

*Xiphinema granatum n. sp. and Longidorus pisi from Iran*

Nem D 11-00121

1  
2  
3 **Molecular and morphological characterisation of *Xiphinema granatum***  
4 ***n. sp.* and *Longidorus pisi* Edward, Misra & Singh, 1964 (Dorylaimida:**  
5 **Longidoridae) from Iran**

6  
7 Majid PEDRAM<sup>1</sup>, Ebrahim POURJAM<sup>1,\*</sup>, Juan E. PALOMARES-RIUS<sup>2</sup>, Azam HOUSHMAND<sup>1</sup>,  
8 Carolina CANTALAPIEDRA-NAVARRETE<sup>2</sup> and Pablo CASTILLO<sup>2</sup>

9  
10 <sup>1</sup> *Department of Plant Pathology, College of Agriculture, Tarbiat Modares University,*  
11 *Tehran, Iran*

12 <sup>2</sup> *Institute for Sustainable Agriculture (IAS), Spanish National Research Council*  
13 *(CSIC), Alameda del Obispo s/n, Apdo. 4084, 14080 Córdoba, Spain*

14  
15  
16 Received: December 2011; revised: March 2012

17 Accepted for publication: March 2012

18  
19  
20  
21  
22  
23 \_\_\_\_\_  
24 \* Corresponding author, e-mail: Pourjame@modares.ac.ir

1 **Summary** – *Xiphinema granatum* n. sp. from pomegranate in Saveh, Markazi province,  
2 central Iran, is described and illustrated. The new species belongs to species group eight  
3 of the genus *Xiphinema sensu* Loof & Luc, 1990. The new species is characterised by a  
4 body 3.5-4.1 mm long, 37-49 µm diam., odontostyle and odontophore 118-132 and 65-  
5 74 µm long, respectively, presence of sperm in the female genital tracts, uterus devoid  
6 of any Z-differentiation or spines, and abundant males with spicules 45-58 µm long.  
7 The polytomous identification codes of the new species are: A4, B4, C5a, D5, E56, F4,  
8 G23, H2, I3, J4, K2, L2. Beside morphological and morphometric data, molecular  
9 analyses of D2-D3 fragments of 28S rRNA gene placed the new species in close  
10 relationship with *X. vuittenezi* and *X. italiae* and some species belonging to *Xiphinema*  
11 morphospecies group 6, but clearly separated from *X. index*. In addition, an Iranian  
12 population of *Longidorus pisi* from the same locality is morphological and molecularly  
13 characterised.

14

15 **Keywords** - dagger nematodes, description, molecular, morphology, morphometrics,  
16 needle nematodes, phylogeny, pomegranate, *Punica granatum*, taxonomy.

17

1           *Xiphinema* Cobb, 1913 tends to be greatly conserved in gross morphology which  
2 makes species identification very challenging because of overlapping morphology and  
3 morphometry. In order to facilitate species identification, Loof and Luc (1990)  
4 introduced eight morphospecies groups within the genus based on the structural  
5 diversity of the female reproductive system, habitus, lip region shape, total spear and  
6 body length, and tail shape of female and juveniles using a polytomous key. They  
7 excluded the *X. americanum* group. Within the proposed groups, members in the  
8 morphospecies group eight are characterised by having two equally developed female  
9 genital branches, a simple uterus devoid of any differentiation, and tail short, conical to  
10 rounded (Loof & Luc, 1990). Currently, morphospecies group eight comprises 21  
11 nominal species, including the virus vector species *X. index* Thorne & Allen, 1950.

12           During a recent nematological survey in Iran, a species of *Xiphinema* belonging  
13 to group eight was collected in the rhizosphere of pomegranate trees in Saveh, Markazi  
14 Province, central Iran. Preliminary morphological observations revealed this species did  
15 not fit with the description of any of the 21 nominal species. In addition, *Longidorus*  
16 *pisi* Edward, Misra & Singh, 1964 recovered in the rhizosphere of apple trees at the  
17 same site. Based on our previous studies on *Xiphinema* in Iran, the only species  
18 belonging to group eight was *X. index* (Pedram *et al.*, 2008; 2009; 2011). The objectives  
19 of this paper were: *i*) to describe *X. granatum* n. sp. and identify *L. pisi*; and *ii*) to  
20 determine the molecular phylogenetic affinities of *X. granatum* n. sp. with closely  
21 related species using the rRNA gene sequence (D2-D3 fragments of 28S gene).

22

## 23 **Material and methods**

24

### 25 NEMATODE POPULATIONS

26

27           Specimens of *X. granatum* n. sp. were obtained from the rhizosphere of  
28 pomegranate trees in Saveh, Markazi Province, central Iran. *Longidorus pisi* came from  
29 the rhizosphere of apple trees at the same site. Nematodes were extracted from soil  
30 samples by the centrifugal-flotation method (Jenkins, 1964).

31           Specimens for light microscopy (LM) were killed by gentle heat, fixed in a  
32 solution of 4% formaldehyde + 1% acetic acid and processed to pure glycerin using De  
33 Grisse's (1969) method. Specimens were examined using a Zeiss III compound  
34 microscope with Nomarski differential interference contrast at magnifications up to

1 ×1000. Measurements were performed using a *camera lucida* attached to a Nikon  
2 Eclipse E600 light microscope. For line drawings, handmade drawings were scanned  
3 and imported to CorelDraw software version 12 and redrawn.

4

#### 5 DNA EXTRACTION, PCR AND SEQUENCING

6

7 Nematode DNA from *X. granatum n. sp.* and *L. pisi* was extracted from single  
8 individuals using proteinase K as described by Castillo *et al.* (2003). Detailed protocols  
9 for PCR and sequencing were as described by Castillo *et al.* (2003). The following  
10 primers were used for amplification D2A (5'- ACAAGTACCGTGAGGGAAAGTTG-  
11 3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') for amplification of D2-D3  
12 regions of 28S (Subbotin *et al.*, 2006).

