Morphological and molecular characterisation of *Longidorus sabalanicus* n. sp. (Nematoda: Longidoridae) from Iran

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Short Title: *Longidorus sabalanicus* n. sp. from Iran

Abstract *Longidorus sabalanicus* n. sp. is described and illustrated based on morphological, morphometric and molecular studies of two populations from northwest Iran. It was recovered from the rhizosphere of meadow grasses in East Azarbaijan province and the rhizosphere of *Astragalus* sp. in Zanjan province. The new species is characterised by having a medium sized body, 3.8–5.1 mm in females and 3.6–4.7 in males; lip region anteriorly flattened, laterally rounded, set off from body contour by a slight depression; amphidial fovea pocket-shaped, without basal lobes; guiding ring at 25–30 µm from anterior end; odontostyle moderately long, 73.0–85.5 µm and odontophore weakly developed, 50.0–67.4 µm long; female reproductive system containing sperm, vulva located at 50–54% of body length; tail bluntly conoid, 41.0–55.0 µm long; males common in both populations, with 54.0–68.5 µm long spicules and seven to ten ventromedian precloacal supplements anterior to the adanal pair; and four juvenile developmental stages present. The alpha-numeric codes for identifying the new species are: A23-B2-C2-D3-E1-F2-G1-H12-I2-J1-K7. Morphologically, the new species most closely resembles to *L. moniloides*, *L. rotundicaudatus*, *L. tabrizicus*, *L. artemisiae*, *L. asiaticus*, *L. behshahrensis* and *L. elongatus*. Morphological and morphometric differences of *Longidorus sabalanicus* n. sp. and the aforementioned species are discussed. In addition to morphological and morphometric data, molecular comparison analyses based on partial 18S rRNA, D2-D3 expansion segments of 28S rRNA and ITS1 region sequences, indicate that *L. sabalanicus* n. sp. is separated from other species and it is related phylogenetically with *L. behshahrensis*.

Keywords rRNA, needle nematodes, new species, phylogeny, taxonomy

Introduction

Longidorus Micoletzky 1922 is the second most diverse and species-rich genus within the family Longidoridae Thorne 1935 (Decraemer and Robbins 2007). Members of the genus *Longidorus* commonly known as needle nematodes and constitutes a globally important group of migratory root-ectoparasitic nematodes that cause serious damage to many plant species (Coomans 1996). In addition, some *Longidorus* species are vectors of economically important plant nepoviruses (Taylor and Brown 1997). Eleven species (*L. apulus* Lamberti & Bleve-Zacheo, 1977, *L. arthensis* Brown et al. 1994, *L. attenuatus* Hooper, 1961, *L. caespiticola* Hooper, 1961, *L. diadecturus* Eveleigh, 1982, *L. elongatus* (de Man, 1876) Micoletzky, 1922, *L. fasciatus* Roca & Lamberti, 1981, *L. leptocephalus* Hooper, 1961, *L. macrosoma* Hooper, 1961, *L. martini* Merny, 1966, and *L. profundorum* Hooper, 1966) 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*) (Taylor and Brown 1997, Decraemer and Robbins 2007). For this reason, it is essential the correct identification of *Longidorus* species in order to establish appropriate control and border inspection measures. *Longidorus* spp. are distributed worldwide, with South America being the less diverse region, followed in increasing order by Oceania, China, South Africa, North America, India, and Europe (Cai et al. 2020a; Decraemer and Robbins 2007; Xu and Zhao 2019). As a result of the increasing numbers of described species, and their geographical distribution being more precisely determined, it is now possible to recognise intraspecies or interspecies relationships from geological influences and geographic distributions (Taylor and Brown 1997). According to last studies, the genus *Longidorus* contains approximately 177 valid species (Gutiérrez-Gutiérrez et al. 2020; Clavero-Camacho et al. 2021). In the last years, the use of integrative taxonomy or polyphasic approach (assemblage of phenotypic, genotypic and phylogenetic data to frame species limits) has been applied to unravel de diversity in plant-parasitic nematodes (Subbotin and Moens 2006; Palomares-Rius et al. 2014) in which the presence of cryptic speciation is frequent (Palomares-Rius et al. 2014). In the case of genus *Longidorus*, several studies have used integrative taxonomy to describe the diversity of this genus (Archidona-Yuste et al. 2016; 2019; Cai et al. 2020a, 2020b, 2020c; Radivojević et al. 2020; Gutiérrez-Gutiérrez et al. 2020). To date, 23 species of

this genus have been reported from Iran (Pedram 2018; Asgari et al. 2019; Bakhshi Amrei et al. 2020).

In recent nematode surveys in natural environments in Iran, two nematode populations of *Longidorus* were found in the rhizosphere of undetermined grasses in a meadow in Haris city, East Azarbaijan province, north-western Iran, and the rhizosphere of *Astragalus* sp. naturally growing in mountains of Tarom city, Zanjan province, north-western Iran. These populations did not fully match any described species of the genus, and this fact prompted us to undertake a detailed morphological and molecular comparative study with already described species. In this study, the objective is to describe a new species using integrative taxonomy herein described as *L. sabalanicus* n. sp. associated with these two locations in the rhizosphere of two different natural host plants.

Materials and methods

Nematode sampling and extraction

A total of 20 soil samples were collected from the rhizosphere of meadow grasses in East Azarbaijan province and the rhizosphere of *Astragalus* sp. in Zanjan province, during the spring season of 2019. Each sample consisted of randomly selecting four to five cores, taken using a standard shovel and considering the upper 5–50 cm depth of soil. Nematodes were extracted from a 500 cm^3 sub-sample of soil by the modified decanting and sieving technique of Cobb (Flegg 1967), and the sugar-floatation/centrifugation method (Jenkins 1964).

