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Xiphinema robbinsi sp. n. (Nematoda, Dorylaimida), an amphimictic species from Iran

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Summary. A new species of *Xiphinema* from north western Iran is described herein. *Xiphinema robbinsi* sp. n. is characterized by its body length (3-3.8 mm), anterior end broadly rounded with a marked depression and high labial region, medium-sized odontostyle length (107.5-127 µm), females with two equally developed genital branches with well developed ovijector and spines in tubular part of uterus, a short convex-conoid tail, abundant males in population and four juvenile stages. The new species most closely resembles *X. aceri* Chizhov, Tiev & Turkina, 1986, *X. aequum* Roca & Lamberti, 1988, *Xipinema riparia* Chizhov, Subbotin, Romanenko & Kruchina, 1991 and *X. macedonicum* Barsi & Lamberti, 1999 and belongs to species group 6 of the polytomous key of Loof & Luc (1990).

Key words: Iran, Longidoridae, *Xiphinema robbinsi* n. sp.

An extensive survey on longidorid nematodes was conducted in East-Azerbaijan province, north western Iran, during November 2006 to September 2007. A number of known species of the genera *Xiphinema* and *Longidorus* as well as one new species of *Xiphinema* were found in soil samples collected from the rhizosphere of naturally growing *Rhamnus catharica* L. shrubs. The new species is described in this article.

MATERIAL AND METHODS

Soil samples were collected at 15-20 cm depth from the rhizosphere of *R. catharica* shrubs that are naturally growing in the mountain region of Goshayesh village, close to Maragheh city. The soil samples were suspended in water and nematodes extracted using 850 µm, 250 µm and 149 µm series of sieves. Specimens were hand picked under a stereomicroscope and transferred to a drop of water to be fixed. The nematodes were fixed by adding boiled 4% formalin solution and processed to anhydrous glycerin with some modifications of De Grisse (1969) method. Permanent microscopic slides were prepared and morphological and morphometric characters of the specimens were studied using an Olympus BX-41 light microscope provided with a drawing tube. Digital photographs were also taken using a DP50 digital Olympus camera. The drawings were sketched using CorelDRAW® software version 12.

DESCRIPTION

Xiphinema robbinsi sp. n. (Figs 1-4)

Measurments: See Table 1.

Females: Body ventrally curved more pronounced in posterior region to an open C shape, rarely spiral, when heat relaxed. Cuticle with two distinct layers, outer 1 µm and inner 2.5 µm thick in post labial region, varying from 1.3 µm to 2.5 µm at mid body to 2.2 µm to 6.3 µm in dorsal part of tail (holotype). Fine transverse striations most obvious in tail region; six body pores present in dorsal and ventral sides in odontostyle region, three pores laterally. Lip region broadly rounded, 5.6-7.5 µm high and marked by a depression. Amphidial slit large, 9.7-10.6 µm wide, located on lip region and amphidial fovea stirrup shaped. Odontostyle 1.5-1.8 µm in diameter, its base furcate, double guiding ring with protruding guiding sheath 5 µm long and rigid guiding ring 4.4-5 µm wide. Odontophore with well developed flanges. Vestigium mostly present and located from far anteriorly to posteriorly in slender part of oesophagus. Nerve ring located at 212-227 µm from anterior end; hemizonid close to

base of odontophore in specimens with retracted stylet. Enlarged part of oesophagus moderately wide; the dorsal nucleus opposite anterior end of platelet region and both ventrosublateral gland nuclei situated about midway and were calculated as described by Loof & Coomans (1972); (DN, 12%, DO, 8%, RS1N, RS1O, 58%, 47% and LS1N, LS1O, 55%, 50 %, respectively) (holotype). Cardia rather small, cone–shaped, surrounded by intestinal tissue. Prerectum 75.5% of anal body width (holotype) varying from 80 to 88% in paratypes. Reproductive system amphidelphic with both branches equally developed, each branch composed of a rather short ovary (anterior 43.7 and posterior 51.2 µm long; holotype), a largely tubular oviduct with enlarged *pars dilatata oviduct* separated from the uterus by a well developed sphincter. Uterus bipartite, with well developed glandular *pars dilatata*, continuing in a narrower, muscular tube-like portion with small to larger spines (7-10 µm), large well developed and clearly offset ovejector (72.5×27 µm) (holotype) occupying nearly the complete body diameter at vulval level; vagina perpendicular to body axis up to 30%-41.5% of corresponding body width long. Sperm usually observed in the ovijector and muscular part of uterus, sometimes showing a concentration near the glandular part of the uterus; sperm may also be present in both wide and narrow parts of oviduct. Tail broadly dorsally convex-conoid (ventral wall almost straight in some specimens), with or without slightly bulging rounded terminus with blind canal. Two to three caudal pores on each side. Paratype females tail variable (Fig. 3).

