

Research Note

**Three species of *Xiphinema americanum*-group complex (Nematoda: Longidoridae),
 from Lorestan province, Iran**

E. BAZGIR, A. NAGHAVI*, Z. ZOLFAGHARI

 Nematology Laboratory, Department of Plant Protection, Faculty of Agriculture, Lorestan University, Khorramabad, Iran,
 *E-mail: ar_ng11@yahoo.com

Article info

 Received May 15, 2020
 Accepted July 14, 2020

Summary

The dagger nematodes of the longidorids can cause diseases of various agronomic and horticultural crops, and are consisted of more than 260 valid species. In a forest survey of ecotypes of longidorid nematodes, from the root zone soil of Brant's oak, (*Quercus brantii* Lindl.) and hawthorn (*Crataegus aronia* L.) trees, three species of *Xiphinema americanum* group namely *Xiphinema pachtaicum*, *X. oxycaudatum* and *X. plesiopachtaicum* were collected and studied based on their morphological and morphometric characters. *X. pachtaicum* is prevalent *Xiphinema* species in Iran. In this paper additional data for *X. oxycaudatum* and *X. plesiopachtaicum* species are presented. *X. plesiopachtaicum* is a new record for nematode fauna of Iran.

Keywords: dagger nematodes; oak; hawthorn; *X. oxycaudatum*; *X. plesiopachtaicum*; new record

Introduction

The tribe Longidorini which is a subfamily of Longidorinae (*Longidorus* spp. and *Paralongidorus* spp.) accompanied with subfamily Xiphineminae (*Xiphinema* spp.) are two major plant parasitic nematode groups. Dagger nematodes of the longidorids comprise of plant-parasitic species that cause damage to a vast range of wild and cultivated plants either directly through feeding on plant root cells or indirectly via vectoring of nepoviruses to a wide range of fruit and vegetable crops (Taylor & Brown, 1997; Gutierrez-Gutierrez *et al.*, 2016). The genus *Xiphinema* has been divided to the *X. americanum* and *X. non-americanum* groups. *X. americanum* morpho-groups can be characterized by a spiral or C-shaped, small body (1 – 3 mm). Odontostyle 60 – 20 µm. Female reproductive system with two equally developed genital branches, short uteri without uterine differentiation. Presence of symbiotic bacteria in intestinal cells of some juveniles and occasionally in the ovaries of adults. Tail short conical to broadly convex-conoid (Lamberti *et al.*, 2000; Orlando *et al.*, 2016).

The *Xiphinema americanum*-group consisted of a complex of about 55 species of plant-ectoparasitic nematodes of plants. This group of plant-parasitic nematodes is one of the most difficult *Xiphinema* species complexes to be diagnosed, because their morphology is very conservative and their morphometric characters are often overlaped (Archidona-Yuste *et al.*, 2016). Ten species of *Xiphinema* have been reported from Iran. They are *X. americanum*, *X. bacaniboia*, *X. brevicollum*, *X. himalayense*, *X. oxycaudatum*, *X. pachtaicum*, *X. pacficum* and *X. rivesi* (Ghaderi *et al.*, 2018), *X. simile* (Naghavi *et al.*, 2018) and *X. primum* (Mobasseri, *et al.*, 2020). In the present nematological survey on longidorids in Khorramabad county, Lorestan province, southwest of Iran, three nematode species of the genus *Xiphinema* namely *X. oxycaudatum*, *X. pachtaicum* and *X. plesiopachtaicum* were collected from several natural environments and were identified based on morphological and morphometrics characters and out of this three nematode species, *X. plesiopachtaicum* is a new record for nematode fauna of Iran.

* – corresponding author

Material and Methods

The present survey carried out in forests of Khorramabad district, Lorestan province, south west of Iran, during spring 2018 till late autumn 2019. Khorramabad is located in the middle parts of Zagros Mountain ranges of Iran, due to receiving more than 500 mm precipitation on average annually, it enjoys a rich diversity of plant coverage, including Oaks and hawthorns forest trees. GPS coordinates of the sampling location were: 33°29' 15" E 48° 21' 15". After removing the upper 5 cm of topsoil, 80 Soil samples were randomly collected from 20 to 50 cm depth of the rhizosphere of Brant's oak (*Quercus brantii* Lindl.) and hawthorn (*Crataegus aronia* L.) forest trees. Keeping 50 cm distance from the trees, four soil samples collected from 4 main geographic directions around the tree stems. The soil samples were thoroughly mixed on clean plastic sheet, and about 1 kg of the mixed soil samples were poured in plastic bag as a composite sample. The bag labeled for location, date and tree species of sample collection. Then the bags transferred to the nematology laboratory of Plant protection Department of Lorestan University and they were stored

