)	(65–80)	(24–29)	(33–44)	(41–58)	(45–70)
at mid-body	100	89.8 ± 12.3	81.2 ± 11.3	27.8 ± 2.3	41.1 ± 4.4	53.4 ± 5.1	65.9 ± 9.0
		(77–108)	(58–96)	(25–33)	(35–52)	(43–62)	(50–79)
at anus	63	56.2 ± 5.5	55.4 ± 5.6	20.2 ± 1.0	31.0 ± 2.4	40.4 ± 2.2	50.0 ± 5.4
		(47–63)	(48–65)	(18–22)	(27–36)	(36–43)	(43–62)
at guiding ring level	31	31.7 ± 1.4	32.5 ± 1.7	15.4 ± 0.6	19.3 ± 1.6	22.5 ± 1.6	26.9 ± 1.1
		(30–34)	(31–34)	(14–16)	(17–23)	(20–26)	(24–28.5)
Prerectum length	600	418.1 ± 161.6	–	–	–	–	–
		(230–700)					

(*Continued*)

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Table 1. (*Continued*)

Character	Holotype	Paratypes					
	Female	Female	Male	Juvenile			
Rectum length	45	41.6 ± 2.1	–	–	–	–	–
		(39–45)					
Tail length	47	44.4 ± 3.6	50.1 ± 4.7	46.6 ± 2.4	44.2 ± 4.2	46.9 ± 4.2	46.4 ± 4.2
		(39–50)	(40–59)	(43–50)	(37–55)	(39–53)	(41–56)
Hyaline tail part length	19	18.5 ± 2.9	19.8 ± 1.6	14.0 ± 1.8	12.0 ± 1.2	14.1 ± 1.9	14.8 ± 1.9
		(14–23)	(18–22)	(12–17)	(9–14)	(11–17)	(11–18)
Spicule length	–	–	75.4 ± 3.3	–	–	–	–
			(68–80)				
Lateral guiding piece length	–	11.2 ± 0.9	–	–	–	–	–
		(10.0–13.0)					

*Abbreviations: n = number of specimens on which measurements are based; L = overall body length; a = body length/greatest body diameter; b = body length/distance from anterior end to pharyngo-intestinal junction; c = body length/tail length; c' = tail length/tail diameter at anus or cloaca; V = distance from body anterior end to vulva expressed as percentage (%) of the body length; T = distance from cloacal aperture to anterior end of testis expressed as percentage (%) of the body length.

Type host and locality

The type population was collected from the rhizosphere of *Astragalus* sp. naturally growing in mountains of Anguran Protected Area, West Mahneshan, Zanjan province, north-western Iran, coordinates: 36º 51' 9.5416" N; 47º 45' 1.2196' 'E; altitude: 1350 m a. s. l.

Type material

Holotype female (slide: 1412-b5), five female paratypes, 11 male paratypes, and 57 juvenile paratypes (slides: 1412-b1-16) were deposited in the nematode collection at the Faculty of Agriculture, University of Zanjan, Zanjan, Iran. Four female paratypes and four male paratypes were deposited in the nematode collection at the Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Cordoba, Spain (IAS_L_2023-2_Ir).

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Molecular characterisation and phylogenetic position of Longidorus zanjanensis sp. nov.

The amplification of D2-D3 segments of 28S rRNA and ITS1 yielded single fragments of *ca.* 900 bp, and 1100 bp, respectively, based on gel electrophoresis. Four identical sequences were obtained for D2-D3 segments of 28S rRNA with 97.3% (22 nucleotides difference), 96.9% (23 nucleotides difference), and 96.4% (27 nucleotides difference) similarity with *L. soosanae* (ON122993), *L. hyrcanus* (OL739253-OL739254), and several sequences of *L. elongatus* (MN123751), respectively. Four ITS1 region sequences with only one nucleotide difference in one sequence were obtained. Similarly to the D2-D3 region, the ITS1 region is similar to *L. soosanae* (ON121993-ON121994), *L. hyrcanus* (OL684817), and several sequences of *L. elongatus* (AF511417), at 87.0–88.5%, 89.2%, and 85.5%, respectively.