Male: Similar to female in all characters except for reproductive system and habitus in which posterior body region more strongly curved after heat relaxation. Three ventromedian precloacal papillae at equal distance from each other, posteriormost one located 89 µm anterior to the adanal pair situated at 20 µm anterior to cloacal opening. Spicules well sclerotized and massive, 12 µm wide at midway; lateral accessory pieces somewhat straight and 15 µm long. Copulatory muscles and spicule protractor and retractor muscles well developed. Sperm cells globular, 7.5×4.5 to 7×5 µm in size. Tail similar to that of holotype female but with 3-5 pores on each side; terminal blind canal present.

Juveniles: Four juvenile stages present. J1 with long tail and replacement odontostyle located within the odontophore (in one of the ventrosublateral sinuses), its tip close to base of functional odontostyle. Tail in J2 slightly shorter and digitate. J3 and J4 with similar tails, *i.e.* short, broadly convex-conoid with a mammiform terminal peg. Replacement odontostyle in J2-J4 located far from base of functional odontostyle. Juvenile stages identified by comparing length of functional and replacement odontostyle according to Robbins *et al.* (1996). Primordium lengths of juvenile stages were measured as: in J2 (22-28 µm), in J3 (42.5-43.5 µm) and in J4 (42-47.5 µm). Relation of body length, functional and replacement odontostyle (func ods and rep ods, respectively) in J1 to mature females is given in Fig. 5.

Differential diagnosis and relationships. *Xiphinema robbinsi* sp. n. belongs to species group 6 of the polytomous identification key of Loof and Luc (1990), characterized in the female by equal genital branches, without Z- or pseudo-Z-differentiation but with uterine spines. It is characterized by a wide and high lip region demarcated from the rest of the body by a depression, medium-sized body length (3.0-3.8 mm), medium sized odontostyle (107.5-127 µm) and short convex-conoid tail. Females with a large muscular ovejector, bipartite uterus with spines in tubular part and glandular *pars dilatata*; males abundant. Males with two to three ventromedian precloacal supplements, equally spaced and well anterior to adanal pair; spicules 52.5-56 µm long. Four juvenile stages. The identification codes according to Loof and Luc (1990) are: A4-B3-C5a-D65-E56-F3(4)-G2-H2-I3(4)-J5a-K2-L2.

The new species belongs to species group 6 *sensu* Loof & Luc (1990) with females characterized by equal genital branches with uterine spines but without Z- or pseudo-Z-differentiation. 18 species are currently known in this group [updated from Loof & Luc (1990) with Chizhov *et al.* (1991), Barsi *et al.* (1998) and Barsi & Lamberti (1999)] – for 9 of them males are unknown and no sperm was observed in females.

It most closely resembles four known species namely *X. aceri* (Chizhov *et al.*, 1986), *X. aequum* (Roca & Lamberti, 1988), *X. riparia* (Chizhov *et al.*, 1991) and *X. macedonicum* (Barsi & Lamberti, 1999). Compared to *X. aceri*, the new species differs by having smaller odontophore (62.5-74.5 *vs.* 72-81 µm), smaller oesophageal bulb (91-105×18-22 *vs.* 115-130×20-26 µm), smaller body width at mid body (42-48 *vs.* 47-61 µm), sperm in uterus of some females, tail shape of J3 (short, dorsally convex with a peg *vs.* long and digitate terminus) (from Arias *et al.* (2005), thicker hyaline part of tail (10.3-17 *vs.* 8-10 µm) and abundant males in population. Further, the new species