at 4 °C until their processing. Nematodes extraction carried out from 500 cm³ of soil samples using the Brown &Boag (1988) modified method. Additional soil samples were collected if needed afterwards, from the same locations to obtain sufficient specimens for morphological characterization. Specimens for light microscopy were killed using gentle heat, then fixed and processed to pure glycerin adopting the De Grisse (1969) method. Morphological and morphometric criteria of specimens were studied using an Olympus BX31 light microscope equipped with a Dino-eye microscope eye-piece camera in conjunction with Dino Capture version 2.0 software. Raw photographs were edited using Picasa 3.9.138.150. Drawings were made using Corel DRAW®, software version 12.

Ethical Approval and/or Informed Consent

The conducted research is neither related to human nor animals use.

Table 1. Morphometrics of the Khorramabad, Iran populations of *Xiphinema* species. All measurements are in μm (except L in mm), and in the form: mean \pm s.d. (range).

| Species | <i>X. oxycaudatum</i> | <i>X. pachtaicum</i> | <i>X. plesiopachtaicum</i> |
|-----------------------------|-----------------------|-------------------------------|-------------------------------|
| Characters ratios* | Female | Female | Female |
| n | 2 | 4 | 4 |
| L | 1.5 , 1.7 | 1.87 – 1.93 (1.90 \pm 0.02) | 1.76 – 1.81 (1.79 \pm 0.01) |
| a | 43 , 43 | 56.5 – 62.5 (58.2 \pm 2.2) | 61.2 – 66.7 (63 \pm 2.1) |
| b | 6 , 6 | 5.5 – 6.2 (5.8 \pm 0.3) | 5.4 – 6 (5.7 \pm 0.3) |
| c | 50 , 54.5 | 63.2 – 65.7 (64.5 \pm 1.1) | 59 – 66 (62.6 \pm 2.5) |
| c' | 1.3 , 1.4 | 1.6 – 1.8 (1.7 \pm 0.1) | 1.4 – 1.6 (1.5 \pm 0.1) |
| V | 51 , 51 | 57 – 58 (57.5 \pm 0.5) | 57 – 59 (58 \pm 0.8) |
| Odontostyle length | 79.5 , 81.3 | 82 – 85.5 (82.8 \pm 0.5) | 73 – 76 (74.4 \pm 1.2) |
| Odontophore length | 43.6 , 46.2 | 44.5 – 49 (47.5 \pm 1.1) | 42 – 43.7 (42.9 \pm 0.7) |
| Spear length | 124.9 , 125.2 | 128 – 133.8 (130 \pm 2.5) | 116 – 118.8 (117.5 \pm 1.1) |
| Lip region diam. | 10.5 , 11.5 | 8.8 – 9 (8.9 \pm 0.1) | 8.5 – 9.2 (9 \pm 0.1) |
| Oral aperture to guide ring | 69.5 , 72 | 75 – 79.5 (77.9 \pm 1.1) | 64.5 – 68 (66.4 \pm 1.1) |
| Pharynx length | 276 , 282.3 | 303.5 – 343 (319 \pm 15) | 297 – 330.5 (310.9 \pm 8.5) |
| Pharyngeal bulb length | 68 , 74 | 67 – 82.5 (74 \pm 6) | 71 – 81 (76.1 \pm 4) |
| Body diam. at phar. base | 31 , 33.5 | 26.5 – 31.3 (28.6 \pm 1.8) | 27 – 29.5 (28.2 \pm 1) |
| at mid-body | 35 , 39.5 | 30 – 33 (31.9 \pm 1.1) | 27 – 29.5 (28.2 \pm 1) |
| at anus | 20 , 24.5 | 16.5 – 17 (16.7 \pm 0.2) | 18.3 – 19.1 (18.7 \pm 0.2) |
| Tail length | 28.5 , 34 | 29 – 30.5 (29.5 \pm 0.3) | 27.5 – 30.6 (28.7 \pm 1.1) |
| Hyaline of tail tip | 9 , 9.5 | 9.5 – 10.7 (10.3 \pm 0.2) | 5.6 – 7 (6.5 \pm 0.4) |

*n = number of females, full body length (L), body length/greatest body width (a), body length/pharyngeal length (b), body length/tail length (c), tail length/tail diameter at anus region (c') and % distance of vulva from anterior end/body length (V).

