Morphological and molecular characterisation of *Longidorus tabrizicus* sp. n. and *L. sturhani* Rubtsova, Subbotin, Brown and Moens, 2001 (Nematoda: Longidoridae) from north-western Iran

Gholamreza Niknam[1], Majid Pedram[1,2], Elmira Ghahremani Nejad[1], Weimin Ye[3], Robert T. Robbins[4] and Zahra Tanha Maafi[5]

[1] Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Tabriz, Iran; e-mail: g_niknam@tabrizu.ac.ir
[2] College of Agriculture, University of Tarbiat Modarres, Tehran, Iran
[3] Nematode Assay Section, North Carolina Department of Agriculture, Raleigh, NC, USA
[4] Department of Plant Pathology, University of Arkansas, Fayetteville, AR, USA
[5] Plant Pests and Diseases Research Institute, P.O. Box 1454-Tehran, 19395, Iran

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Summary. *Longidorus tabrizicus* sp. n., an amphimictic species is described based on two populations recovered from soil samples collected from the rhizosphere of apple trees in Namin, Ardebil province and the rhizosphere of *Rosa* sp. from Maragheh, East Azarbaijan province, Iran in a population mixed with *L. sturhani*. The new species is characterised by having anteriorly flattened lip region separated from the rest of the body by a shallow depression, medium body length (4.2-6.1 mm), pocket-shaped amphidial pouches without distinct basal lobes, short odontostyle (61.5-69.0 µm) and odontophore (43.0-54.0), a bluntly conoid tail, three juvenile stages and males common equal in number to females. The new species most closely resembles 11 known species, namely *L. moniloides, L. globulicauda, L. ampullatus, L. auratus, L. makatinus, L. kakamus, L. rotundicaudatus, L. pini, L. sylphus, L. bernardi* and *L. sturhani*. Phylogenetic trees based on sequences of the 18S and ITS1 rDNA regions representing the relationships of *L. tabrizicus* n. sp. and all other species of *Longidorus* Micoletzky, 1922 with available sequences of the same regions are presented. *Longidorus sturhani* is the closest to the new species regarding both morphological and molecular characters. The Iranian population of *L. sturhani* is similar to the European populations (according to original description) but has a wider diameter at mid-body and smaller a ratio.

Key words: 18S rDNA, ITS1, Longidoridae, nematodes, phylogeny.

During 2006 and 2007, an extensive survey for collecting members of Longidoridae in north-western Iran was conducted. As a result, several known species of the genera *Longidorus* Micoletzky, 1922 and *Xiphinema* Cobb, 1913 were identified including *L. crassus* (Thorne, 1974) Robbins & Brown, 1995, *L. elongatus* (de Man, 1876) Thorne & Swanger, 1936, *L. euonymus* Mali & Hooper, 1974, *L. leptocephalus* Hooper, 1961, *L. profundorum* Hooper, 1965, *L. protae* Lamberti & Bleve-Zacheo, 1977, *L. proximus* Sturhan & Argo, 1983, *X. aceri* Chizhov, Tiev & Turkina, 1986, *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939, *X. index* Thorne & Allen, 1950, *X. macedonicum* Barsi & Lamberti, 1999, *X. montenegrinum* Barsi, Lamberti & Agostinelli, 1998, *X. pyrenaicum* Dalmasso, 1969 and *X. vuittenezi* Luc, Lima, Weischer & Flegg, 1964. Two new species of *Xiphinema* (*X. robbinsi* Pedram, Niknam & Decraemer, 2008; *X. iranicum* Pedram, Niknam, Robbins, Ye & Karegar, 2009) and one new species of *Longidorus* (*L. kheirii* Pedram, Niknam, Robbins, Ye & Karegar, 2008) from this survey were previously described (Pedram *et al*., 2008a, b, 2009).

One sample from Goshayeesh village near Maragheh city, East Azarbaijan, north western Iran yielded two species of *Longidorus* separated on the basis of odontostyle length and presence of males. Another sample was collected later from Namin, Ardebil province, north western Iran, from the rhizosphere of apple trees. This population was found to be similar to one of Maragheh mixed population. The new species *Longidorus tabrizicus* n. sp. was erected on the basis of the Namin

population, since it was not mixed and included females, males and juveniles. The Maragheh population was considered to be a second population.

In this article, the morphological and molecular characters of the new species and the Iranian population of *L. sturhani* are provided and their phylogenetic relationships with each other and all other *Longidorus* species for which sequences are available, are discussed.

MATERIAL AND METHODS

Soil samples were collected from the rhizosphere of apple trees from Namin at a depth of 20-30 cm during 2007-2008 and from the rhizosphere of wild *Rosa* sp. at the same depth during 2006-2007. Nematodes were extracted by suspending the soil samples in water and collected by using sieves with 60 (250 µm aperture), 100 (150 µm aperture), and 200 (74 µm aperture) meshes. For morphological studies nematodes collected on the last two sieves were hand picked under a stereomicroscope and fixed by adding boiling 4% formaldehyde solution. The fixed nematodes were processed to anhydrous glycerin according to the method of De Grisse (1969) and mounted on permanent slides. Measurements were obtained using an Olympus BX-41 light microscope. All measurements are in micrometers, except for the body length and the distance from the anterior end to the vulva, which are in millimeters. Digital images were captured using a DP50 digital camera attached to the microscope. Photos were processed by Adobe® Photoshop® CS2 software. Drawings were done using Corel® Draw (12) and Microsoft® Office® Picture Manager software.

For molecular study, a single nematode was picked into distilled water and its morphological identity was confirmed with light microscopy before being placed into 50µl of worm lysis buffer (WLB) containing Proteinase K for DNA extraction (Williams et al., 1992) and then crushed with a pipette tip. DNA samples were stored at -20°C until used as a PCR template. Primers for DNA amplification are the same as described in Pedram et al. (2008 a, b, 2009). The 25-µl PCR contained 12.5-µl 2x GoTaq DNA polymerase mix (Promega Corporation, Madison, WI, USA), 1 µl each of a 0.4 µM forward and reverse primers solution, and 1 µl of DNA template. The thermal cycling programme was as follows: denaturation at 95ºC for 6 min, followed by 35 cycles of denaturation at 94ºC for 30 s, annealing at 55ºC for 30 s, and extension at 72ºC for 1 min. A final extension was performed at 72ºC for 10 min. DNA sequencing was performed using PCR primers for direct sequencing by dideoxynucleotide chain termination using an ABI PRISM BigDye terminator cycle sequencing ready reaction kit in an Applied Biosystems 377 automated sequencer (both Applied Biosystems, Foster City, CA, USA) by MWG-BIOTECH Inc. (Huntsville, Alabama, USA). The sequences were deposited into the GenBank database. DNA sequences were aligned by Clustal W (http://workbench.sdsc.edu, Bioinformatics and Computational Biology group, Dept. Bioengineering, UC San Diego, CA). The molecular sequences of *Longidorus tabrizicus* sp. n. were compared with those of the other nematode species available at the GenBank sequence database using the BLAST homology search program. The model of base substitution was evaluated using MODELTEST (Posada & Crandall, 1998; Huelsenbeck & Ronquist, 2001). The Akaike-supported model, the base frequencies, the proportion of invariable sites and the gamma distribution shape parameters and substitution rates were used in phylogenetic analyses. Bayesian analysis was performed to confirm the tree topology for each gene separately using MrBayes 3.1.0 (Huelsenbeck & Ronquist, 2001) running the chain for 1 x 10^6 generations and setting the 'burnin' at 1,000. The Markov Chain Monte Carlo (MCMC) method was used within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget & Simon, 1999) using 50% majority rule. Only the last Bayesian tree (10001st tree) was selected to represent the phylogenetic relationships with branch length and support level from consensus tree.