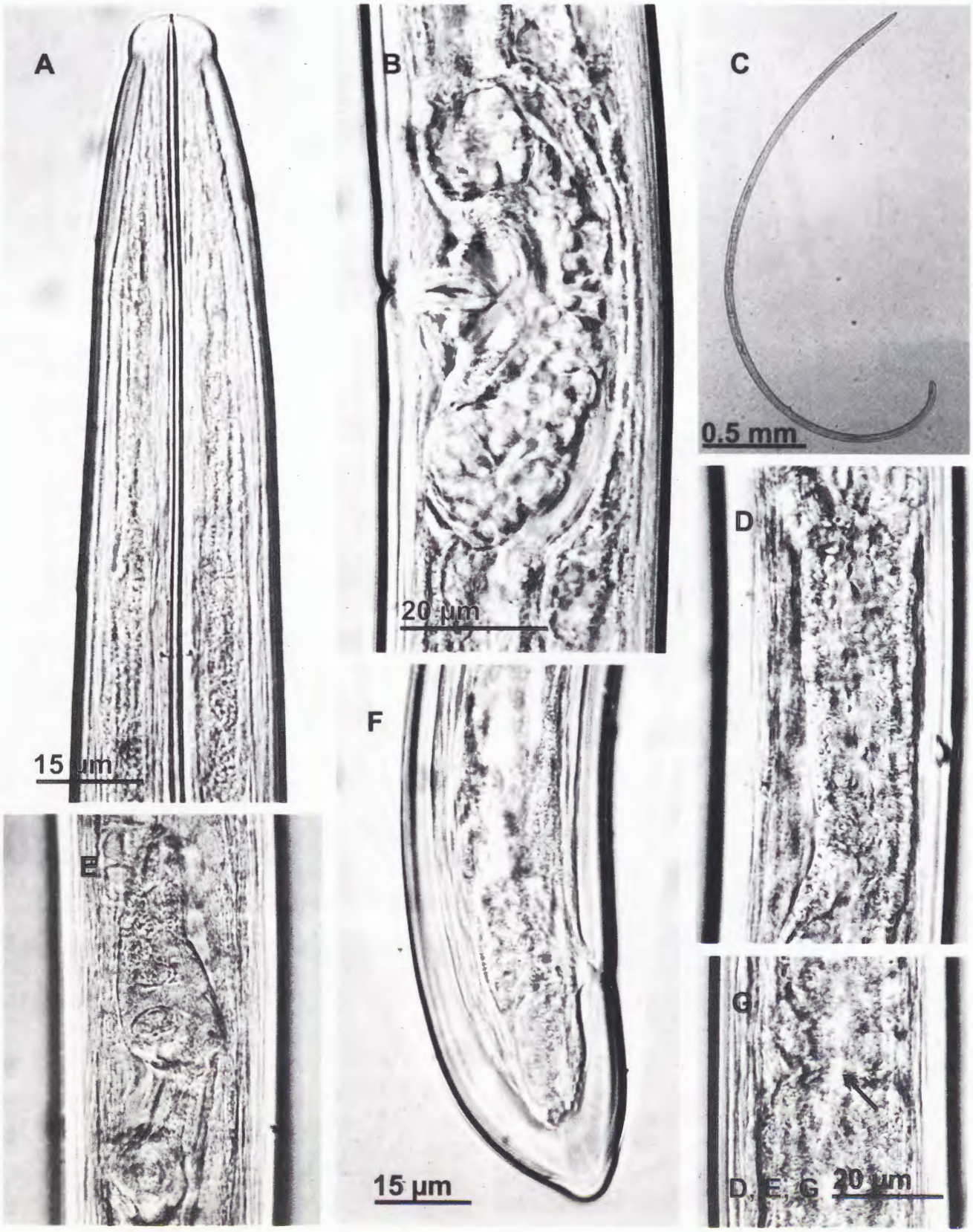

Fig. 1. *Xiphinema robbinsi* sp. n., holotype female. A: Anterior end; B: Ovejector with sperm; C: Whole body; D: Uterus portion with spines; E: Ovary; F: Tail region; G: Sphincter (arrow).