13 PCR products were purified after amplification with GeneClean turbo (Q-  
14 BIOgene SA, Illkirch Cedex, France) or QIAquick (Qiagen, USA) gel extraction kits,  
15 quantified using a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington,  
16 DE, USA) and used for direct sequencing in both directions with the PCR primers. The  
17 resulting products were purified and run on a DNA multicapillary sequencer (Model  
18 3100 genetic analyser; Applied Biosystems, Foster City, CA, USA) at the STABVIDA  
19 sequencing facilities (Monte da Caparica, Portugal). The newly obtained sequences  
20 were submitted to the GenBank database under accession numbers JQ240273 and  
21 JQ240274 as indicated on the phylogenetic trees.

22

#### 23 PHYLOGENETIC ANALYSES

24

25 DNA sequences of D2-D3 expansion segments of 28S of *X. granatum n. sp.* and  
26 other sequences from GenBank were used for phylogenetic reconstruction. Outgroup  
27 taxa were chosen according to previous published data (Vovlas *et al.*, 2008). The  
28 sequences were aligned using ClustalW (Thompson *et al.*, 1997) with default  
29 parameters. Sequence alignments were manually edited using BioEdit (Hall, 1999).  
30 Phylogenetic analysis of the sequence data sets were performed with maximum  
31 likelihood (ML) using PAUP \* 4b10 (Swofford, 2003) and Bayesian inference (BI)  
32 using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). The best fit model of DNA  
33 evolution was obtained using the program jModelTest ver. 0.1.1 (Posada, 2008) with  
34 the Akaike Information Criterion (AIC). The Akaike-supported model, the base

1 frequency, the proportion of invariable sites and the gamma distribution shape  
2 parameters and substitution rates were used in phylogenetic analyses. BI analysis under  
3 GTR + I + G model was initiated with a random starting tree and was run with four  
4 chains for  $4.0 \times 10^6$  generations. The Markov chains were sampled at intervals of 100  
5 generations. Two runs were performed for each analysis. After discarding burn-in  
6 samples and evaluating convergence, the remaining samples were retained for further  
7 analysis. The topologies were used to generate a 50% majority rule consensus tree.  
8 Posterior probabilities (PP) are given on appropriate clades. Trees were visualised using  
9 TreeView program (Page, 1996). In ML analysis, the estimation of the support for each  
10 node was made using a bootstrap analysis with 100 fast-step replicates.

11

## 12 **Results**

13

### 14 *Xiphinema granatum*\* n. sp.

15 (Figs 1, 2)

16

#### 17 MEASUREMENTS

18

19 See Table 1.

20

#### 21 DESCRIPTION

22

##### 23 *Female*

24

25 Body cylindrical, tapering very slightly towards anterior end, J-shaped upon  
26 fixation. Cuticle with very fine transverse striations more visible in tail region, 2.5-3.0  
27  $\mu\text{m}$  thick in anterior end, varying from 2-3  $\mu\text{m}$  at mid-body to 4-6  $\mu\text{m}$  in posterior tail  
28 region. Lip region anteriorly more or less flat, separated from body contour by a  
29 constriction and 1.8-2.0 times as high as broad. In a single specimen an asymmetry in  
30 the lip region was detected (Fig. 2). Amphidial fovea cup-shaped; aperture extending for  
31 66.5-68.0% of lip region breadth and located slightly anterior to depression of head and  
32 remainder of body. Body pores not seen in anterior end. Odontostyle typical of genus,

---

\*The species epithet refers to the host-plant (*Punica granatum* L.) in the rhizosphere of which the species was found.

1 long and slender, 8.5-11.0 lip region diam. or 1.5-2.0 odontophore lengths long.  
2 Odontophore with well developed flanges. Guiding ring double, guiding sheath 10-15  
3  $\mu\text{m}$  long depending on degree of protraction/retraction of stylet. Pharynx consisting of  
4 an anterior slender narrow part extending to a terminal pharyngeal bulb occupying 18-  
5 25% of total pharynx length with three nuclei. Nucleus of dorsal gland (DN) large,  
6 located at 12.0-13.5% of pharyngeal bulb length, being larger than both ventro-  
7 sublateral nuclei (S1N) which are located at 52-58% of terminal bulb length (location of  
8 gland nuclei according to Loof & Coomans, 1972). Tip of reserve odontostyle  
9 (vestigium) 3-5  $\mu\text{m}$  in size, directed anteriorly (in one female directed to posterior body)  
10 observed in isthmus in almost all individuals. Cardia conoid, 9-12  $\times$  10-11  $\mu\text{m}$  in size,  
11 Intestine simple, prerectum 15.0-23.5 and rectum 0.8-1.2 of anal body diam. Female  
12 reproductive system didelphic-amphidelphic with branches about equally developed.  
13 Each branch composed of a 47-82  $\mu\text{m}$  long ovary, a 87-137  $\mu\text{m}$  long reflexed oviduct  
14 with well developed *pars dilatata oviductus*, a sphincter (barely visible) and a 237-287  
15  $\mu\text{m}$  long bipartite uterus composed of *pars dilatata uteri* and tubular part without any  
16 differentiation. Ovejector well developed, 65-80  $\times$  25-30  $\mu\text{m}$ , vagina perpendicular to  
17 body axis, extending for 47-57% of corresponding body diam., vulva a transverse slit.  
18 Tail dorsally rounded/convex, ventrally slightly convex with a distinct terminal blind  
19 canal and a terminal peg (in one female conical shaped; Fig. 1N). Caudal pores not  
20 clearly seen.

21

#### 22 *Male*

23

24 Common, almost as abundant as female. General morphology similar to that of  
25 the female except for posterior end more bent ventrally and sexual features. Testis  
26 paired, spicules massive, 4-5 times longer than wide, lateral accessory pieces 11-12  $\mu\text{m}$   
27 long. Precloacal supplements composed of an adanal pair located at 14-17  $\mu\text{m}$  from  
28 cloacal opening and a series of three to four ventromedian supplements ending over a  
29 57-77  $\mu\text{m}$  distance from adanal pair.