Morphological characterisation

The collected nematode suspension of each soil sample was examined under a stereo microscope and longidorid specimens were individually picked out, killed by gentle heat, fixed in an aqueous solution of 4% formaldehyde + 1% glycerol, and then transferred to anhydrous glycerine using De Grisse (1969) method. The processed nematode specimens were mounted on permanent glass slides using paraffin wax and used for microscopy. Morphometric values and photomicrographs were taken using a Dino-Eye digital eyepiece camera (Model AM7023, bundled with the 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, and then redrawn and prepared for publication using CorelDRAW® software version 16. Morphological comparisons were performed using the polytomous identification keys for the identification of *Longidorus* species (Chen et al. 1997; Loof and Chen 1999) and with the descriptions of all other characterised species up to the present. The position of pharyngeal gland nuclei was calculated according to Loof and Coomans (1972) and the juvenile developmental stages were identified according to Robbins et al. (1995). All measurements were recorded in micrometer (µm), except for body length (in mm) and ratios.

Molecular characterisation

Following morphological confirmation, four live nematode specimens of *L. sabalanicus* n. sp. from the both populations, were used for DNA extraction. Each specimen was transferred to an Eppendorf tube containing 10 µl ddH_2O, 8 µl lysis buffer (125 mM KCl, 25 mM Tris-Cl pH 8.3, 3.75 mM $MgCl_2$, 2.5 mM DTT, 1.125% Tween 20, 0.025% gelatine) and 2 µl proteinase K (600 µg/ml), and crushed during 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), four µl of extracted DNA was added to the polymerase chain reaction (PCR) mixture in a 0.2 ml Eppendorf tube containing: 20 µl 2x Master mix (Ampliqon, Denmark), 2 µl of each primer (10 pMol/µl) and 12 µl ddH_2O, 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 portion of 18S rRNA was amplified as two overlapping fragments, using forward 988F (5′–CTCAAAGATTAAGCCATGC–3′) and reverse 1912R (5′–TTTACGGTCAGAACTAGGG–3′) primers for the first fragment, and forward 1813F (5′–CTGCGTGAGAGGTGAAAT–3′) and reverse 2646R (5′–GCTACCTTGTTACGACTTTT–3′) primers for the second fragment (Holterman et al. 2006). The ITS1 region was amplified using forward primer 18S (5′–TTGATTACGTCCCTGCCCTTT–3′) (Vrain et al. 1992) and reverse primer rDNA1 (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 54 °C (SSU), 57 °C (LSU), 55 °C (ITS1) for 30 s, and extension at 72 °C for 3 min; and finally elongation step at 72 °C for 10 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 these accession numbers (partial 18S rRNA: MZ474674, D2-D3 expansion segments of 28S rRNA: MZ474667-MZ474670, and ITS1 region: MZ474672-MZ474673).

Phylogenetic analyses

The newly obtained sequences of *L. sabalanicus* n. sp. (D2-D3 expansion segments of 28S rRNA, 18S 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, only sequence similarity comparisons were studied with the 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. 2019; Cai et al. 2020b). 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 manually visualized using BioEdit (Hall, 1999) and edited by Gblocks ver. 0.91b (Castresana 2000) in the Castresana Laboratory server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) using options for a less stringent selection (minimum number of sequences for a conserved or a flanking position: 50% of the number of sequences +1; maximum number of contiguous non-conserved positions: 8; minimum length of a block: 5; allowed gap positions: with half). 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 obtained using JModelTest V.2.1.7 (Darriba et al. 2012) with the Akaike Information Criterion (AIC). The best-fit model, the base frequency, the proportion of invariable sites, and the 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 partial 18S 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 detect four known species of the genus *Longidorus* namely *L. orientalis* Loof 1982; *L. aetnaeus* Roca et al. 1986; *L. iranicus* Sturhan and Barooti 1983; *L. proximus* Sturhan and Argo 1983, and to verify an undescribed species of the genus herein as *L. sabalanicus* n. sp.

Description of *Longidorus sabalanicus*[1] n. sp. (Figs. 1–4, Table 1)

Female Body moderately thin, gradually tapering towards both extremities, curved into a C or spiral shape when relaxed. Cuticle thin appearing smooth under low magnifications, 2.4–3.6 µm thick at mid-body, and 12–16 µm at tail hyaline part. Lateral chords 13.5–18.0 µm wide at mid-body, occupying 21.9–26.6% of the corresponding body width. Lip region anteriorly flattened, laterally rounded, 12.5–14.5 µm wide and 4.0–5.0 µm high, separated from rest of body by a shallow depression. (Figs. 1 and 2). Amphidial fovea pocket-shaped without lobes at base. Guiding ring single, anteriorly positioned, located approximately *ca* 2 times the lip region width from anterior end. Odontostyle moderately

[1]The specific epithet refers to the Sabalan mountain, where the new species was collected.