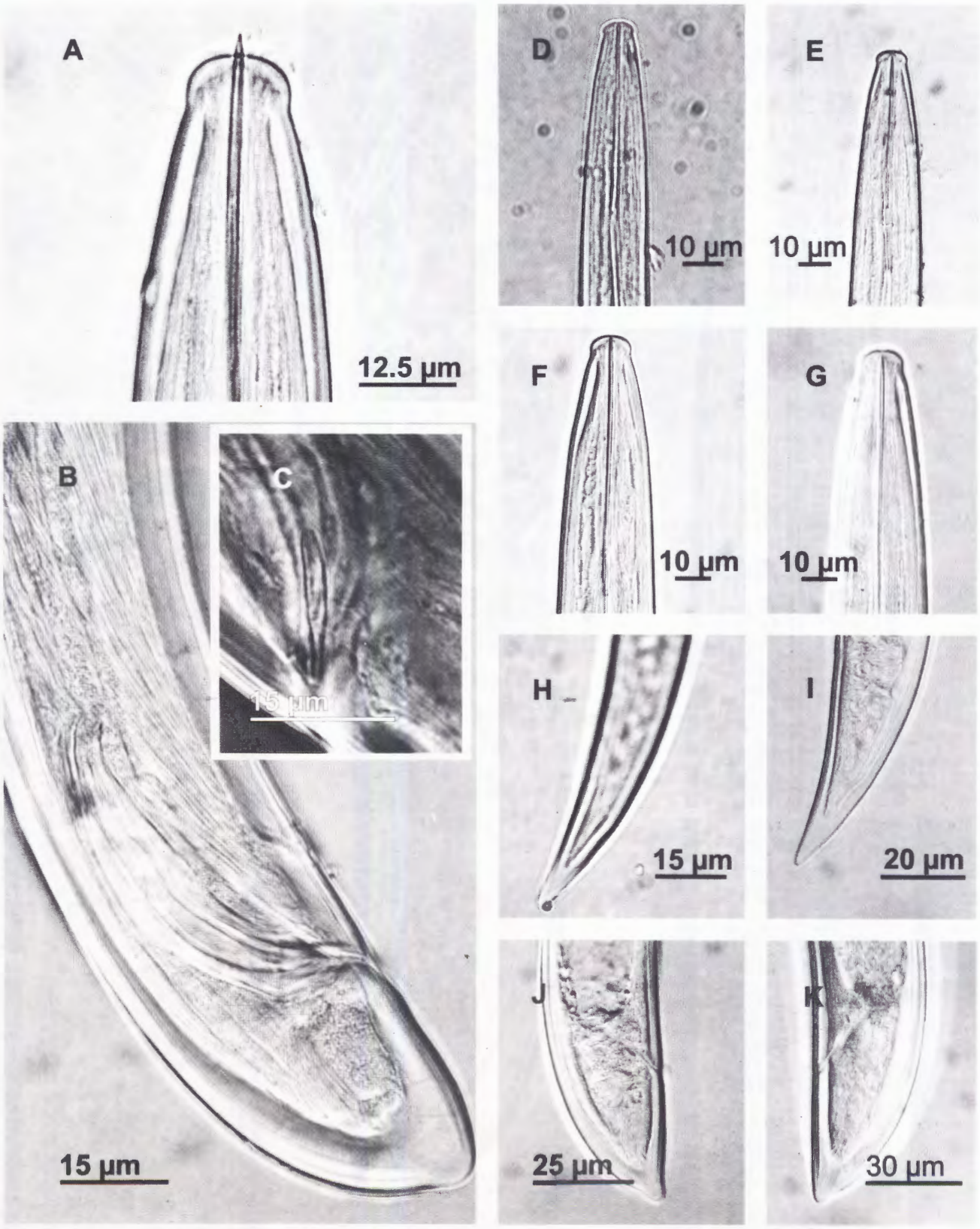

Fig. 2. *Xiphinema robbinsi* sp. n., male and juveniles. A-C: Male; A: Anterior end; B: Posterior end with copulatory apparatus; C: Lateral accessory pieces; D-K: Juveniles; D-G: Anterior end; H-K: Posterior end of J1-J4, respectively.