30

#### 31 *Juvenile stages*

32

33 Four juvenile stages were identified according to Robbins *et al.* (1996).

34 Correlation between body length, replacement and functional odontostyle is given in

1 Figure 3. Lip region in all juvenile stages similar to that of female. J1 characterised by  
2 replacement odontostyle tip being close to base of functional odontostyle and located at  
3 level of odontophore and a conical tail. J2 tail conical and slightly bent ventrally, J3 tail  
4 conical, dorsally convex, ventrally more or less flat with a slightly developed mucro and  
5 tail of J4 similar to that of female, *i.e.*, dorsally more convex and ventrally flat with a  
6 mucro and a distinct terminal blind canal.

7

8 TYPE HOST AND LOCALITY

9

10 Clay soil around pomegranate trees (*Punica granatum* L.) from Saveh, Markazi  
11 Province, central Iran.

12

13 TYPE MATERIAL

14

15 Holotype female, five female and five male paratypes deposited in the Nematode  
16 Collection of the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.  
17 Three females and male paratypes on separate slides deposited at each of the following  
18 collections: USDA Nematode Collection, Beltsville, MD, USA, Department of  
19 Nematology, University of California, Riverside; CABI Bioscience, UK Centre, Egham,  
20 TW20 9TY, UK.

21

22 DIAGNOSIS AND RELATIONSHIPS

23

24 *Xiphinema granatum* n. sp. is a new member of morphospecies group 8 *sensu*  
25 Loof and Luc (1990) and is characterised by the medium-size body length (3.5-4.1  
26 mm), lip region anteriorly more or less flat, separated from body contour by a  
27 constriction, body diam. 37-49  $\mu$ m, odontostyle and odontophore 118-132 and 65-74  
28  $\mu$ m long, respectively, female reproductive system didelphic with equally developed  
29 branches, uterus bipartite without any Z-differentiation and with sperm, tail dorsally  
30 rounded/convex, ventrally slightly convex, males common with spicules 45-58  $\mu$ m long,  
31 and a unique D2-D3 sequence (GenBank accession number JQ240273). The  
32 polytomous identification code of the new species is: A4, B4, C5a, D5, E56, F4, G23,  
33 H2, I3, J4, K2, L2.

1           Based on the molecular and morphological similarities, *X. granatum* n. sp. is  
2 closely related to *X. index*, *X. barensense* Lamberti, Roca, Agostinelli & Bleve-Zacheo,  
3 1986, *X. basilgoodeyi* Coomans, 1965, *X. histriiae* Lamberti, Coiro & Agostinelli, 1993,  
4 *X. italiae* Meyl, 1953, *X. mammillatum* Schuurmans Stekhoven & Teunissen, 1938, *X.*  
5 *papuanum* Heyns & Coomans, 1983, *X. tarjani* Luc, 1975, *X. variurum* Barsi &  
6 Lamberti, 1998, and *X. vuittenezi* Luc, Lima, Weischer & Flegg, 1964. From *X. index*,  
7 the new species can be differentiated by longer body (3.5-4.1 vs 2.9-3.4 mm), higher  
8 ratio a (74.5-99.5 vs 54-66), higher ratio c (87-114 vs 75-98), higher ratio V (47.5-53.0  
9 vs 38-42), and common males and sperm in female genital tracts vs very rare (Hunt,  
10 1993). From *X. barensense*, the new species differs by shorter odontostyle in J1, J2, J3, J4  
11 and female (50-57, 64-68, 84-90, 103-110, 118-132 vs 80-84, 81-94, 111-112, 1112-  
12 119, 129-138  $\mu$ m, respectively), higher ratio c' in J1, J2, J3, J4 and female (2.7-4.5, 2.5-  
13 3.5, 2.0-2.5, 1.5-2.2, 1.2-1.5 vs 1.4-1.9, 1.2-1.8, 1.2-1.3, 1.0-1.3, 0.8-1.1, respectively),  
14 and shorter spicules (45-58 vs 65-71  $\mu$ m). Furthermore, the tail in the J1 and J2 of the  
15 two species differ (conical in the new species vs dorsally convex and ventrally more or  
16 less flat with a mucro). From *X. basilgoodeyi*, the new species differs by longer body  
17 (3.5-4.1 vs 2.5-3.3 mm), higher ratio a (74.5-99.5 vs 46-59), higher ratio c' (1.2-1.5 vs  
18 0.8-1.0), and common males and sperm in female genital tracts vs males absent. From  
19 *X. histriiae*, the new species differs by shorter body (3.5-4.1 vs 4.0-4.5 mm), higher ratio  
20 a (74.5-99.5 vs 64.1-69.4), higher ratio c' (1.2-1.5 vs 0.9-1.0), higher ratio V (47.5-53.0  
21 vs 44-45), shorter odontostyle (118-132 vs 142.9-156.5  $\mu$ m), and shorter spicules (45-58  
22 vs 82.3-85.2  $\mu$ m). From *X. mammillatum*, the new species differs by longer body (3.5-  
23 4.1 vs 2.3 mm), higher ratio a (74.5-99.5 vs 56.9), higher ratio b (8.5-11.0 vs 5.8), higher  
24 ratio c (87-114 vs 41), and higher ratio V (47.5-53.0 vs 39.5). From *X. papuanum*, the  
25 new species differs by longer body (3.5-4.1 vs 2.8-2.9 mm), higher ratio a (74.5-99.5 vs  
26 60-63), higher ratio c' (1.2-1.5 vs 0.7-0.9), longer odontostyle (118-132 vs 113-116  
27  $\mu$ m), and functional and abundant males vs absent. From *X. tarjani*, the new species  
28 differs by longer body (3.5-4.1 vs 1.6-2.4 mm), higher ratio a (74.5-99.5 vs 35.1-46.2),  
29 higher ratio c (87-114 vs 42.1-60.5), and abundant and functional males vs absent. From  
30 *X. variurum*, the new species differs by higher ratio c' (1.2-1.5 vs 0.8-1.1), higher ratio  
31 V (47.5-53.0 vs 31.5-36.3), mucro in tail end vs rarely without bulge or with a rounded  
32 peg, and abundant males with spicules 45-58  $\mu$ m long vs rare with 72.5  $\mu$ m long  
33 spicules. From *X. vuittenezi*, the new species basically differs by having a uterus  
34 devoided of any Z-differentiation vs having spines, longer body (3.5-4.1 vs 2.6-3.8



