Characterisation of *Longidorus iranicus* Sturhan & Barooti, 1983 (Nematoda: Longidoridae) from Iran and synonymisation of *L. moesicus* Lamberti, Choleva & Agostinelli, 1983

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Summary. The needle nematode, *Longidorus iranicus* Sturhan & Barooti, 1983 has been described from Iran and not reported from any other country so far. Based on the studies of a population from the rhizosphere of raspberry in the north of Iran a detailed description is presented and males are described for the first time. Among the total of more than 150 *Longidorus* species, *L. iranicus* is morphologically most close to *L. moesicus*. Comparative morphological and morphometric characters are given for three populations of *L. iranicus* and populations of *L. moesicus*, including the original descriptions of both species and other references. Morphological and molecular studies indicate species identity and synonymy of *L. moesicus* with *L. iranicus* is proposed in the present paper.

Key words: morphology, morphometrics, needle nematode, synonymy, taxonomy.

The needle nematodes of the genus *Longidorus* Micoletzky, 1922 comprise more than 150 valid species (Decraemer & Robbins, 2007). Presently, fifteen species have been reported from Iran and three of them were only found in Iran: *L. iranicus* Sturhan & Barooti, 1983, *L. kheirii* Pedram, Niknam, Robbins, Ye & Karegar, 2008 and *L. tabrizicus* Niknam, Pedram, Nejad, Ye, Robbins & Maafi, 2010.

The species *L. africanus* Merny, 1966, *L. pisi* Edward, Misra & Singh, 1964 and *L. vineacola* Sturhan & Weischer, 1964 were reported from the rhizosphere of pistachio, pomegranate, citrus and watermelon in southeast and north of Iran (Kheiri & Barooti, 1984). *Longidorus crassus* Thorne, 1974, *L. elongatus* (de Man, 1876) Micoletzky, 1922, *L. protae* Lamberti & Bleve-Zacheo, 1977 and *L. proximus* Sturhan & Argo, 1983 were found in the northwest from crops and grapevine (Ghaderi *et al*., 2012). Four species of *Longidorus*: *L. leptocephalus* Hooper, 1961, *L. profundorum* Hooper, 1966, *L. euonymus* Mali & Hooper, 1974 and *L. sturhani* Rubtsova, Subbotin, Brown & Moens, 2001 were isolated from woody plants in the northwest (Pedram *et al*., 2008). *Longidorus orientalis* Loof, 1983 was mainly recorded from date palm in the southeast and south of Iran (Noruzi & Barooti, 2005; Gharakhani *et al*., 2007; Palomares-Rius *et al*., 2010).

Longidorus iranicus is one of the three species described only from Iran. This species was first reported from the rhizosphere of several crops, *i.e.*, grapevine, olive, apple, pear, rice, cabbage and tomato, in the provinces of Markazi, Ghazvin, Mazandaran, Gilan, East Azerbaijan and West Azerbaijan (Sturhan & Barooti, 1983) located in the central, north and northwest of Iran. *Longidorus iranicus* was also recorded in the southern part of the country from the rhizosphere of tomato, citrus and date palm (Ghaderi *et al*., 2012). It was also

reported from soil around fruit trees: apple, pear, plum and apricot in the northwest of Iran (Fadaei Tehrani & Kheiri, 2004) and peach, sweet cherry, hazelnut from Ardabil (Ghaderi *et al*., 2012). Based on the studies of a population from the rhizosphere of raspberry in Mazandaran province a detailed description is presented and males are described for the first time in this study.

Longidorus moesicus was firstly described by Lamberti *et al*. (1983) from the rhizosphere of black currant (*Ribes nigrum*) at Kostinbrod near Sofia, Bulgaria. Afterwards it was reported from different regions of Serbia from the rhizosphere of many plants, such as *Populus* sp., *Trifolium* sp., pear and grapevine, *Urtica* sp. and *Rubus* sp. (Barsi & Lamberti, 2004). More recently, *L. moesicus* was reported from Slovenia in a vineyard soil sample and characterised morphologically and molecularly along with five other *Longidorus* species (Širca & Urek, 2009). The Crete Island in Greece was the latest locality where *L. moesicus* was found in the rhizosphere of grapevine and morphologically and molecularly characterised (Tzortzakakis *et al*., 2014).