Phylogenetic relationships among *Longidorus* species inferred from analyses of D2-D3 expansion segments of 28S rRNA and ITS1 sequences using BI are given in Figures 5 and 6, respectively. The D2-D3 expansion segments of the 28S rRNA tree of *Longidorus* spp. are based on a multiple edited alignment including 121 sequences and 760 total characters, revealing four major clades, three of them highly supported (PP = 1.00) and the other with moderate support (PP = 0.97) (Figure 5). *Longidorus zanjanensis* sp. nov. (OR509844-OR509847) and *L. hyrcanus* (OL739254) clustered in a moderately supported clade (PP = 0.96). These two species are related to *L. intermedius* Kozlowska & Seinhorst, 1979 (AY593058), *L. elongatus* (AF480078), and *L. soosanae* (ON122993) in a relatively well-supported clade (PP = 0.98); they are related in another relatively well-supported clade (PP = 0.96) to *L. carpathicus* Lišková *et al.*, 1997 (AF480072), *L. uroshis* Krnjaić *et al.*, 2000 (EF538754), *L. piceicola* Lišková, *et al.*, 1997 (KY086070), and *L. artemisiae* (KX137849). These species from both clades are related in a highly supported clade (PP = 1.00).

For the ITS1 region sequences, the 50% majority rule consensus BI tree of a multiple sequence alignment containing 12 sequences and 928 characters is showed in Figure 6. *Longidorus zanjanensis* sp. nov. (OR509848-OR509851) clustered with *L. soosanae* (ON121994) in a low supported clade (PP = 0.83). These two species clustered with *L. hyrcanus* in a highly supported clade (PP = 0.99). Additionally, *L. elongatus* (AF511417) is closely related to these three species in a low-supported clade (PP = 0.90) sp. nov. These species are related with *L. piceicola* (LT669803) and *L. intermedius* (KT308890) in a highly supported clade (PP = 1.00).

This new species increases the knowledge of the biodiversity of this genus in Iran, including molecular markers for its unequivocal identification. Other species from Iran are closely related to our species (*L. soosane* and *L. hyrcanus*), but clearly separated using our integrative taxonomy. This species is clearly described using an integrative taxonomical approach (combination of morphology-morphometry and molecular data). The high diversity of this genus in Iran points to this region as a high diversity location for this group of nematodes.

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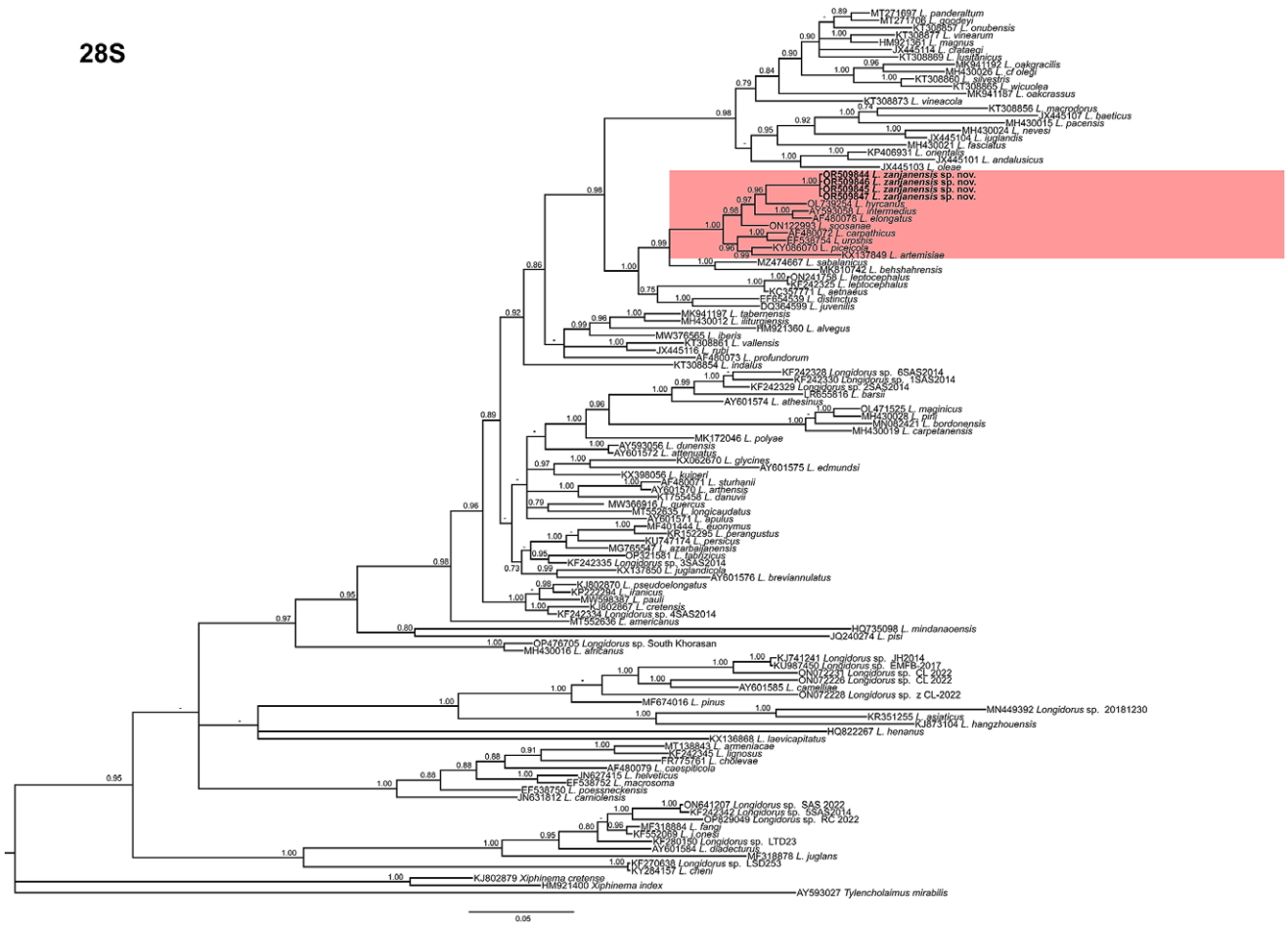