1 mm), higher ratio a (74.5-99.5 vs 57-79), higher ratio c' (1.2-1.5 vs 0.8-1.1), tail shape  
2 (dorsally rounded/convex, ventrally slightly convex vs rounded with short peg) and  
3 common males and sperm in female genital tracts vs very rare. From *X. italiae*, the new  
4 species can be differentiated by longer body (3.5-4.1 vs 2.3-3.5 mm), higher ratio c (87-  
5 114 vs 30-56), higher ratio V (47.5-53.0 vs 41-50), longer odontostyle (118-132 vs 87-  
6 112 µm), tail shape (dorsally rounded/convex, ventrally slightly convex vs elongate-  
7 conoid), common males and sperm in female genital tracts vs rare, and longer spicules  
8 (45-58 vs 44-48 µm).

9  
10 ***Longidorus pisi* Edward, Misra & Singh, 1964**

11 = *Longidorus latocephalus* Lamberti, Choleva & Agostinelli, 1983

12 (Fig. 4; Table 2)

13  
14 REMARKS

15  
16 Measurements and morphology of the Iranian population of *L. pisi* agree with  
17 those of the original description by Edward *et al.* (1964), except for a slightly longer  
18 body and odontostyle (3.7-4.5 vs 2.7-3.6 mm; 68-78 vs 56-61 µm, respectively), and  
19 with the description of *L. latocephalus*, except for a higher ratio c' (2.3.2.9 vs 1.9-2.3).  
20 They also agree with another Iranian population of *L. pisi* collected in Haji Abad  
21 (Hormozgan province, Iran) by Fadaei Tehrani and Kheiri (2005), except for a slightly  
22 longer body and odontostyle (3.7-4.5 vs 3.5-3.7 mm; 68-78 vs 64.5-69.0 µm,  
23 respectively), higher ratio c' (2.3.2.9 vs 1.9-2.1) and longer tail (43-47 vs 37-40 µm).  
24 According to Robbins *et al.*, (1995), the three juvenile developmental stages of *L. pisi*  
25 could be separated by comparing the length of the functional and replacement  
26 odontostyle as well as their body length. The range for the mentioned characters of  
27 juveniles of the Iranian population agrees well with the ranges for the same characters  
28 given by Robbins *et al.*, (1995). Molecular analysis of the D2-D3 region of 28S  
29 (GenBank accession JQ240274) showed a similarity of 99% (differing by 1 nucleotide  
30 and 0 or 1 gaps) from the two unique sequences of *L. latocephalus* deposited in  
31 GenBank (AY601569 and AF480073). However, the taxonomic status of *L.*  
32 *latocephalus* is questionable (Choleva *et al.*, 1991; Chen *et al.*, 1997), and Loof and  
33 Chen (1999) regarded *L. latocephalus* as a junior synonym of *L. pisi*. Consequently, we

1 refer to this population as *L. pisi*, and sequences of *L. latocephalus* deposited in  
2 GenBank, should be also considered as *L. pisi*.

3

4 PHYLOGENETIC POSITION OF *XIPHINEMA GRANATUM* N. SP. WITHIN THE GENUS

5

6 The primer pairs of D2A and D3B, amplified a PCR product 900 bp in length  
7 based on gel images. Sequencing of the ITS and 18S was not successful despite several  
8 attempts. The D2-D3 sequences of *X. granatum* n. sp. is unique and different from the  
9 most closely related species, *X. italiae* (HM921351) by 53 nucleotides (94% similarity,  
10 773/826 identities) and 18 gaps (2%, 18/826); from *X. vuittenezi* (EF614266) by 43  
11 nucleotides (95% similarity, 749/792 identities) and 14 gaps (2%, 14/792); from *X.*  
12 *hispanum* Lamberti, Castillo, Gómez Barcina & Agostinelli, 1992 (GU725074) by 61  
13 nucleotides (93% similarity, 759/820 identities) and 16 gaps (2%, 16/820), from *X.*  
14 *adenohysterum* Lamberti, Castillo, Gómez Barcina & Agostinelli, 1992 (GU725075) by  
15 65 nucleotides (92% similarity, 742/807 identities) and 19 gaps (2%, 19/807); from *X.*  
16 *sphaerocephalum* Lamberti, Castillo, Gómez Barcina & Agostinelli, 1992 (GU725076)  
17 by 60 nucleotides (92% similarity, 733/793 identities) and 22 gaps (3%, 22/793); from  
18 *X. nuragicum* Lamberti, Castillo, Gómez Barcina & Agostinelli, 1992 (GU725067) by  
19 62 nucleotides (92% similarity, 763/825 identities) and 21 gaps (3%, 21/825), from *X.*  
20 *pyrenaicum* Dalmaso, 1969 (GU725073) by 71 nucleotides (91% similarity, 752/823  
21 identities) and 25 gaps (3%, 25/823); and from *X. hispidum* Roca & Bravo, 1994  
22 (HM921346) by 61 nucleotides (92% similarity, 667/728 identities) and 16 gaps (2%,  
23 16/728).

