Description of a new dagger nematode, *Xiphinema barooghii* n. sp. (*Nematoda: Longidoridae*) and additional data on the three known species of the genus from northwest of Iran

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Abstract

*Xiphinema barooghii* n. sp. collected from the rhizosphere of common wheat (*Triticum aestivum* L.) in Roodghat area, Sufiyan, East-Azarbaijan province, northwest of Iran, is described on the basis of the morphological, morphometric and molecular data. The new species belongs to morphospecies group 6 of the polytomous key prepared by Loof and Luc, 1990. *Xiphinema barooghii* n. sp. is characterized by having two almost equally developed female reproductive branches with spines in the tubular portion of the uterus, a body length of 3.67–4.25 mm, a flat lip region, rounded cephalic region, separated from body contour by a shallow depression, a spear 215–225 µm long, mid-body diameter of 60–79 µm, vulva (46–48%), a short tail (30–38 µm, c = 103–133, c’ = 0.7–0.9), conoid, dorsally convex, ventrally directed with a small terminal peg and a distinct terminal blind canal, the presence of four juvenile stages and the absence of males. The polytomous identification codes of the new species are: A4, B3, C5a, D6, E5, F4, G3, H2, I3, J4, K2, L1. In addition to morphological and morphometric data, molecular analyses of the D2–D3 expansion regions of the 28S rDNA gene placed the new species as a sister species of *X. herakliense* (Group 5) with 65% Bayesian posterior probability and further separated this species from the other members in group 6. In this study, *X. index*, *X. pachtaicum* and *X. vuittenezi* were also collected and additional data for the species were provided.

Key words

D2–D3 of 28S rDNA, Longidorids, Molecular analysis, Morphology, Morphometrics, New species, Taxonomy, *Xiphinema index*, *X. pachtaicum* and *X. vuittenezi*.

The genus *Xiphinema*, excluding the *X. americanum* group, comprises a complex of more than 220 species divided into eight morphospecies groups (Loof and Luc, 1990), based on the structural diversity of the female reproductive system, body habitus, lip region shape, total spear, body length and tail shape of female and juveniles using a polytomous key. Within the proposed groups, members of the group 6 (*X. pyrenaicum* species complex) are characterised by having two equally developed female genital branches with the presence of spines in the tubular part of the uterus and a rounded tail with or without a conspicuous projecting bulge.

According to Ghaderi et al. (2018), 26 species of the genus *Xiphinema* have been reported from Iran. Later on, some other species including *X. simile* (Lamberti et al., 1983; Naghavi et al., 2018), *X. macroacanthum*
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(Lamberti et al., 1989), and *X. utahense* (Lamberti and Bleve-Zacheo, 1979; Jabbari et al., 2018) have also been added to our country’s nematode fauna.

In a recent nematological survey on longidorids in Roodghat area, Sufiyan, East-Azarbaijan province in northwest of Iran, three known and one new species of the genus *Xiphinema* were found in several agroecosystems. The new species belonging to morphospecies group 6 of the genus was recovered from soil samples collected from the rhizosphere of common wheat and is described herein as *X. barooghii* n. sp. This is the fifth species belonging to morphospecies group 6 originally described from Iran, the other species being *X. robbinsi* (Pedram et al., 2008), *X. iranicum* (Pedram et al., 2009), *X. mazandaranense* (Pedram, Pourjam, Robbins, Ye, Atighi, and Decramer, 2012; Pedram, Pourjam, Palomares-Rius, Ghaemi, Cantalapiedra-Navarrete and Castillo, 2012) and *X. zagrosense* (Ghaemi et al., 2012), and except for *X. robbinsi*, the remaining three species have only been reported from Iran.

**Materials and Methods**

**Sampling, extraction, mounting, and drawing**

During a survey on nematode diversity in agroecosystems of Roodghat area, Sufiyan, East-Azarbaijan province in northwest of Iran, several soil samples were collected during 2016 and 2017 and processed at the Nematology Laboratory, University of Tabriz, Tabriz, Iran. The samples were taken from a depth of 5–30 cm. Nematodes were extracted using a tray (Whitehead and Hemming, 1965) and posteriorly killed, fixed and transferred to anhydrous glycerin using the method proposed by De Grisse (1969). Temporary and permanent microscopic slides of the nematodes were prepared to study morphological and morphometric characters. Morphometric data were obtained using a drawing tube attached to an Olympus BX41 light microscope. Photographs were taken by a DP50 digital camera system connected to the microscope. Raw photographs were edited using Adobe® Photoshop® CS. Drawings were made by Corel DRAW®, software version 12.

**DNA extraction, PCR, and sequencing**

For DNA extraction from *X. barooghii* n. sp. and *X. index*, a live adult nematode from each was hand-picked and separately placed in a small drop of distilled water or worm lysis buffer on a clean slide and crushed by a sterilized scalpel. The suspension was transferred to an eppendorf tube containing 25.65 μl ddH2O, 2.85 μl 10x PCR buffer and 1.5 μl proteinase K (600 μg/ml) (Promega, Benelux, The Netherlands). The tubes were stored at −80°C for 1 hr, incubated at 65°C for 1 hr and heated for 15 min at 95°C to inactivate the proteinase. The DNA sample was stored at −20°C until used as the PCR template. The D2–D3 domain region of LSU was amplified with the forward primer D2A (5′-ACAA-GTACCGTGAGGGAAAGTTG-3′) and the reverse primer D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Nunn, 1992). The 25 μl PCR reaction mixture was composed by 10 μl ddH2O, 12.5 μl PCR master mix (Ampiliqon, Denmark), 0.75 μl of each forward and reverse primers and 1 μl of DNA template. The process was carried out using a Thermocycler Machine in accordance with Archidona-Yuste et al. (2016). PCR cycle conditions were as follows: denaturation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 30 s, annealing of primers at 55°C for 45 s and extension at 72°C for 3 min followed by a final elongation step at 72°C for 10 min. PCR products were purified and sequenced by the Applied Biosystems® 3730/3730xl DNA Analyzer in South Korea. The recently obtained sequences of the new species and *X. index* were deposited in GenBank database under accession numbers MH884067 for *X. barooghii* n. sp. and MH879782 for *X. index* as indicated on the phylogenetic tree (Fig. 5).