Figure 5. Phylogenetic relationships of *Longidorus zanjanensis* sp. nov. within the genus *Longidorus.* Bayesian 50% majority rule consensus tree as inferred from D2 and D3 expansion domains of 28S rRNA sequence alignment under the general time-reversible model of sequence evolution with correction for invariable sites and a gamma-shaped distribution (GTR + I+ G). Posterior probabilities more than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in boldface type, and coloured box indicates clade association of the new species. Scale bar = expected changes per site.

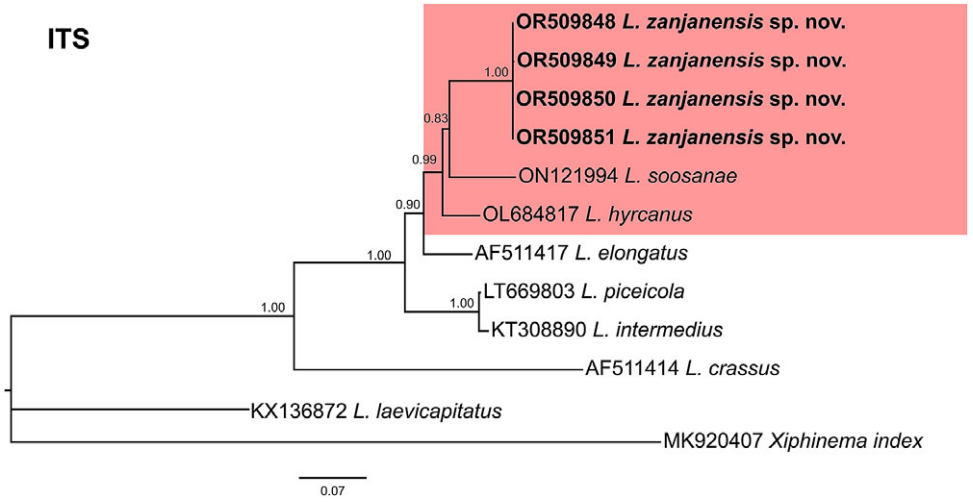

Figure 6. Phylogenetic relationships of *Longidorus zanjanensis* sp. nov. within the genus *Longidorus.* Bayesian 50% majority rule consensus tree as inferred from ITS1 region sequence alignment under the GTR + G model. Posterior probabilities more than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in boldface type, and coloured box indicates clade association of the new species. Scale bar = expected changes per site.

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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

Andrássy I (2009). *Free-living nematodes of Hungary (Nematoda errantia), Volume* **III**. Budapest: Hungarian Natural History Museum and Systematic Zoology Research Group of the Hungarian Academy of Sciences, 608p.