Morphological and molecular studies of the populations of *L. iranicus* and comparison of the data with the available records for *L. moesicus* were the main purposes of this study.

MATERIALS AND METHODS

Soil samples were collected from the rhizosphere of raspberry, citrus (Mazandaran province), walnut, raspberry, alder (*Alnus* sp.) (Golestan province) and oak (Ilam province). Nematodes were extracted from soil and fixed using the tray method (Whitehead & Hemming, 1965), fixed in formalin, transferred in dehydrated glycerin and processed onto permanent slides (De Grisse, 1969). Females and males were subsequently used for morphological studies. Morphometric features were measured by using a camera lucida installed on a light microscope (Olympus BH-2). The measurements are expressed in micrometers (mm for body length and µm for other characters) and presented as a mean and the standard deviation of the mean with the range in parentheses. Photomicrographs were taken by digital camera (Dino Capture 2.0) via a light microscope Olympus BH-2 with Nomarski differential interference contrast.

DNA was extracted from single females. Protocols for DNA extraction, PCR and sequencing were described by Tanha Maafi *et al*. (2003). The following primers were used for amplification of two rRNA gene fragments. The ITS1 region using forward primer 18S (5'-TTGATTACGTCCCTGCC CTTT-3') and reverse primer rDNA1 (5'-ACGAGC CGAGTGATCCACCG-3') as described in Wang *et al.* (2003), the D2-D3 expansion segments of 28S rRNA gene with the forward D2A (5'-ACAAGTAC CGTGAGGGAAAGTTG-3') and the reverse D3B (5'-TCGGAAGGAACCAGCTACTA-3') primers (Rubtsova *et al*., 2001). The PCR products were sequenced at MWG Biotech (Ebersberg, Munich, Germany) and the sequences were submitted to the GenBank database under the accession numbers KP222294 and KP222295.

DESCRIPTION

Longidorus iranicus Sturhan & Barooti, 1983 (Figs 1 & 2)

Measurements. Table 1.

Female. Body long, variously shaped after fixation: ventrally curved to open C, open coiled to irregular (Figs 1O & P). Lip region rounded and narrow, continuous to slightly offset from the rest of the body. Amphidial pouches long, more than half to 70% of the distance from oral opening to guiding ring, slightly bilobed at base, with lobes of almost equal length (Fig. 2). Guiding ring typical of the genus. Odontostyle long and slender, odontophore less than half its length. Nerve ring located about equivalent to the corresponding body diameter posterior to odontophore. Pharynx typical dorylaimoid, Pharyngeal bulb muscular, dorsal pharyngeal gland nucleus location is variable from one-third of pharyngeal bulb length to mid-bulb, nuclei of the two ventro-sublateral glands located at the posterior third part of the bulb. Pharyngeal intestinal valve rounded. Rectum almost equal to body width at anus. Genital tract didelphic, amphidelphic with reflexed ovaries, occupying 7-8% of the total body length, oviduct as a narrow tube separated from the uterus by a sphincter, uterus long, without sperm. Vulva a transverse slit, vagina occupying more than half the corresponding body width with conspicuous sphincter muscles. Tail bluntly convex conoid, short, slightly less than one anal body width long, one pair of caudal pores on each side visible.

Male. Body long, curved ventrally after fixation, the posterior part of body being complete spiral specially near tail (Figs 1M & N). Lip region rounded, slightly offset from the rest of the body. Amphidial pouches similar to female with small lobes, guiding ring conspicuous and located less than three corresponding lip region widths from the oral aperture, odontostyle long and slender, odontophore less than half of odontostyle length,

nerve ring located posterior to odontophore. Dorsal pharyngeal gland nucleus located almost in the anterior part of pharyngeal bulb and at one-third of its length, nuclei of the two ventro-sublateral glands located at 62-64% of the bulb length. Testis diorchic, opposed, with conspicuous fusiform to oval sperm, posterior testis reflexed, T = 38% and 53%, anterior testis longer, T = 45% and 61%. Spicules curved ventrally, heavily sclerotised, lateral guiding pieces (crura) sclerotised, 15 and 17 µm long. An adanal pair plus a ventral series of about 8 to 10 supplementary papillae present. Tail conoid, dorsally convex, terminus rounded, with two pairs of caudal pores.

