SHORT COMMUNICATION

First report of the nematodes *Filenchus orientalis* and *Hemicriconemoides californianus* on faba bean in IranS. Azimi¹, E. Mahdikhani-Moghadam^{1*}, H. Rouhani¹ and H. Rajabi Memari²

Summary During a survey in Iran, two known species of plant-parasitic nematodes of the families Tylenchidae and Criconematidae were reported for the first time. The morphological and morphometric characters of Iranian populations of the two recovered species are discussed and illustrated based on morphological and morphometrics data. Iranian population of *Filenchus orientalis* is characterized by having a 601-755 µm body length, stylet length of 9.0-11.3 µm, lateral field with four incisures, tail length of 100-118 µm and males with 15-21 µm long spicules. *Hemicriconemoides californianus* population is characterized by having a body length of 430-550 µm, lip region with two annuli, stylet length of 75-83 µm and tail length of 20-28 µm. The morphological and morphometric characters of both species are in agreement with those in original descriptions.

Additional keywords: *Hemicriconemoides*, *Filenchus*, first record, , morphology, morphometric, plant-parasitic

Introduction

Plant parasitic nematodes cause severe damage to faba bean (*Vicia faba* L.) in many countries (Sikora and Greco, 1990). The root lesion nematodes (*Pratylenchus thornei*, *P. neglectus*, *P. pinguicaudatus*) and *Ditylenchus dipsaci* are major pests of this crop in the Mediterranean basin (Troccoli and Di Vito, 2002). Greco *et al.* (1984) recovered *Helicotylenchus* sp., *Tylenchus* sp. and *Tylenchorhynchus* sp. from soil samples of broad bean in Syria. *Tylenchorhynchus dubius*, *Merlinius brevidens*, *Pratylenchus* spp. and *Heterodera trifolii* have been reported on faba bean in Poland (Skwiercz *et al.*, 1990). Di Vito *et al.* (1994) studied nematodes of cool-season food legumes in North Africa and found *Heterodera goettingiana* which occurs in high densities in faba bean fields. Faba bean can be attacked by *Ditylenchus dipsaci*, *H. goet-*

tingiana, *Pratylenchus* spp. and *Meloidogyne* spp. but the most common and harmful parasitic nematode to this crop is the stem nematode *D. dipsaci* (Sillero *et al.*, 2010). *Ditylenchus gigas* has been recorded from broad bean with economic importance in Italy, Spain and Lebanon (Vovlas *et al.*, 2011).

During nematode surveys in Iran conducted during 2011-2014, we recovered seven species of the genus *Pratylenchus* associated with root and soil samples of faba bean (Azimi & Mahdikhani-Moghadam, 2013) and two species belonging to the rare genus, *Apratylenchoides* from the rhizosphere of faba bean (Azimi *et al.*, 2014). Additional data about two species of the genera *Filenchus* Andrassy, 1954 and *Hemicriconemoides* Chitwood and Birchfield, 1957 associated with faba bean fields in Iran are presented herein.

Materials and methods

Soil samples were collected from the rhizosphere of faba bean fields in Khuzestan province, south-western Iran during 2011-2014. Nematodes were extracted from soil samples using the Jenkins (1964) method.

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The collected specimens were killed in hot 4% formaldehyde solution, transferred to anhydrous glycerin according to De Grisse's (1969) method. Nematodes were mounted in a small drop of glycerin on permanent slides. Observations and measurements were done using a Leitz SM-LUX light microscope equipped with a drawing tube. Some of the best-preserved specimens were photographed using an Olympus DP72 digital camera attached to an Olympus BX51 light microscope. Nematode species were identified based on morphological and morphometric characters (Geraert, 2008, 2010).