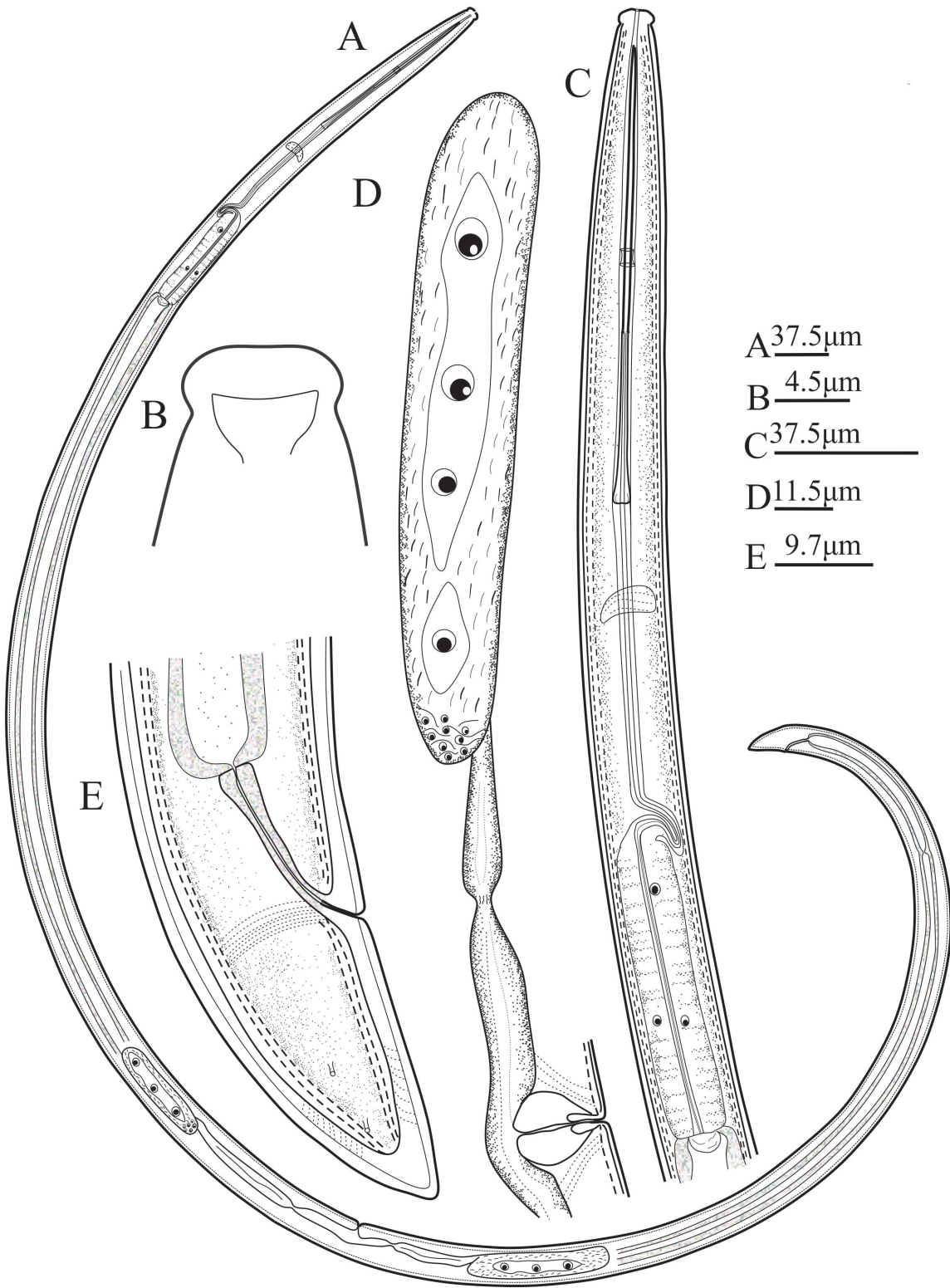


Fig. 1. *Xiphinema plesiopachtaicum* Archidona-Yuste, Navas-Cortes, Cantalapiedra-Navarrete, Palomares-Rius and Castillo, 2016: A: Entire body, B: Amphid, C: Head, Stylet and Pharynx, D: Anterior genital branch of the female reproductive system and symbiotic bacteria in ovary, E: Posterior end of body.

Results and Discussion

Xiphinema plesiopachtaicum Archidona-Yuste, Navas-Cortes, Cantalapiedra-Navarrete, Palomares-Rius and Castillo, 2016

(Figs. 1, 3)

Measurements

See Table 1.

