

## Study of *Psilenchus* species in pistachio gardens of Khorasan Razavi Province, Iran

R. Hadadfar<sup>1</sup>, E. Mahdikhani-Moghadam<sup>2†</sup>, S. Baghaee<sup>2</sup> and M. S. Bajestani<sup>1</sup>

<sup>1</sup>Department of Plant Protection, Ferdowsi University of Mashhad, Iran

<sup>2</sup>Plant Pathology Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran

†Corresponding author: Mahdikhani\_e@yahoo.com

### Abstract

To identify *Psilenchus* species in pistachio gardens of Khorasan Razavi Province of Iran, 50 soil and plant samples from pistachio roots rhizosphere (Depth 30-50 cm) were collected during years 2016-2017. Samples were transferred on ice to laboratory and nematodes were extracted by centrifugal methods. *Psilenchus* species were identified on morphological and morphometrical characters based on recent valid keys. Seven species from *Psilenchus* genus were identified viz., *Psilenchus curcumerus*, *P. aestuarius*, *P. iranicus*, *P. hilarus*, *P. bilineatus*, *P. pratensis* and *P. bahiablancae* of which *P. bilineatus*, *P. pratensis* and *P. bahiablancae* are new reports of these species for Iranian nematode fauna.

**Keywords:** *Psilenchus*, Pistachio, Iran, Khorasan Razavi Province.

**P**istachio is one of the important gardening and export product of Iran. Pistachio tree (*Pistacia vera* L.) is from *Anacardiaceae* family with vertical roots that penetrate into soil down to about 5-6 meters; the conditions of the soil have a major impact on its growth, health and fertility (Tous & Ferguson, 1996). Pistachio trees found naturally in vast areas of Central and Western Asia (Zohary, 1952). The main pistachio is located in the Northeastern Khorasan (Khorasan Razavi province of Iran) from Khajeh Sarakhs forest (Nakhaee Nejad, 2007). According to data released by the United Nations Food and Agriculture Organization (FAO) in 2014, Iranian exports of pistachio were more than 191,000 tons and Iran is the world's largest exporter of this product (<http://www.fao.org>).

Khorasan Razavi province is one of the most strategically located areas for the production of

pistachio. Based on reported data from Agricultural Statistics Yearbook in 2015, more than 20 percent of total production is contributed by this province Siddiqi (2000) included the genus *Psilenchus* in the Psilenchidae family while De Ley *et al.*, (2006) in the modified classification of Phylum Nematoda on molecular studies placed the genus under Tylenchidae family. Pistachio plant suffered by several diseases that directly or indirectly caused by number of pests, including plant parasitic nematodes (Neshat *et al.*, 2011). About 50 species of nematodes have been identified in pistachio fields throughout the world (Fatemy, 2009). From California pistachio gardens, *Pratylenchus* spp., *Meloidogyne* spp. and *Xiphinema americanum* were reported (Kodira & Westerdahl, 1995). Yildiz *et al.*, (2007) studied the nematodes associated with pistachio farm of Turkey and reported *Rotylenchulus*

*macrosomus*, *Criconema* spp., *Paratylenchus* spp., *Geocenamus* spp., *Trophurus* spp., *Trichodorus* spp. *Tylenchorhynchus* spp. and *Pratylenchoides* spp. High population of *Pratylenchus vulnus* and *Pratylenchus terebinthus* was reported from pistachio roots in Spain (Pinochet *et al.*, 1992). In another study *Paralongidorus litoralis* was described in pistachio gardens from Spain (Palomares-Rius *et al.*, 2008). A report in Italy showed that the *P. lentiscus* and *P. vera* pistachio cultivars are very sensitive to *Heterodera mediterranea* and are also highly infected with the *Rotylenchulus macrodoratus* (Vovlas & Inserra, 1983). In addition, *Meloidogyne marioni*, *Meloidogyne javanica*, *Pratylenchus neglectus* and *Xiphinema* spp. were isolated from the pistachio gardens of Sicily, Italy (Greco & Nucifora, 1999).