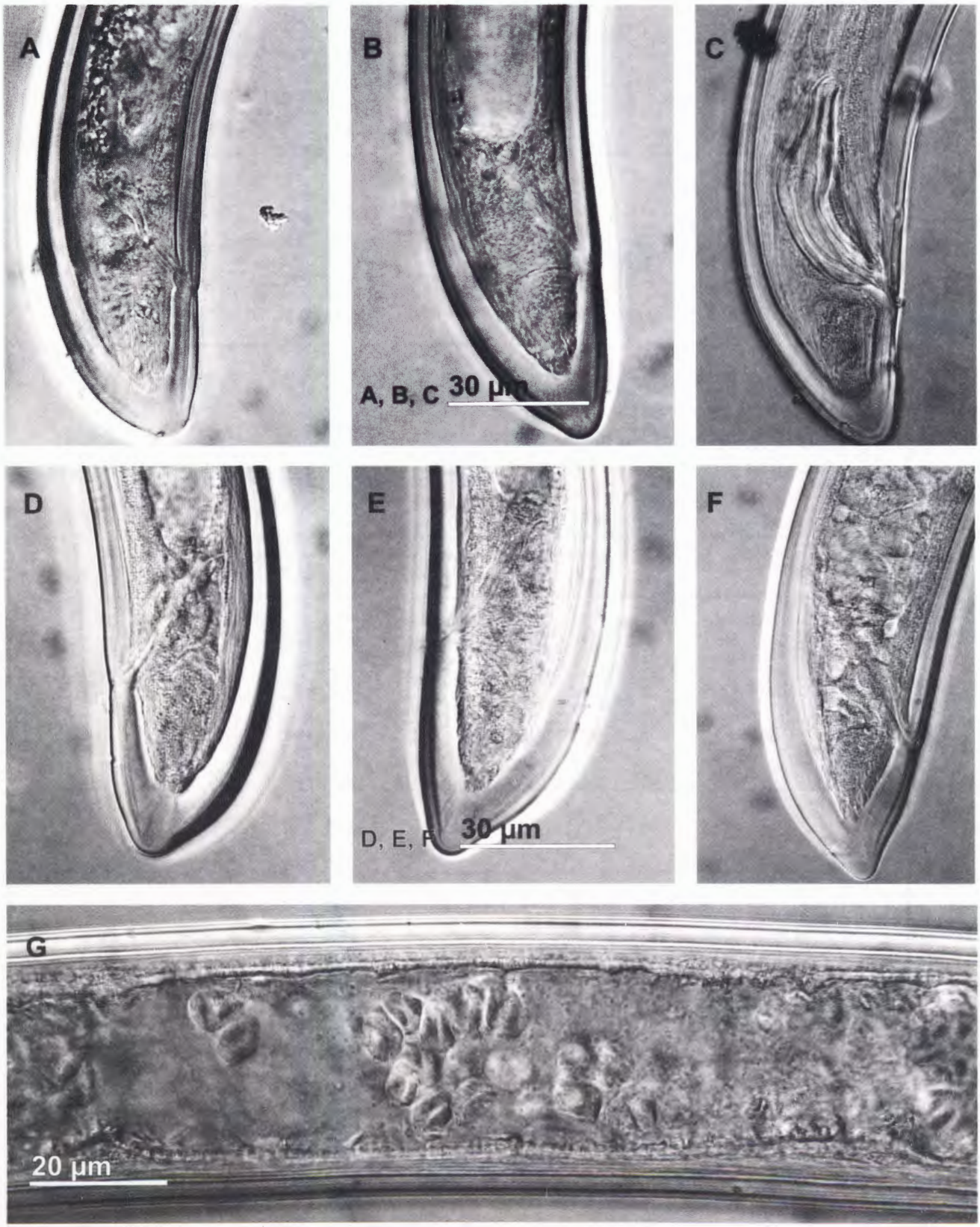

Fig. 3. *Xiphinema robbinsi* sp. n.; A-B: Female tail variations; C: Male paratype tail (this shape found only in one individual); D-F: Common tail shape in paratype females; G: Sperm in uterus of a paratype female.

Table 1. Morphometrics of *Xiphinema robbinsi* sp. n. Measurements are in µm (except of L and anterior end to vulva) and in the form: mean ± SD (range).