Results and discussion

Filenchus orientalis Xie and Feng, 1996 (Figs 1 & 2)

MEASUREMENTS

See Table 1

DESCRIPTION

Female

Body slightly ventrally arcuate after fixation. Lateral field slightly more than one-fourth of maximum body diameter, with smooth four lines. Lip region rounded, smooth, continuous with body contour or separated with slight constriction. Amphidial apertures in form of longitudinal slits on lateral sides of lip region. Stylet delicate, 9-11 μm long, conus about one-third of entire stylet length, knobs directed posteriorly. Dorsal gland orifice opens at 1.5-2.0 μm posterior to stylet base. Procorpus narrow, cylindrical, median bulb oval with relatively prominent valve, posterior glandular region pyriform, not overlapping intestine. Excretory pore obvious, at 70-85 μm distance from anterior end. Reproductive system monodelphic-prodelphic, composed of an ovary which tip of the germinal zone sometimes reaching to terminal bulb and oocytes mostly in single row, short oviduct, spermatheca longer than wide, 35-41 $\times$ 11-14 μm size, filled with spheroid sperm cells, crustaformeria well visible, uterus with distinct lumen, post vulval uterine sac shorter than body width, vagina perpendicular to body axis and vulva

a small transverse slit. Tail long-conical, narrowing evenly to filiform part and straight to slightly curved.

Male

General morphology similar to that of female except for character states associated with sexual differences. Body straight to slightly ventrally arcuate after fixation. Excretory pore 82-91 μm from anterior end. Testis a single continuous tube. Spicules slightly curved ventrad, 15-21 μm long. Gubernaculum simple. Tail narrowing evenly, straight to slightly curved, its end filiform as in female.

REMARKS

The species was originally described by

Figure 1. *Filenchus orientalis*. A: Male; B: Female; C: Anterior region of female; D: Anterior region of male; E: Female lateral field at mid-body; F: Part of reproductive system; G: Female tail; H: Male tail.

Figure 2. *Filenchus orientalis*. A: Anterior region of female; B: Female lateral field at mid-body; C: Vulval region; D: Female tail; E: Male tail (Scale bars: 10 μ m).

Xie and Feng (1996) from soil around roots of water spinach (*Ipomoea aquatica*) in China. The morphological and morphometrics character of the recovered species are in agreement with those of original description. This population is characterized by having lateral field with four lines, head continuous to slightly constricted, 9-11 μ m long stylet, and filiform tail. It can be distinguished from *F. vulgaris* (Brzeski, 1963) Lownsbery and Lownsbery (1985) by the shape of head region (rounded, from continuous to slightly constricted, smooth vs mostly trapezoid in outline, sometimes mostly rounded, continuous with 4-5 annuli), the range of T/VA ratio (1-1.1 vs 1.2-1.7) and common of males. This species was recovered in 6.66% of soil samples (15 females and 8 males) from the

Table 1. Morphometrics of *Filenchus orientalis* Xie and Feng, 1996 from Iranian population and their comparison with the type population (data according to Geraert, 2008). All measurements are in μ m and in the form: mean $\pm$ s.d. (range).