Twenty three plant parasitic nematode species have been described from pistachio so far from Iran (Barooti & Alavi 2002). The first

report of root-knot nematodes on pistachio was made in 1987 from Rafsanjan city of Iran (Farivar Mihan, 1987). Several species of root-knot nematode have been reported from Iran (Akhiani *et al.*, 1984; Madani 1996; Hossainipour, 1991). Neshat *et al.*, in 2011 conducted research on pistachio nematode fauna in the Sirjan City, Iran and reported 23 species belonging to 6 genera. *Rotylenchus whiteheadi* was reported as a new record for Iranian nematode fauna on pistachio from Kerman province (Barooti & Neshat, 2010). *Longidorus africanus* was reported for the first time in pistachio garden in 1984 by Khairi & Barooti. *Xiphinema index* was reported from Iran by Mojtahedi *et al.*, 1980. *Psilenchus* species reported so far in Iran, are given in Table 1.

The objective of this study was to identify the species of genus *Psilenchus* in pistachio gardens in Khorasan Razavi Province of Iran.

**Table 1. List of *Psilenchus* species previously reported from Iran.**

Nematode species	Sampling areas	Plant	Reported by
<i>Psilenchus aestuarius</i>	Khorasan Razavi Province	Unknown	Mokarram Hesar <i>et al.</i> , 2010
<i>Psilenchus mixus</i>	Khorasan Razavi Province	Unknown	Mokarram Hesar <i>et al.</i> , 2010
<i>Psilenchus vinciguerrae</i>	Khorasan Shomali Province	Gardening plants	Atighi <i>et al.</i> , 2011
<i>Psilenchus curcumerus</i>	Khorasan Shomali Province	Gardening plants	Atighi <i>et al.</i> , 2011
<i>Psilenchus hilarus</i>	Phars Province	Beet	Ebrahimi <i>et al.</i> , 2002
<i>Psilenchus minor</i>	Hamedan city	Potato	Gity <i>et al.</i> , 2001
<i>Psilenchus terextremus</i>	Sistan and Balochistan Province	Agricultural plants	Seraji <i>et al.</i> , 2001
<i>Psilenchus hilarulus</i>	Alborz city	Peanut	Kheiri, 1972
<i>Psilenchus iranicus</i>	Alborz & Esfahan Cities	Rye, Alfalfa	Kheiri, 1970

### Material and Methods

**Soil sampling:** Soil samples were collected from the rhizospheres of pistachio gardens in Khorasan Razavi province of Iran. Fifty soil samples were taken from the depth of 30-50 cm, placed in polyethylene bags, labeled properly and brought to the laboratory for further processing.

**Processing of samples:** Nematodes were extracted from soil samples by using the Jenkins (1964) method, and killed and fixed as per De Grisse (1969). Genera and species were identified based on morphological and morphometric characters (Siddiqi, 2000; Geraert, 2008).

**Measurements and drawings:** Measurements were taken with an ocular micrometer of

“Olympus BH2” model microscope. Drawings were made with a drawing tube attached to the compound microscope.

### Results and discussion

Nematological survey of pistachio gardens in Khorasan Razavi Province revealed the presence of many plant-parasitic nematode genera and species. However, the present study focused only on *Psilenchus* species. Seven species from *Psilenchus* genus were identified viz., *Psilenchus curcumerus*, *P. aestuarius*, *P. iranicus*, *P. hilarus*, *P. bilineatus*, *P. pratensis* and *P. bahiablanceae* of which three species viz., *P. bilineatus*, *P. pratensis* and *P. bahiablanceae* are new reports for Iranian nematode fauna (Table 2). Their morphometric data, brief description and illustrations have been incorporated herein.