Developmental stage	Holotype female	♀	Allotype male	♂	J_1	J_2	J_3	J_4
n	1	10	1	10	1	5	7	3
L (mm)	3.2	3.3±0.2 (3.0-3.6)	3.0	3.5±0.3 (3.0-3.8)	1.0	1.8±0.5 (1.3-2.2)	2.6±0.0 (2.5-2.7)	2.6±0.0 (2.5-2.8)
a	73.0	75.1±5.5 (67.0-83.0)	78.4	89.0±7.5 (78.5-99.0)	75.6	65±8.5 (55-76)	69.0±4.1 (64.3-75.0)	76.7±14.5 (67.7-93.5)
b	6.8	7.8±0.58 (6.8-8.4)	7.6	7.8±0.5 (7.0-8.5)	-	5.3±0.6 (4.5-6)	6.3±0.2 (6.0-6.6)	6.5±0.5 (6.0-6.8)
c	88.6	100.7±9.2 (85.2-112.2)	94.6	98.5±7.8 (87.5-110.5)	21.2	37.8±7.8 (29-44.5)	64.3±5.2 (58.3-73)	69±5.3 (63.5-75.6)
c'	1.0	1.0±0.0 (1.0-1.2)	1.0	1.0±0.1 (0.8-1.2)	3.6	2.2±0.2 (2-2.7)	1.4±0.1 (1.2-1.5)	1.3±0.0 (1.2-1.3)
V	48.0	49.1±2.4 (48.0-56.0)	–	–	–	–	–	–
Anterior end to vulva (mm)	1.5	1.6±0.0 (1.5-1.7)	–	–	–	–	–	–
Anterior end to base of terminal bulb	466.7	429.2±28.7 (384-466.7)	412.6	438.5±28.0 (412.6-495.0)	–	337.7±42.0 (282.5-381.0)	410.5±13.8 (400.0-438.0)	404.7±2.2 (403.0-406.3)
Anterior end to nerve ring	250.0	233.0±13.0 (219-250)	212.5	222.0±12 (212-238)	–	–	–	–
Odontostyle	121.3	119.7±5.7 (107.5-127.0)	120.6	119.7±5.0 (111.0-126.0)	51.3	75.8±11.7 (61.0-87.5)	99.0±2.6 (95.6-102.5)	106.0±2 (103.7-107.5)
Odontophore	72.0	70.0±3.7 (62.5-74.4)	66.3	69.0±3.4 (62.5-73.0)	37.0	50.8±6.8 (43.0-56.2)	59.7±2.2 (58.0-63.7)	60.6±4.3 (55.6-63.0)
Replacement odontostyle	–	–	–	–	64.0	95±10.7 (81.2-104.3)	119.4±0.0 (117.0-122.5)	128.0±1.0 (126.8-128.7)
Odo / Rep ods	–	–	–	–	0.8	0.8±0.03 (0.75-0.83)	0.8±0.02 (0.78-0.83)	0.82±0.01 (0.80-0.83)
Spear	193.0	190±7 (180.0-198.0)	186.8	188.7±8.5 (173.7-199.5)	88.0	138.4±7.5 (133.0-143.7)	158.6±4.0 (155.0-166.0)	166.5±4.5 (161.8-170.5)
Body width at base of terminal bulb	37.5	38.7±2.2 (35.6-43.7)	36.3	36.0±1.3 (35.0-38.7)	20.6	28.0±3.8 (23.1-31.8)	34.7±1.8 (31.8-37.5)	36.4±0.3 (36.0-36.8)
Body width at anterior of terminal bulb	36.3	37.6±2.3 (34.4-42.5)	35.6	35.7±1.0 (35-37.5)	19.3	27.7±3.7 (23.0-32.0)	33.6±1.4 (31.2-35.6)	35.4±0.3 (35.0-35.6)
Length of terminla bulb	102.0	98.2±4.1 (91.0-105.0)	92.0	94.8±4.5 (90.5-104.5)	64.3	81.5±9.6 (73.0-92.5)	92.4±7.7 (80.6-104.7)	87.2±4.7 (83.0-92.5)
Width of terminal bulb	18.0	19.7±1.1 (18.0-22.0)	18.0	18.7±1.0 (17.5-20.6)	12.5	16.0±1.3 (15.0-17.5)	16.8±1.1 (15.0-18.7)	18.5±1.2 (17.5-20.0)
Body width at anus level	31.3	32.7±1.6 (31.3-35.6)	33.0	34.0±2.4 (32.5-40.0)	14.0	21.1±3.0 (16.8-25.0)	28.3±1.2 (26.8-30.0)	29.0±1.0 (28.1-30)
Tail	35.6	34.0±2.3 (30.5-38.0)	33.0	35.4±2.3 (32.0-38.7)	51.0	47.6±1.7 (45.6-50.0)	40.8±2.8 (36.8-44.3)	38.0±0.6 (37.5-38.7)
Body width	43.0	44.4±2.7 (41.9-47.8)	40.0	38.5±2 (36.2-40.6)	20.6	27.6±4.3 (23.0-33.0)	38±2.3 (35.0-41.2)	29.3±1.0 (38.7-40.6)
Flange width	11.9	11.4±1.0 (10.0-12.5)	12.0	11.0±1.0 (9.4-12.5)	7.5	8.7±1.3 (7.0-10.6)	9.8±0.8 (8.0-10.6)	11.6±0.7 (11.0-11.5)
Width of lip region	12.5	13.0±0.5 (12.5-13.7)	12.5	13.0±0.5 (12.5-13.7)	8.0	9.7±0.5 (8.7-10.0)	11.3±0.2 (11.2-11.8)	11.0±0.0 (11.0-11.2)
Body width at guiding ring level	31.3	31.7±2.0 (28.7-37.0)	30.0	29.4±1.3 (27.5-31.2)	16.3	21.7±2.0 (19.3-23.7)	26.6±1.0 (25-28.0)	26.8±0.5 (26.5-27.5)
Anterior end to guiding ring	96.3	95.7±5.7 (88.0-108.0)	93.8	97.3±5.7 (90.0-104.5)	395.0	61.8±7.8 (50.6-68.7)	79.8±3.5 (75.6-85.6)	83.7±1.5 (82.5-85.6)
Hyaline part of tail	13.0	14.5±2.0 (10.3-17.0)	12.5	13.0±1.7 (12-16.8)	9.5	13.0±1.0 (12.0-13.8)	12.2±1.6 (10.6-13.7)	15.0±0.6 (14.3-15.5)
Spicules	–	–	53.0	54.0±1.2 (52.5-56.2)	–	–	–	–
Male copulatory supplements	–	–	3	2 - 3	–	–	–	–