Character	Present study		Xie and Feng, 1996	
	Females	Males	Females	Males
n	15	8	-	-
L	643.3 $\pm$ 39.1 (601 -755)	616.6 $\pm$ 5.4 (610-626)	610-740	-
a	32.8 $\pm$ 1.2 (30.0-37.1)	34.2 $\pm$ 2.1 (31.7-38.5)	29-36	-
b	5.9 $\pm$ 0.3 (5.5-6.4)	5.7 $\pm$ 0.4 (5.1-6.2)	-	-
c	5.4 $\pm$ 1.1 (4.3-6.9)	4.4 $\pm$ 0.5 (3.8-5.5)	5.6-7.0	-
c'	10.3 $\pm$ 1.2 (8.1-13.0)	11.6 $\pm$ 1.4 (9.1-13.3)	7.5-11.0	-
V	63.6 $\pm$ 4.5 (58.6-69.7)	-	63-70	-
V'	78.4 $\pm$ 2.2 (76.2-81.3)	-	76-81	-
Stylet length	9.9 $\pm$ 0.9 (9.0-11.3)	9.9 $\pm$ 0.9 (9-11)	10-11	-
M	33.0 $\pm$ 2.4 (30-36)	33.0 $\pm$ 1.4 (31-36)	-	-
DGO	1.5 $\pm$ 0.2 (1.5-2.2)	1.5 $\pm$ 0.3 (1.4-2.1)	-	-
MB	37.3 $\pm$ 1.9 (34.0-40.7)	44.1 $\pm$ 4.6 (37.9-51.8)	37-54	-
Body width	19.2 $\pm$ 1.5 (16.7-21.5)	17.9 $\pm$ 1.1 (16.2-19.2)	-	-
S. E. pore	80.3 $\pm$ 5.5 (70.0-85.5)	88.4 $\pm$ 4.9 (82.1-90.8)	67.6-91.0	-
Vulval body width	16 $\pm$ 1.9 (14.5-20.3)	-	-	-
Post vulval uterine sac	13 $\pm$ 2.9 (9.6-17.7)	-	-	-
Vulva-anus	113.9 $\pm$ 6.7 (101 -120)	-	-	-
Anal body width	11.4 $\pm$ 1.3 (9.6-13.1)	12.2 $\pm$ 0.8 (11.1-13.5)	-	-
Tail length	112.3 $\pm$ 6.0 (100.3-118.0)	113.0 $\pm$ 4.0 (106-118)	106-112	-
T/VA	1.1 $\pm$ 0.1 (0.9-1.3)	-	1.0-1.1	-
Spicule length	-	17.3 $\pm$ 2.3 (15-21)	-	15.5-21.0
Gubernaculum length	-	6.6 $\pm$ 0.7 (5.5-7.7)	-	7-8
Bursa length	-	35.5 $\pm$ 2.8 (30.0-38.5)	-	-

rhizosphere of faba bean fields in the vicinity of Shooshtar city in Khuzestan province, south-western Iran. This is the first report of the species from Iran, and the second report of it after the original description.

***Hemicriconemoides californianus* Pinochet and Raski, 1975**

(Figs 3 & 4)

MEASUREMENTS

See Table 2

DESCRIPTION

Female

Body slightly ventrally arcuate after fixation. Cuticular sheath attached to body at anterior end, and vulva generally closely fitting. Body annuli 3.5-5.0 μm wide at mid-body. Lip region 11.5-12.5 μm wide and 5-6 μm high, anteriorly flatted, with two annuli, first annulus slightly smaller in diameter than second. Stylet very long, its conus com-

prising ca. 84% of total stylet length, knobs anchor-shaped, indented anteriad, rounded at base, 5.5-7.0 μm across. Dorsal pharyngeal gland orifice opens close to knobs. Excretory pore at 124-130 μm from anterior end. Pharynx typical of the genus. Nerve ring encircling isthmus. Reproductive system monodelphic-prodelphic, outstretched, composed of ovary with oocytes arranged in one or two rows, short oviduct, crustaformeric, oval spermatheca, 22-25 $\times$ 12-15 μm size, with spheroid sperm cells, vulval slit lacking flaps. Tail conoid with bluntly rounded terminus.

Male

Not found

REMARKS

The species was originally described by Pinochet and Raski (1975) from soil around roots of grape (*Vitis vinifera* L.) in California. Ye and Robbins (2000) examined 13 populations of this species from different hosts in California, no morphological or morphometric differences were found among examined populations. The general morphology of the recovered species closely resembles

Figure 3. Female of *Hemicriconemoides californianus*. A: Entire body; B: Anterior region showing pharynx; C-F: Posterior end showing vulva and tail.

Figure 4. Female of *Hemicriconemoides californianus*. A, B: Anterior end; C: Pharynx, D: Vulval region and tail (Scale bars: 10 μm).