Bakhshi Amrei S, **Peneva V**, **Rakhshandehroo F**, **Pedram M** (2020). Morphological and molecular description of *Longidorus behshahrensis* sp. n. (Nematoda: Longidoridae) from natural forests of Abbas Abad, north of Iran. *European Journal of Plant Pathology* **156**, **1**, 387–398. https://doi.org/10.1007/s10658-019-01884-y

Bakhshi Amrei S, **Peneva V**, **Rakhshandehroo F**, **Pedram M** (2022). Description of *Longidorus armeniacae* n. sp. (Nematoda: Longidoridae), associated with *Prunus armeniaca* L. in Semnan province, Iran. *European Journal of Plant Pathology* **162**, **1**, 739–750. https://doi.org/10.1007/s10658-021-02435-0

Archidona-Yuste A, **Navas-Cortés JA**, **Cantalapiedra-Navarrete C**, **Palomares-Rius JE**, **Castillo P** (2016a). Remarkable diversity and prevalence of dagger nematodes of the genus *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae) in olives revealed by integrative approaches. *PLoS ONE* **11**, **11**, e0165412. https://doi.org/10.1371/journal.pone.0165412

Archidona-Yuste A, **Navas-Cortés JA**, **Cantalapiedra-Navarrete C**, **Palomares-Rius JE**, **Castillo P** (2016b). Unravelling the biodiversity and molecular phylogeny of needle nematodes of the genus *Longidorus* (Nematoda: Longidoridae) in olive and a description of six new species. *PLoS ONE* **11**, **1**, e0147689. https://doi.org/10.1371/journal.pone.0147689

Archidona-Yuste A, **Navas-Cortés JA**, **Cantalapiedra-Navarrete C**, **Palomares-Rius JE**, **Castillo P** (2016c). Cryptic diversity and species delimitation in the *Xiphinema americanum*-group complex (Nematoda: Longidoridae) as inferred from morphometrics and molecular markers. *Zoological Journal of the Linnean Society* **176**, **2**, 231–265. https://doi.org/10.1111/zoj.12316

Archidona-Yuste A, **Navas-Cortés JA**, **Cantalapiedra-Navarrete C**, **Palomares-Rius JE**, **Castillo P** (2016d). Molecular phylogenetic analysis and comparative morphology resolve two new species of olive-tree soil related dagger nematodes of the genus *Xiphinema* (Dorylaimida: Longidoridae) from Spain. *Invertebrate Systematics* **30**, **6**, 547–565. https://doi.org/10.1071/IS16002

Archidona-Yuste A, **Wiegand T**, **Castillo P**, **Navas-Cortés JA** (2019a). Dataset on the diversity of plant-parasitic nematodes in cultivated olive trees in southern Spain. *Data in Brief* **27**, 104658. https://doi.org/10.1016/j.dib.2019.104658

Archidona-Yuste A, **Cantalapiedra-Navarrete C**, **Castillo P**, **Palomares-Rius JE** (2019b). Molecular phylogenetic analysis and comparative morphology reveals the diversity and distribution of needle nematodes of the genus *Longidorus* (Dorylaimida: Longidoridae) from Spain. *Contributions to Zoology* **88**, 1–41. https://doi.org/10.1163/18759866-20191345

Asgari M, **Eskandari A**, **Castillo P**, **Palomares-Rius JE** (2022). Morphological and molecular characterisation of *Longidorus sabalanicus* n. sp. (Nematoda: Longidoridae) from Iran. *European Journal of Plant Pathology* **163**, **1**, 19–34. https://doi.org/10.1007/s10658-021-02454-x

Brown DJF, **Lamberti F**, **Taylor CE**, **Trudgill DL** (1988). Nematode-virus plant interactions. *Nematologia Mediterranea* **16**, **2**, 153–158.

Brown DJF, **Grunder J**, **Hooper DJ**, **Klingler J**, **Kunz P** (1994). *Longidorus arthensis* sp. n. (Nematoda: Longidoridae) a vector of cherry rosette disease caused by a new nepovirus in cherry trees in Switzerland. *Nematologica* **40**, **1–4**, 133–149. https://doi.org/10.1163/003525994X00094

Cai R, **Archidona-Yuste A**, **Cantalapiedra-Navarrete C**, **Palomares-Rius JE**, **Castillo P** (2020a). Integrative descriptions and molecular phylogeny of two new needle nematodes of the genus *Longidorus* (Nematoda: Longidoridae) from Spain. *European Journal of Plant Pathology* **156**, **1**, 67–86. https://doi.org/10.1007/s10658-019-01862-4

Cai R, **Prior T**, **Lawson B**, **Cantalapiedra-Navarrete C**, **Palomares-Rius JE**, **Castillo P**, **Archidona-Yuste A** (2020b). An integrative taxonomic study of the needle nematode complex *Longidorus goodeyi* Hooper, 1961 (Nematoda: Longidoridae) with description of a new species. *European Journal of Plant Pathology* **158**, **1**, 59–81. https://doi.org/10.1007/s10658-020-02055-0

Chen QW, **Hooper, DJ**, **Loof PAA**, **Xu J** (1997). A revised polytomous key for the identification of species of the genus *Longidorus* Micoletzky, 1922 (Nematoda: Dorylaimoidea). *Fundamental and Applied Nematology* **20**, **1**, 15–28.