long, 1.1–1.6 times as long as odontophore, straight or slightly arcuate. Odontophore weakly developed, with rather weak basal swellings (Figs. 1 and 2). Nerve ring surrounding the slender portion of the pharynx behind the odontophore base, located at 145–168 μm from anterior end. Basal pharyngeal bulb cylindrical, 3.4–4.5 times longer than wide. Arrangement of pharyngeal glands normal, their nuclei of approximately the same size, dorsal gland nucleus (DN) and two subventral nuclei (SVN), situated at 25–35% and 50–60% of distance from anterior end of basal pharyngeal bulb, respectively. Cardia conoid to rounded, 7.0–15.5 μm long. Intestine simple. Reproductive system didelphic-amphidelphic, with both genital branches almost equally developed, each branch 286–538 μm long, with reflexed ovaries very variable in length, anterior ovary 107–162 μm long and posterior ovary 85–90 μm long. Oviducts slightly longer than ovaries. Uteri cylindrical, quite variable in length, anterior uterus 136–210 μm long and posterior uterus 141–192 μm long. Sphincter separating oviduct and uterus well developed. Sperm commonly found in the uteri. Vulva in form of a transverse slit, located about mid-body or slightly posterior (50.0–54.2%). Vagina perpendicular to body axis, 37–44 μm long or 54.7–67.8% of corresponding body width and surrounded by well-developed constrictor muscles; *pars distalis vaginae* 12–18 μm long, *pars proximalis vaginae* measuring 15–24 μm long. Prerectum visible, variable in length, 6–13 times longer than anal body width, rectum simple, 0.6–0.8 times as long as tail length. Tail almost equivalent to anal body width in length, bluntly conoid in shape, dorsally convex and ventrally almost straight or slightly concave, terminus widely rounded with distinct radial lines in hyaline region, caudal pores not observed. Hyaline part of tail almost as long as wide.

Male Common and functional, equal to females in number. Morphologically similar to female except for reproductive system, posterior end more ventrally curved. Male reproductive system diorchic with opposed testes, containing multiple rows of different stages of spermatogonia. Spicules arcuate, robust, about 1/5 times longer than tail length, lateral guiding pieces more or less straight. Adanal supplements paired, preceded anteriorly by a row of 7–10 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 Four developmental juvenile stages were found and distinguished based on morphometric values of body, odontostyle and replacement odontostyle length, and tail shape (Table 1, Fig. 3). Habitus more or less an open C-shape. Morphologically similar to females, except for their size and sexual characteristics. The relation of the functional and replacement odontostyle length to the body length is given in Fig. 4. The labial region in all juvenile stages were similar to that of females. First-stage juveniles (J1) were characterised by a convex-conoid to conical tail with distinctly digitate or subdigitate terminus (c′ = 1.5, 1.9), shorter distance from anterior end to stylet guiding ring than that in adult stages, and the replacement odontostyle being inserted into odontophore base. 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.

Type habitat and locality

The type population was collected from the rhizosphere of undetermined grasses in a meadow in Haris city, East Azarbaijan province, north-western Iran, by M. Asgari on May 16, 2019 (GPS coordinates: 38º 15' 20.623" N, 47º 14' 31.284" E; altitude: 2550 m a.s.l.).

Other habitat and locality

The second population was collected from the rhizosphere of *Astragalus* sp. naturally growing in mountains of Tarom city, Zanjan province, north-western Iran, by M. Asgari on April 11, 2019 (GPS coordinates: 36º 49' 23.977" N, 48º 43' 42.546' 'E; altitude: 2296 m a.s.l.).

Referenced specimens and nomenclatural registration

Holotype female, seven paratype females, four paratype males, thirteen paratype juveniles, together with two females and four males from the second population; mounted in pure glycerin and deposited in the nematode collection at Faculty of Agriculture, University of Zanjan, Zanjan, Iran (slide numbers: 9908A-K); one female and one male

paratypes, together with one female and two males from the second population; deposited in the nematode collection at Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Córdoba, Spain (IAS_L_2021-2_Ir).

The new species binomial has been registered in the ZooBank database (zoobank.org) under the identifier: urn:lsid:zoobank.org:act:E8099BE3-2D90-446B-B419-59A3EC872837. The LSID for the publication is: urn:lsid:zoobank.org:pub:E071D8F1-E494-4D1F-9DDF-782D6BDD3CCF.

Diagnosis and relationships

Longidorus sabalanicus n. sp. is characterised by a moderately long body, 3.8–5.1 mm; lip region anteriorly flattened, laterally rounded and separated from body contour by a shallow depression, 12.5–14.5 µm wide; amphidial fovea pocket-shaped, not bilobed and extending about 1/2 part of oral aperture-guiding ring distance; moderately long odontostyle, odontophore and total stylet, 73.0–85.5, 50.0–67.5 and 128–148 µm, respectively; guiding ring located at 25.0–30.5 µm from anterior end; vulva located at 50–54% of body length; female tail bluntly conoid, dorsally convex and ventrally almost straight to slightly concave, ending in a rounded terminus, 41–51 µm long. Males common with 54.0–68.5 µm long spicules, one pair of adanal supplement plus 7–10 pairs of ventromedian supplements. Four developmental juvenile stages were identified, the tail of the first-stage juvenile conoid with a digitate or subdigitate rounded terminus. *Longidorus sabalanicus* n. sp. has also specific D2-D3 of 28S rRNA, ITS1, and partial 18S rRNA sequences (GenBank accession numbers: MZ474667-MZ474670, MZ474672-MZ474673, and MZ474674, respectively). According to the polytomous key by Chen et al. 1997, supplement by Loof and Chen 1999 and the addition of some characters by Peneva et al. 2013; codes for the new species are: A23-B2-C2-D3-E1-F2-G1-H12-I2-J1-K7.