**Phylogenetic analyses**

The newly obtained sequences were aligned using MEGA6 (Tamura et al., 2013) and compared with other *Xiphinema* D2–D3 expansion segment of 28S rDNA gene sequences available in GenBank using the Nblast homology search program. *Longidorus helveticus* (Lamberti et al., 2001) (AY601566) was chosen as out group. The best-fitted model of DNA evolution was obtained using MrModeltest 2.3 (Nylander, 2004) with the Akaike Information Criterion (AIC). Phylogenetic analysis of the sequence data sets was performed based on Bayesian Inference algorithm implemented in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) under the general time-reversible model with invariable sites and a gamma-shaped distribution (GTR+I+G). After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analyses. Posterior probabilities (PP) are given on appropriate clades. Tree was visualised using Fig tree 1.4.3 v.
Results

Systematics

*Xiphinema barooghii* n. sp. (Figs. 1–3; Tables 1–3).

Description

Female

It is characterized by having a cylindrical body, gradually tapered towards both ends, ventrally curved, open C to G-shape upon fixation. It has a two-layered cuticle and very fine transverse striations are visible more in tail region, 3.0–4.0 μm wide at mid-body and 11–16 μm at the tail tip. Lateral pores are present along the body, with four dorsal and five ventral located between anterior end and guiding ring. Lateral chords of 14–17 μm or those occupying one-fifth of the mid-body diameter are present. The lip region is flat and the cephalic region is rounded, separated from body contour by a shallow depression, 1.7–2.5 times as broad as high and one-fourth to one-fifth (21–28%) of body diameter at neck base. Amphidial fovea is cup shaped, with aperture occupying 52–59% of the corresponding lip region diameter, located slightly anterior to depression of head, remainder of body and pouches typical of the genus. Odontostyle is long and slender, furcates at junction with odontophore, 8.8–9.4 times lip region diameter or 1.5–1.8 times odontophore length. Odontophore with well-developed basal flanges, 14–18 μm wide, exists. A double guiding ring and a guiding sheath of 3–30 μm length, depending on the degree of protrusion/retraction of stylet, is present. Esophagus is slender with a weak muscular narrow part extending to a cylindrical terminal esophageal bulb with three nuclei. The esophageal basal bulb is 128–153 μm long, occupying about 24–29% of total esophagus length and 24–31 μm width. The nucleus of dorsal esophageal gland (DN) is located at the beginning of basal bulb (8–11%), 3.7–5.1 μm in diameter, dorsal gland esophageal orifice (D GEO), 4.7–6.5 μm from anterior end, and two smaller ventrosublateral nuclei (SVN) located at 52–57% of the terminal bulb length. The esophageal intestinal valve is rounded conoid with 12–14 μm length. The tip of reserve odontostyle (vestigium) is observed in isthmus in some specimens. The nerve ring is positioned at 55–68% length from anterior end and intestine is simple. The female reproductive system is didelphic–amphidelphic with equally developed genital branches (428–575) μm and (387–512) μm long, respectively. Each branch is composed of a 64–117 μm long reflexed ovary, not reaching the oviduct–uterus junction; oocytes are arranged first in several rows and then in a single row; oviduct is 70–160 μm long with developed *pars dilatata oviductus* near the sphincter, joining the ovary terminally; oviduct–uterus junction is marked by a poorly developed sphincter and a 256–297 μm long bipartite uterus composed of *pars dilatata uteri* close to sphincter and a tubular part containing spines 2–5 μm long, spindle-shaped and scattered between the enlarged distal portion and the ovejector, with a lack of sperm in the genital tract. Ovejector is well developed (71–84 × 15–28 μm); vagina is perpendicular to body axis, 31–37 μm long or 42–52% of corresponding body diameter in lateral view and surrounded by robust muscles. Vulva, a transverse slit, pre-equatorial in position, is present. Pre-rectum variable is 503–700 μm in length and the rectum length is 0.7–1.1 times anal body diameter. A short tail, conoid
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**Juveniles**

All four juvenile stages were identified using morphological characters such as body length, length of replacement and functional odontostyle (Robbins et al., 1996). The scatter diagram representing the relationships between body length, functional and replacement odontostyle of females and juveniles.

**Male**

Not found.

and dorsally convex, ventrally directed with a small terminal peg, 4.5–7.5 μm long, and a distinct terminal blind canal, exists. Three to four caudal pores are present on each side.