Description

Female

Body medium-sized 1.7 – 1.8 mm, ventrally arcuate upon fixation, tapering toward both ends, habitus usually open C shaped after fixation. Labial region 3.6 – 3.8 μm high, expanded, frontally flattened, laterally rounded, separated from the rest of the body by constriction. Amphid stirrup-shaped 5.5 – 6 μm wide and 63 – 66 % of the corresponding lip region diameter. Odontostyle 8, 8.4 times lip region diameter, odontophore 0.5 times odontostyle with weak flanges 6 – 7.5 μm wide diameter. Pharynx dorylaimoid with basal bulb occupying ca 23 – 25 % of the total length, muscular bulb measuring 11.4 – 12.3 \times 74 – 81 μm . The female reproductive system didelphic-amphidelphic, reflexed, both branches apparently equally developed, anterior and posterior genital branch 225 – 285 μm and 230 – 280 μm long, respectively, ovaries filled with symbiotic bacteria, vulva equatorial, transverse, slit-like, vagina with short distal part and very well developed proximal part, 57 – 60 % of corresponding body diam. Prerectum 415 – 490 μm long. Rectum 18.5 – 20 μm long. Tail short, dorsoventral depression at hyaline region level, two pairs of caudal pores are present on each side.

Male: Not found.

Distribution

Faculty of Agriculture and Natural Resources, Khorramabad County, Lorestan province, Iran, (GPS coordinates: N 33° 26' 17" E 48° 15' 41", altitude 1769 m a.s.l.), in the root zone soil of oak (*Quercus brantii* Lindl.).

Remarks

X. plesiopachtaicum, is reported for the second time since its original description and for the first time outside Spain. The species *X. plesiopachtaicum* described by Archidona-Yuste *et al.*, 2016 from the rhizosphere of olive trees in southern Spain for the first time. The present population fits well with the type specimens in most morphological and morphometric characteristics, only slight differences were observed viz shorter odontostyle (73 – 76 vs 77 – 89 μm) and longer tail (27.5 – 30 vs 23.5 – 28.5 μm). Based on the morphological similarities, *X. plesiopachtaicum* is closely related to *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951, *X. madeirense* Brown, Faria, Lamberti, Halbrendt, Agostinelli and Jones, 1988, *X. parapachydermum* Gutierrez-Gutierrez, Cantalapiedra-Navarrete, Decramer, Vovlas, Prior, Palomarres &

Castillo, 2012 and *X. peruvianum* Lamberti and Bleve-Zacheo, 1979. *X. plesiopachtaicum* could be separated from *X. pachtaicum* in having a smaller *a* (61.2 – 66.7 vs 64 – 74) and *c'* (1.4 – 1.6 vs 1.7 – 2) ratio and shorter odontostyle (73 – 76 vs 77.6 – 81.6 μm). It can also be differentiated from *X. madeirense* via a shorter body length (1.7 – 1.8 vs 2 – 2.4 mm), anteriorly located guiding ring (64.5 – 68 vs 82 – 98 μm), shorter odontostyle (73 – 76 vs 100 – 109 μm) and shorter tail (27.5 – 30.6 vs 33 – 44 μm). It differs from *X. parapachydermum* by smaller *c'* value (1.4 – 1.6 vs 1.5 – 2.3), shorter odontostyle (73 – 76 vs 70 – 87.5 μm) and odontophore (42 – 43.7 vs 36.5 – 54.5 μm). Finally, *X. plesiopachtaicum* can be differentiated from *X. peruvianum* by greater *a* value (61.2 – 66.7 vs 45 – 56), shorter odontostyle (73 – 76 vs 85 – 92 μm) and odontophore (42 – 43.5 vs 46 – 52 μm), narrower lip region (8.5 – 9.2 vs 9 – 10.5 μm) and anteriorly located guiding ring (64.5 – 68 vs 67 – 78 μm).

Xiphinema oxycaudatum Lamberti & Bleve-Zacheo, 1979

(Figs. 2, 3)

Measurements

See Table 1.