DESCRIPTION

Longidorus tabrizicus sp. n. (Figs. 1-5; Table 1)

Female (holotype). When heat relaxed, body assuming an open C shape, cylindrical, and tapering very slightly anteriorly. Cuticle with very fine transverse striations most clearly visible at tail region and distinctly two-layered, outer layer 1.3 µm and inner one 2.0 µm thick at postlabial (mid-distance between anterior end and guiding ring) region varying to 1.7 µm and 0.8 µm at mid-body (dorsal part at vulva region) and 1.2 µm and 2.5 µm at post-anal region, ventrally. Body pores not seen. Lip region anteriorly flattened and separated from rest of body by very shallow depression. Amphidial pouches pocket-shaped without lobes at base. Guiding ring 3.8 µm wide. Odontostyle short,

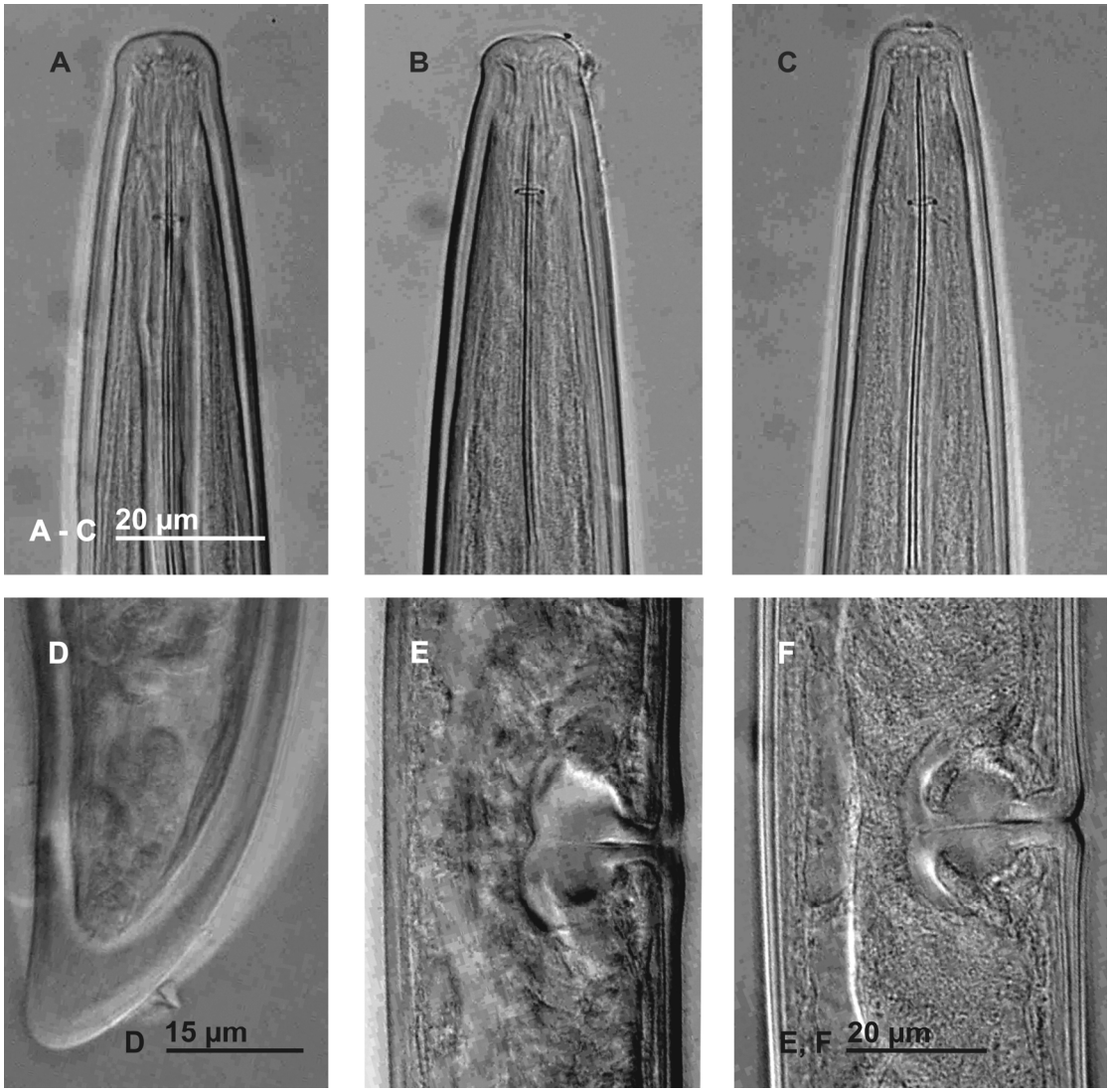

Fig.1. *Longidorus tabrizicus* sp. n. A - C: Anterior end; A: Holotype female, B: Paratype male, C: Paratype female (Maragheh population); D: Tail of holotype female; E & F: Two parts of the vagina; E: Paratype female (Maragheh population), F: Holotype female.

1.3 µm wide at base, odontophore base without swellings. Nerve ring 153 µm from anterior end. Hemizonid at level of odontophore base. Oesophagus dorylaimoid. The DN located at 36.3% and RS1N and LS1N at relatively same level and 54.0% of length of oesophageal bulb (Loof & Coomans, 1972). Cardia semispherical. Vulva a transverse slit, vagina expands inwardly up to 57.4% of corresponding body width and composed of two parts (De Ley et al., 1993). *Pars proximalis vaginae* (proximal section with hyaline walls) 12.5 x 15.5, and *pars distalis vaginae* (distal section continuous with body cuticle) 11.2 µm long. Female reproductive system amphidelphic, each branch includes wide tubular thin-walled uterus (sperm observed in some paratype females in both populations), sphincter between *pars dilatata uteri* and *pars dilatata oviductus*, reflexed oviduct and ovary 76 µm long (posterior tract). Pre-rectum 179.5 µm and rectum 20 µm long. Tail bluntly conoid, dorsally convex and ventrally almost straight to slightly concave. Caudal pores not observed.

Male. Functional, equal to females in number, similar to females in general morphology, except for the reproductive system and posterior end being more strongly ventrally curved. The reproductive system composed of two reflexed testes, massive sclerotized spicules 8-14 µm wide and lateral accessory piece 10.5-14.0 µm. Tail conical, dorsally convex and ventrally concave, variable in shape (Fig. 3, D-F). Two precloacal pairs preceded

Fig. 2. *Longidorus tabrizicus* sp. n. Juvenile stages (J1-J3); A-C: Anterior end and D-F: Tail shapes.