Figure 2: *Xiphinema barooghii* n. sp. Female (A, B) anterior end; (C, G) uterine differentiation spines; (D) entire body; (E) reproductive system; (F) vagina; (H) tail; (I) caudal pores in lateral optical view; (J) amphidial pouch; (K) pharyngeal expansion. (Scale bars: A–K = 10 μm, D = 70 μm).
is given in Figure 4. Juveniles are similar to adults in gross morphology, except for their smaller size, longer tail, and undeveloped reproductive organs. J1 is characterized by the lip region being separated from body contour by a deep depression, replacement odontostyle tip being close to base of functional odontostyle and located at the level of odontophore, and tail conoid and dorsally convex, directed ventrally, has a depression on dorsal and ventral sides at hyaline level, with a curved finger like cuticular extension and blind canal at the end. The lip region in J2 is separated from body contour by a depression but in J3 and J4, it is similar to that of female, i.e., flat with the cephalic region rounded and separated from body contour by a shallow depression. In J2–J4, replacement odontostyle is located at some distance from odontophore.
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Table 1. Morphometrics of *Xiphinema barooghii* n. sp. All measurements are in μm (except L in mm), and in the form: mean ± s.d. (range).

| Characters          | J1     | J2     | J3     | J4     | Holotype | Paratypes |
|---------------------|--------|--------|--------|--------|----------|-----------|
| n                   | 3      | 1      | 5      | 5      | –        | 8         |
| L                   | 1.15 ± 0.01 | 1.73 ± 0.12 | 3.07 ± 0.17 | 4.10 ± 0.21 |
| a                   | 44.0 ± 2.3 | 55.0 ± 3.9 | 58.0 ± 5.0 | 55 ± 5.0 |
| b                   | 3.7 ± 0.1 | 5.1 ± 0.3 | 6.5 ± 0.6 | 8.2 ± 0.3 |
| c                   | 18.0 ± 0.7 | 44.0 ± 2.0 | 66.0 ± 6.1 | 132 ± 10 |
| c′                  | 3.2 ± 0.1 | 4.02 ± 0.21 | 5.1 ± 0.3 | 7.7 ± 0.3 |
| V                   | –      | –      | –      | –      | 47 ± 0.9 |
| Lip region diam.    | 10.5 ± 0.3 | 12.2 ± 0.2 | 14.0 ± 1.1 | 15 ± 1.0 |
| Odontostyle length | 58.5 ± 0.2 | 95.0 ± 2.8 | 113.0 ± 3.3 | 139 ± 2.7 |
| Odontophore length | 45.0 ± 1.4 | 63.0 ± 3.0 | 76.0 ± 6.3 | 82 ± 3.2 |
| Spear length        | 104.0 ± 0.4 | 158.0 ± 4.8 | 189.0 ± 5.7 | 221 ± 3.3 |
| Replacement odontostyle | 72.0 ± 1.6 | 115.0 ± 2.9 | 137.0 ± 1.7 | – ± 0.0 |
| Oral aperture to guide ring | 51.0 ± 0.5 | 82.0 ± 3.1 | 101.0 ± 2.4 | 129 ± 4.3 |
| Pharynx length      | 311.0 ± 3.4 | 423 ± 37 | 447 ± 31 | 500 ± 19 |
| Pharyngeal bulb length | 88.0 ± 0.7 | 112.0 ± 8.6 | 122.0 ± 2.5 | 144 ± 9.3 |
| Body diam. at phar. base | 24.0 ± 1.6 | 36.0 ± 3.6 | 45.0 ± 4.1 | 65 ± 5.0 |
| mid-body            | 24.0 ± 2.1 | 40.0 ± 3.4 | 52.0 ± 5.0 | 74 ± 6.7 |
| anus                | 18.0 ± 0.4 | 30.0 ± 0.8 | 40.0 ± 2.7 | 41 ± 1.8 |
| G1                  | –      | –      | –      | –      | 13 ± 0.4 |
| G2                  | –      | –      | –      | –      | 13 ± 1.3 |
| Prerectum length    | 265 ± 16 | 412 ± 28 | 572 ± 40 | 603 ± 74 |

Continued
|                     |       |       |       |       |       |       |       |       |       |       |
|---------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| **Rectum length**   | 14.0  | 26.0  | 33.0  | 43.0  |       |       |       |       |       |       |
| (13.0 – 15.0)       | (22.0 – 28.0) | (31.0 – 35.0) | (35.0 – 51.0) |       |       |       |       |       |       |
| **Tail length**     | 61.0  | 49.0  | 46.0  | 33.0  |       |       |       |       |       |       |
| (59.0 – 63.0)       | (48.0 – 51.0) | (44.0 – 48.0) | (30.0 – 38.0) |       |       |       |       |       |       |
| **Hyaline part of tail** | 8.4   | 13.0  | 14.2  | 13.0  |       |       |       |       |       |       |
| (8.0 – 10.0)        | (12.0 – 15.0) | (13.0 – 15.0) | (11.0 – 16.0) |       |       |       |       |       |       |

In J2, the tail is conoid and dorsally convex, slightly bent ventrally and with a dorsal depression at hyaline region level; in J3, tail is conoid, dorsally convex, ventrally more or less flat with a slightly developed mucro and tail of J4 is similar to that of female.

**Diagnosis and relationships**

*Xiphinema barooghii* n. sp. belongs to morphospecies group 6 *sensu* Loof and Luc (1990). It is an apparently parthenogenetic species characterised by a medium-to-moderate long body of 3.67–4.25 mm; a C to G-shape upon fixation; a flat lip region, a rounded cephalic region, separated from body contour by a shallow depression of 14–15 μm width; along and slender odontostyle having 132–139 μm length; a guide ring being located at 117–132 μm from anterior end; a female reproductive system being didelphic with two opposite almost equally developed reproductive branches with spines in the tubular part of the uterus; a short tail, conoid and dorsally convex, ventrally