Cherry T, **Szalanski AL**, **Todd TC**, **Powers TO** (1997). The internal transcribed spacer region of *Belonolaimus* (Nemata: Belonolaimidae). *Journal of Nematology* **29**, **1**, 23–29.

Clavero-Camacho I, **Liébanas G**, **Escuer M**, **Cantalapiedra-Navarrete C**, **Archidona-Yuste A**, **Castillo P**, **Palomares-Rius JE** (2021). Studies on *Longidorus iberis* (Escuer & Arias, 1997) n. comb. (Nematoda: Longidoridae) from Spain. *Nematology* **23**, **8**, 871–885. https://doi.org/10.1163/15685411-bja10082.

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

Coomans A (1996). Phylogeny of the Longidoridae. *Russian Journal of Nematology* **4**, **1**, 51–60.

Coomans A, **Huys R**, **Heyns J**, **Luc M** (2001). Character analysis, phylogeny, and biogeography of the genus *Xiphinema* Cobb, 1913 (Nematoda, Longidoridae). *Annales Sciences Zoologiques, Musée Royal de l'Afrique Centrale, Tervuren, Belgique* **287**, 1–239.

Darriba D, **Taboada GL**, **Doallo R**, **Posada D** (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, **8**, 772. https://doi.org/10.1038/nmeth.2109

Decraemer W, **Robbins RT** (2007). The who, what and where of Longidoridae and Trichodoridae. *Journal of Nematology* **39**, **4**, 295–297.

Decraemer W, **Geaert E** (2013). Ectoparasitic nematodes. In Perry R, Moens M (eds), *Plant Nematology*, 2nd Edition. Wallingford, UK: CABI, 179–214.

De Grisse A (1969). Redescription ou modifications de quelques techniques utililisées dans l'étude des nematodes phytoparasitaires. *Mededelingen van de Rijksfaculteit Landbouwwetenschappen Gent* **34**, 351–369.

De Luca F, **Reyes A**, **Grunder J**, **Kunz P**, **Agostinelli A**, **De Giorgi C**, **Lamberti F** (2004). Characterization and sequence variation in the rDNA region of six nematode species of the genus *Longidorus* (Nematoda). *Journal of Nematology* **36**, **2**, 147–152.

De Man JG (1876). Onderzoekingen over vrij in de aarde levende nematoden. *Tijdschrift Der Nederlandsche Dierkundige Vereeniging* **2**, 78–196.

Edward JC, **Misra SL**, **Singh GR** (1964). *Longidorus pisi* n. sp. (Nematoda, Dorylaimoidea) associated with the rhizosphere of *Pisum sativum*, from Uttar Pradesh, India. *Japanese Journal of Applied Entomology and Zoology* **8**, 310–312. https://doi.org/10.1303/jjaez.8.310

Esmaeili M, **Heydari R**, **Archidona-Yuste A**, **Castillo P**, **Palomares-Rius JE** (2017). A new needle nematode, *Longidorus persicus* n. sp. (Nematoda: Longidoridae), from Kermanshah province, western Iran. *European Journal of Plant Pathology* **147**, **1**, 27–41. https://doi.org/10.1007/s10658-016-0976-9

Eveleigh ES, **Allen WR** (1982). Description of *Longidorus diadecturus* n. sp. (Nematoda: Longidoridae), a vector of the peach rosette mosaic virus in peach orchards in southwestern Ontario, Canada. *Canadian Journal of Zoology* **60**, **2**, 112–115. https://doi.org/10.1139/z82-014.