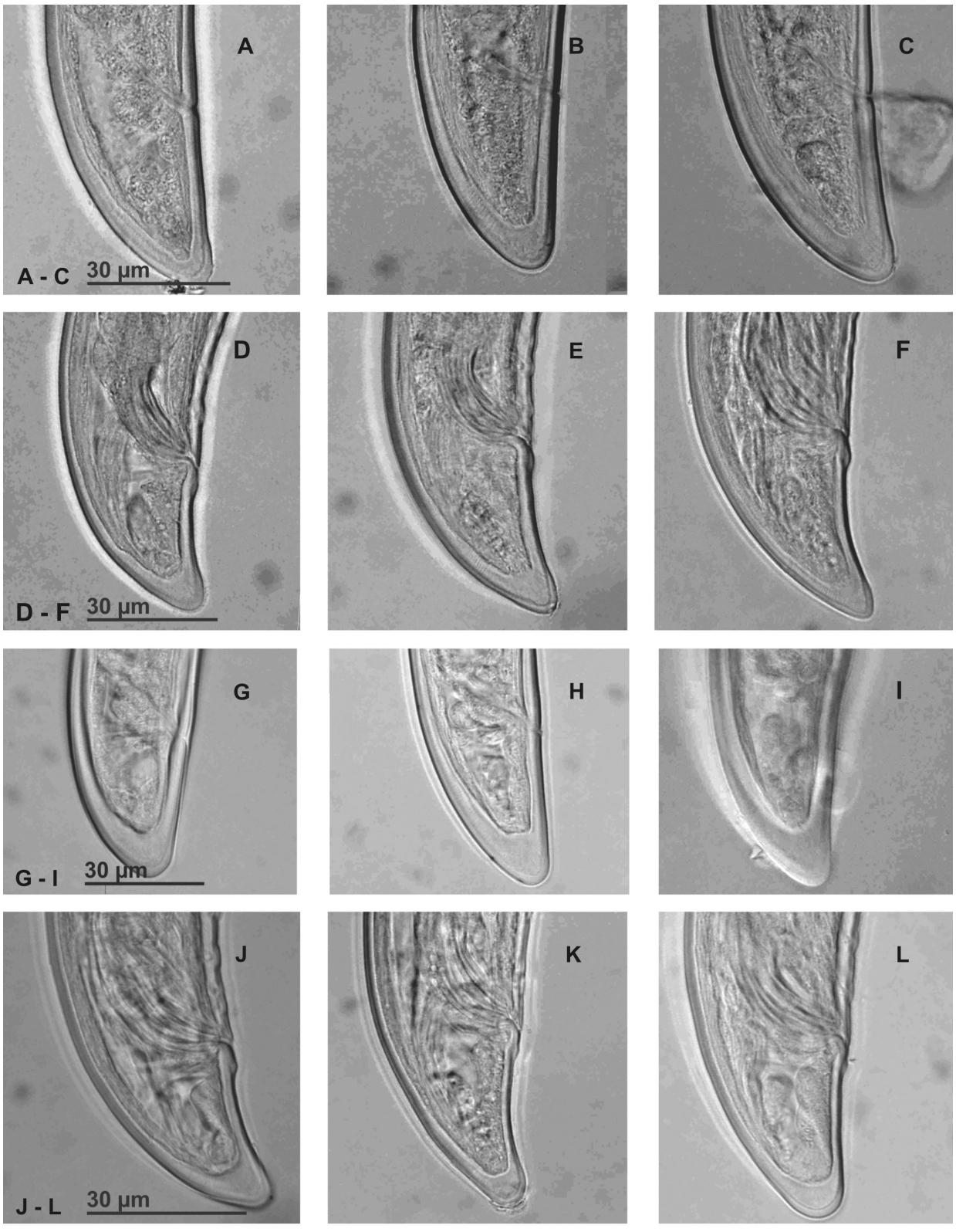

Fig. 3. *Longidorus tabrizicus* sp. n. Tail variations in two populations. A-F: Common tail shapes in Namin population, A-C: Female tails; D-F: Male tails, G-L: Common tail shapes in Maragheh population, G-I: Female tails, J-L: Male tails.

Table 1. Morphometrics of *Longidorus tabrizicus* n. sp. All measurements in µm (except given characters in mm), and in the form: mean±s.d. (range). VM: Ventromedian supplements.