**Table 2. Specific α-numeric code of each *Xiphinema* spp. belonging to X. non-americanum morphospecies Group 6 according to Loof and Luc (1990).**

| Xiphinema spp.   | A | B | C | D | E | F | G | H | I | J | K | L | References |
|------------------|---|---|---|---|---|---|---|---|---|---|---|---|-------------|
| spinuterus       | 4 | 3 | 1 | 1 | 4 | 3 | 3 | 2 | 12| – | – | 2 | 1           |
| mluci            | 4 | 3 | 2 | 123| 45 | (3)45| 23 | 2 | 34| 2 | – | 1 | 1           |
| xenovariabile    | 4 | 3 | 23| 34 | 56 | 23 | 1 | 2 | 3(4)| 2 | – | 2 | 1           |
| diannae          | 4 | 3 | 3 | 4  | 45 | 3  | 12| 2 | 23| 3 | – | 2 | 1           |
| coomansi         | 4 | 3 | 3 | 45 | 456| 3  | 2 | 2 | 3 | 3 | – | 2 | 1           |
| lacrimaspinae    | 4 | 3 | 4 | 4  | 4  | 3  | 2 | 2 | 3 | 34| 2 | 1 | 1           |
| barbercheckae    | 4 | 3 | 4 | 5  | 56 | 3  | 2 | 2 | 23| 3 | – | 1 | 1           |
| mammatum         | 4 | 3 | 5a | 4 | 4  | 3  | 2 | 2 | 3 | 5 | 2 | 2 | 1           |
| aequum           | 4 | 3 | 5a | 5  | 5  | 45 | 3 | 2 | 3 | 5 | 2 | 2 | 1           |
| aceri            | 4 | 3 | 6a | 56 | 5  | 4  | 23| 3 | 3 | 6 | – | 1 | 1           |
| robbinsi         | 4 | 3 | 5a | 65 | 56 | 3(4)| 2 | 2 | 3(4)| 5a| 2 | 2 | 2           |
| iranicum         | 4 | 3 | 5a, b| 6 | 6  | 4  | 3(4)| 2 | 3 | 5 | 4 | 1 | 3           |
| nuragicum        | 4 | 3 | 7 | 6  | 56 | 345| 3 | 2 | 3 | 7 | 2 | 1 | 4           |
| pyrenaicum       | 4 | 3 | 6 | 6  | 56 | 345| 3 | 2 | 3 | 6 | 2 | 1 | 4           |
| adenohystherum   | 4 | 3 | 7 | 6  | 56 | 45 | 3 | 2 | 3 | 7 | – | 1 | 4           |
| sphaerocephalum  | 4 | 3 | 5 | 6  | 56 | 34 | 3 | 2 | 3 | 5 | 2 | 1 | 4           |
| mazandaranense   | 4 | 3 | 7b | 6 | 456| 45 | 2 | 2 | 23| 7b| 2 | 1 | 5           |
| zagrosense       | 4 | 3 | 6 | 6  | 456| 45 | 34| 2 | 3 | 5 | 2 | 1 | 6           |
| vuittenezi       | 4 | 3 | 5(7b)| 56 | 56 | 34 | 23| 2 | 3 | 45| 2 | 1 | 7           |
| barooghii n. sp. | 4 | 3 | 5a | 6 | 5  | 4  | 3 | 2 | 3 | 4 | 2 | 1 | 8           |
Table 3. *Xiphinema* species, locality, associated host and sequences used in this study.

| Species                  | Locality                                                                 | Host-plant                                                                 | Accession number |
|--------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------------------|------------------|
| *X. abrantinum*          | –              | –                                                                          | AY601625         |
| *X. adenohystherrum*     | Bollullos par del Condado, Huelva province, Spain                       | *Vitis vinifera* L.                                                      | GU725075         |
| *X. andalusiense*        | Arévalo de la Sierra, Soria province, Spain                             | Holly tree                                                               | KC567164         |
| *X. baetica*             | Manzanilla, Huelva province, Spain                                       | Grapevine                                                                | KC567167         |
| *X. bakeri*              | –              | –                                                                          | AY601623         |
| *X. barense*             | Apulian region, Torre Pozzella, Brindisi province, southern Italy        | Wild olive (*Olea europaea sylvestris* L.)                                | KM199690         |
| *X. barense*             | Apulian region, Torre Pozzella, Brindisi Province, southern Italy        | Wild olive (*Olea europaea sylvestris* L.)                                | KM199691         |
| *X. barense*             | Apulian region, Torre Pozzella, Brindisi Province, southern Italy        | Wild olive (*Olea europaea sylvestris* L.)                                | KM199692         |
| *X. barooghii* n. sp.    | Roodghat area, Sufiyan, East-Azarbaijan province, northwest of Iran      | Common wheat (*Triticum aestivum* L.)                                     | MH884067         |
| *X. basiri*              | –              | –                                                                          | AY601630         |
| *X. belmontense*         | Merza, Coruña province, Spain                                            | Pedunculate oak                                                          | KC567172         |
| *X. brasiliense*         | Shenzhen, China                                                         | –                                                                          | KP793050         |
| *X. brasiliense*         | –              | –                                                                          | AY601616         |
| *X. castilloi*           | Sefid Rud River near Rasht, Gilan province, Iran                        | Ash tree                                                                  | KF444655         |
| *X. cadavalense*         | Espiel ,Cordoba province, Spain                                          | Cultivated olive                                                         | KX244900         |
| *X. celtiense*           | Peñafar, Sevilla province, Spain                                         | Wild olive                                                               | KX244889         |
| *X. chambersi*           | Florida, USA                                                            | –                                                                          | DQ299512         |
| *X. chambersi*           | –              | –                                                                          | AY601617         |
| *X. citricolum*          | Florida, USA                                                            | –                                                                          | DQ285668         |
| *X. cohni*               | El Puerto de Santa María, Cádiz province, Spain                          | Stone pine                                                               | KC567173         |
| *X. conurum*             | Uleila del Campo, Almeria province, Spain                               | cultivated olive                                                         | KX244902         |
| *X. cretense*            | Hersonisos, Heraklion province, Crete, Greece                           | Olive (*Olea europaea* L. subsp. *europaea*)                              | KJ802878         |
| *X. costaricense*        | Pacayitas, La Suiza de Turrialba, Cartago, Costa Rica                   | Sugarcane                                                                | KX931059         |
| *X. coxi*                | Hinojos, Huelva province, Spain                                          | Carob tree                                                               | KC567175         |
| *X. dentatum*            | Czech Republic                                                          | Carpinus betulus and *Acer platanoides*                                  | EU781538         |
| *X. diversicaudatum*     | –              | –                                                                          | EF538755         |
| *X. diversicaudatum*     | Marchegg, Austria                                                       | –                                                                          | JQ780366         |
| *X. elongatum*           | China                                                                   | –                                                                          | EF140790         |
| *X. floridae*            | Florida, USA                                                            | –                                                                          | DQ299507         |