24 The D2-D3 alignment consisted of 66 sequences with 824 total characters. The  
25 50% majority rule consensus phylogenetic tree by BI analysis under the GTR + I + G  
26 model is presented in Figure 5. The tree topologies between ML and BI were congruent.  
27 This tree topology was similar to that obtained by Gutiérrez-Gutiérrez *et al.* (2011) with  
28 the phylogeny of dagger and needle nematodes infesting vineyards in southern Spain.  
29 Small differences may be due to the different phylogenetic methods and additional  
30 sequences added in our study. The phylogenetic tree resolved two major clades: *i*) *X.*  
31 *americanum* group; and *ii*) the rest of the species. Both clades were well supported in  
32 our analysis. *Xiphinema granatum* n. sp. was placed in the non-*X. americanum* group  
33 and was in a well supported clade with *X. vuittenezi* by ML and BI analysis. This clade  
34 is in a well supported major clade formed by *X. italiae*, *X. hispidum* and the species of

1 the *X. pyrenaicum* group (*X. hispanum*, *X. adenoysterum*, *X. sphaerocephalum*, *X.*  
2 *nuragicum*, *X. pyrenaicum*), yet clearly separated from *X. index* (Fig. 5).

3

#### 4 **Acknowledgements**

5

6 The authors thank Mohammad Reza Atighi, Razieh Ghaemi and Farideh  
7 Moharrek for their kind help and the Iranian National Science Foundation (INSF) and  
8 Tarbiat Modares University (Iran) for financial support, and the excellent technical  
9 assistance of J. Martín Barbarroja (IAS-CSIC).

10

#### 11 **References**

12

13 BARSİ, L. & LAMBERTI, F. (1998). *Xiphinema variurum* sp. n. (Nematoda: Dorylaimida)  
14 from Montenegro, Yugoslavia. *Nematologia Mediterranea* 26, 151-156.

15 CASTILLO, P., VOVLAS, N., SUBBOTIN, S. & TROCCOLI, A. (2003). A new root-knot  
16 nematode, *Meloidogyne baetica* n. sp. (Nematoda: Heteroderidae), parasitizing  
17 wild olive in Southern Spain. *Phytopathology* 93, 1093-1102.

18 CHEN, Q.W., HOOPER, D.J., LOOF, P.A.A. & XU, J. (1997). A revised polytomous key  
19 for the identification of species of the genus *Longidorus* Micoletzky, 1922  
20 (Nematoda: Dorylaimoidea). *Fundamental and applied Nematology* 20, 15-28.

21 CHOLEVA, B., PENEVA, V. & BROWN, D.J.F. (1991). *Longidorus latocephalus* Lamberti,  
22 Choleva & Agostinelli, 1983, a junior synonym of *L. pisi* Edward, Misra & Singh,  
23 1964 (Nematoda: Dorylaimida). *Revue de Nématologie* 14, 505-509.

24 COOMANS, A. (1963). *Xiphinema basilgoodeyi* n. sp. with observations on its larval  
25 stages (Nematoda: Dorylaimina). *Nematologica* 10, 581-593.

26 DE GRISSE, A.T. (1969). Redescription ou modifications de quelques techniques  
27 utilisées dans l'étude des nématodes phytoparasitaires. *Mededelingen Faculteit*  
28 *Landbouwwetenschappen Rijksuniversiteit Gent* 34, 351-369.

29 EDWARD, J.C., MISRA, S.L. & SINGH, G.R. (1964). *Longidorus pisi* n. sp. (Nematoda,  
30 Dorylaimoidea) associated with the rhizosphere of *Pisum sativum*, from Uttar  
31 Pradesh, India. *Japanese Journal of Applied Entomology and Zoology* 8, 310-312.

32 FADAEI TEHRANI, A.A. & KHEIRI, A. (2005). Some species of *Longidorus* from Iran.  
33 *Journal of Crop Production and Processing* 9, 239-249.

- 1 GUTIÉRREZ-GUTIÉRREZ, C., PALOMARES-RIUS, J.E., CANTALAPIEDRA-NAVARRETE, C.,  
2 LANDA, B.B. & CASTILLO, P. (2011). Prevalence, polyphasic identification, and  
3 molecular phylogeny of dagger and needle nematodes infesting vineyards in  
4 southern Spain. *European Journal of Plant Pathology* 129, 427-453.
- 5 HALL, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and  
6 analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series* 41, 95-  
7 98.
- 8 HEYNS, J., & COOMANS, A. (1983). Three *Xiphinema* species from Papua New Guinea  
9 and The Solomon Islands (Nematoda: Longidoridae). *Nematologica* 29, 1-10.
- 10 HUELSENBECK, J.P. & RONQUIST, F. (2001). MrBAYES: Bayesian inference of  
11 phylogenetic trees. *Bioinformatics* 17, 754-755.
- 12 HUNT, D.J. (1993). *Aphelenchida, Longidoridae and Trichodoridae: their systematics*  
13 *and bionomics*. Wallingford, UK, CABI Publishing, 352 pp.
- 14 JAIRAJPURI, M.S. & AHMAD, W. (1992). *Dorylaimida. Freelifving, predaceous and*  
15 *plant-parasitic nematodes*. New Delhi, India, Oxford & IBH Publishing Co, 458  
16 pp.
- 17 JENKINS, W.R. (1964). A rapid centrifugal flotation technique for separating nematodes  
18 from soil. *Plant Disease Reporter* 48, 692.
- 19 LAMBERTI, F., CHOLEVA, B. & AGOSTINELLI, A. (1983). Longidoridae from Bulgaria  
20 (Nematoda, Dorylaimida) with descriptions of three new species of *Longidorus* and  
21 two new species of *Xiphinema*. *Nematologia Mediterranea* 11, 49-72.
- 22 LAMBERTI, F., COIRO M.I. & AGOSTINELLI A. (1993). *Xiphinema histriae* (Nematoda:  
23 Dorylaimida) a new species from Northern Italy. *Nematologia Mediterranea* 21,  
24 247-250.
- 25 LAMBERTI, F., ROCA, F., AGOSTINELLI, A. & BLEVE-ZACHEO, T. (1986). *Xiphinema*  
26 *barense* sp. n. (Nematoda: Dorylaimida) from Italy. *Nematologia Mediterranea* 14,  
27 101-106.
- 28 LOOF, P.A.A. & CHEN, Q. (1999). A revised polytomous key for the identification of the  
29 species of the genus *Longidorus* Micoletzky, 1922 (Nematoda: Dorylaimoidea).  
30 *Nematology* 1, 55-59.
- 31 LOOF, P.A.A. & COOMANS, A. (1972). The oesophageal gland nuclei of Longidoridae  
32 (Dorylaimida). *Nematologica* 18, 213-233.