Description

Female

Body medium-sized 1.5, 1.7 mm, tapering gradually toward the extremities. Labial region rounded separated by a constriction from the rest of the body. Amphidial pouches stirrup-shaped, with slit-like aperture, 4.2, 4.5 μm wide, 39, 40 % of the corresponding lip region diameter. Odontostylet, odontophore and guiding apparatus typical for *X. americanum*-group, odontostyle robust, 7, 7.5 times lip region diameter, odontophore 0.5 times odontostyle with weak flanges, 8.6, 9 μm wide diameter. The oesophageal basal bulb occupies ca 24, 26 % of the oesophagus total length, muscular bulb measuring 15.6, 18.8 \times 68.7, 70 μm . The female reproductive system didelphic-amphidelphic with equally developed genital branches, anterior and posterior gonad 200, 264 μm and 239, 255 μm long, respectively, reflexed ovary filled with symbiotic bacteria, vulva transverse, equatorial, vagina occupying 47, 48 % of corresponding body diam. Tail, conoid, dorsally convex, ventrally straight to slightly arcuate with pointed terminus, bearing two caudal pores on each side.

Male: Not found

Distribution

Faculty of Agriculture and Natural Resources, Khorramabad County, Lorestan province, Iran, (GPS coordinates: N 33° 26' 17" E 48° 15' 41", altitude 1769 m a.s.l.), in the root zone soil of oak (*Quercus brantii* Lindl.).