According to the body and odontostyle length, distance of guiding ring from anterior body end, lip region, amphidial fovea and tail shape, and the abundance of males; *L. sabalanicus* n. sp. comes close to seven known *Longidorus* species, namely *L. moniloides* Heyns 1966, *L. rotundicaudatus* Jacobs and Heyns 1987, *L. tabrizicus* Niknam et al. 2010, *L. artemisiae* Rubtsova et al. 1999, *L. asiaticus* Trisciuzzi et al. 2015, *L. elongatus* (de Man 1876) Micoletzky 1922, and *L. behshahrensis* Bakhshi Amrei et al. 2020. The new species differs from *L. moniloides* by having a different amphidial fovea shape

(pocket-shaped, not bilobed *vs* symmetrically bilobed at base), slightly longer body (3.8–5.1 *vs* 3.2–4.1 mm), a longer odontostyle, odontophore, spicule and tail (73.0–85.5 *vs* 56–63 µm, 50.0–67.5 *vs* 25–33 µm, 54.0–68.5 *vs* 52–53 µm, and 41–55 *vs* 27–37 µm, respectively), and a lower number of ventromedian supplements in the male tail (7–10 *vs* 13–18). It differs from *L. rotundicaudatus* by having a different amphidial fovea shape (not bilobed *vs* bilobed at base), lower oral aperture-guiding ring distance (25.0–30.5 *vs* 33–38 µm), lower a and c ratios (60.1–84.5 *vs* 91–115, and 80.8–108.9 *vs* 140–197, respectively), longer tail (41–55 *vs* 23–37 µm), and a longer spicule (54.0–68.5 *vs* 49 µm). From *L. tabrizicus* it differs by having a longer odontostyle and odontophore (73.0–85.5 *vs* 61.5–70.0 µm, and 50.0–67.0 *vs* 43–54 µm, respectively), lower a ratio (60.1–84.5 *vs* 81–135), longer spicule (54.0–68.5 *vs* 36–49 µm), and higher oral aperture-guiding ring distance (25.0–30.5 *vs* 19–27 µm). From *L. artemisiae* it differs by a smaller body (3.8–5.1 *vs* 5.1–6.5 mm), different lip region shape (separated from body contour by shallow *vs* by distinct depression), shorter odontostyle (73.0–85.5 *vs* 84–98 µm), and lower a and c ratios (60.1–84.5 *vs* 109–155, and 80.8–108.9 *vs* 120–207, respectively). From *Longidorus asiaticus* the new species differs mainly by having a longer body in females (3.8–5.1 *vs* 2.7–3.5 mm), higher a and c′ ratios (60.1–84.5 *vs* 42.6–58.5, and 0.9–1.2 *vs* 0.6–0.9, respectively), a different lip region shape (separated from body contour by a shallow depression *vs* continuous) and the presence *vs* absence of males. From *Longidorus elongatus* the new species differs mainly by having a slightly shorter body (3.8–5.1 *vs* 4.5–6.4 mm), different amphidial fovea shape (not bilobed *vs* slightly bilobed at base), and a different tail shape in J1 (convex-conoid to conical with a distinctly digitate or subdigitate terminus *vs* slender conical, without a digitate or subdigitate terminus). In addition, *L. sabalanicus* n. sp. is molecularly related to *L. behshahrensis*, from which it can be mainly differentiated by a shorter body (3.8–5.1 *vs* 6.8–8.4 mm), lower a and c ratios (60.1–84.5 *vs* 97.2–157.9, and 80.8–108.9 *vs* 132.1–205.4, respectively) and a wider lip region (12.5–14.5 *vs* 9–10 µm).

Molecular characterisation and phylogenetic position of *Longidorus sabalanicus* n. sp.

The amplification of D2-D3 segments of 28S rRNA, ITS1, and partial 18S rRNA yielded single fragments of *ca* 900 bp, 1100 bp and 1800 bp, respectively, based on gel electrophoresis. Only one sequence was obtained for partial 18S rRNA with 99.5%, 99.42%, and 99.42% similarity with *L. elongatus* (de Man 1876) Micoletzky 1922

(EU503141), *L. uroshis* Krnjaić et al. 2000 (EF538760) and *L. piceicola* Lišková et al. 1997 (AY687993), respectively. Three and one D2-D3 segments of 28S rRNA sequences were obtained for East Azarbaijan province and Zanjan province populations, respectively. Molecular similarities between both populations were between 99.3–99.4% and 4–5 nucleotides differences, and 0 indels. D2-D3 segments of 28S rRNA for *L. sabalanicus* n. sp. is similar to *L. behshahrensis* (MK810742), *L. elongatus* (MN123755), *L. intermedius* Kozlowska and Seinhorst 1979 (AY593058) and *L. uroshis* (EF538754) in 94.3%, 94.1%, 93.5% and 93.3%, respectively. Two sequences were obtained for ITS1 region for the East Azarbaijan province population without intraspecific variation. Only *L. behshahrensis* (MK810738-MK810740) is the only species with similarity with *L. sabalanicus* n. sp. with 82.2–82.7% similarity (with 93% coverage). Other species have partial similarities in some regions of the sequence with even lower similarities. For this reason, the phylogenetic position of *L. sabalanicus* n. sp. was not studied for this molecular marker.

Phylogenetic relationships among *Longidorus* species inferred from analyses of D2-D3 expansion segments of 28S rRNA and partial 18S rRNA sequences using BI are given in Figures 5 and 6, respectively. The D2-D3 expansion segments of 28S rRNA tree of *Longidorus* spp. based on a multiple edited alignment including 109 sequences and 744 total characters revealed three major clades, two of them highly supported (PP = 1.00) and the another with low-support (PP = 0.84) (Fig. 5). *Longidorus sabalanicus* n. sp. (MZ474667-MZ474670) and *L. behshahrensis* (MK810742) clustered in a high-supported clade (PP = 1.00). These two species are related to *L. elongatus* (AF480078), *L. intermedius* (AY593058), *L. artemisiae* (KX137849), *L. piceicola* (KY086070), *L. uroshis* (EF538754) and *L. carpathicus* Lišková et al. 1997 (AF480072) in a moderately supported clade (PP = 0.96).