- 1 LOOF, P.A.A. & LUC, M. (1990). A revised polytomous key for the indentification of  
2 species of the genus *Xiphinema* Cobb, 1913 (Nematoda:Longidoridae) with  
3 exclusion of the *X. americanum*-group. *Systematic Parasitology* 16, 35-66.
- 4 LUC, M. (1975). Trois nouvelles especes de *Xiphinema* Cobb, 1913 a queue arrondie-  
5 mucronée (Nematoda: Longidoridae). *Cahiers ORSTOM, Série Biologie* 10, 293-  
6 302.
- 7 LUC, M., LIMA, M.B., WEISCHER, B. & FLEGG, J.J.M. (1964). *Xiphinema vuittenezi* n. sp.  
8 (Nematoda: Dorylaimidae). *Nematologica* 10, 151-163.
- 9 MEYL, A. H. (1953). Beiträge zur Kenntnis der nematodenfauna vulkanisch erhitzter  
10 biotope. I Mitt., die terrikolen nematoden im Bereich von Fumarolen auf der Insel  
11 Ischia. *Zeitschrift Fur Morphologie und Ökologie Der Tiere* 42, 67-116.
- 12 PAGE, R.D.M. (1996). TREEVIEW: an application to display phylogenetic trees on  
13 personal computers. *Computer Applications in the Biosciences* 12, 357-358.
- 14 PEDRAM, M, NIKNAM, G. & DECRAEMER, W. (2008). *Xiphinema robbinsi* sp. n.  
15 (Nematoda, Dorylaimida), an amphimictic species from Iran. *Russian Journal of*  
16 *Nematology* 16, 49-57.
- 17 PEDRAM, M., NIKNAM, G., ROBBINS, R.T., YE, W. & KAREGAR, A. (2009). *Xiphinema*  
18 *iranicum* n. sp. (Nematoda: Longidoridae) from north-western Iran. *Nematology*  
19 11, 11-21.
- 20 PEDRAM, M., POURJAM, E., ROBBINS, R.T., YE, W., ATIGHI, M.R. & DECRAEMER, W.  
21 (2012). Morphological and molecular characterisation of *Xiphinema*  
22 *mazandaranense* n. sp. (Dorylaimida: Longidoridae), a new member of the  
23 *Xiphinema pyrenaicum* species complex. *Nematology* 14, 109-119.
- 24 POSADA, D. (2008). jModelTest: Phylogenetic model averaging. *Molecular Biology and*  
25 *Evolution* 25, 1253-1256.
- 26 ROBBINS, R.T., BROWN, D.J.F., HALBRENDT, J.M. & VRAIN, T.C. (1995). Compendium  
27 of *Longidorus* juvenile stages with observations on *L. pisi*, *L. taniwha*, and *L.*  
28 *diadecturus* (Nematoda: Longidoridae). *Systematic Parasitology* 32, 33-52.
- 29 ROBBINS, R.T., BROWN, D.J.F., HALBRENDT, J.M. & VRAIN, T.C. (1996). Compendium  
30 of juvenile stages of *Xiphinema* species (Nematoda: Longidoridae). *Russian*  
31 *Journal of Nematology* 4, 163-171.
- 32 SCHUURMANS-STEKHOVEN J.H. & TEUNISSEN, R.J.H. (1938). Nématodes libres  
33 terrestres. Fasc. (22) Mission De Witte (1933-35) Exploration du Parc national  
34 Albert, 229 p.

- 1 SUBBOTIN, S.A., STURHAN, D., CHIZHOV, V.N., VOVLAS, N. & BALDWIN, J.G. (2006).  
2 Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3  
3 expansion fragments of the 28S rRNA gene sequences. *Nematology* 8, 455-474.
- 4 SWOFFORD, D.L. (2003). PAUP\*: Phylogenetic analysis using parsimony (\*and other  
5 methods), version 4.0b 10. Sunderland, MA, USA, Sinauer Associates.
- 6 THOMPSON, J.D., GIBSON, T.J., PLEWNIAK, F., JEANMOUGIN, F. & HIGGINS, D.G. (1997).  
7 The CLUSTAL\_X windows interface: flexible strategies for multiple sequence  
8 alignment aided by quality analysis tools. *Nucleic Acids Research* 25, 4876-4882.
- 9 VOVLAS, N., SUBBOTIN, S.A., TROCCOLI, A., LIÉBANAS, G. & CASTILLO, P. (2008).  
10 Molecular phylogeny of the genus *Rotylenchus* (Nematoda, Tylenchida) and  
11 description of a new species. *Zoologica Scripta* 37, 521-537.
- 12

1 **Table 1.** Morphometrics of *Xiphinema granatum n. sp.* All measurements are in  $\mu\text{m}$  and in the form: mean  $\pm$  s.d. (range).