Table 2. Morphometrics of *Hemicriconemoides californianus* Pinochet and Raski, 1975 females from Iranian population and their comparison with the type population. All measurements are in μm and in the form: mean $\pm$ s.d. (range).

Character	Present study	Pinochet and Raski, 1975	Ye and Robbins, 2000	
n	20	10	5	10
L	469.8 $\pm$ 38.4 (430-550)	440.0 (410-460)	436.0 $\pm$ 28.1 (400-480)	467.8 $\pm$ 23.8 (425-500)
a	17.4 $\pm$ 1.3 (15.6-20.0)	17.0 (16.0-19.0)	14.8 $\pm$ 1.5 (12.5-17.1)	15.4 $\pm$ 1.4 (13.5-17.8)
b	4.2 $\pm$ 0.4 (3.4-4.7)	4.1 (3.4-4.6)	3.6 $\pm$ 0.3 (3.1-3.9)	3.6 $\pm$ 0.1 (3.4-3.8)
c	21.9 $\pm$ 1.9 (19-26)	21.0 (17-25)	24.4 $\pm$ 4.8 (19.2-30.3)	24.2 $\pm$ 2.8 (20.2-27.8)
c'	1.3 $\pm$ 0.1 (1.0-1.4)	-	1.1 $\pm$ 0.1 (0.9-1.3)	1.1 $\pm$ 0.1 (1.0-1.3)
V	92.0 $\pm$ 0.7 (91.0-93.1)	91.0 (90-92)	91.8 $\pm$ 0.9 (90.3-92.8)	91.7 $\pm$ 0.6 (90.8-92.4)
Stylet length	80.0 $\pm$ 3.4 (75-83)	80.0 (77-83)	77.6 $\pm$ 4.3 (74.0-86.0)	84.4 $\pm$ 1.6 (82-86)
m	86.3 $\pm$ 2.2 (83.1-89.7)	-	86.2 $\pm$ 3.3 (81.4-89.3)	89.3 $\pm$ 1.5 (86.9-91.6)
DGO	4.1 $\pm$ 0.5 (3.5-4.8)	-	-	-
MB	66.2 $\pm$ 1.5 (63.7-68.0)	-	-	-
Body width	30.6 $\pm$ 1.7 (28.4-32.5)	-	29.5 $\pm$ 1.9 (26.5-32.0)	30.4 $\pm$ 2.5 (27.5-35.0)
S. E. pore	126.7 $\pm$ 2.2 (124.1-130.2)	-	126.6 $\pm$ 5.1 (120-135)	138.3 $\pm$ 8.2 (127-153)
Vulval body width	92.0 $\pm$ 1.3 (91.0-93.1)	-	-	-
Vulva-anus	15.0 $\pm$ 1.6 (13-18)	-	-	-
Anal body width	18.5 $\pm$ 2.4 (16-22)	-	-	-
Tail length	24.4 $\pm$ 3.1 (20-28)	-	-	-
R	126.8 $\pm$ 6.4 (120-135)	119.0 (112-127)	114.6 $\pm$ 6.1 (103-121)	119.9 $\pm$ 2.0 (117-124)
RSt	20.7 $\pm$ 0.8 (20-22)	-	20.8 $\pm$ 1.1 (20-23)	23.0 $\pm$ 0.6 (22-24)
Roes	32.8 $\pm$ 3.1 (28-38)	-	30.0 $\pm$ 0.6 (29-31)	33.3 $\pm$ 2.0 (31-37)
Rex	34.2 $\pm$ 2.9 (30-39)	35.0 (34-37)	32.6 $\pm$ 1.7 (30-34)	36.2 $\pm$ 2.3 (33-40)
RV	12.2 $\pm$ 1.0 (11-13)	11.0 (10-12)	12.4 $\pm$ 1.0 (11-14)	11.8 $\pm$ 0.6 (11-13)
RVan	4.2 $\pm$ 0.5 (4-5)	6.0 (5-6)	4.8 $\pm$ 0.7 (4-6)	5.1 $\pm$ 0.5 (4-6)
Ran	6.6 $\pm$ 0.8 (6-8)	5.0 (4-6)	7.6 $\pm$ 0.8 (7-9)	6.7 $\pm$ 0.6 (6-8)
VL/VB	1.6 $\pm$ 0.1 (1.4-1.8)	1.7 (1.3-1.8)	1.5 $\pm$ 0.1 (1.4-1.8)	1.6 $\pm$ 0.1 (1.4-1.9)