For the partial 18S rRNA sequences, the 50% majority rule consensus BI tree of a multiple sequence alignment containing 85 sequences and 1665 characters is showed in Fig 6. *Longidorus sabalanicus* n. sp. (MZ474674) clustered with *L. aetnaeus* (KF242287) in a low supported clade (PP = 0.78). The position of these two species inside other clades are not well-supported in the phylogenetic tree. Unfortunately, no sequences of *L. behshahrensis* are available for partial 18S rRNA. Additionally, *L. behshahrensis* is closely related to *L. sabalanicus* n. sp. in the ITS1 region as it is the unique species with a clear similarity in this region.

This new species increases the diversity of this genus in Iran, including molecular markers for its unequivocal identification. *Longidorus sabalanicus* n. sp. has been found in two localities at high altitude in mountains (more than 2000 m.a.s.l.), and not in other parts of Iran. This suggests that this species could be adapted to high altitude environments and putative host plants adapted to this habitat. Other species of this genus have been also only found at high altitude (i. e. *L. panderaltum* Cai et al. 2020) (Cai et al., 2020c) and also some species of the genus *Xiphinema* (*Xiphinema subbaetense* Cai et al. 2020) (Cai et al., 2020b). Other species from Iran are closely related to our species (*L. behshahrensis*), but clearly separated using our integrative taxonomy (Bakhshi Amrei et al. 2020). The use of topotypes for molecular analysis could help to resolve many cryptic species in the future inside the family Longidoridae as it has been proven for other species inside the frame of species complexes as in the case of *L. iliturgiensis* Archidona-Yuste et al. 2019 and *X. hispanum* Lamberti et al. 1992 complexes (Cai et al., 2020b) or *Longidorus goodeyi* Hooper 1961 complex (Cai et al., 2020c).

In conclusion, this paper describes a new species from Iran. This species is clearly described using an integrative taxonomical approach (combination of morphology-morphometry and molecular data). *L. sabalanicus* n. sp. is clearly related molecularly to other species described in Iran as *L. behshahrensis*.

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Declarations

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Conflicts of interest/Competing interests

All authors certify that 1) they do not have any actual or potential conflict of interest, 2) the study described is original and has not been published previously, and is not under consideration for publication elsewhere, 3) all prevailing local, national and international regulations and conventions, and normal scientific ethical practices, have been respected.

Availability of data and material

Holotype female, seven paratype females, four paratype males, thirteen paratype juveniles, together with two females and four males from the second population; mounted in pure glycerin and deposited in the nematode collection at Faculty of Agriculture, University of Zanjan, Zanjan, Iran (slide numbers: 9908A-K); one female and one male paratypes, together with one female and two males from the second population; deposited in the nematode collection at Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Córdoba, Spain (IAS_L_2021-2_Ir).

The new species binomial has been registered in the ZooBank database (zoobank.org) under the identifier: urn:lsid:zoobank.org:act:E8099BE3-2D90-446B-B419-59A3EC872837. The LSID for the publication is: urn:lsid:zoobank.org:pub:E071D8F1-E494-4D1F-9DDF-782D6BDD3CCF.

Longidorus sabalanicus n. sp. GenBank accession number sequences: MZ474667-MZ474670, MZ474672-MZ474673, and MZ474674.

Code availability

Not applicable

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors. All authors read and approved the final manuscript.

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

The authors certify that 1) no special permits were required for the fieldwork investigations, 2) soil samplings did not involve any species endangered or protected in Iran, 3) the soil samples analyzed in this study were obtained from natural public areas that are not under protection in any way.

Ethics approval

All the authors certify that the work carried out in this research followed the principles of ethical and professional conduct have been followed.

Consent to participate

We also certify that all authors have reviewed the manuscript and approved the final version of manuscript before submission.

Consent for publication

We also certify that all authors have reviewed the manuscript and approved the final version for publication in European Journal of Plant Pathology.

References

Archidona-Yuste, A., Navas-Cortés, J. A., Cantalapiedra-Navarrete, C., Palomares-Rius, J. E., & Castillo, P. (2016) 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, e0147689. https://doi.org/10.1371/journal.pone.0147689.

Archidona-Yuste, A., Cantalapiedra-Navarrete, C., Castillo, P., & Palomares-Rius, J. E. (2019). 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. (2019). First report of *Longidorus artemisiae* Rubtsova, Chizov & Subbotin, 1999 from Iran. *1st Iranian Congress of Nematology, Tehran, Iran*, p. 21.

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

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

Cai, R., Archidona-Yuste, A., Cantalapiedra-Navarrete, C., Palomares-Rius, J. E., & 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*, 67–86. https://doi.org/10.1007/s10658-019-01862-4

Cai, R., Archidona-Yuste, A., Cantalapiedra-Navarrete, C., Palomares-Rius, J. E., & Castillo, P. (2020b). New evidence of cryptic speciation in the family Longidoridae (Nematoda: Dorylaimida). *Journal of Zoological Systematics and Evolutionary Research, 58*, 869–899. https://doi.org/10.1111/jzs.12393

Cai, R., Prior, T., Lawson, B., Cantalapiedra-Navarrete, C., Palomares-Rius, J. E., P. Castillo, & Archidona-Yuste, A. (2020c). 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, 59-81. https://doi.org/10.1007/s10658-020-02055-0.

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution, 17*, 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Chen, Q.-W., Hooper, D. J., Loof, P. A. A., & 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*, 15–28.

Cherry, T., Szalanski, A. L., Todd, T. C., & Powers, T. O. (1997). The internal transcribed spacer region of *Belonolaimus* (Nemata: Belonolaimidae). *Journal of Nematology, 29*, 23–29.