Character	Namin population						Maragheh population	
	Holotype ♀	Paratype females	Paratype males	J1	J2	J3	Females	Males
N	1	18	15	2	15	8	5	5
L (mm)	4.6	5.0±0.5(4.2-6.1)	4.7±0.4(4.3-5.6)	1.7, 2.0	2.2±0.3(1.7-2.7)	3.5±0.3(3.2-4.0)	4.3±0.2(4.1-4.7)	4.7±0.3(4.2-5.2)
a	121.7	107.0±12.4(81.5-135.0)	107.0±12.5(81.5-134.0)	67.3, 71.4	73.0±6.5(65.5-88.5)	83.7±7.0(73-92)	101.5±4.5(95-105)	104.5±8.2(91-113)
b	14.7	15.6±1.4(13.5-18.5)	15.0±1.8(12.5-18.5)	7.0, 8.2	8.5±1.0(7-11)	12.8±1.8(10-15)	13.0±1.1(11.7-14.5)	13.3±0.9 (12-14)
c	109	116±18(91-155)	109±12(92.7-131.0)	43.0, 44.5	55±8(46-75)	83.5±9(68.7-94)	114.3±11.5(100.5-132.0)	110±16(90.5-129.0)
c`	1.5	1.4±0.2(1.1-1.7)	1.4±0.2(1.2-1.8)	2, 2	1.7±0.2(1.4-2.0)	1.5±0.2(1.1-1.8)	1.3±0.1(1.1-1.4)	1.3±0.1(1.2-1.4)
V	48.4	49.4±2.3(41.0-53.5)	–	–	–	–	47.3±1.0(45.5-48.3)	–
Body width at base of oesophageal bulb	33	36.7±3.4(30.5-45.0)	35.0±2.6(30.5-40.0)	23, 27	26.0±2.5(23.0-32.5)	35±5(30-42)	34.7±1.7(32.0-36.3)	35.0±1.2(33.5-36.5)
Body width at anterior of oesophageal bulb	32	35.5±3.0(30.5-43.0)	34.2±2.7(29.5-39.0)	22.5, 26.5	25.0±2.5(23-32)	34±4(30.5-40.5	34.0±1.3(32-35)	34.0±0.9(32.5-35.0)
Anterior end to vulva (mm)	2.2	2.5±0.3(1.9-3.0)	–	–	–	–	2.0±0.1(2.0-2.2)	–
Anterior end to base of oesophageal bulb	314.5	330.0±40.5(252.5-451.0)	333.5±38.5(259-400)	238.0, 241.5	241±18(232-289)	294±36(254-343)	332.5±19.0(319-365)	351.5±20.0(327-375)
Odontostyle	63	65.5±2.5(61.5-69.0)	66.0±1.8(63.0-70.5)	40.0, 41.5	48±2.8(45.5-54)	57±3(52-60)	68.5±1.6(66.5-70.0)	70.0±1.2(69-72)
Odontophore	54	48±3(43-54)	46.7±4.5(38-52)	39.5	40±3.0(39.5-49.5)	45.5±2.5(42.5-49.0)	51.0±3.5(46.5-55.0)	54±3(50.5-57.5)
Replacement odontostyle	-	–	–	49.5, 50.5	52.5±3.5(50-62)	64.0±2.5(60-67)	–	–
Anterior end to guiding ring	23	23.2±1.8(19.5-27.0)	23.5±1.2(21.3-25.0	15, 16.5	16.5±1.5(15.0-19.5)	20.5±1.0(19.5-22.0)	25±2(22.5-27.5)	23.5±1.7(20.7-25.0)
Body width at guiding ring	17.5	18.2±1.0(16.0-20.5)	17.5±0.7(16.3-19.0)	13.8	13.5±0.5(13-15.5)	16.0±0.7(15-19)	19.0±0.7(18-20)	18.5±0.6(18.0-19.5)
Body width at mid-body	38	47.2±5.7(38-63)	42.0±3.4(38.0-49.5)	-	33±2.7(29.5-35)	43.5±4.5(38-50)	43.0±3.8(39-47)	44.5±3.7(40.5-50.4)
Width of lip region	12	12.0±0.7(10.5-13.0)	12.2±0.4(11.3-12.5)	8.8, 9.5	9.0±0.5(8.5-10)	10.5±0.5(10-11.5)	12.5±0 (12.5)	12.7±0.7(12-14)
Width of oesophageal bulb	15	18.3±2.0(15.0-22.5)	17.0±1.6(15-20)	12.5, 14.5	14.0±1.5(12.5-17.0)	15.5±1.5(13.5-17.0)	17.5±1.5(15.5-19.0)	17.0±1.3(15.5-19.0)
Length of oesophageal bulb	75	81.0±8.7(64-98)	80.0±8.5(68-99)	56.5, 57.5	59.5±7.0(50.5-75.0)	71.0±7.5(63-80)	70.0±2.5(67-73)	80.5±4.0(77-87)
Body width at anus level	28.8	31.5±2.7(27.5-37.5)	30.5±1.5(28-33)	19.5, 21.5	20.5±1.8(19-25)	29.5±3.0(26-34)	29.5±1.5(28-32)	33.0±0.5(32.5-34.0)
Tail length	42.5	43.5±4.5(34-53)	43.2±4.0(38.0-50.5)	39.5, 44.0	38±3(35.5-48.0)	44.0±6.5(38.0-51.5)	38.0±3.8(34-43)	43±4(39.0-47.5)
Length of hyaline part of tail	10	10.0±1.5(7.5-12.5)	10.0±2.3(7.5-14.0)	6.5, 6.5	7.5±1.0(5.5-9.0)	9.0±1.5(7.0-11.5)	12.5±1.2(11.5-14.5)	9.0±2.5(5.5-12.0)
Length of spicules	–	–	45.2±2.3(41.5-49.0)	–	–	–	–	44.0±4.5(36.5-47.5)
Copulatory supplements	–	–	2 precloacal pairs + 8-10 VM	–	–	–	–	2 precloacal pairs + 7-11 VM

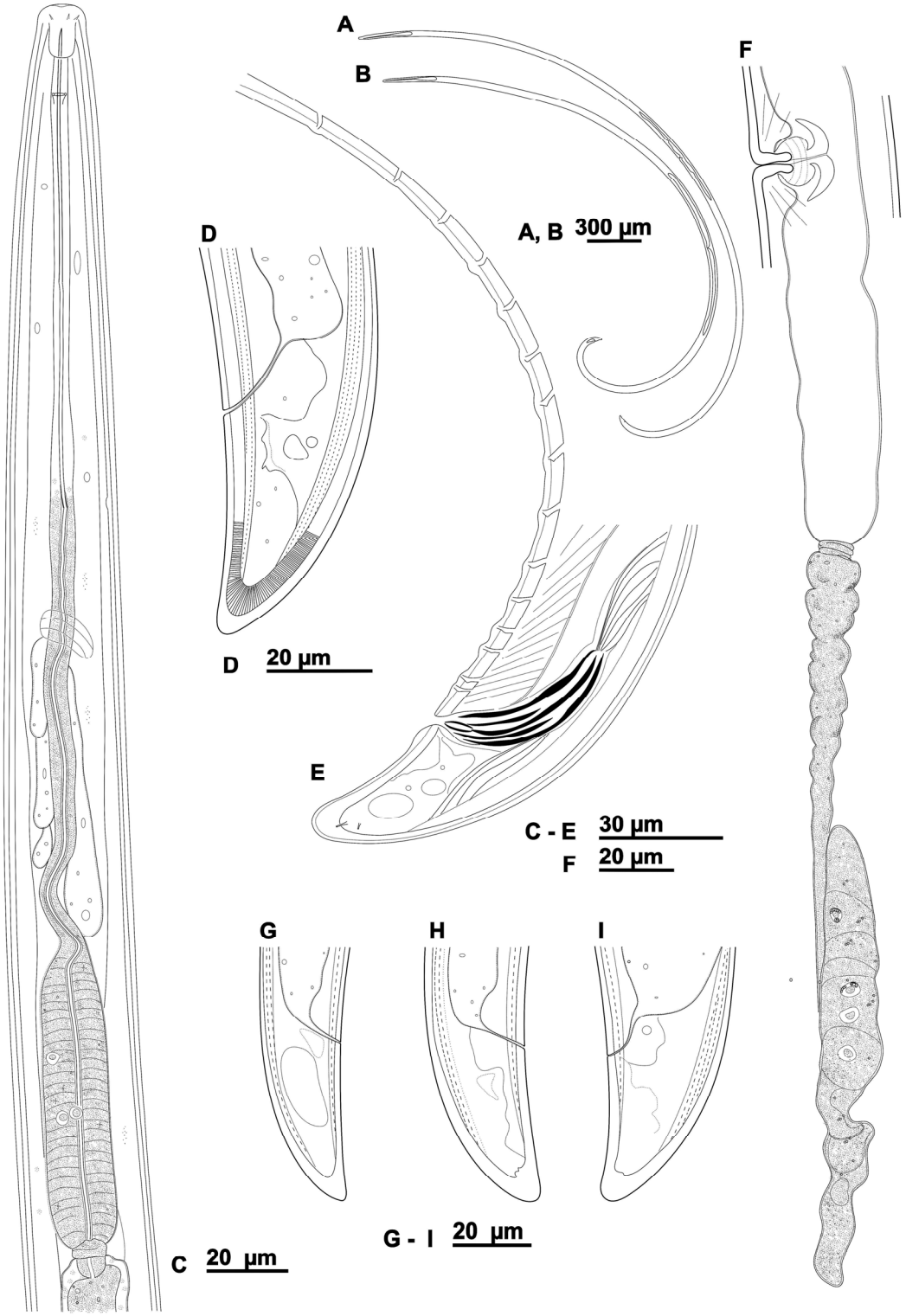

Fig. 4. *Longidorus tabrizicus* sp. n. A & B: Habitus, A: Holotype female and B: Paratype male, C, D & F: Holotype female; C: Anterior part, D: Tail and F: Posterior genital tract, E: Posterior part of paratype male, G-I: Tails of J1-J3, respectively.