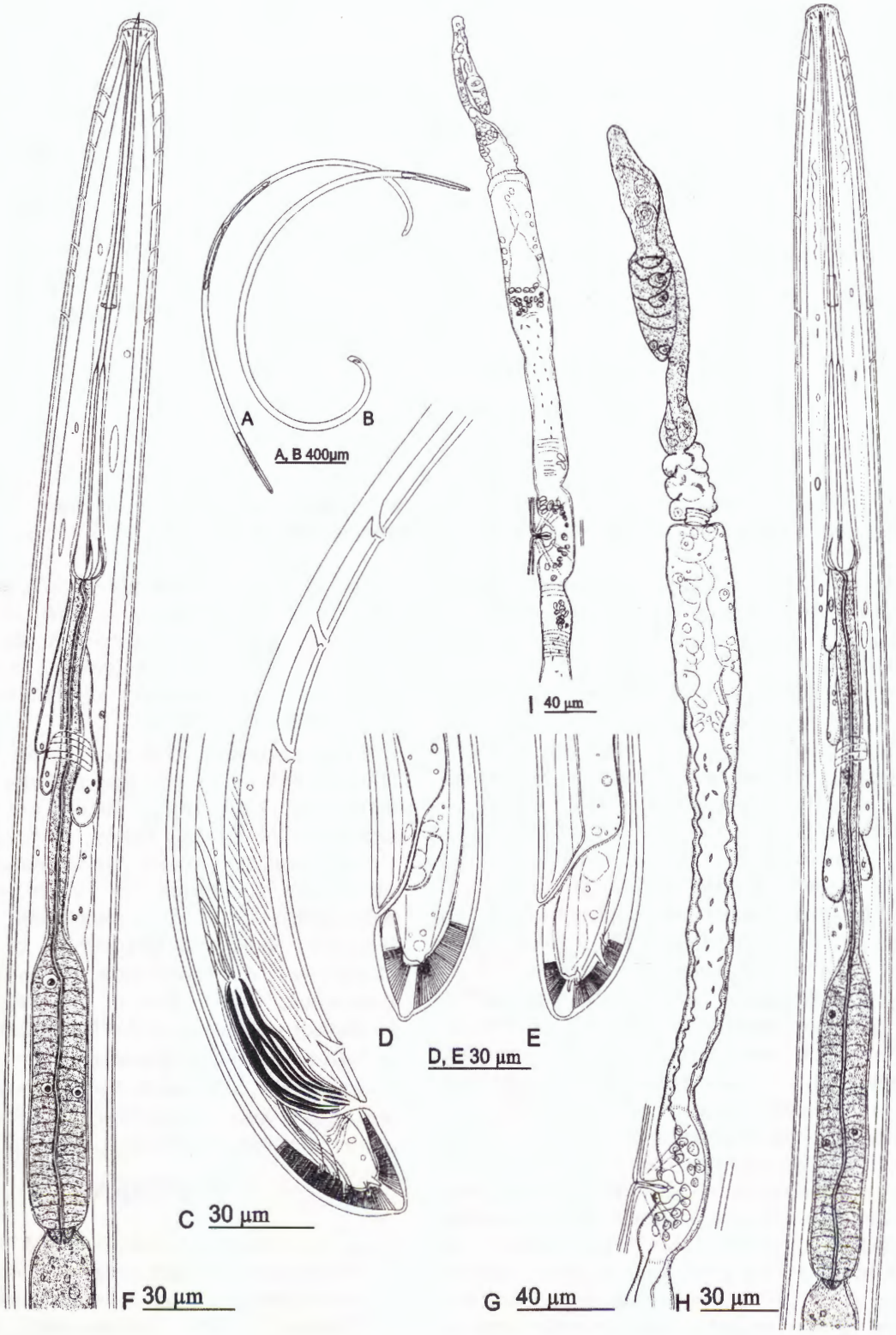

Fig. 4. *Xiphinema robbinsi* sp. n. A, B: Female and male habitus; C: Male tail; D, E: Tail in female paratype and holotype, respectively; F: Anterior end of paratype male, neck region; G: Holotype female, anterior genital branch and vagina region; H: Anterior end of holotype female; I: Paratype female uterus with sperm and spines.