Clavero-Camacho, I., Liébanas, G., Escuer, M., Cantalapiedra-Navarrete, C., Archidona-Yuste, A., Castillo, P., & Palomares-Rius, J. E. (2021). Studies on *Longidorus iberis* (Escuer & Arias, 1997) n. comb. (Nematoda: Longidoridae) from Spain. *Nematology* (published online ahead of print 2021). https://doi.org/10.1163/15685411-bja10082

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

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

De Grisse, A. T. (1969). Redescription ou modifications de quelques techniques utilisées dans l'étude des nématodes phytoparasitaires. *Mededelingen van de Rijksfaculteit Landbouwwetenschappen te Gent, 34*, 351–369.

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

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

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

Gutiérrez-Gutiérrez, C., Cantalapiedra-Navarrete, C., Montes-Borrego, M., Palomares-Rius, J. E., & 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*, 473–500. https://doi.org/10.1111/zoj.12019

Gutiérrez-Gutiérrez, C., Teixeira Santos, M., Inácio, M. L., Eisenback, J. D., & 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*, 175–193. https://doi.org/10.3897/zse.96.49022

Hall, T. A. (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, S. A., Rubtsova, T. V., Lamberti, F., Brown, D. J. F., & Moens, M. (2005). A molecular phylogenetic approach to Longidoridae (Nematoda: Dorylaimida). *Nematology, 7*, 111–124. https://doi.org/10.1163/1568541054192108

Heyns, J. (1966). Further studies on South African Longidoridae (Nematoda). *South African Journal of Agricultural Science, 9*, 927–944.

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*, 1792–1800. https://doi.org/10.1093/molbev/msl044

Jacobs, P. J. F., & Heyns, J. (1987). Eight new and two known species of *Longidorus* from South Africa (Nematoda: Longidoridae). *Phytophylactica, 19*, 15–33.

Jenkins, W. R. (1964). A rapid centrifugal flotation technique for separating nematodes from soil. *Plant Disease Reporter, 48*, 692.

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

Kozlowska, J., & Seinhorst, J. W. (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*, 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*, 235–248.

Lamberti, F., Castillo, P., Gomez-Barcina, A., & Agostinelli, A. (1992). Descriptions of six new species of *Xiphinema* (Nematoda, Dorylaimida) from the Mediterranean region. *Nematologia Mediterranea*, *20*, 125–139.

Lišková, M., Robbins, R. T., & Brown, D. J. F. (1997). Descriptions of three new *Longidorus* species from Slovakia (Nemata: Longidoridae). *Journal of Nematology, 29*, 336–348.

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

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

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

Micoletzky, H. (1922). Die freilebenden Erd-Nematoden mit besonderer Berücksichtigung der Steiermark und der Bukowina, zugleich mit einer Revision sämtlicher, nicht mariner, freilebender Nematoden in Form von Genus-Beschreibungen und Bestimmungschlüsseln. *Archiv für Naturgeschichte, 87A* (8/9) (1921), 1–650.

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

Nunn, G. B. (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, J. E., Subbotin, S. A., Landa, B. B., 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*, 87–101. https://doi.org/10.1163/156854108783360186

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

Pedram, M. (2018). Nematodes of the family Longidoridae. In R. Ghaderi, L. Kashi, & A. Karegar (Eds.), *Plant-parasitic nematodes in Iran*. pp. 627–667. Science reference in collaboration with the Iranian Society of Nematology (in Persian).

Peneva V. K., Lazarova S. S., De Luca, F., & Brown, D. J. F. (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

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*, 57–64.

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

Subbotin S. A., & Moens, M. (2006). Molecular taxonomy and phylogeny. In: Perry, R. N., Moens, M., editors. Plant Nematology. Wallingford, UK: CABI; pp. 33–58.

Radivojević, M., Barsi, L., Fanelli, E., & De Luca, F. (2020). Description of *Longidorus barsii* Radivojevic & De Luca sp. n. (Nematoda: Longidoridae) from Serbia and observations on some taxonomic characters. *Nematology* 22, 555-576. https://doi.org/10.1163/15685411-00003323.

Rambaut, A. (2018). FigTree v1.4.4. Available online at https://github.com/rambaut/figtree/releases/tag/v1.4.4

Robbins, R. T., Brown, D. J. F., Halbrendt, J. M., & Vrain, T. C. (1995). Compendium of *Longidorus* juvenile stages with observations on *L. pisi*, *L. taniwha* and *L. diadecturus* (Nematoda: Longidoridae). *Systematic Parasitology, 32*, 33–52. https://doi.org/10.1007/BF00009466

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

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

Rubtsova, T. V., Chizhov, V. N., & Subbotin, S. A. (1999). *Longidorus artemisiae* sp. n. (Nematoda: Longidoridae) from roots of *Artemisia* sp., Rostov region, Russia. *Russian Journal of Nematology, 7*, 33–38.

Subbotin, S., Waeyenberge, A. L., & Moens, M. (2000). Identification of cyst forming nematodes of the genus *Heterodera* (Nematoda: Heteroderidae) based on the ribosomal DNA-RFLPs. *Nematology, 2*, 153–164. https://doi.org/10.1163/156854100509042

Taylor, C. E., & Brown, D. J. F. (1997). *Nematode vectors of plant viruses*. Wallingford, UK: CAB International.

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

Trisciuzzi, N., Archidona-Yuste, A., Troccoli, A., Fanelli, E., De Luca, F., Vovlas, N., & Castillo, P. (2015). Description of new needle nematode, *Longidorus asiaticus* n. sp. (Nematoda: Longidoridae), from the rhizosphere of crape myrtle (*Lagerstroemia indica*) bonsai trees imported into Italy from China. *European Journal of Plant Pathology*, *143*, 567–580. https://doi.org/10.1007/S10658-015-0710-Z

Vrain, T. C., Wakarchuk, D. A., Lévesque, A. C., & Hamilton, R. I. (1992). Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology, 15*, 563–573.