**Table 2. List of *Psilenchus* species from Pistachio garden in Khorasan Razavi province east of Iran with UTM and name of sampling areas.**

Nematode species	Sampling areas	UTM*	First description
<i>Psilenchus aestuarius</i>	Feiz Abad City	665031 - 3878147	Andrassy, 1962
<i>Psilenchus curcumerus</i>	Bardaskan City	594560 - 3890373	Rahaman, Ahmad & Jairajpuri, 1994
<i>Psilenchus iranicus</i>	Rokn Abad Village	593414 -3891102	Kheiri, 1970
<i>Psilenchus hilarus</i>	Shams Abad Village	642024 - 3878403	Siddiqi, 1963
<i>Psilenchus bilineatus</i> **	Bardaskan City	594560 - 3890373	Mizukubo & Nakasono, 1987
<i>Psilenchus pratensis</i> **	Dooq Abad Village & Bardaskan City	665044 - 3878097	Doucet, 1996
<i>Psilenchus bahiablanceae</i> **	Kheir Abad Village	656749 - 3878142	Doucet, 1996

\*UTM = Universal Transverse Mercator; \*\* New record species for Iran

#### ***Psilenchus bahiablanceae* Doucet, 1996 (Fig. 1, Table 3)**

**Description:** Body slightly curved ventrally. Cuticle with fine transverse striation about 1µm wide at mid-body Lateral field with four

equidistant incisures, not areolated, 35% of body width. Labial area rounded, slightly flattened, smooth, 4±1.8 µm high and 7.6±0.5 µm wide, set off from body by a narrowing of the body contour. Stylet with thin walls, with-out thickenings at its base. Median bulb ovoid,

21.6±5.28 µm in length, 14.3±3.96 µm in width. Basal bulb 22.3±1.48 µm in length, 19.1±9.15 µm in width. Dierids just above the level of the excretory pore. Spermatheca axial, short, with spermatozoa, round to square rectangular in shape. Tail conical, straight or slightly curved ventrally or dorsally, tapering gradually to a clavate tip; terminal devoid of annulation. Phasmid located at 32±4.5µm behind the anus.

**Remarks:** In our study the specimens of *P. bahiablancae* were collected from soil around roots of pistachio gardens of Khorasan Razavi province. *P. bahiablancae* showed a close resemblance in the morphometric measurements to the original description given by Doucet, 1996 with slight variations. This species was reported for the first time in Iran.