Remarks

This species was previously recorded from rhizosphere of oil palm

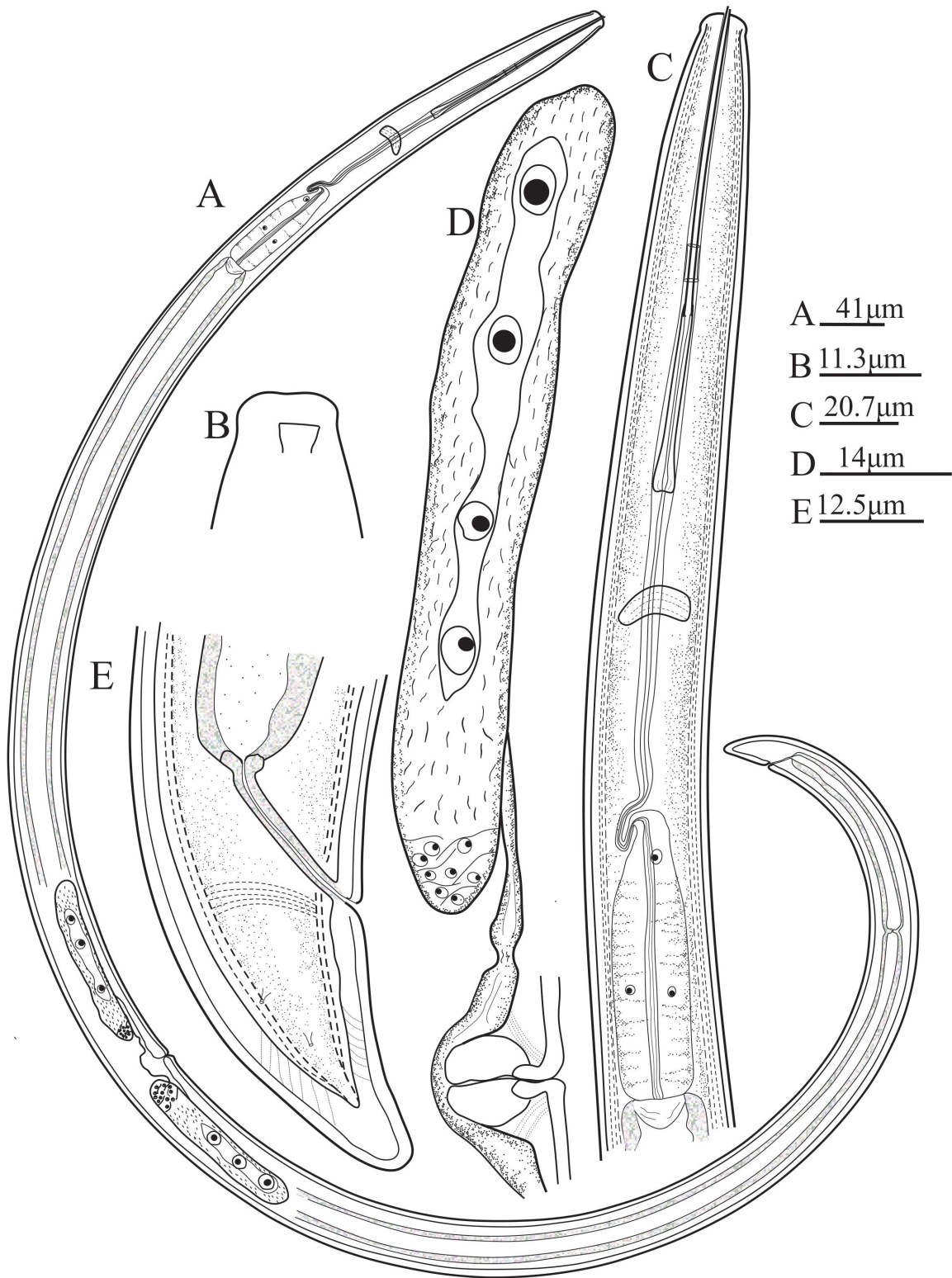


Fig. 2. *Xiphinema oxycaudatum* Lamberti & Bleve-Zacheo, 1979: A: Entire body, B: Amphid, C: Head, Stylet and Pharynx, D: Anterior genital branch of the female reproductive system and symbiotic bacteria in ovary, E: Posterior end of body.

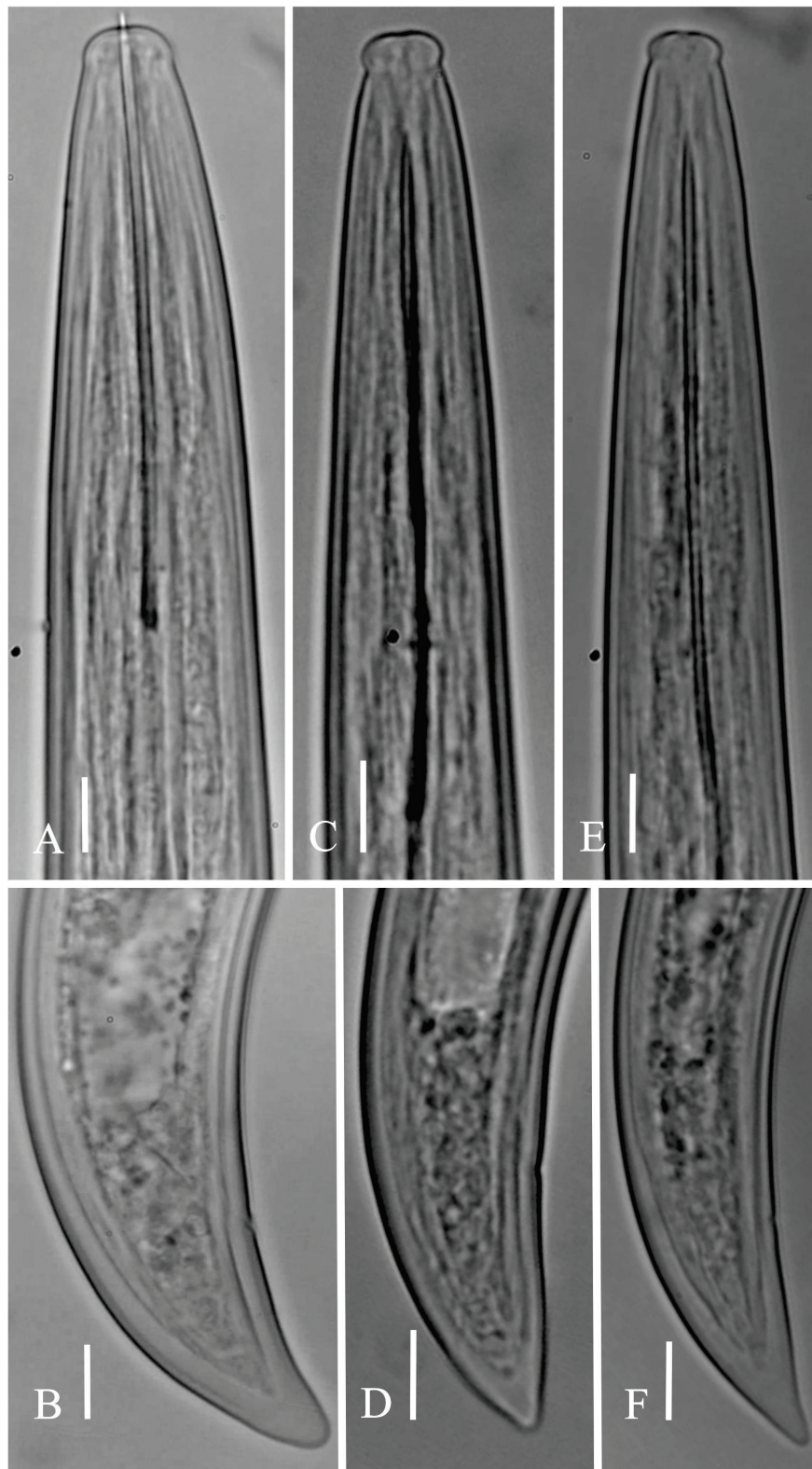


Fig. 3. A and B: *X. oxycaudatum* Lamberti & Bleve-Zacheo, 1979, C and D: *X. plesiopachtaicum* Archidona-Yuste, Navas-Cortes, Cantalapedra-Navarrete, Palomares-Rius and Castillo, 2016, E and F: *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951 (Scale bar: A-F=10 μ m).