to the characters given in the original description and the data given by Ye and Robbins, 2000 (see Table 2). However, the body length is slightly longer (430-550 vs 400-500 μm) and the number of body annuli is greater (120-135 vs 112-127). These differences can be attributed to the intraspecies variations due to geographical differences. The recovered population of the species is closely related to *H. chitwoodi*, Esser, 1960, *H. gaddi* (Loos, 1949) Chitwood & Birchfield, 1957 and *H. phoenicis*, Van den Berg, Tiedt, Insera, Stanley, Vovlas, Palomares-Rius, Castillo and Subbotin (2015).

Our population differs from *H. chitwoodi*

di by lip region having the first annulus smaller than the second one, more broadly conoid tail with bluntly rounded terminus (vs conoid, gradually tapering to slender conoid outline, with finely rounded terminus). In our population, the first lip annulus is slightly smaller in diameter than the second, whereas it is always much smaller than the second in *H. gaddi*. Also, VL/VB ratio is 1.3-1.9 (vs 2.1-2.7). It can be distinguished from *H. phoenicis* by lacking the coarse cuticular ridges and grooves of the annuli.

Hemicriconemoides californianus has been reported from North Korea (Kornobis and Dobosz, 1997) and Taiwan (Chen et al.,

2007). This species was recovered in 4.0% of soil samples (20 females) from the rhizosphere of faba bean fields in the vicinity of Dizful city in Khuzestan province, south-western Iran. This is the first report of *H. californianus* from Iran and the first report of the species in association with faba bean. The aim of the present study was originally to identify the plant parasitic fauna in Iran, therefore no further study was performed regarding the pathogenicity of the aforementioned plant parasitic nematode species found in association with faba bean roots. Some stunting and other symptoms such as yellowing, weakening etc, were observed on the plants, but, assigning these symptoms specifically to nematode damage needs further pathogenicity testing.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη αναφορά των νηματωδών *Filenchus orientalis* και *Hemicriconemoides californianus* σε κουκιά στο Ιράν

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Περίληψη Σε μία επισκόπηση στο Ιράν αναφέρθηκαν για πρώτη φορά δύο ήδη γνωστά είδη φυτο-παρασιτικών νηματωδών, τα οποία ανήκουν στις Οικογένειες Tylenchidae και Criconematidae. Γίνεται συζήτηση και παρουσίαση των μορφολογικών και μορφομετρικών χαρακτήρων των Ιρανικών πληθυσμών των δύο ειδών με βάση μορφολογικά και μορφομετρικά δεδομένα. Ο Ιρανικός πληθυσμός του *Filenchus orientalis* χαρακτηρίζεται από μήκος σώματος 601-755μm, μήκος στίλετου 9.0-11.3 μm, πλάγιο πεδίο με τέσσερις εγκολπώσεις, μήκος ουράς 100-118 μm και μήκος συζευκτικών ακάνθων αρσενικών 15-21 μm. Ο πληθυσμός του *Hemicriconemoides californianus* χαρακτηρίζεται από μήκος σώματος 430-550μm, περιοχή χειλέων με δύο ραβδώσεις, μήκος στίλετου 75-83μm και μήκος ουράς 20-28 μm. Οι μορφολογικοί και μορφομετρικοί χαρακτήρες και των δύο ειδών είναι σε συμφωνία με αυτούς των αρχικών περιγραφών.

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