Continued
| Species          | Location                                                                 | Planta or Pathogen                          | Accession Number |
|------------------|---------------------------------------------------------------------------|---------------------------------------------|------------------|
| X. gersoni       | Almonte, Huelva province, Spain                                           | Eucalyptus                                  | KC567180         |
| X. georgianum    | Florida, USA                                                              | –                                           | DQ299497         |
| X. globosum      | Valdeinfierno in the Los Alcornocales Regional Park, Alcalá de los Gazules, Cádiz province, southern Spain | Black alder, Alnus glutinosa, and river bank grapevine, *Vitis riparia* | GU549474         |
| X. granatum      | Saveh, Markazi province, Iran                                            | Pomegranate trees (*Punica granatum* L.)    | JQ240273         |
| X. hangzhouense  | Hangzhou, Zhejiang Province, China                                       | Magnolia grandiflora L.                    | MF538772         |
| X. herakliense   | Vathy Rema, Heraklion province, Crete, Greece                            | Olive tree (*Olea europaea* subsp. *sylvestris*) | KM586345         |
| X. herakliense   | Hersonisos, northeast Heraklion province, Crete, Greece                  | Olive (Olea europaea subsp. *europaea* L.)  | KM586351         |
| X. herakliense   | Agiofarago, south west Heraklion province, Crete, Greece                 | Olive                                       | KM586347         |
| X. herakliense   | Agiofarago, south west Heraklion province, Crete, Greece                 | Olive                                       | KM586348         |
| X. herakliense   | Agiofarago, south west Heraklion province, Crete, Greece                 | Olive                                       | KM586349         |
| X. herakliense   | Hersonisos, northeast Heraklion province, Crete, Greece                  | Olive (Olea europaea subsp. *europaea* L.)  | KM586352         |
| X. hispanum      | Andujar, Jaen province, Spain                                            | *Cistus albidus* L.                         | GU725074         |
| X. hispidum      | Bollulos par del Condado, Huelva province, Spain                         | Grapevine (*Vitis vinifera* L.)             | HM921346         |
| X. hunaniense    | Shenzhen, China                                                           | –                                           | KP793045         |
| X. hunaniense    | Shenzhen, China                                                           | –                                           | KP793048         |
| X. index         | Córdoba province, Spain                                                  | Grapevine                                  | HM921398         |
| X. index         | Kentri, Greece                                                           | Olive                                       | KJ802882         |
| X. index         | Córdoba province, Spain                                                  | Grapevine                                  | HM921399         |
| X. index         | Córdoba province, Spain                                                  | Grapevine                                  | HM921400         |
| X. index         | Cádiz province, Spain                                                    | Grapevine                                  | HM921401         |
| X. index         | Roodghat area, Sufiyan, East-Azarbaijan province, northwest of Iran       | Apple (*Malus domestica* L.) variety Red delicious | MH879782         |
| X. index         | Chogha Kaboud village, Harsin, Kermanshah province, Iran                 | *Astragalus* sp.                           | KJ956388         |
| X. insigne       | –                                                                        | –                                           | AY601619         |
| X. israeliae     | Roufas, Greece                                                           | Olive                                       | KJ802883         |
| X. israeliae     | Agiofarago, Greece                                                      | Wild olive                                 | KJ802884         |
| X. italae        | Cabra, Córdoba province, Spain                                          | Grapevine                                  | KC567182         |
| X. italae        | –                                                                        | –                                           | AY601613         |
| X. iznajarense   | Iznaajar, Cordoba province, Spain                                        | Cultivated olive                           | KX244892         |
| X. japonicum     | Japan                                                                    | *Podocarpus macrophyllus* L.               | KY131240         |