Flegg JJM (1967). Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Cobb's decanting and sieving technique. *Annals of Applied Biology* **60**, **3**, 429–437. https://doi.org/10.1111/j.1744-7348.1967.tb04497.x

Gutiérrez-Gutiérrez C, **Cantalapiedra-Navarrete C**, **Montes-Borrego M**, **Palomares-Rius JE**, **Castillo P** (2013). Molecular phylogeny of the nematode genus *Longidorus* (Nematoda: Longidoridae) with description of three new

species. *Zoological Journal of the Linnean Society* **167**, **4**, 473–500. https://doi.org/10.1111/zoj.12019

Gutiérrez-Gutiérrez C, **Teixeira Santos M**, **Inácio, ML**, **Eisenback JD**, **Mota M** (2020). Description of *Longidorus bordonensis* sp. nov. from Portugal, with systematics and molecular phylogeny of the genus (Nematoda, Longidoridae). *Zoosystematics and Evolution* **96**, **1**, 175–193. https://doi.org/10.3897/zse.96.49022

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.

He Y, **Subbotin SA**, **Rubtsova TV**, **Lamberti F**, **Brown DJF**, **Moens, M** (2005). A molecular phylogenetic approach to Longidoridae (Nematoda: Dorylaimida). *Nematology* **7**, **1**, 111–124. https://doi.org/10.1163/1568541054192108

Holterman M, **van der Wurff A**, **van den Elsen S**, **van Megen H**, **Bongers T**, **Holovachov O**, **Bakker J**, **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**, **9**, 1792–1800. https://doi.org/10.1093/molbev/msl044

Hooper DJ (1961). A redescription of *Longidorus elongatus* (de Man, 1876) Thorne & Swanger, 1936 (Nematoda, Dorylaimidae) and descriptions of five new species of *Longidorus* from Great Britain. *Nematologica* **6**, **3**, 237–257. https://doi.org/10.1163/187529261X00072

Hooper DJ (1966). *Longidorus profundorum* n. sp. (Nematoda: Dorylaimidae). *Nematologica* **11**, **4**, 489–495. https://doi.org/10.1163/187529265X00672

Janssen J, **Karssen G**, **Orlando V**, **Subbotin SA**, **Bert W** (2017). Molecular characterization and species delimiting of plant-parasitic nematodes of the genus *Pratylenchus* from the *penetrans* group (Nematoda: Pratylenchidae). *Molecular Phylogenetics and Evolution* **117**, 30–48. https://doi.org/10.1016/j.ympev.2017.07.027

Jairajpuri MS. **Ahmad W** (1992). *Dorylaimida: Free-living, Predaceous and Plant-Parasitic Nematodes*. New Delhi: Oxford and IBH Publishing Co. Pvt. Ltd.

Katoh K, **Rozewicki J**, **Yamada KD** (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* **20**, **4**, 1160–1166. https://doi.org/10.1093/bib/bbx108

Kozlowska J, **Seinhorst JW** (1979). *Longidorus elongatus* and closely related species in The Netherlands and Lower Saxony (Germany), with the description of two new species, *L. cylindricaudatus* and *L. intermedius* (Nematoda: Dorylaimida). *Nematologica* **25**, **1**, 42–53. https://doi.org/10.1163/187529279X00361

Krnjaić D, **Lamberti F**, **Krnjaić S**, **Agostinelli A**, **Radicci V** (2000). Three new longidorids (Nematoda: Dorylaimida) from Montenegro. *Nematologia Mediterranea* **28**, **2**, 235–248.

Lamberti F, **Bleve-Zacheo T** (1977). Two new species of *Longidorus* (Nematoda: Longidoridae) from Italy. *Nematologia Mediterranea* **5**, **1**, 73–83.

Lamberti F, **Molinari S**, **De Luca F**, **Agostinelli A**, **Di Vito M** (1999). Longidorids (Nematoda, Dorylaimida) from Syria with description of *Longidorus pauli* sp. n. and *Paralongidorus halepensis* sp. n. with SOD isozymes and PCR-RFLP profiles. *Nematologia Mediterranea* **27**, **1**, 63–78.

Lišková M, **Robbins RT**, **Brown DJF** (1997). Descriptions of three new *Longidorus* species from Slovakia (Nemata: Longidoridae). *Journal of Nematology* **29**, **3**, 336–348.