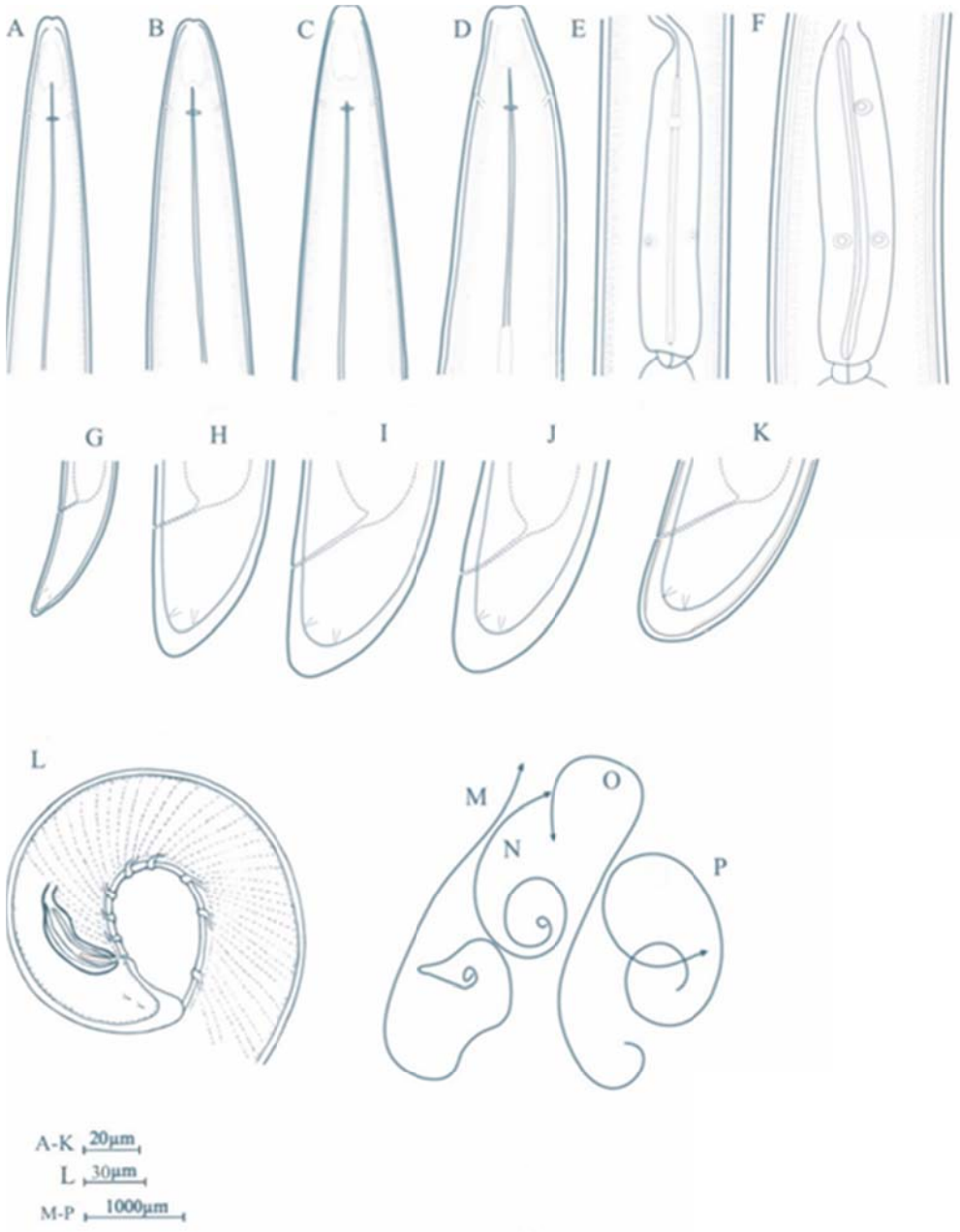

Fig. 1. *Longidorus iranicus*. A, B, F-K, O, P: female; C-E, L-N: male. A-D: anterior end; F: pharyngeal bulb; G-I: tail of three juvenile stages; J-K: female tail; O, P: shape of fixed-relaxed female; C, D: anterior end, E-F: pharyngeal bulb, L: male posterior part; M, N: shape of fixed-relaxed male.