Xu, Y.-M., & Zhao, Z.-Q. (2019). *Longidoridae and Trichodoridae (Nematoda: Dorylaimida and Triplonchida*). *Fauna of New Zealand, 79*. Lincoln, New Zealand: Landcare Research.

Table 1 Morphometrics of *Longidorus sabalanicus* n. sp. from north-western Iran. All measurements are in µm (except L in mm), and in the form: mean ± standard deviation (range).

Characters/ratios *	East Azarbaijan population			Zanjan population		East Azarbaijan population			
	Female		Male	Female	Male	Juvenile Paratypes			
	Holotype	Paratypes	Paratypes			J1	J2	J3	J4
n		8	5	3	6	2	6	2	3
L	4.4	4.4 ± 0.37 (4.0–5.1)	4.4 ± 0.18 (4.2–4.7)	4.0 ± 0.34 (3.8–4.4)	3.8 ± 0.21 (3.6–4.2)	1.8 ± 0.00 (1.8–1.8)	2.5 ± 0.2 (2.2–2.8)	3.2 ± 0.00 (3.2–3.2)	3.6 ± 0.03 (3.6–3.7)
a	76.6	68.6 ± 4.68 (61.6–75.9)	80.4 ± 4.26 (74.1–84.5)	66.1 ± 2.05 (63.7–67.3)	71.3 ± 9.15 (60.1–82.4)	51.2 ± 7.77 (45.7–56.7)	61.0 ± 4.8 (55.4–66.4)	66.3 ± 12.23 (57.6–74.9)	70.0 ± 6.96 (65.8–78.1)
b	11.7	10.3 ± 1.38 (8.7–13.0)	10.0 ± 0.49 (9.4–10.7)	9.5 ± 0.64 (8.9–10.2)	9.2 ± 0.23 (8.9–9.6)	6.0 ± 0.71 (5.5–6.5)	7.7 ± 0.83 (6.4–8.7)	10.6 ± 1.34 (9.6–11.5)	9.7 ± 0.31 (9.3–10.0)
c	105.9	98.7 ± 6.24 (89.8–108.9)	90.8 ± 7.77 (80.8–99.6)	97.8 ± 7.32 (93.2–106.2)	86.5 ± 5.67 (81.6–97.3)	41.8 ± 1.63 (40.6–42.9)	54.9 ± 5.15 (48.7–62.1)	72.8 ± 3.67 (70.2–75.4)	75.1 ± 9.93 (64.0–82.9)
c′	1.0	1.1 ± 0.07 (1.0–1.2)	1.1 ± 0.06 (1.1–1.2)	1.0 ± 0.06 (0.9–1.1)	1.1 ± 0.08 (1.0–1.2)	1.7 ± 0.28 (1.5–1.9)	1.5 ± 0.16 (1.4–1.7)	1.25 ± 0.07 (1.2–1.3)	1.2 ± 0.27 (1.1–1.5)
V or T	51.5	51.2 ± 1.36 (50.0–54.2)	47.8 ± 1.74 (45.8–50.2)	51.7 ± 1.00 (50.8–52.7)	–	–	–	–	–
Lip region width	14	13.6 ± 0.46 (13.0–14.5)	14.0 ± 0.43 (13.5–14.5)	12.9 ± 0.40 (12.5–13.5)	13.0 ± 0.58 (12.5–14.0)	10.0 ± 1.41 (9–11)	11 ± 0.63 (10–12)	12.8 ± 0.35 (12.5–13.0)	13.1 ± 0.22 (13.0–13.5)
Odontostyle length	78	81.2 ± 2.96 (75.5–85.5)	78.0 ± 3.75 (73–83)	79.4 ± 1.35 (78–81)	76.6 ± 2.07 (74.0–78.5)	57.0 ± 4.24 (54–60)	60.8 ± 3.14 (56–65)	66.5 ± 3.53 (64–69)	71.9 ± 2.09 (70–73)
Odontophore length	54	58.1 ± 5.05 (50–65)	56.2 ± 4.49 (50–60)	62.0 ± 4.76 (58.5–67.5)	56.9 ± 4.25 (51.5–61.0)	44.0 ± 1.41 (43–45)	47.3 ± 5.21 (40–54)	50.5 ± 3.53 (48–53)	51.3 ± 5.59 (45–55)
Replacement odontostyle length	–	–	–	–	–	65.0 ± 2.83 (63–67)	71.4 ± 4.35 (66–76)	74.0 ± 0.00 (74–74)	80.1 ± 1.92 (78–82)
Total stylet length	132	139.4 ± 6.15 (130–147)	134.2 ± 2.70 (131.0–137.5)	141.4 ± 5.8 (138–148)	133.5 ± 5.13 (128–139)	101.0 ± 2.83 (99–103)	108.2 ± 5.85 (100–115)	117.0 ± 0.00 (117–117)	123.2 ± 7.52 (115–128)
Oral aperture-guiding ring distance	28	28.6 ± 0.69 (28–30)	29.3 ± 0.93 (28.0–30.5)	27.4 ± 1.85 (26.0–29.5)	26.8 ± 0.98 (25–28)	18.8 ± 0.35 (18.5–19.0)	21.9 ± 0.72 (21.5–22.5)	23.3 ± 2.47 (21.5–25.0)	26.3 ± 0.93 (26.0–27.5)
Pharynx length	380	430.1 ± 45.65 (348–485)	445.2 ± 7.04 (438–455)	425.6 ± 9.20 (417–435)	418.0 ± 25.52 (390–458)	304.0 ± 33.94 (280–328)	336.0 ± 31.13 (305–388)	310.0 ± 42.42 (280–340)	379.6 ± 16.65 (362–395)