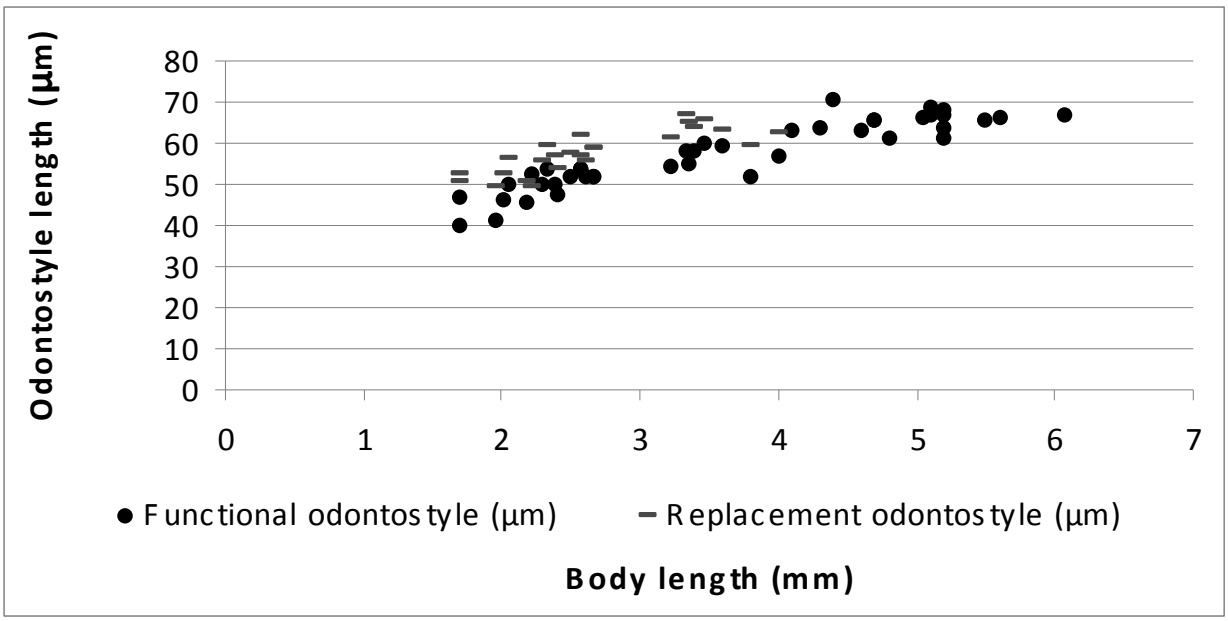

Fig. 5. *Longidorus tabrizicus* sp. n. Scatter plot of the functional and replacement odontostyle length in relation to the body length of the juvenile stages and females.

by a row of 8-10 ventromedian supplements in ventral region.

Juveniles. The common method for identifying the juvenile stages of *Longidorus* (Robbins et al., 1995) was used to separate juvenile stages of the new species. The new species has three juvenile stages, and the relation of the functional and replacement odontostyle length to the body length is given in Fig. 5. First-stage juveniles (J1) are identified by the tip of the replacement odontostyle being embedded in the odontophore base; the length of the replacement odontostyle in each stage is relatively equal with the length of functional odontostyle of the following stage; for the rest of the juvenile stages, the replacement odontostyle is located at some distance posterior to the odontophore base. The labial region in all juvenile stages similar to that of mature females; tails conical in shape; in J1 and J2 tails are similar but narrower in J1 and without a terminal peg; tail terminus broader in J3 than in J2.

Molecular characterization and relationship with other species. Nearly full length of 18S and ITS1 rDNA of *L. tabrizicus* sp. n. (M52 in the trees) and *L. sturhani* (M51) collected from the same sample as well as the Maragheh population of the new species (M30) were sequenced for molecular analysis. All other *Longidorus* species with sequences of the same regions available in the GenBank were used for generating phylogenetic trees. Fig. 7 represents a phylogenic tree based on a nearly full length of 18S rDNA from a multiple alignment of 1,787 total characters. This dataset has 1,618 constant characters (90.5%), 78 parsimony-uninformative sites (4.4%) and 91 parsimony-informative sites (5.1%). Two populations of *L. tabrizicus* n. sp. have 6 bp differences, 1bp insertion from 705 bp sequenced fragment and are grouped in a highly supported clade with 100% posterior probability. This species comes closest to *L. sturhani* with 69% support. *Longidorus tabrizicus* sp. n. is also close to *L. kheirii* and *L. profundorum*. All four species (collected in Iran in our previous survey (Pedram *et al*., 2008b)) are in a monophyletic clade with 56% support next to *L. euonymus*. Figure 8 represents a phylogenic tree based on ITS1 rDNA from a multiple alignment of 1,866 total characters. This DNA fragment is much more variable than 18S. The dataset has only 638 constant characters (34.2%), 242 parsimony-uninformative sites (13.0%) and 986 parsimony-informative sites (52.8%). This tree resolved more highly supported monophyletic clades than 18S tree. The sequences of ITS1 were identical in the two populations of *L. tabrizicus* n. sp., the closest inferred relative was also *L. sturhani* in a clade with 100% support. *Longidorus kheirii* is separated from the two populations of *L. tabrizicus* n. sp. and is a sister to this clade. This tree revealed that *L. elongatus* and

L. profundorum were closer to *L. tabrizicus* n. sp. than the rest of the available *Longidorus* species.

Diagnosis. The new species is unique in the genus by having the following set of characters: medium body length (4.2-6.1 mm), short odontostyle (61.5-69.0 µm) and odontophore (43-53 µm), lip region flattened and separated from rest of the body by a very slight depression, amphidial pouch not bilobed at the base, tail bluntly conoid, males functional with two precloacal supplement pairs and distinct sequences of 18S and ITS1 rDNA regions. The identification codes according to the polytomous key of Chen *et al.* 1997 are: A2, B12, C12, D3, E1, F2(3), G2(3), H23, I2.

Type-locality and habitat. The type population was collected by third author from the rhizosphere of apple trees in Namin city, Ardebil province, north-western Iran, co-ordinates: 38º25,704'N; 8º27.303' E. The second population was collected from the rhizosphere of *Rosa* sp. naturally growing in mountains of Goshayesh village close to Maragheh city, East Azarbaijan province, north-western Iran, co-ordinates: 37º19.322′N; 46º20. 517′E.

Type-material. Holotype female (Acc. No. IRL 101), ten paratype females, ten paratype males and juveniles are deposited in the Nematode Collection of Faculty of Agriculture, University of Tabriz, Tabriz, Iran. Two paratype females and one paratype male are deposited at the USDA Nematode Collection, Beltsville, Maryland, USA. One female and one male are in the University of California Nematode Collection, Riverside, California, USA; one paratype female is in the Canadian National Nematode Collection, Ottawa, Canada; two paratype females and one male are in the CABI Bioscience, UK Centre, Surrey, UK. One female and one male from the *Rosa* sp. specimens from near Goshayesh village are also deposited in the USDA collection.