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| Species               | Location/Host                  | Identification Code |
|-----------------------|--------------------------------|---------------------|
| *X. laevistriatum*    | Florida, USA                   | DQ299505            |
| *X. lambertii*        | India                          | HM163211            |
| *X. lupini*           | Bollullos par del Condado, Huelva province, Spain | Grapevine (Vitis vinifera L.) HM921352 |
| *X. lupini*           | Hinojos, Huelva province, Spain | Grapevine           |
| *X. macroacanthum*    | Southern Italy                 |                     |
| *X. macrodora*        | La Granjuela, Córdoba province, Spain | Cultivated olive KU171040 |
| *X. mengibarense*     | Mengibar, Jaen province, Spain | Cultivated olive    |
| *X. meridianum*       | Sbitla, Kasserine, Tunisia     | Cultivated olive    |
| *X. naturale*         | Florida, USA                   | DQ299515            |
| *X. nuragicum*        | Marchena, Seville province, Spain | Olea europaea sp. europaea L. GU725071 |
| *X. nuragicum*        | Puente Genil, Cordoba province, Spain | Vitis vinifera L. GU725067 |
| *X. oleae*            | Tarifa, Cádiz province, Spain  | KU171037            |
| *X. poasense*         | Toro Amarillo, Valverde Vega, San Carlos Alajuela, Costa Rica | Eucalyptus, cypress and fountain grass MF461347 |
| *X. pseudocoxi*       | Alcaracejos, Cordoba province, Spain | Wild olive KK244915  |
| *X. pyrenaicum*       | Cahors, Midi-Pyrenees province, France | Vitis vinifera L. GU725073 |
| *X. rivesi*           | Bollullos par del Condado, Huelva province, Spain | Grapevine (Vitis vinifera L.) HM921358 |
| *X. robbinsi*         | Sbitla, Kasserine, Tunisia     |                     |
| *X. robbinsi*         | Abida, Kairouan, Tunisia       |                     |
| *X. santos*           | –                              |                     |
| *X. savanica*         | –                              |                     |
| *X. setariae*         | –                              |                     |
| *X. sphaerocephalum*  | Coto Rios, Jaen province, Spain | Quercus faginea L. GU725076 |
| *X. tarjanense*       |                                 | DQ299511            |
| *X. tica*             | Chirraca, San Ignacio de Acosta, San José, Costa Rica | Vine KY623485       |
| *X. turicum*          | Sanlúcar de Barrameda, Cádiz province, Spain | Grapevine KC567185  |
| *X. turdetanensis*    | Sanlúcar de Barrameda, Cádiz province, Spain | Stone pine KC567186  |
| *X. vuittenezi*       | Czech Republic                 |                     |
| *X. vuittenezi*       | –                              |                     |
| *X. vulgare*          | Florida, USA                   |                     |
| *X. zagrosense*       | Madavan village, Kohgiluyeh and Boyer-Ahmad province, Iran | Grasses JN153101    |

Directed with a small terminal peg, 4.5–7.5 μm long, and a distinct terminal blind canal; lack of males in population, having four juvenile stages and J1 tail having a depression on dorsal and ventral sides at hyaline level, with a curved finger like cuticular extension at the end.

The identification codes for the new species, according to the polytomous key of Loof and Luc (1990), are: A4, B3, C5a, D6, E5, F4, G3, H2, I3, J4, K2, L1.

Based on the molecular and morphological similarities, the new species is closely related to *X. aceri* (Chizhov et al., 1986); *X. granatum* (Pedram, Pourjam,
Palomares-Rius, Ghaemi, Cantalapiedra-Navarrete and Castillo, 2012; X. herakliense (Tzortzakakis et al., 2015); X. zagrosense (Ghaemi et al., 2012), and X. vuittenezi (Luc et al., 1964), but it can be separated using the morphometric data, characters of the juveniles, especially the shape of their tail in the first stage and partial sequences of 28S rDNA (except X. aceri as it currently lacks molecular data of D2–D3 expansion part of 28S rDNA).

Compared to X. aceri, the new species has a shorter body (3.67–4.25 vs 4.90–5.50 mm), smaller a (53–63 vs 75–83) and V values (46–48 vs 48–51), shorter odontophore (75–85 vs 83–101 μm) and juvenile characters as well, e.g., shorter tail length (59–63 vs 67.2–68.8 μm) and tail characters in J1. Xiphinema barooghii n. sp. differs mainly from X. granatum by having lower a (53–63 vs 74–99), b (7.0–8.5 vs 8.5–11.0) and c’ (0.7–0.9 vs 1.2–1.5) values, longer odontostyle (132–139 vs 118–132 μm), odontophore (75–85 vs 65–74 μm) and spear (215–225 vs 189–204 μm), esophageal (493–553 vs 360–460 μm) and esophageal basal bulb length (128–153 vs 77–104 μm), posteriorly located guiding ring (117–132 vs 100–116 μm), absence of males, uterus with spines vs devoid of any Z-differentiation or spines and juvenile characters. In addition, X. barooghii n. sp. is similar to X. herakliense but differs by a longer esophageal basal bulb (128–153 vs 94–121 μm), absence vs presence of a pseudo-Z-organ and cuticular bodies in the uterus. Furthermore, X. herakliense is an amphimictic species and has functional males and sperm in the female reproductive system compared to the absence of males in the new species. The new species differs from X. zagrosense by a shorter odontostyle (132–139 vs 151–169 μm), odontophore (75–85 vs 94–105 μm) and spear length (215–225 vs 215–225 vs 246–274 μm), slightly smaller lip region width (14–15 vs 15–18 μm), the shape of tail (conoid, dorsally convex, ventrally directed with a small terminal peg and distinct terminal blind canal vs conoid and dorsally convex, with rounded end lacking a mucro or cuticular projection) and differences in juvenile characters. Finally, X. barooghii n. sp. can be differentiated from X. vuittenezi (according to original description) by a slightly longer body in the females (3.67–4.25 vs 2.63–3.83 mm), longer spear (215–225 vs 183–212 μm), wider body diameter at neck base (52–69 vs 44–μm) and mid-body or vulva level (60–79 vs 39–58 μm), cuticle two vs three layered, cephalic region separated from body contour by a shallow depression and 21–28% of body diameter at neck base vs clearly depression and 31% of body diameter at neck base, greater length and width of esophageal basal bulb, 128–153 × 24–31 vs 109–126 × 18–23 μm, cardia 12–14 μm long and rounded conoid vs 6.5 μm (calculated from the image) conoid. In addition, there are a number of differences in juvenile characters, in J1: greater body (1.14–1.16 vs 0.78–1.03 mm), odontostyle (58–59 vs 47–53 μm), odontophore (44–47 vs 34–40 μm), spear (103–105 vs 81–93 μm), replacement odontostyle (71–74 vs 62–71 μm) length, oral aperture to guide ring (50–52 vs 43–50 μm), tail length (59–63 vs 40–52 μm) and shape (presence a depression on dorsal and ventral sides at hyaline level, with a curved finger like cuticular extension at the end vs absence); in J2: longer body (1.73 vs 1.07–1.49 mm), odontophore (54 vs 42–50 μm), spear (124 vs 107–121 μm), replacement odontostyle (93 vs 80–87 μm), oral aperture to guide ring (65 vs 56–59 μm) and tail length (60 vs 43–50 μm), larger body diameter at mid-body (42 vs 22–34 μm) at anus level (26 vs 15–23 μm); in J3: higher body length (2.01–2.31 vs 1.47–1.95 mm), odontostyle (93–100 vs 79–89 μm) and spear (150–167 vs 130–146 μm) length and a value (51–62 vs 37–48); in J4: longer body (2.87–3.34 vs 2.01–2.74 μm), replacement odontostyle (135–139 vs 121–135 μm) and a value (51–64 vs 44–50).