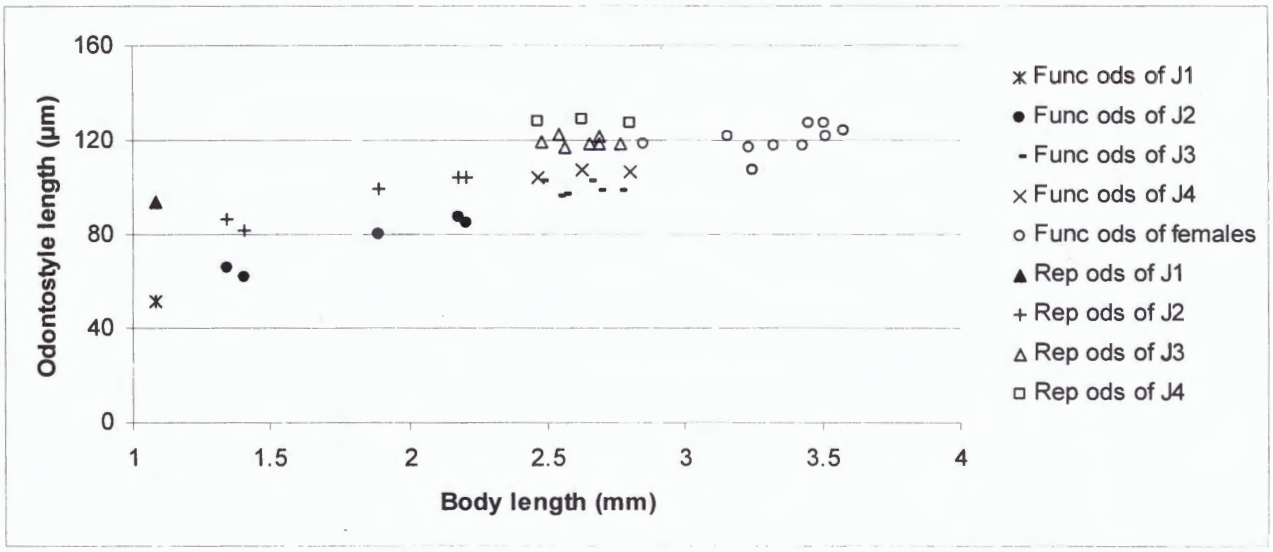

Fig. 5. Relation of body length with length of functional and replacement odontostyle (func ods and rep ods, respectively) length in all developmental stages from J1 to mature females of *Xiphinema robbinsi* sp. n.

differs from *X. aequum* (Roca & Lamberti, 1988) by its short body (3-3.8 *vs.* 4.1-5.3 mm), smaller b ratio (6.8-8.4 *vs.* 8.4-11.4 µm), shorter odontostyle (107.5-127 *vs.* 139.3-150.6 µm), shorter tail (30.5-38 *vs.* 46.6-52.0 µm), shorter spicules (52.5-56 *vs.* 66.6-86.6 µm) and shape of tail (absence of terminal peg *vs.* presence of that in both sexes). Compared to *X. riparia*, *X. robbinsi* sp. n. has a slightly longer body length (3.0-3.8 *vs.* 2.7-3.24 mm), larger a ratio (67-83 *vs.* 45.1-68.4), larger c' ratio (1.0-1.2 *vs.* 0.6-0.8), larger V value (48-56 *vs.* 43-47), shorter odontostyle (107.5-127 *vs.* 128-140 µm), shorter odontophore (62.5-74.5 *vs.* 78-88 µm), shorter spicules (52.5-56 *vs.* 59-69 µm) and different tail shape in mature individuals (broadly convex-conoid with ventral wall almost straight in some specimens and with or without slightly bulging rounded terminus *vs.* hemispherical to rounded with a distinctly terminal peg) and finally the new species differs from *X. macedonicum* by having larger V value (48-56 *vs.* 45.8-48.9), shorter odontophore (62.5-74.5 *vs.* 72-82 µm), shorter spicules (52.5-56 *vs.* 59-62 µm), shorter distance between anterior end to guiding ring (88-108 *vs.* 115.6-126.9 µm), shorter tail (30.5-38 *vs.* 35.3-52.3 µm), smaller body width at lip region (12.5-13.7 *vs.* 14.4-15 µm) and different tail shape of mature individuals (broadly convex-conoid with ventral wall almost straight in some specimens and with or without slightly bulging rounded terminus *vs.* convex-conoid, with a ventrally directed 4.4-10 µm long conical peg).

Type host and locality. All specimens were collected during November 2006 to September 2007 from the rhizosphere of *R. catharica* L. in mountain region of Goshayesh village close to Maragheh city, East Azarbaijan, north west of Iran. The GPS position of sampling point is: N 37°19.324 E 46°20.323.

Type materials. Holotype female, ten paratype females, ten males and juveniles are deposited in Nematode Collection, Faculty of Agriculture, University of Tabriz, Tabriz, Iran. Two paratype females and two males on separate microscopic slides are deposited in each of following collections: USDA Nematode Collection, Beltsville, Maryland, Department of Nematology, University of California, Riverside; CABI Bioscience, UK Center, Surrey, UK and Nematode collection of the Royal Belgian Institute of Natural Sciences, Brussels.

Etymology. The name *Xiphinema robbinsi* sp. n. was chosen honoring of Prof. Robert T. Robbins, a pioneer and well known taxonomist of longidorids.

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Pedram M., Niknam G., Decraemer W. *Xiphinema robbinsi* sp. n. (Nematoda: Dorylaimida) амфиктический вид из Ирана.

Резюме. В статье приводится описание нового вида рода *Xiphinema* из северо-западного Ирана. *Xiphinema robbinsi* sp. n. имеет характерную длину тела (3-3.8 mm); округленный передний конец с хорошо выраженным сужением и выступающим губным отделом; среднюю длину одонтостиля (107.5-127 µm); половую систему самок с двумя равно выраженными ветвями половой трубки и хорошо развитым яйцеметом; шипики в трубчатой части матки; короткий конический хвостовой конец. В популяции всегда присутствуют многочисленные самцы. Описаны 4 личиночные стадии. Новый вид наиболее близок к *X. aceri* Chizhov, Tiev & Turkina, 1986, *X. aequum* Roca & Lamberti, 1988, *Xipinema riparia* Chizhov, Subbotin, Romanenko & Kruchina, 1991 и *X. macedonicum* Barsi & Lamberti, 1999 и относится к группе «шесть» из политомического ключа Loof & Luc (1990).