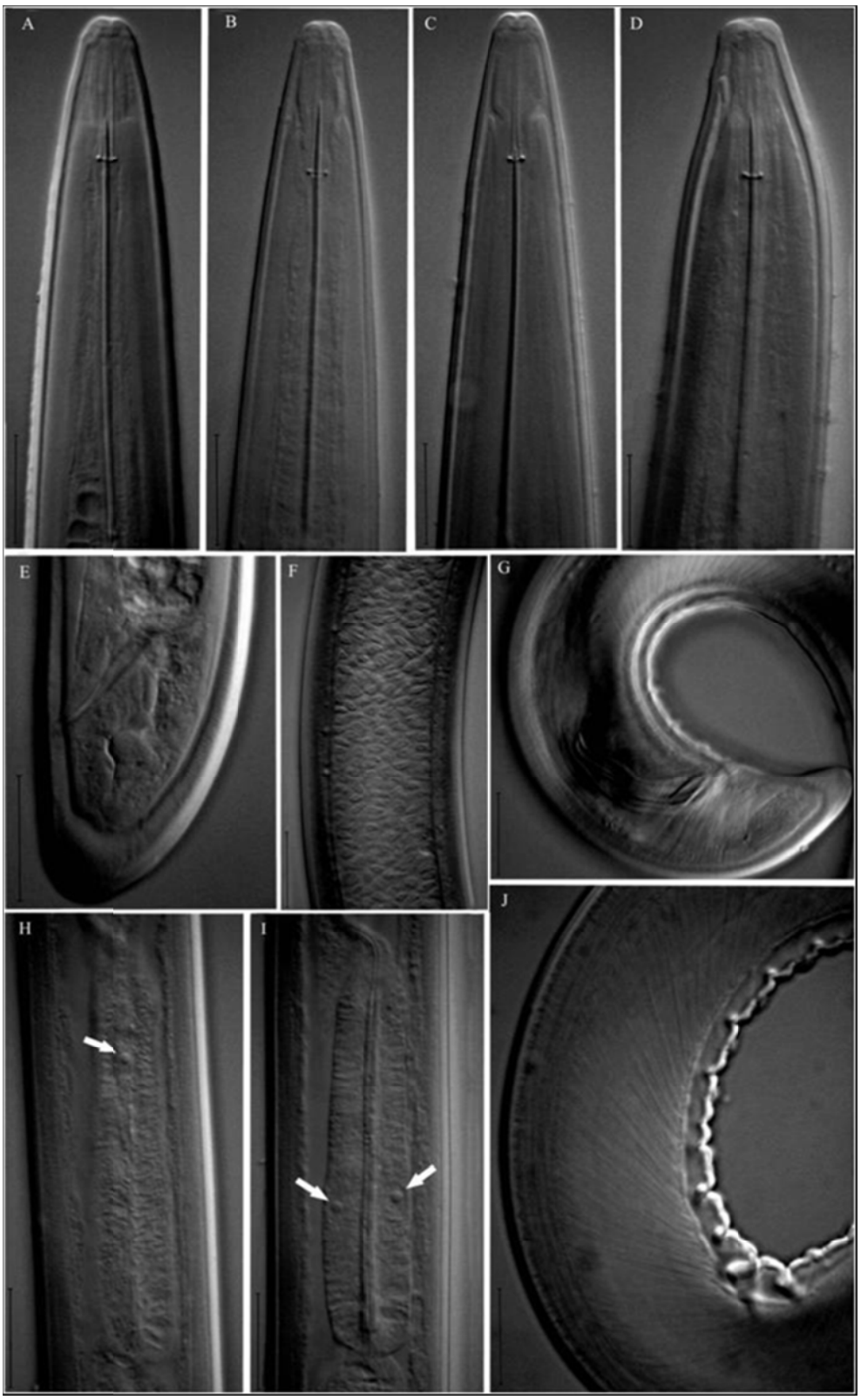

Fig. 2. *Longidorus iranicus* photomicrographs. A, B, E: female; C, D, F-J: male. A-D: anterior end; E: tail; F: sperm in testis, H, I: pharyngeal bulb showing pharyngeal ventro-sublateral glands nuclei; G, J: tail showing spicules and preanal supplementary papillae.