Etymology. Named after Azam Shojaee Tabrizi respecting her kind help while our sampling.

DISCUSSION

By morphometric characters of its two studied populations, *L. tabrizicus* sp. n. closely resembles 11 known *Longidorus* species, namely *L. moniloides* Heyns, 1966, *L. globulicauda* Dalmasso, 1969, *L. ampullatus* Jacobs & Heyns, 1987, *L. auratus* Jacobs & Heyns, 1987, *L. makatinus* Jacobs & Heyns, 1987, *L. kakamus* Jacobs & Heyns, 1987, *L. rotundicaudatus* Jacobs & Heyns, 1987, *L. pini* Andres & Arias, 1987, *L. sylphus* (Thorne, 1939) Robbins & Brown, 1995, *L. bernardi* Robbins & Brown, 1996 and *L. sturhani* Rubtsova, Subbotin, Brown & Moens, 2001. The most similar to the new species is *L. sturhani,* which could be distinguished from it by having a longer odontostyle (77-96 *vs* 61.5-69.0 µm) and absence *vs* presence of functional males (sperm observed in the female genital tracts) in two populations of the new species. Also, juveniles of *L. sturhani* (J1 and J4) have longer functional and replacement odontostyles when compared to the JI and J3 of *L. tabrizicus* n. sp.

In comparison with *L. moniloides*, the new species has a longer body (4.2-6.1 *vs* 3.25-4.14 mm), odontophore (43-54 *vs* 25-33 µm) and tail (34-53 *vs* 27-37 µm), a higher a ratio (81.5-135.0 *vs* 68-88), and differently shaped tail in males (males of *L. moniloides* have a short broad peg in their tail end). Compared to *L. globulicauda*, the new species has amphidial pouches without lobes *vs* symmetrically bilobed, larger c ratio (91-155 *vs* 78-92), shorter odontostyle (61.5-69.0 *vs* 72-80 µm), shorter odontophore (43-54 *vs* 54-60 µm), anteriorly located guiding ring (19.5-27.0 *vs* 29-32 µm), functional males in population *vs* no males and differences in juvenile characters. Amphidial pouches without lobes *vs* symmetrically bilobed, longer body (4.2-6.1 *vs* 2.88 mm), larger c ratio (91-155 *vs* 70), shorter odontostyle (61.5-69.0 *vs* 76 µm) and two precloacal (adanal) paired supplements in males *vs* one pair of supplements separate the new species from *L. ampullatus*. Also, *Longidorus auratus* has a shorter body (3.01-4.51 *vs* 4.2-6.1 mm), longer odontostyle (74-100 *vs* 61.5-69.0 µm), smaller anal body width (18-25 *vs* 27.5-37.5 µm), no males in population and sperm in female genital tract whereas *L. tabrizicus* sp. n. has functional males in population and sperm in genital tracts of paratype females in both populations. *Longidorus makatinus* can be distinguished from the new species by having a shorter body (3.06-4.33 *vs* 4.2-6.1 mm) and tail (23-34 *vs* 34-53 µm, in females and 27, 28 *vs* 38.0-50.5 µm, in males), a smaller body width at cloacal region in males (23, 24 *vs* 28-33 µm) and different number of ventromedian supplements (one precloacal pair plus seven ventromedian series *vs* two pairs plus 8-10). The new species, *L. tabrizicus* sp. n. could be differentiated from *L. kakamus* by having a shorter body length (4.2-6.1 *vs* 5.91-7.22 mm), smaller a ratio (81.5-135.0 *vs* 234-238), smaller c ratio (91-155 *vs* 178-189) and males with two precloacal paired supplements *vs* one pair.

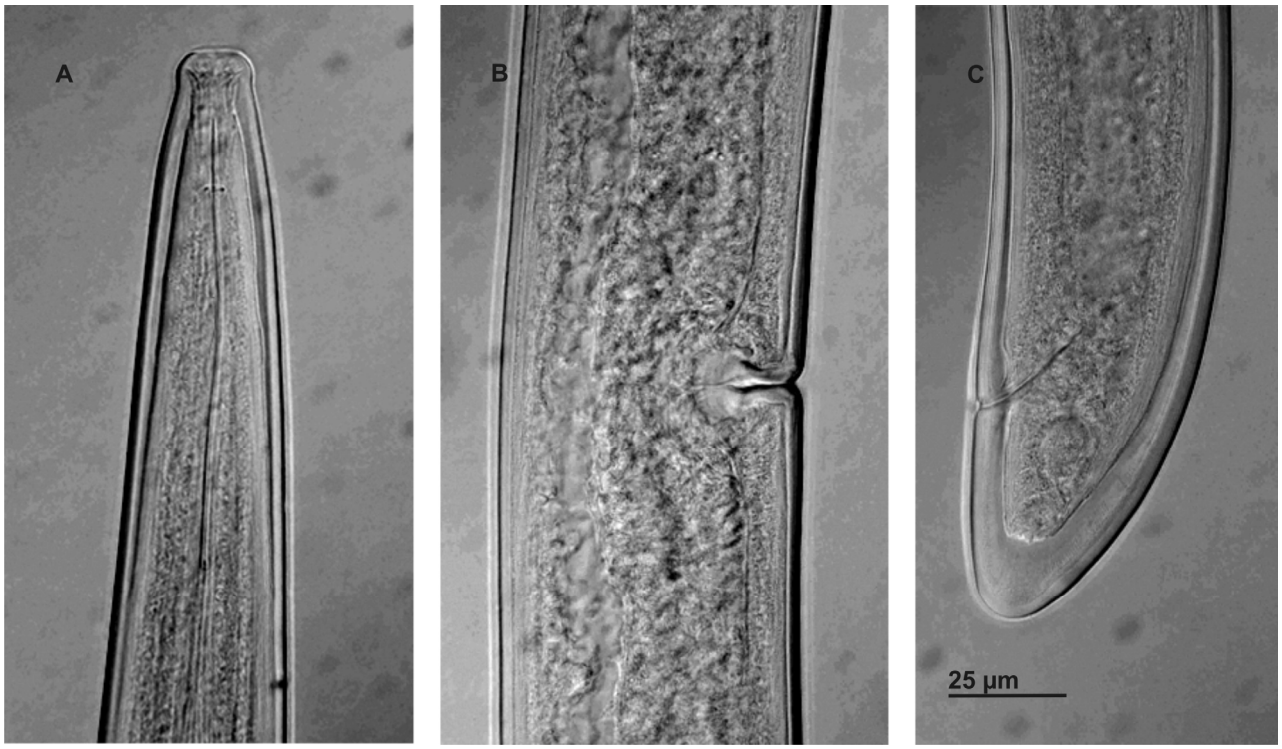

Fig. 6. *Longidorus sturhani*. A: anterior end, B: Vagina, C: Tail.