***Psilenchus bilineatus*  
Mizukubo & Nakasono, 1987  
(Fig. 2, Table 3)**

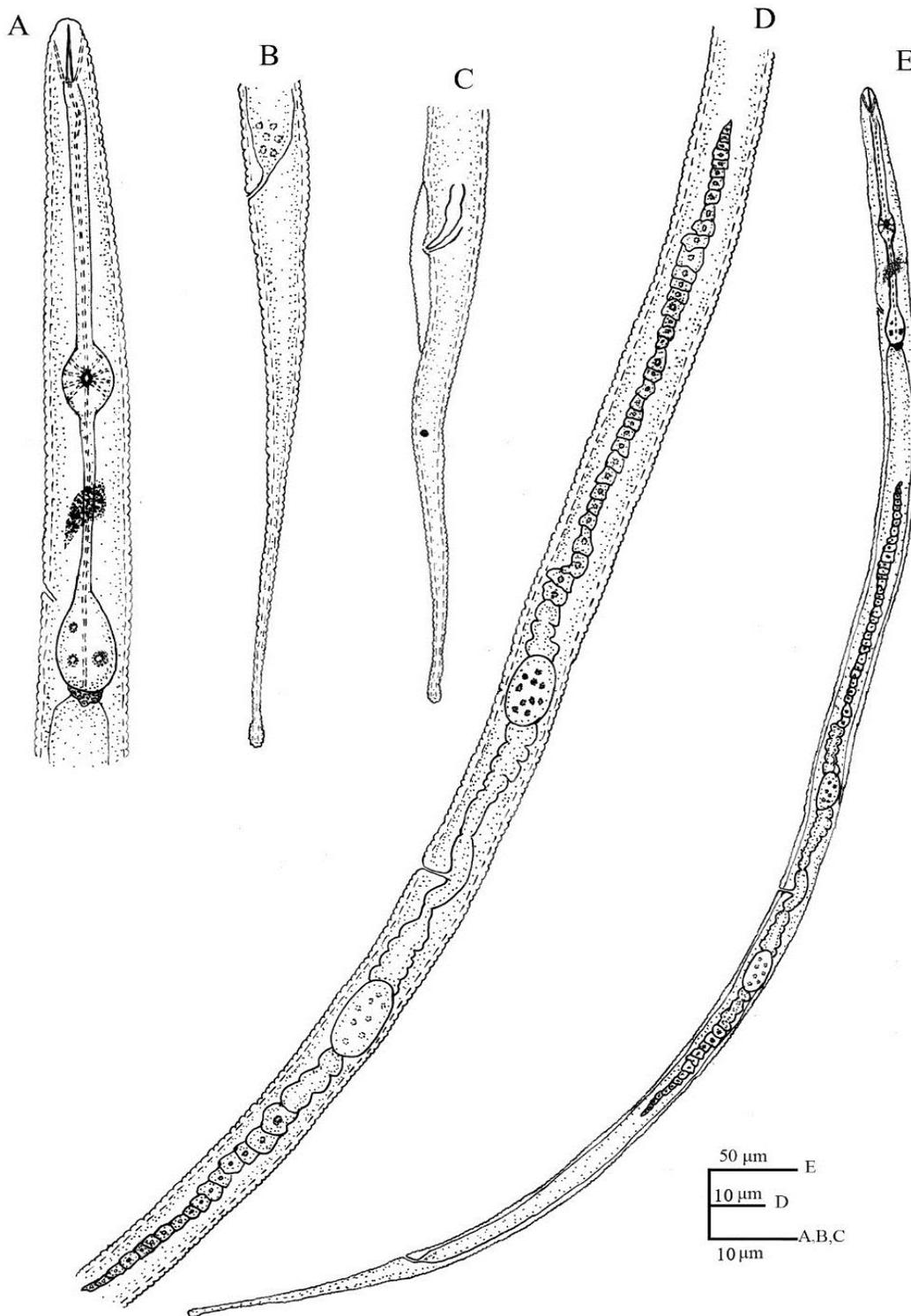
**Description:** Body from straight to arcuate. Annuli width at mid-body 0.7-1.2 µm, at median bulb 1-1.3 µm, at tail in 1.2-2.2 µm. Lateral field plain, without inner two incisures, with weakly crenate margins, width 6.5-9.5 µm. Head elevated, rounded, not set off from body, height 4.8±1.2 µm, width 7.2±1.2 µm, smooth but rarely annulated up to amphidial level. Amphidial slits about 1/3<sup>rd</sup> as wide as head base. Stylet delicate, without knobs. Excretory pore varies in position from slightly behind median bulb to posterior half of isthmus. Nerve ring just behind median oesophageal bulb. Spermatheca 19.3±3.0 µm long. Usually tail clavate, which is rarely annulated.

**Remarks:** Morphological and morphometric measurements of *Psilenchus bilineatus* specimens have been in close agreement to the original measurements given by Mizukubo & Nakasono, 1987. This species was collected from pistachio gardens of Khorasan Razavi Province as a new record from Iran.