**Type habitat and locality**

Soil samples were collected from the rhizosphere of common wheat (*Triticum aestivum* L.) in Roodghat area, Sufiyan, East-Azarbaijan province, northwest of Iran, during 2016 and 2017 (GPS coordinates: N 38°22′ 10″, E 46° 07′ 26″, altitude 1808 m a.s.l.).

**Type material**

Holotype and paratype females and juveniles were deposited at Nematology Collection of the Department of Plant Protection, Faculty of Agricul-
Xiphinema index Thorne and Allen (1950)
(Fig. 6 A–E; Tables 3, 4)

Distribution
Iran, East-Azerbaijan province, Sufiyan, Roodghat area (GPS coordinates: N 38° 19’ 59” E 46° 07’ 00”, altitude 1582 m a.s.l.), in the rhizosphere of apple (Malus domestica L.) variety Red delicious.

Remarks
Xiphinema index is a soil dweller, its major economic host is grapevine and acts as vector of Grapevine Fanleaf Virus (GFLV), very well known, worldwide spread species, recorded from many countries of Africa, America, Australia, Asia and Europe. In Iran, this species was first observed by Mojtahedi et al. (1980) in cultivated soils and in natural woodland as well and then reported from different parts of Iran. Comparison of our population sequence with the GenBank database showed that Iranian X. index (MH879782) has 99% identity with other populations of the species. Phylogenetic analysis placed our population with other populations of X. index in a clade with 0.87 PP values. Males were not found but a few females contain sperm cells in their ovejector and pars dilatata oviductus. This Iranian population of females of X. index is within the morphometrical ranges recorded for the species (for comparative purposes, see Thorne and Allen, 1950; Mojtahedi et al., 1980; Lamberti et al., 1987; Barsi and Lamberti, 2000a; Jawhar et al., 2006; Gutiérrez-Gutiérrez et al., 2011; Meza et al., 2012).

Xiphinema pachtaicum (Tulaganov, 1938; Kirjanova, 1951)
(Fig. 6 F–H; Table 4)

Distribution
It was collected in 2016 and 2017 from Iran, East-Azerbaijan province, Sufiyan, Roodghat area (GPS coordinates: N 38° 22’ 10”, E 46° 07’ 26”, altitude 1808 m a.s.l.), from the rhizosphere of common wheat (Triticum aestivum L.).

Remarks
This species is widely distributed in the rhizosphere of different plants in agricultural lands and reported from several localities in the world including Africa, America, Australia, Asia and Europe. Little is known
Figure 5: Bayesian tree inferred under the GTR + I + G model from 28S rDNA D2–D3 expansion domains of X. barooghii n. sp. and X. index (--lnL = 9,085.8555; AIC = 18,191.7109; freqA = 0.2402; freqC = 0.2341; freqG = 0.2885; freqT = 0.2373; R(a) = 1.0503; R(b) = 2.8325; R(c) = 2.6784; R(d) = 0.5047; R(e) = 4.0878; R(f) = 1.0000). Posterior probabilities are given for appropriate clades. Newly obtained sequences are indicated by bold letters.
Decription of a new dagger nematode, *Xiphinema barooghii* n. sp. (Nematoda: Longidoridae)

**Figure 6**: (A–E) *Xiphinema index* Thorne and Allen, 1950, (A) Anterior end; (B) Tubular part of uterus; (C) Vagina; (D) Pars dilatata oviductus; (E) Tail, (F–H) *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951, (F) Anterior end; (G) Anterior genital branch; (H) Tail, (I–N) *X. vuittenezi* Luc, Lima, Weischer and Flegg, 1964, (I) Anterior end; (J, L) Uterine differentiation spines; (K) Vagina; (M, N) Tail. (Scale bars =10 μm).

About its role as a plant pathogen and it has not been recorded as a vector of plant viruses. Mojtabehdi et al. (1980) reported *X. pachtaicum* for the first time from vineyards in Iran; later on, it was obtained from different localities in the country from the rhizosphere of different plants. The Iranian specimens fit, morphologically and morphometrically, well to earlier descriptions of the species (Fadaei et al., 2003; Getaneh et al., 2015; Lazarova et al., 2016).

*Fig. 6 I–N; Table 4*.