The other similar species, *Longidorus rotundicaudatus* can be separated from *L. tabrizicus* sp. n. by having a relatively shorter body length (3.97-4.59 *vs* 4.2-6.1 mm) and tail (23-29 *vs* 34-53), a smaller b (9.3-11.9 *vs* 13.5-18.5), higher c ratio (140-197 *vs* 91-155), longer odontostyle (70-81 *vs* 61.5-69.0 µm), more posteriorly located guiding ring (33-36 *vs* 19.5-27.0 µm) and a shorter (37 *vs* 38-50.5 µm) and differently shaped tail in males with one precloacal pair *vs* two pairs. *L. tabrizicus* sp. n. has a pouch-like amphids *vs* symmetrically bilobed ones, larger c ratio (91-155 *vs* 69-81), smaller c' ratio (1.1-1.7 vs 2.0-2.8) and wider lip region (10.5-13.0 *vs* 8.5-9.5 µm) compared with *L. pini.* Also the new species is an amphimictic species with functional males whereas males in *L. pini* are absent. Longer body (4.2-6.1 *vs* 4.08-4.20 µm), shorter odontostyle (61.5-69.0 *vs* 72-80 µm), larger c ratio (91-155 *vs* 84-98) and presence *vs* absence of males differ the new species from *L. sylphus*. Finally, *L. tabrizicus* sp. n. has a longer body (4.2-6.1 *vs* 3.51-4.60 mm), shorter odontostyle (61.5-69.0 *vs* 72-80 µm), more posteriorly located guiding ring (at 19.5-27.0 *vs* 16.0-20.5 µm from anterior end), functional males *vs* no males and differences in juvenile characters in comparison to *L. bernardi*.

MORPHOLOGICAL AND MOLECULAR CHARACTERS OF IRANIAN POPULATION OF *L. STURHANI*.

The Iranian population of *L. sturhani* has the morphology (Fig. 6) and morphometric characters (Table 2) similar to the populations cited in the original description (Rubtsova *et al.*, 2001) except for the body width at mid-body and as a result, a smaller a ratio (81-88 *vs* 99-138). As previously discussed, *L. sturhani* is the closest species to *L. tabrizicus* n. sp. and this is well supported by its position in the phylogenetic trees inferred from sequencing of 18S rDNA and ITS1 (Figs 7, 8).

As given by Roubtsova *et al.* (2001) in Fig. 4, *L. profundorum* is the closest species to *L. sturhani* based on the analysis of D2-D3 sequences. Our results (Figs 7, 8) confirm their relationships. However, the two species clearly differ in their morphological characters. Males were absent in the Iranian population as in the type and other population in the original description. This is the first report of *L. sturhani* from Iran.

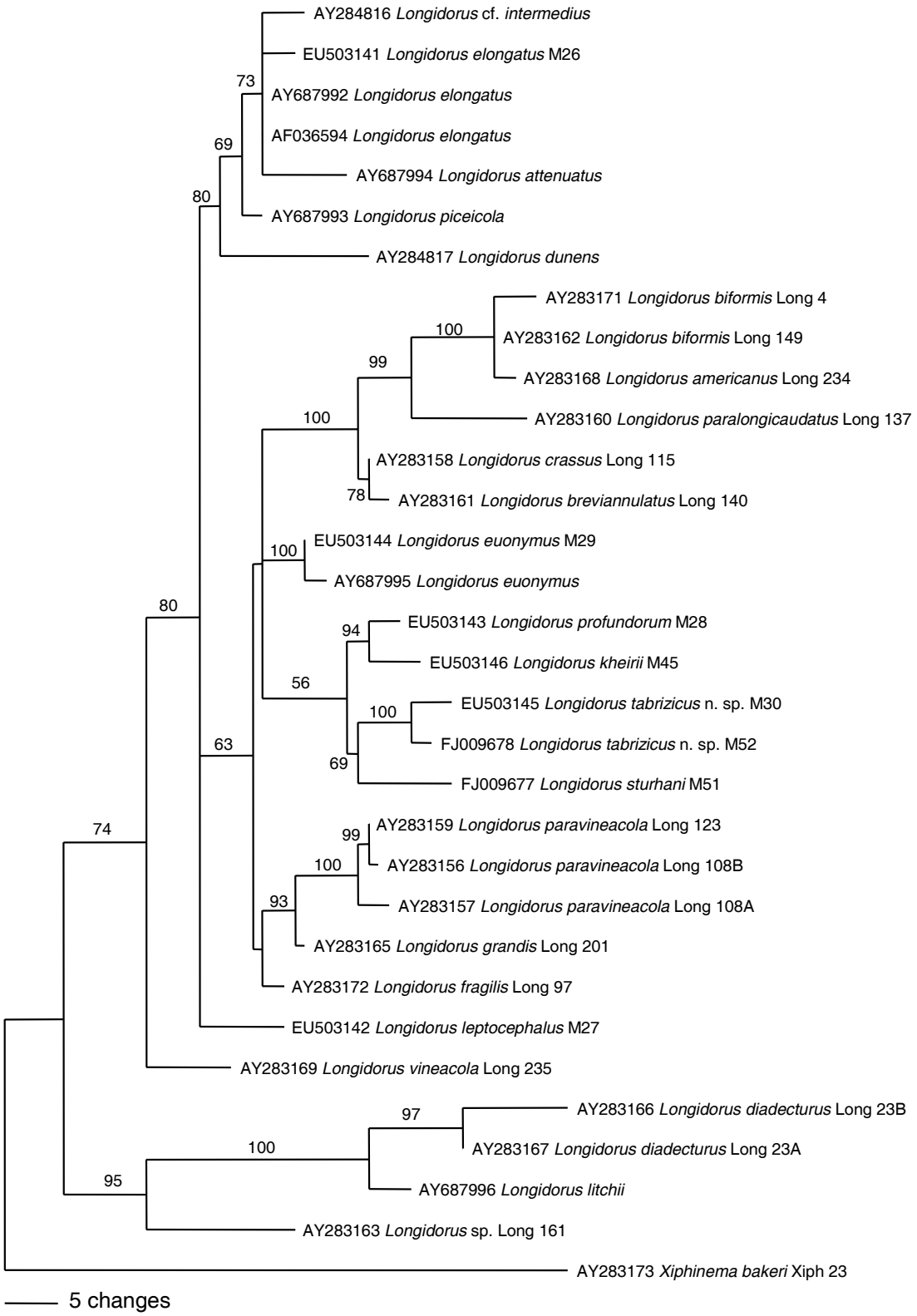

Fig. 7. The 10001st Bayesian tree inferred from 18S under GTR+I+G model (lnL=4337.1733; freqA=0.2766; freqC=0.208; freqG=0.2604; freqT=0.2549; R(a)=1.0324; R(b)=4.3901; R(c)=2.4235; R(d)=0.7873; R(e)=7.0219; R(f)=1; Pinva=0.8158; Shape=0.8044). Posterior probability values exceeding 50% are given on appropriate clades.