Table 1. Morphometric characters of *Longidous iranicus* and *L. moesicus* (measurements in mm for body length and in µm for other characters except ratios)

Characters	*L. iranicus*				*L. moesicus*			
	Sturhan & Barooti (1983)	Fadaei Tehrani & Kheiri (2004)	The present study		Lamberti *et al.* (1983)	Barsi & Lamberti (2004)	Širca & Urek (2009)	Tzortzakakis *et al.* (2014)
n	8 females	10 females	11 females	2 males	10 females	15 females	17 females	2 females (P. Elias population)
L	5.09 (5.22-7.1)	5.7 (5-6.5)	6.28±0.6 (5.1-7.14)	4.92, 7.5	7.2 (6.4-8)	6.5±0.63 (5.7-7.56)	6.9±0.7 (5.9-8.4)	5.06, 5.61
a	103 (92-115)	104.6 (100-112)	90.3±9.8 (78.4-109.8)	82.1, 124.4	120 (96-147)	124.4±6.46 (115.4-139.5)	128.6±7.4 (117-145.5)	97.5, 105.9
b	12.7 (9-14.9)	12.6 (10-15.2)	13.5±1.8 (11.5-17.2)	10.9, 18.7	15.8 (13.8-18.8)	13.8±1.3 (11.5-16.4)	15.8±1.4 (13.1-18.3)	13.5, 16.3
c	180 (151-220)	162.5 (150-182)	183.4±30.3 (137.6-237.8)	111.9, 196.4	170 (146-186)	166.8±15.28 (141-197)	164.3±18 (129-200.8)	137.0, 160.4
c’	0.84 (0.78-1)	0.92 (0.81-1.2)	0.8±0.1 (0.7-0.9)	0.9, 1	1 (0.8-1.2)	0.94±0.09 (0.79-1.06)	1.05±0.08 (0.90-1.23)	0.9, 1.0
V%	50.6 (48.8-52)	50.7 (49-53)	49.4±2.3 (44.5-52.9)	–	53 (50-54)	48.9±1.73 (44.8-51.5)	52.7±1.4 (49.6-54.6)	48, 49
Stylet length	–	–	143.7±6.2 (131-150)	136, 144	–	174.3±5.05 (163.8-183.8)	155.8±5.9 (143.1-161)	-
Odontostyle length	112 (106-118)	104.5 (100-110)	104.5±4.1 (97-111)	96, 103	119 (115-124)	114±5.60 (102.5-125)	107.1±4.4 (96.3-114.6)	97, 104
Odontophore length	43 (40-48)	65 (58-72)	41.7±3.1 (34-49)	40, 41	63 (59-66)	60.3±3.70 (55-67.5)	48.7±4 (42-56.4)	51, 55
Oral aperture to guide ring	35 (33.5-36.5)	35.3 (30-40)	35.1±1 (33-37)	38	34 (32-38)	37.6±1.80 (34.4-41.9)	35.6±1.6 (32.8-39.1)	30, 34.5
Body diameter at lip region	–	–	14.3±1.1 (13-16)	14, 16	12 (11-13)	13.1±0.36 (12.8-13.8)	12.4±1 (10.5-13.9)	10.5, 11.5
Body diameter at guide ring	–	–	27.7±3.2 (23-34)	25, 33	25 (24-26)	25.1±0.81 (23.4-26.3)	26.6±1.1 (24.2-28.4)	–
Body diameter at base of pharynx	–	–	55.5±3.9 (50-62)	50, 64	50 (45-61)	44.4±2.50 (40-49.7)	46±2.9 (41.9-50.8)	–
Distance from oral aperture to the base of pharynx	–	–	459±26.3 (410-495)	387, 425	–	–	437.6±27.5 (381-466)	–
Body diameter at vulva	–	–	68.2±3.9 (62-75)	–	61 (52-71)	52.1±3.04 (48.1-58.4)	53.6±3.7 (48.4-59.9)	–
Body diameter at anus		–	43±2 (41-46)	–	42 (39-49)	40.5±1.67 (37.8-43.1)	40.2±3 (36.3-46.1)	–
J (hyaline portion of tail)	–	–	12.7±1.8 (10-15)	–	13 (12-13)	11.3±1.31 (9.4-13.8)	–	–
Body diameter at beginning of J	–	–	28.5±2.2 (24-32)	–	27 (24-30)	24.2±1.60 (21.3-28.3)	–	–
Tail length	34 (29-41)	–	33.4±3.2 (28-38)	–	43 (40-49)	39±2.45 (34.3-42.5)	42.2±3.8 (36.2-49.4)	35, 37
Spicule length				55, 58				