in Nigeria (Lamberti & Bleve-Zacheo, 1979; Bos & Loof, 1984) and from soil under old mango and baoba tree in Kenya (Coomans & Hens, 1997). In Iran, this species was first observed by Fadaei *et al.* (2003) from rhizosphere of citrus trees in Hormozgan. The present Iranian females conformed well in most morphological and morphometric characteristics to *X. oxycaudatum* described by Lamberti & Bleve-Zacheo (1979) except in having wider lip region (10.5 – 11.5 vs 9 – 10 µm). The main features of the specimens, also fit perfectly to those populations described by Fadaei *et al.* (2003) except in having slightly greater c ratio (50 – 54 vs 37 – 51.5) and moderately greater c' value (1.3 – 1.4 vs 1.5 – 1.8).

***Xiphinema pachtaicum* (Tulaganov, 1938) Kirjanova, 1951**

(Figs. 3)

Measurements

See Table 1.

Distribution

Robat Namaki village, Robat area, Khorramabad County, Lorestan province, Iran, (GPS coordinates: N 33° 36' 31" E 48° 18' 16", altitude 1332 m a.s.l.), in the root zone soil of Hawthorn (*Crataegus aronia* L.).

Remarks

Xiphinema pachtaicum is a well-known species of worldwide distribution and is probably the most cosmopolitan representative of the *X. americanum*-group. This species is widely distributed throughout the Mediterranean Basin and in Central Asia. It has also been collected from California and Switzerland, England, and South Africa (Lamberti & Bleve-Zacheo, 1979). Furthermore it is widespread in several countries of Central and Eastern Europe, such as Bulgaria, Croatia, the Czech Republic, Macedonia, Montenegro and Serbia, Moldavia and Ukraine and Slovakia (Repaci *et al.*, 2008). In Iran, *X. pachtaicum* was first reported from vineyards in East Azarbaijan, West Azarbaijan, Tehran, Esfahan, Lorestan, Khorasan, Sistan and Baluchestan by Mojtahedi *et al.* (1980), later this species was collected from Ardabil, Kerman, Markazi, Alborz, Kurdistan, Gilan, Zanjan and Hormozgan of Iran from the root zone soil of Beet, Fruit trees, Olive, Potatoe, Tomatoe, Alfalfa, Pistachio, Apricot, Pine, Bean, Walnut and Forest trees (Ghaderi *et al.*, 2018). The Khorramabad population entirely matches with the earlier described populations by Lazarova *et al.*, 2016.

References

ARCHIDONA-YUSTE, A., NAVAS-CORTES, J.A., CANTALAPIEDRA-NAVARRETE, C., PALOMARES-RIUS, J.E., CASTILLO, P. (2016): Cryptic diversity and species delimitation in the *Xiphinema americanum*-group complex (Nematoda: Longidoridae) as inferred from morphometrics and molecular markers. *Zool J Linn Soc.*, 176(2): 231 – 265. DOI: 10.1111/zooj.12316

BOS, W.S., LOOF, P.A.A. (1984): Nigerian species of the genus *Xiphinema* Cobb, 1913 (Nematoda: Dorylaimida): part I. *Nematologica*, 30(4): 395 – 418.

BROWN, D.J.F., BOAG, B. (1988): An examination of methods used to extract virus vector nematodes (Nematoda: Longidoridae and Trichodoridae) from soil samples. *Nematol Mediterr.*, 16(1): 93 – 99

BROWN, D.J.F., FARIA, A.A., LAMBERTI, F., HALBRENDT, S.M., AGOSTINELLI, A., JONES, A.T. (1992): A description of *Xiphinema madeirense* n. sp. and the occurrence and virus vector potential of *X. diversicaudatum* (Nematoda, Dorylaimida) from Santana, Madeira. *Nematol Mediterr.*, 20(1992): 251 – 259

COOMANS, A., HEYNS, J. (1997): Three species of the *Xiphinema americanum*-group (Nematoda: Longidoridae) from Kenya. *Nematologica*, 43(5): 259 – 274. DOI: 10.1163/005025997X00021

DE GRISSE, A.T. (1969): Redescription ou modification de quelques techniques utilisées dans l'étude des nematodes phytoparasitaires [Redescription or modification of some techniques used in the study of phytoparasitic nematodes]. *Mededelingen Rijksfaculteit Landbouwwetenschappen, Gent*, 34: 351 – 369 (In French)

FADAEI, A., COOMANS, A., KHEIRI, A. (2003): Three species of the *Xiphinema americanum* lineage (Nematoda: Longidoridae) from Iran. *Nematol.*, 5(3): 453–461. DOI: 10.1163/156854103769224430

GHADERI, R., KASHI L., KAREGAR, A. (2018): *Plant-parasitic nematodes in Iran*. Marja-e-elm with Iranian Society of Nematology. 754 pp.