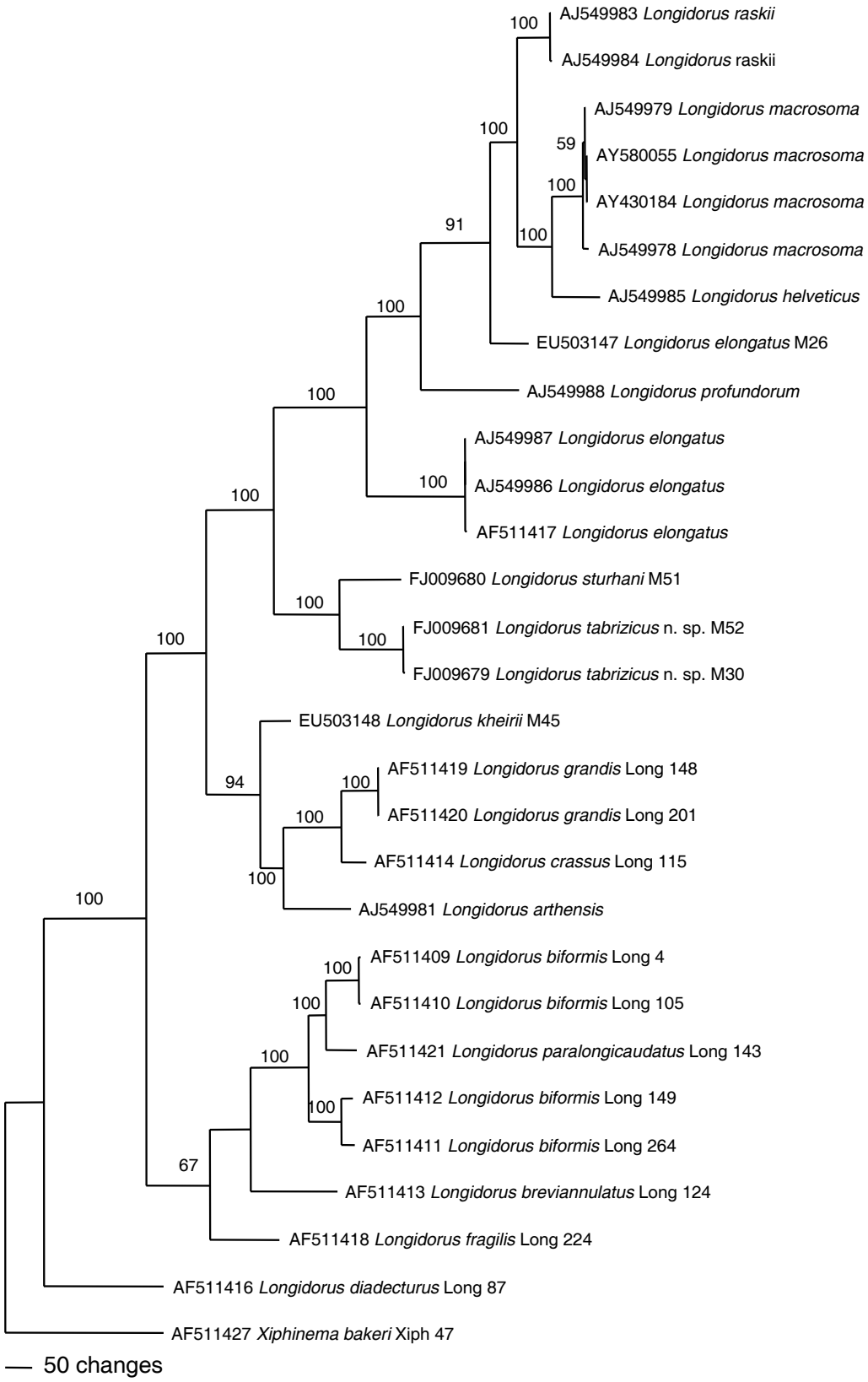

Fig. 8. The 10001st Bayesian tree inferred from ITS1 under GTR+I+G model (lnL=16574.0312; freqA=0.2722; freqC=0.2048; freqG=0.256; freqT=0.267; R(a)=0.8624; R(b)=2.2892; R(c)=1.2998; R(d)=0.7133; R(e)=3.088; R(f)=1; Pinvar=0.16; Shape=1.6836). Posterior probability values exceeding 50% are given on appropriate clades.

Table 2. Morphometrics of Iranian and European populations of *Longidorus sturhani.* All measurements in µm (except characters given in mm), and in the form: mean ± s.d. (range) for Iranian population and total range, from original description.

	Maragheh population	Original description (Rubtsova *et al*., 2001)
N	3	60
L (mm)	5.3±0.5 (4.8-5.7)	(3.9-6.4)
a	85.7±4.3 (81-88)	(99-138)
b	12.5±1.0 (12.0-13.5)	(9.8-17)
c	111.0±3.2 (109.0-114.7)	(83-155)
c`	1.1±0.0(1.1)	(1.0-1.6)
V	47.5±0.5 (47-48)	(46-54)
Body width at base of oesophageal bulb	52±3(49-55)	–
Body width at anterior of oesophageal bulb	50.5±2.8 (47.5-53.0)	–
Anterior end to vulva (mm)	2.5±0.2(2.3-2.8)	–
Anterior end to base of oesophageal bulb	429.5±20.7 (409.5-451.0)	(272-490)
Odontostyle	96.5±3.5 (92.5-99.5)	(77-96)
Odontophore	52±10 (40.5-59.0)	(40-78)
Anterior end to guiding ring	30±0.0(30)	(24-31)
Body width at guiding ring	24.0±0.7 (23.0-24.5)	(16-22)
Body width at mid-body	62.0±2.7 (60-65)	(38-51)
Width of lip region	16.5±1.0 (15.5-17.5)	(12-17)
Width of oesophageal bulb	24.0±2.8 (21.5-27.0)	(14-19)
Length of oesophageal bulb	85±6(78-90)	(77-101)
Body width at anus level	43.5±4.5 (40.5-49.0)	(29-40)
Tail length	48.0±4.2 (44.5-52.5)	(37-50)
Length of hyaline part of tail	17.0±0.5 (17.0-17.5)	(5.8-16.0)

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Резюме. Дано описание амфимиктического *Longidorus tabrizicus* sp. n. по обнаруженным двум популяциям: из ризосферы яблони в Намин, провинция Эрдебиль и из ризосферы *Rosa* sp. из Марагхех, провинция Восточный Азербайджан. В последней пробе вместе с нематодами нового вида были обнаружены представители вида *L. sturhani*. Новый вид характеризуется уплощенной фронтальной поверхностью губного региона, отделенного от остального тела неглубокой перетяжкой, средней длиной тела (4.2-6.1 mm), кармановидными амфидами без заметных базальных долей, коротким одонтостилем (61.5-69.0 мкм) и одонтофором (43.0-54.0 мкм), тупоконическим хвостовым концом, наличием трех личиночных стадий, соотношением самцов и самок, близким к 1:1. Новый вид близок к 11 известным видам: *L. moniloides, L. globulicauda, L. ampullatus, L. auratus, L. makatinus, L. kakamus, L. rotundicaudatus, L. pini, L. sylphus, L. bernardi* и *L. sturhani*. Даны основанные на анализе 18S и ITS1 rDNA последовательностей филограммы, отражающие взаимоотношения *L. tabrizicus* sp. n. с другими видами рода *Longidorus* Micoletzky, 1922, для которых известны подобные последовательности. Ближайший по морфологическим и молекулярным признакам вид - *Longidorus sturhani*. Иранская популяция *L. sturhani* близка к европейской, однако отличается большим диаметром тела и значением индекса a.