**Xiphinema vuittenezi** Luc et al. (1964)
Table 4. Morphometrics of the Iranian populations of *Xiphinema* species. All measurements are in μm (except L in mm), and in the form: mean ± s.d. (range).

| Characters                  | *X. index* Female | *X. pachtaicum* Female | *X. vuittenezi* Female |
|-----------------------------|-------------------|------------------------|------------------------|
| n                           | 5                 | 5                      | 6                      |
| L                           | 2.98 ± 0.18 (2.68 – 3.13) | 2.04 ± 0.08 (1.95 – 2.17) | 3.51 ± 0.16 (3.25 – 3.76) |
| a                           | 55.0 ± 4.2 (50.0 – 60.0) | 61.0 ± 8.7 (62.0 – 74.0) | 69.0 ± 5.0 (63.0 – 78.0) |
| b                           | 6.7 ± 0.3 (6.3 – 7.3) | 6.7 ± 0.6 (6.3 – 7.8) | 7.6 ± 0.1 (7.3 – 7.9) |
| c                           | 76.0 ± 2.4 (74.0 – 80.0) | 69.0 ± 3.6 (64.0 – 73.0) | 89.0 ± 3.6 (77.0 – 95.0) |
| c’                          | 1.04 ± 0.05 (1.00 – 1.10) | 1.50 ± 0.10 (1.40 – 1.70) | 1.10 ± 0.08 (1.00 – 1.20) |
| V                           | 37.0 ± 0.7 (36.0 – 38.0) | 54.0 ± 1.2 (53.0 – 56.0) | 49.0 ± 0.4 (47.0 – 50.0) |
| Lip region diam.            | 13.0 ± 0.8 (12.0 – 14.0) | 9.4 ± 0.5 (9.0 – 10.0) | 13.0 ± 0.7 (12.0 – 14.0) |
| Odontostyle length          | 121.0 ± 2.7 (118.0 – 125.0) | 83.0 ± 2.6 (81.0 – 87.0) | 122.0 ± 3.7 (118.0 – 128.0) |
| Odontophore length          | 70.0 ± 1.7 (69.0 – 73.0) | 50.0 ± 3.3 (44.0 – 54.0) | 68.0 ± 4.1 (51.0 – 75.0) |
| Spear length                | 192.0 ± 2.7 (189.0 – 195.0) | 133.0 ± 2.7 (130.0 – 137.0) | 191.0 ± 6.8 (175.0 – 200.0) |
| Oral aperture to guide ring | 114.0 ± 4.3 (106.0 – 119.0) | 70.0 ± 3.6 (68.0 – 77.0) | 108.0 ± 4.3 (100.0 – 113.0) |
| Pharynx length              | 437 ± 11 (421 – 450) | 303 ± 20 (250 – 328) | 457 ± 11 (443 – 475) |
| Pharyngeal bulb length      | 99.0 ± 6.4 (87.0 – 110.0) | 77.0 ± 4.6 (72.0 – 84.0) | 114 ± 10 (100 – 131) |
| Body diam. at phar. base    | 47.0 ± 3.4 (43.0 – 52.0) | 29.0 ± 2.2 (26.0 – 32.0) | 41.0 ± 2.7 (37.0 – 45.0) |
| mid-body                    | 54.0 ± 3.8 (45.0 – 59.0) | 33.0 ± 2.4 (28.0 – 37.0) | 49.0 ± 5.3 (41.0 – 56.0) |
| anus                        | 35.0 ± 3.5 (31.0 – 40.0) | 19.0 ± 1.4 (17.0 – 21.0) | 35.0 ± 2.1 (32.0 – 38.0) |
| G1                          | 13.2 ± 0.4 (13.0 – 14.0) | 12.0 ± 1.2 (11.0 – 14.0) | 12.6 ± 0.3 (12 – 13) |
| G2                          | 12.0 ± 1.3 (11.0 – 14.0) | 12.0 ± 1.9 (10.0 – 14.0) | 12.0 ± 0.8 (11.0 – 13.0) |
| Prerectum length            | 320 ± 46 (250 – 381) | 168 ± 17 (134 – 187) | 496 ± 58 (431 – 575) |
| Rectum length               | 30.0 ± 1.9 (27.0 – 32.0) | 24.0 ± 3.5 (23.0 – 28.0) | 31.0 ± 1.2 (29.0 – 33.0) |
| Tail length                 | 39.0 ± 0.9 (36.0 – 41.0) | 29.0 ± 1.1 (28.0 – 31.0) | 39.0 ± 1.4 (38.0 – 41.0) |
| Hyaline part of tail        | 17.0 ± 1.4 (15.0 – 19.0) | 10.0 ± 0.8 (9.0 – 11.0) | 14.0 ± 1.8 (12.0 – 16.0) |

**Distribution**

It was collected from Iran, East-Azarbaijan province, Sufiyan, Roodghat area (GPS coordinates: N 38° 22’ 10” E 46° 07’ 26”, altitude 1481 m a.s.l.), from the rhizosphere of common wheat (*Triticum aestivum* L.).

**Remarks**

This species is widely spread in Europe. Besides, it has been recorded from Asia, North America, South America and Australia. It inhabits vineyards and various orchards (Andrássy, 2009). The original descrip-
tion of X. vuittenezi did not consider the presence of spines in the uteri and such structures were not mentioned in the revised polytomy key to the species of Xiphinema by Loof and Luc (1990), although distinct spindle-shaped structures of variable size and number were observed in the tubular portion of the uterus of an Iranian population of X. vuittenezi by Mojtahedi et al. (1980). For the first time in Iran, this species has been reported by Mojtahedi et al. (1980) from different regions of the country. The present Iranian specimens correspond well with the earlier descriptions in their general morphology and morphometrics as the relevant measurements and values are totally coincident or widely overlapping (Luc et al., 1964; Barsi and Lamberti, 2000b; Kumari and Decraemer, 2006).

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