Basal pharyngeal bulb length	84	103.6 ± 8.84 (85–111)	105.2 ± 6.79 (95–111)	94.1 ± 2.61 (91–97)	93.5 ± 7.17 (82–102)	68.5 ± 2.12 (67–70)	76.3 ± 6.85 (67–86)	91.5 ± 19.09 (78–105)	90.5 ± 5.23 (85–95)
Basal pharyngeal bulb width	24	25.4 ± 1.75 (23.5–29.0)	23.4 ± 1.14 (22–25)	23.2 ± 0.80 (22–24)	21.5 ± 1.54 (19–23)	15.5 ± 0.71 (15–16)	21.5 ± 2.17 (18–22)	22.0 ± 1.41 (21–23)	23.4 ± 1.33 (23–25)
Body diameter at pharynx base	48	50.9 ± 3.78 (47–56)	47.9 ± 1.96 (45–49)	48.1 ± 4.10 (45–53)	46.2 ± 6.34 (40–55)	30.5 ± 0.70 (30–31)	37.6 ± 2.52 (34–40)	42.0 ± 2.83 (40–44)	45.1 ± 4.36 (41–49)
-at mid-body	58	64.6 ± 5.98 (57.5–73.0)	55.6 ± 3.64 (51–60)	61.6 ± 7.25 (57–70)	55.2 ± 8.66 (46.0–66.5)	36.0 ± 5.65 (32–40)	42.5 ± 3.1 (38.5–46.0)	49.8 ± 8.13 (44.0–55.5)	52.9 ± 4.66 (47.5–56.0)
-at anus	42	41.8 ± 1.64 (39.0–43.5)	42.4 ± 2.07 (40–45)	39.4 ± 2.68 (37.5–42.5)	39.4 ± 4.29 (35–45)	25.3 ± 4.60 (22.0–28.5)	30.4 ± 3.18 (27.5–35.0)	34.5 ± 0.71 (34–35)	38.5 ± 2.27 (36.5–41.0)
-at guiding ring level	24	23.5 ± 0.54 (22.5–24.0)	23.8 ± 0.20 (23.5–24.0)	22.6 ± 1.26 (21.5–24.0)	22.0 ± 0.95 (20.5–23.5)	16.8 ± 3.18 (14.5–19.0)	18.1 ± 0.84 (17–19)	20.5 ± 0.71 (20–21)	20.2 ± 0.52 (20–21)
Prerectum length	393	396.0 ± 84.83 (250–507)	–	311.0 ± 76.36 (257–365)	–	–	–	–	–
Rectum length	33	32.5 ± 2.81 (29–38)	–	30.5 ± 0.70 (30–31)	–	–	–	–	–
Tail length	42	44.8 ± 3.02 (41–51)	49.3 ± 3.85 (45–55)	41.5 ± 0.75 (41–42)	44.8 ± 2.20 (41.5–48.5)	43.5 ± 2.12 (42–45)	47.3 ± 2.95 (43–51)	45.0 ± 1.41 (44–46)	49.5 ± 6.54 (45–57)
Hyaline tail region length	16	14.5 ± 1.27 (12–16)	15.6 ± 1.51 (14–18)	13.6 ± 0.28 (13.5–14.0)	12.8 ± 3.14 (10–18)	19.0 ± 1.41 (18–20)	11.1 ± 0.83 (10.0–12.5)	12.5 ± 0.71 (12–13)	10.4 ± 0.46 (10–11)
Spicule length	–	–	63.5 ± 3.60 (60.0–68.5)	–	57.6 ± 2.52 (54–61)	–	–	–	–

*Abbreviations: n = number of examined specimens; 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 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.

Figure legends

Fig. 1 Line drawings of *Longidorus sabalanicus* n. sp. A: Female anterior region. B: Female reproductive system. C: Anterior end showing amphidial fovea. D: Male posterior body region. E, F: Female tail. G: J1 tail. H: J2 tail. I: J3 tail. J: J4 tail. Abbreviations: Scale bars A, B, D-J= 40 μm; C = 10 μm.

Fig. 2 Photomicrographs of *Longidorus sabalanicus* n. sp. A, B: Complete female and male. C: Female anterior region. D: Female anterior region with detail of odontostyle. E: Female lip region with detail of amphidial fovea. F: Detail of female basal bulb. G: Vulval region. H: Female posterior region showing prerectum. I: Female tail. J: Male posterior region. K: Male tail. L: Detail of sperm cells. Abbreviations: a = anus; af = amphidial fovea; as = adanal supplement; bb = basal bulb; c = cloaca; gr = guiding ring; Odt = odontostyle; sp = spicules; spc = sperm cells; svn = ventrosublateral nuclei; V = vulva. (Scale bars: A-L = 20 μm).

Fig. 3 Photomicrographs of *Longidorus sabalanicus* n. sp. A-D: tails of J1, J2, J3, and J4. Abbreviations: a = anus. (Scale bars: A-D = 10 μm).

Fig. 4 Relationship between body length, and functional and replacement odontostyle length in all developmental stages and mature adults of *Longidorus sabalanicus* n. sp.

Fig. 5 Phylogenetic relationships of *Longidorus sabalanicus* n. sp. 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 bold. Scale bar = expected changes per site.

Fig. 6 Phylogenetic relationships of *Longidorus sabalanicus* n. sp. within the genus *Longidorus*. Bayesian 50% majority rule consensus tree as inferred from 18S rRNA sequence alignment under the GTR + I+ G model. Posterior probabilities more than 0.70 are given for

appropriate clades. Newly obtained sequences in this study are shown in bold. Scale bar = expected changes per site.