2

	J1	J2	J3	J4	Female		Male
	Paratypes	Paratypes	Paratypes	Paratypes	Holotype	Paratypes	Paratypes
n	10	5	8	8	–	18	10
L	990 $\pm$ 41 (935-1055)	1335 $\pm$ 38.5 (1282-1375)	1899 $\pm$ 117.5 (1660-2035)	2804 $\pm$ 66.5 (2749-2955)	3687	3807 $\pm$ 160 (3540-4050)	3744 $\pm$ 216 (3420-4087)
a	55 $\pm$ 3 (50.0-61.5)	58 $\pm$ 9 (47.0-65.5)	66 $\pm$ 5 (56.5-74.5)	81.0 $\pm$ 5.5 (74.5-89.5)	99.7	87.5 $\pm$ 6.5 (74.5-99.5)	90.0 $\pm$ 8.5 (81.0-103.5)
b	4.0 $\pm$ 0.2 (4.0-4.5)	4.5 $\pm$ 0.5 (4.0-5.5)	5.5 $\pm$ 0.5 (5.0-6.5)	7.0 $\pm$ 0.3 (6.5-7.0)	8.5	9.0 $\pm$ 0.5 (8.5-11.0)	8.5 $\pm$ 0.5 (8.0-9.5)
c	23.7 $\pm$ 4.0 (21-34)	28.5 $\pm$ 1.0 (27.5-29.5)	40.5 $\pm$ 3.0 (37-45)	61 $\pm$ 4 (55.0-67.5)	92.2	99.5 $\pm$ 7.5 (87-114)	95.0 $\pm$ 8.5 (85.5-111.5)
c'	3.5 $\pm$ 0.5 (2.7-4.5)	3.2 $\pm$ 0.3 (2.5-3.5)	2.5 $\pm$ 0.1 (2.0-2.5)	1.8 $\pm$ 0.2 (1.5-2.2)	1.5	1.5 $\pm$ 0.1 (1.2-1.5)	1.3 $\pm$ 0.1 (1.1-1.5)
V	–	–	–	–	47.8	49.5 $\pm$ 1.5 (47.5-53.0)	–
Anterior end to vulva	–	–	–	–	1761	1891 $\pm$ 114 (1690-2107)	–
Lip region diam.	8.0 $\pm$ 0.5 (8-9)	9.0 $\pm$ 0.0 (9-9)	10.0 $\pm$ 0.5 (10-11)	11.5 $\pm$ 0.5 (10-12)	12.5	13 $\pm$ 1 (11.5-15.0)	12.5 $\pm$ 0.5 (12-14)
Lip region height	3.5 $\pm$ 0.5 (3-4)	4.5 $\pm$ 0.5 (4-5)	5.5 $\pm$ 0.5 (5-6)	6.0 $\pm$ 0.0 (6-6)	6	7.0 $\pm$ 0.5 (5.5-8.0)	6.5 $\pm$ 1.0 (5-8)
Odontostyle	54 $\pm$ 2.0 (50-57)	66 $\pm$ 1.5 (64-68)	87 $\pm$ 2.0 (84-90)	107 $\pm$ 2.5 (103-110)	124	125 $\pm$ 3.5 (118-132)	125 $\pm$ 3 (120-129)
Odontophore	37 $\pm$ 1.5 (35-39)	45 $\pm$ 3.5 (41-48)	52 $\pm$ 4 (47-60)	60 $\pm$ 3.5 (52-64)	65	70 $\pm$ 3 (65-74)	69 $\pm$ 4.0 (62-74)
Stylet total length	91 $\pm$ 2.0 (89-95)	111 $\pm$ 2 (109-114)	139 $\pm$ 4.5 (134-147)	168 $\pm$ 4.5 (160-172)	189	195 $\pm$ 4 (189-204)	194 $\pm$ 6.0 (185-203)
Replacement odontostyle	65 $\pm$ 1.5 (63-68)	85 $\pm$ 2.0 (83-88)	103 $\pm$ 2.5 (100-107)	124 $\pm$ 4.5 (115-129)	–	–	–
Guiding ring from ant. end	44 $\pm$ 2 (40-46)	49 $\pm$ 6.5 (42-57)	70 $\pm$ 3.5 (67-77)	86 $\pm$ 3.0 (82-90)	103	107 $\pm$ 5.0 (100-116)	104 $\pm$ 3 (99-108)
Flange width	7.0 $\pm$ 0.5 (6-8)	8 $\pm$ 1 (7-9)	9.5 $\pm$ 0.5 (8-10)	10.5 $\pm$ 1.0 (9-12)	11	11.5 $\pm$ 1.0 (10-13)	11.5 $\pm$ 1.5 (9-13)
Pharynx length	241 $\pm$ 9 (225-251)	288 $\pm$ 35 (235-321)	338 $\pm$ 19 (312-367)	410 $\pm$ 15.5 (385-430)	432.5	429 $\pm$ 22.5 (360-460)	437 $\pm$ 19 (412-467)
Pharyngeal expansion length	54 $\pm$ 3.5 (48-58)	65 $\pm$ 7.5 (56-72)	68 $\pm$ 6 (60-77)	76 $\pm$ 6.5 (65-85)	88	90 $\pm$ 7.0 (77-104)	89 $\pm$ 6 (80-100)
Pharyngeal expansion diam.	10.5 $\pm$ 1.5 (9-13)	14.5 $\pm$ 2.0 (13-18)	17.5 $\pm$ 3.5 (15-26)	17.5 $\pm$ 1.0 (17-19)	18	20.5 $\pm$ 2.0 (17-24)	21.0 $\pm$ 2 (17-24)
Diam. at pharyngeal base	18.0 $\pm$ 1.5 (17-21)	23.5 $\pm$ 3.0 (20-27)	28.0 $\pm$ 2 (25-30)	33.0 $\pm$ 2 (30-35)	33.5	38.5 $\pm$ 3.0 (33.5-44.0)	39.0 $\pm$ 3 (33-43)
– at mid-body	18.0 $\pm$ 1.5	23.5 $\pm$ 4.0	29.0 $\pm$ 3	35.0 $\pm$ 2.5	37	43.5 $\pm$ 3.5	42.0 $\pm$ 4.5