Loof PAA (1982). Two new species of Longidoridae (Dorylaimida) from Saudi Arabia. *Nematologica* **28**, **3**, 307–317. https://doi.org/10.1163/187529282X00358

Loof PAA, **Coomans A** (1972). The oesophageal gland nuclei of Longidoridae (Dorylaimida). *Nematologica* **18**, **2**, 213–233. https://doi.org/10.1163/187529272X00458

Loof PAA, **Chen QW** (1999) A revised polytomous key for the identification of species of the genus *Longidorus* Micoletzky, 1922 (Nematoda: Dorylaimoidea). Supplement 1. *Nematology* **1**, **1**, 55–59. https://doi.org/10.1163/156854199507974

Macfarlane SA (2003). Molecular determinants of the transmission of plant viruses by nematodes. *Molecular Plant Pathology* **4**, **3**, 211–215. https://doi.org/10.1046/j.1364-3703.2003.00164.x

Mali VR, **Hooper DJ** (1974). Observations on *Longidorus euonymus* n. sp. and *Xiphinema vuittenezi* Luc *et al* 1964 (Nematoda: Dorylaimida) associated with spindle trees infected with euonymus mosaic virus in Czechoslovakia. *Nematologica* **19**, **4**, 459–467. https://doi.org/10.1163/187529273X00457

Merny G (1966). Nématodes d'Afrique tropicale: un nouveau *Paratylenchus* (Criconematidae), deux nouveaux *Longidorus* et observations sur *Longidorus Laevicapitatus* Williams, 1959 (Dorylaimidae). *Nematologica* **12**, **3**, 385–395. https://doi.org/10.1163/187529266X00860

Micoletzky H (1922). Die freilebenden Erd-Nematoden mit besonderer Berucksichtigung der Steiermark und der Bukowina, zugleich mit einer Revision sämtlicher, nicht mariner, freilebender Nematoden in Form von Genus-Beschreibungen und Bestimmungsschlüsseln. *Archiv für Naturgeschichte* **87A**, 1–650.

Mobasseri M, **Pourjam E**, **Farashiani ME**, **Pedram M** (2023). Morpho-molecular characterization of a new nematode species of the genus *Longidorus* Micoletzky, 1922 (Nematoda: Longidoridae) from northern Iran. *European Journal of Plant Pathology* **165**, **1**, 241–254. https://doi.org/10.1007/s10658-022-02603-w

Niknam G, **Pedram M**, **Ghahremani Nejad E**, **Ye W**, **Robbins RT**, **Tanha Maafi Z** (2010). Morphological and molecular characterization of *Longidorus tabrizicus* sp. n. and *L.* sturhani Rubtsova, Subbotin, Brown & Moens, 2001 (Nematoda: Longidoridae) from north-western Iran. *Russian Journal of Nematology* **18**, **2**, 127–140.

Nunn GB (1992). *Nematode Molecular Evolution. An Investigation of Evolutionary Patterns among Nematodes Based upon DNA Sequences*. Ph.D. Thesis, University of Nottingham: Nottingham, UK.

Palomares-Rius JE, **Cantalapiedra-Navarrete C**, **Castillo P** (2014). Cryptic species in plant-parasitic nematodes. *Nematology* **16**, **10**, 1105–1118. https://doi.org/10.1163/15685411-00002831

Palomares-Rius JE, **Escobar C**, **Cabrera J**, **Vovlas A**, **Castillo P** (2017). Anatomical alterations in plant tissues induced by plant-parasitic nematodes. *Frontiers in Plant Science* **8**, 1987. https://doi.org/10.3389/fpls.2017.01987

Palomares-Rius JE, **Subbotin SA**, **Landa BB**, **Vovlas N**, **Castillo P** (2008). Description and molecular characterisation of *Paralongidorus litoralis* sp. n. and *P. paramaximus* Heyns, 1965 (Nematoda: Longidoridae) from Spain. *Nematology* **10**, **1**, 87–101. https://doi.org/10.1163/156854108783360186

Pearse AS (1942). *Introduction to Parasitology*, 1st edn. Springfield-Baltimore: Charles C. Thomas Company.

Pedram M, **Niknam G**, **Robbins RT**, **Ye W**, **Karegar A** (2008). *Longidorus kheirii* n. sp. (Nematoda: Longidoridae) from Iran. *Systematic Parasitology* **71**, **3**, 199–211. https://doi.org/10.1007/s11230-008-9148-4

Peneva VK, **Lazarova SS**, **De Luca F**, **Brown DJF** (2013). Description of *Longidorus cholevae* sp. n. (Nematoda, Dorylaimida) from a riparian habitat in the Rila Mountains, Bulgaria. *ZooKeys* **330**, 1–26. https://doi.org/10.3897/zookeys.330.5750

Pour Ehtesham N, **Pedram M**, **Atighi, MR**, **Jahanshahi Afshar F** (2023). Morphological and molecular phylogenetic study of *Longidorus soosanae* n. sp. (Nematoda: Longidoridae) from Golestan province, northern Iran. *European Journal of Plant Pathology* **166**, **2**, 227–239. https://doi.org/10.1007/s10658-023-02657-4

Rambaut A (2018). FigTree v1.4.4, a Graphical Viewer of Phylogenetic Trees. Available online: http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 27 July 2023).