Molecular characterisation. A comparison of the results of the obtained sequences of the D2-D3 expansion segments of the 28S rRNA gene from *L. iranicus* with those from *L. moesicus* from Slovenia and Crete, Greece, showed 99% similarity and differed by only two nucleotides, with one of the differences being in the poly T and A regions, which are rather common regions for mutations. The ITS1 of rRNA gene sequence of *L. iranicus* contained several ambiguous nucleotide positions and it was different from that of the Crete *L. moesicus* at least by one nucleotide.

DISCUSSION

Chen *et al*. (1997) indicated morphological similarities of four species of *Longidorus* including *L. iranicus*, *L. iuglandis* Roca, Lamberti & Agostinelli, 1984, *L. moesicus* and *L. olegi* Kankina & Metlitskaya, 1983 and pointed out the difficulties of their separation due to sharing morphological and morphometrics characters, *i.e*., similar body and odontostyle length, rounded lip region, symmetrical bilobed amphidial pouches and width of the lip region.

The morphometric characters of three populations of *L. iranicus* and of three populations of *L. moesicus*, including the original descriptions of both species and of the recently reported Cretan population are presented and compared in Table 1. The morphometric characters of both species are similar, with the same codes of the polytomous key of Chen *et al.* (1997): A345, B12, C3, D1, E23, F-3, G23, H12, which is also in agreement with Cretan population (Tzortzakakis *et al*., 2014) except for the key code H. The only noticeable difference between the two species appears to be the presence of males in *L. moesicus*, while no male was available for the original description of *L. iranicus* and no sperm was observed in the genital tracts of the females. However, in our present study two males were recovered from the soil sample collected from the rhizosphere of raspberry and alder at Minoodasht in Golestan province. Although, in surveys from Serbia and Crete Island males were not found in *L. moesicus* (Barsi & Lamberti, 2004; Tzortzakakis *et al*., 2014).

In addition to morphological, morphometric and molecular similarities, the type habitats of both species are also similar; both species were found mainly in vineyard soil and the rhizosphere of fruit trees as well as the bush plants raspberry and black currant.

Based on the morphological and molecular similarities of *L. iranicus* and *L. moesicus*, we suggest synonymy of these two species. Because of the journal issue with the description of *L. iranicus* appeared 15/03/1983, and the journal issue containing the description of *L. moesicus* was published 15/06/1983, the name *L. iranicus* has the priority over *L moesicus*; thus, *L moesicus* is suggested as its junior synonym.

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Резюме. Нематода *Longidorus iranicus* Sturhan & Barooti, 1983 до настоящего времени не была отмечена нигде кроме Ирана. Представлено подробное описание этого вида по особям из популяции, выделенной из ризосферы малины в Сев. Иране. Впервые приводится описание самцов. Среди более чем 150 видов *Longidorus*, *L. iranicus* морфологически ближе всего к *L. moesicus*. Морфологические и морфометрические параметры трех популяций *L. iranicus* и одной популяции *L. moesicus* приводятся в сравнительном аспекте, включая данные первоописаний и других находок. На основе сравнения морфологических и молекулярных данных предлагается синонимизация *L. moesicus* и *L. iranicus*.