GUTIÉRREZ-GUTIÉRREZ, C., BRAVOS, M.A., SANTOS, M.T., VIEIRA, P., MOTA, M. (2016): An update on the genera *Longidorus*, *Paralongidorus* and *Xiphinema* (Family Longidoridae) in Portugal. *Zootaxa*, 4189: 99 – 114. DOI: 10.11646/zootaxa.4189.1.4

GUTIÉRREZ-GUTIÉRREZ, C., CANTALAPIEDRA-NAVARRETE, C., DEGRAEMER, W., VOVLAS, N., PRIOR, T., PALOMARES-RIUS, J.E., CASTILLO, P. (2012): Phylogeny, diversity, and species delimitation in some species of the *Xiphinema americanum*-group complex (Nematoda: Longidoridae), as inferred from nuclear and mitochondrial DNA sequences and morphology. *Eur. J. Plant Pathol.*, 134(3): 561 – 597. DOI: 10.1007/s10658-012-0039-9

LAMBERTI, F., BLEVE-ZACHEO, T. (1979): Studies on *Xiphinema americanum* sensu lato with description of 15 new species (Nematoda, Longidoridae). *Nematol Mediterr.*, 7: 51 – 106

LAMBERTI, F., MOLINARI, S., MOENS, M., BROWN, D.J.F. (2000): The *Xiphinema americanum* group. I. Putative species, their geographical occurrence and distribution, and regional polytomous identification keys for the group. *Russ J Nematol.*, 8(1): 65 – 84

LAZAROVA, S., PENEVA, V., KUMARI, S. (2016): Morphological and molecular characterisation, and phylogenetic position of *X. browni* sp. n., *X. penevi* sp. n. and two known species of *Xiphinema americanum*-group (Nematoda, Longidoridae). *ZooKeys*, 574: 1 – 42. DOI: 10.3897/zookeys.574.8037

MOBASSERI, M., HUTCHINSON, M.C., AFSHAR, F.J., PEDRAM, M. (2020): New evidence of nematode-endosymbiont bacteria coevolution based on one new and one known dagger nematode species of *Xiphinema americanum*-group (Nematoda, Longidoridae). *PloS one*, 14(6): 1 – 33. DOI: 10.1371/journal.pone.0217506

- MOJTAHEDI, H., STURHAN, D., AKHIANI, A., BAROOTI, S. (1980): *Xiphinema* species in Iranian vineyards. *Nematol Mediterr.*, 8: 165 – 170
- NAGHAVI, A., NIKNAM, G., VAZIFEH, N. (2018): Thirteen species of Longidoridae family from East Azarbaijan province. Iran. *Proceeding of 23st Iranian Plant Protection Congress, Volume II Plant Diseases, Gorgan University of Agricultural Sciences and Natural Resources*, 797 – 798
- ORLANDO, V., CHITAMBAR, J.J., DONG, K., CHIZHOV, V.N., MOLLOV, D., BERT, W., SUBBOTIN, S.A. (2016): Molecular and morphological characterisation of *Xiphinema americanum*-group species (Nematoda: Dorylaimida) from California, USA, and other regions, and co-evolution of bacteria from the genus *Candidatus xiphinematobacter* with nematodes. *Nematol.*, 18(9): 1015 – 43. DOI: 10.1163/15685411-00003012
- REPASI, V., AGOSTINELLI, A., NAGY, P., COIRO, M.I., HECKER, K., LAMBERTI, F. (2008): Distribution and morphometrical characterization of *Xiphinema pachtaicum*, *X. simile* and *X. brevicollum* from Hungary. *Helminthologia*, 45: 96 – 102. DOI: 10.2478/s11687-008-0018-z
- TAYLOR, C.A., BROWN, D.J.F. (1997): *Nematode vectors of plant viruses*. 2nd Edition, Wallingford, UK, CABI Publishing, 635 pp.