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

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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 overlapped (Archidona-Yuste et al., 2016). Ten species of *Xiphinema* have been reported from Iran. They are *X. americanum*, *X. bacaniiboia*, *X. brevicollum*, *X. himalayense*, *X. oxycaudatum*, *X. pachtaicum*, *X. pacificum* 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.

Article info

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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
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 48º 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 Lindi.) 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 cm$^3$ 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.

| Species | X. oxycaudatum | X. pachtaicum | X. plesiopachtaicum |
|---------|----------------|---------------|---------------------|
| Characters ratios* | | | |
| n | Female | Female | Female |
| L | 1.5 , 1.7 | 1.87 – 1.93 (1.90 ± 0.02) | 1.76 – 1.81 (1.79 ± 0.01) |
| a | 43 , 43 | 56.5 – 62.5 (58.2 ± 2.2) | 61.2 – 66.7 (63 ± 2.1) |
| b | 6 , 6 | 5.5 – 6.2 (5.8 ± 0.3) | 5.4 – 6 (5.7 ± 0.3) |
| c | 50 , 54.5 | 63.2 – 65.7 (64.5 ± 1.1) | 59 – 66 (62.6 ± 2.5) |
| c’ | 1.3 , 1.4 | 1.6 – 1.8 (1.7 ± 0.1) | 1.4 – 1.6 (1.5 ± 0.1) |
| V | 51 , 51 | 57 – 58 (57.5 ± 0.5) | 57 – 59 (58 ± 0.8) |
| Odontostyle length | 79.5 , 81.3 | 82 – 85.5 (82.8 ± 0.5) | 73 – 76 (74.4 ± 1.2) |
| Odontophore length | 43.6 , 46.2 | 44.5 – 49 (47.5 ± 1.1) | 42 – 43.7 (42.9 ± 0.7) |
| Spear length | 124.9 , 125.2 | 128 – 133.8 (130 ± 2.5) | 116 – 118.8 (117.5 ± 1.1) |
| Lip region diam. | 10.5 , 11.5 | 8.8 – 9 (8.9 ± 0.1) | 8.5 – 9.2 (9 ± 0.1) |
| Oral aperture to guide ring | 69.5 , 72 | 75 – 79.5 (77.9 ± 1.1) | 64.5 – 68 (66.4 ± 1.1) |
| Pharynx length | 276 , 282.3 | 303.5 – 343 (319 ± 15) | 297 – 330.5 (310.9 ± 8.5) |
| Pharyngeal bulb length | 68 , 74 | 67 – 82.5 (74 ± 6) | 71 – 81 (76.1 ± 4) |
| Body diam. at phar. base | 31 , 33.5 | 26.5 – 31.3 (28.6 ± 1.8) | 27 – 29.5 (28.2 ± 1) |
| at mid-body | 35 , 39.5 | 30 – 33 (31.9 ± 1.1) | 27 – 29.5 (28.2 ± 1) |
| at anus | 20 , 24.5 | 16.5 – 17 (16.7 ± 0.2) | 18.3 – 19.1 (18.7 ± 0.2) |
| Tail length | 28.5 , 34 | 29 – 30.5 (29.5 ± 0.3) | 27.5 – 30.6 (28.7 ± 1.1) |
| Hyaline of tail tip | 9 , 9.5 | 9.5 – 10.7 (10.3 ± 0.2) | 5.6 – 7 (6.5 ± 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 tapering toward both ends, habitus usually open C shaped after constriction. Amphid stirrup-shaped 5.5 – 6 µm wide and 63 – 66 % flattened, laterally rounded, separated from the rest of the body by a constriction. Amphidial pouches stirrup-shaped, with slit-like aperture, 4.2, 4.5 µm wide, 39, 40 % 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 – 12.3×74 – 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′′, 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. madeirensense* Brown, Faria, Lamberti, Halbrendt, Agostinelli and Jones, 1988, *X. parapachydermum* Gutierrez-Gutierrez, Cantalapiedra-Navarrete, Decramer, Vovlas, Prior, Palomares & 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. madeirensense* 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. Odontostyle, 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 × 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, 25 % of the total length, muscular bulb measuring 11.4 – 12.3×74 – 81 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′′, 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, Cantalapiedra-Navarrete, Palomares-Rius and Castillo, 2016, E and F: *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951 (Scale bar: A-F=10 μm).
in Nigeria (Lamberti & Bleve-Zacheo, 1979; Bos & Loof, 1984) and from soil under old mango and baobab 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)

Measrements

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-Zaheoa, 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 Azerbaijan, West Azerbaijan, Tehran, Esfahan, Lorestan, Khorasan, Sistan and Baluchestan by Mojahedsi 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.

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