*Xiphinema granatum n. sp. and Longidorus pisi from Iran*

	(16-21)	(20-29)	(25-34)	(31-38)		(37-49)	(33-48)
– at anus	12.0 ± 1.5	15.0 ± 1.5	20.0 ± 1	25.0 ± 1	27.0	28.5 ± 1.5	31.0 ± 1.5
	(10-16)	(14-17)	(18-21)	(23-27)		(27-31)	(29-34)
– at guiding ring level	15.0 ± 0.5	19.5 ± 1.5	22.5 ± 1.0	26.5 ± 1.5	30.0	31.0 ± 1.5	32.0 ± 2
	(15-16)	(18-21)	(21-24)	(25-30)		(29-35)	(30-34)
Prerectum length	–	–	–	–	–	559 ± 94	–
						(437-652)	
Rectum length	12.0 ± 2	15.0 ± 2	18.0 ± 1.5	24.0 ± 1.5	27.0	30.5 ± 3.0	–
	(9-13)	(12-17)	(16-19)	(21-25)		(25-35)	
Tail	42 ± 4.5	47 ± 2	47 ± 3	45 ± 3.0	40	38 ± 3.0	39 ± 3.5
	(31-47)	(44-49)	(44-52)	(42-51)		(32-43)	(34-45)
Hyaline portion of tail	7.5 ± 1.0	9.0 ± 1	11.5 ± 1.0	14.5 ± 1.5	19	16.5 ± 2.0	16.0 ± 2
	(6-9)	(8-10)	(9-12)	(13-17)		(14-20)	(13-18)
Spicules	–	–	–	–	–	–	51 ± 4
							(45-58)

1



1 **Table 2.** Morphometrics of Longidorus pisi Edward, Misra & Singh, 1964. All  
 2 measurements are in  $\mu\text{m}$  and in the form: mean  $\pm$  s.d. (range).

3

	J1	J2	J3	Female
n	2	1	2	5
L	(1182, 1182)	1787.5	(2717, 2430)	4046 $\pm$ 364 (3698-4556)
a	(71.5, 72.0)	99.3	(104.5, 105.5)	139.4 $\pm$ 3.5 (134.8-144.6)
b	(5.6, 5.6)	8.5	(10.0, 10.5)	12.3 $\pm$ 3.0 (7.6-15.1)
c	(33, 35)	42.6	(55.0, 55.5)	90.4 $\pm$ 7.9 (80.3-101.3)
c'	(2.8, 3.0)	3.2	(2.9, 2.9)	2.5 $\pm$ 0.3 (2.3-2.9)
V	–	–	–	50.3 $\pm$ 1.1 (49-52)
Lip region diam.	(7.0, 8.0)	8.0	(9.0, 9.5)	9.9 $\pm$ 0.2 (9.5-10.0)
Lip region height	(3.0, 4.0)	4.5	(4.0, 4.0)	4.8 $\pm$ 0.4 (4.0-5.0)
Odontostyle	(47, 48)	50.0	(60, 62)	74 $\pm$ 4.1 (68-78)
Odontophore	–	40.0	(44, 49)	46 $\pm$ 1.4 (44-48)
Styilet total length	–	90	(104, 111)	120 $\pm$ 4.4 (114-124)
Replacement odontostyle	(50.0, 51.5)	61.0	(74, 74)	–
Guiding ring from ant. end	(22.5, 24.0)	29.0	(35, 36)	41.5 $\pm$ 1.6 (40-44)
Pharynx length	(211, 212)	210.0	(171, 175)	345 $\pm$ 90.1 (259-498)
Pharyngeal expansion length	(43, 44)	48.0	(45, 47)	64 $\pm$ 6.1 (56-70)
Pharyngeal expansion diam.	(10.0, 11.0)	13.0	(12.0, 13.0)	13.2 $\pm$ 1.8 (11-16)
Diam. at pharyngeal base	(16.5, 17.0)	18.0	(22, 23)	24.4 $\pm$ 1.1 (23-26)
– at mid-body	(16.5, 17.0)	18.0	(23, 26)	29.0 $\pm$ 2.2 (26.5-31.5)
– at anus	(12.0, 12.5)	13.0	(15.0, 17.0)	18.1 $\pm$ 1.3 (16-19)
– at guiding ring	(11.5, 12.0)	13.0	(15, 15)	15.7 $\pm$ 0.4 (15-16)
Prerectum length	(212, 214)	-	-	401 $\pm$ 12.7 (392-410)
Rectum length	(12, 13)	13.0	(14, 23)	18.0 $\pm$ 2.3 (15-20)
Tail	(34, 36)	42.0	(44, 49)	45 $\pm$ 1.8 (43-47)

4

5

1 **Figure legends**

2

3 **Fig. 1.** *Xiphinema granatum n. sp.* A: Anterior region in lateral optical view; B: Female  
4 anterior genital branch (fresh female); C: Detail of odontophore and guiding ring; D,  
5 E: Lip region; F, G: Entire body, male and female; H-K: Tail of juveniles from J1-J4,  
6 respectively; L-N: Female tail region.

7

8 **Fig. 2.** Photomicrographs of *Xiphinema granatum n. sp.* A-C: Female anterior body  
9 region; D: Detail of pharyngeal bulb; E: Female anterior genital branch; F: Detail of  
10 uterus without Z-differentiation; G: Detail of vulval region; H, I: Female tail; J: Male  
11 tail. Abbreviations: a = anus; n = nucleus of pharyngeal glands; V = vulva; vp =  
12 ventromedian papillae. (Scale bars: A, D, E = 50  $\mu$ m; B, C = 10  $\mu$ m; F-J = 20  $\mu$ m.)

13

14 **Fig. 3.** Relation of body length with length of functional and replacement odontostyle  
15 (ost and rost, respectively) length in all developmental stages from J1 to mature females  
16 of *Xiphinema granatum n. sp.* from pomegranate, in Saveh, Markazi province, Iran.

17

18 **Fig. 4.** Photomicrographs of *Longidorus pisi* Edward, Misra & Singh, 1964. A, B:  
19 Female anterior body region; C: Detail of pharyngeal bulb; D: Vulval region; E:  
20 Female tail; F: First-stage juvenile tail. Abbreviations: a = anus; gr = guiding ring; n  
21 = nucleus of pharyngeal glands. (Scale bars: A = 50  $\mu$ m; B-F = 20  $\mu$ m.)

22

23 **Fig. 5.** The 50% majority rule consensus trees from Bayesian analysis generated from  
24 the D2-D3 of 28S rRNA gene dataset with the GTR + I + G model. Posterior  
25 probabilities more than 65% are given for appropriate clades; bootstrap values greater  
26 than 50% are given on appropriate clades in ML analysis. Newly obtained sequence is  
27 underlined.

28