Robbins RT, **Brown DJF**, **Halbrendt JM**, **Vrain TC** (1995). Compendium of *Longidorus* juvenile stages with observations on *L. pisi*, *L. taniwha*, and *L. diadecturus* (Nematoda: Longidoridae). *Systematic Parasitology* **32**, **1**, 33–52. https://doi.org/10.1007/BF00009466

Robbins RT, **Ye W**, **Pedram M** (2009). *Longidorus ferrisi* n. sp. from California Citrus. *Journal of Nematology* **41**, **2**, 104–110.

Roca F, **Lamberti F** (1981). *Longidorus fasciatus* sp. n. from Greece and Italy. *Nematologia Mediterranea* **9**, **2**, 175–179.

Roca F, **Lamberti F**, **Agostinelli A**, **Vinciguerra MT** (1986). *Longidorus aetnaeus*, a new Longidoridae species from Italy. *Nematologia Mediterranea* **14**, **1**, 1–5.

Ronquist F, **Huelsenbeck JP** (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, **12**, 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Roshan-Bakhsh A, **Pourjam E**, **Pedram M** (2016). Description of *Longidorus perangustus* sp. n. (Dorylaimida: Longidoridae), an amphimictic species from Iran. *European Journal of Plant Pathology* **144**, **3**, 581–594. https://doi.org/10.1007/s10658-015-0796-3

Rubtsova TV, **Chizhov VN**, **Subbotin SA** (1999). *Longidorus artemisiae* sp. n. (Nematoda: Longidoridae) from roots of *Artemisia* sp., Rostov region, Russia. *Russian Journal of Nematology* **7**, **1**, 33–38.

Rubtsova TV, **Subbotin SA**, **Brown DJF**, **Moens M** (2001). Description of *Longidorus sturhani* sp. n (Nematoda: Longidoridae) and molecular characterization of several longidorid species from Western Europe. *Russian Journal of Nematology* **9**, **2**, 127–136.

Sturhan D, **Argo D** (1983). Studies on *Longidorus closelongatus* Stoyanov and *L. cohni* Heyns, with description of *L. proximus* sp. nov. *(Nematoda, Dorylaimida). Revue de Nématologie* **6**, **1**, 57–64.

Sturhan D, **Barooti S** (1983). *Longidorus iranicus* n. sp. (Nematoda: Dorylaimida). *Systematic Parasitology* **5**, **1**, 21–24. https://doi.org/10.1007/BF00010982

Sturhan D, **Weischer B** (1964). *Longidorus vineacola* n. sp. (Nematoda: Dorylaimidae). *Nematologica* **10**, **2**, 335–341. https://doi.org/10.1163/187529264X00114

Subbotin SA, **Halford PD**, **Warry A**, **Perry RN** (2000). Variations in ribosomal DNA sequences and phylogeny of *Globodera* parasitising solanaceous plants. *Nematology* **2**, **6**, 591–604. https://doi.org/10.1163/156854100509484

Tan G, **Muffato M**, **Ledergerber C**, **Herrero J**, **Goldman N**, **Gil M**, **Dessimoz C** (2015). Current methods for automated filtering of multiple sequence alignments frequently worsen single-gene phylogenetic inference. *Systematic Biology* **64**, **5**, 778–791. https://doi.org/10.1093/sysbio/syv033

Taylor CE, **Brown DJF** (1997). *Nematode Vectors of Plant Viruses*. 1st edn. Wallingford: CAB International.

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

Thorne G (1974). Nematodes of the northern great plains. Part II. Dorylaimoidea in part (Nemata: Adenophorea). *Agricultural Experiment Station Technical Bulletins* **41**, 1–120.

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

Vrain TC, **Wakarchuk DA**, **Levesque AC**, **Hamilton RI** (1992). Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology* **15**, **6**, 563–573.

Whitehead AF, **Hemming JR** (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* **55**, **1**, 25–38. https://doi.org/10.1111/j.1744-7348.1965.tb07864.x

Ye W, **Robbins RT** (2003). *Longidorus paravineacola* n. sp. (Nematoda: Longidoridae), a new species from Arkansas. *Journal of Nematology* **35**, **4**, 388–394.

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