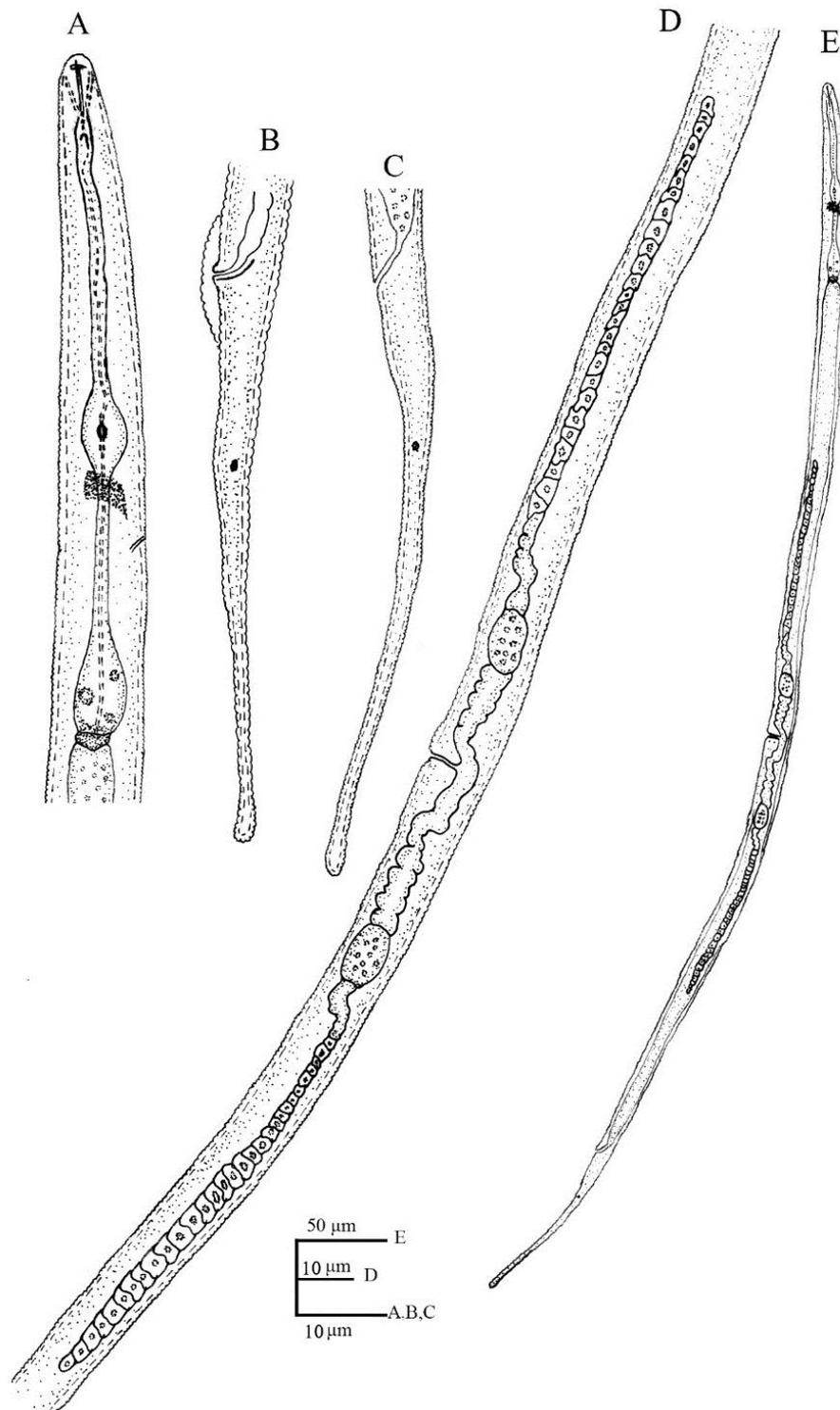
***Psilenchus pratensis* Doucet, 1996  
(Fig. 3, Table 3)**

**Description:** Body medium sized and thin, straight or slightly curved. Cuticle with fine transverse annulation about 1µm wide at mid-body. Lateral field marked by four incisures, not areolated, 32.2% of body width, delimiting three bands of equal width. Labial area conical flattened, smooth, 5.8±0.8 µm in height, 8.8±1.1 µm in width, slightly set off from body. Amphidial apertures dorso-ventrally elongated. Stylet with thin walls, without thickening at its base. Median bulb ovoid, 16.8±1.3 µm in length, 11±2.5 µm in width and located 76.3±4.6 µm from anterior end. Basal bulb 20.8±2.9 µm in length, 11.6±2.1 µm in width. Deirids clearly visible at level of excretory pore. Spermatheca full of spermatozoa, mostly rounded, sometimes oval or rectangular in shape. Tail conical, slightly curved ventrally, gradually tapering to a clavate tip; terminal third of the tail devoid of annulation. Phasmid at 40±8.3 µm from anus.

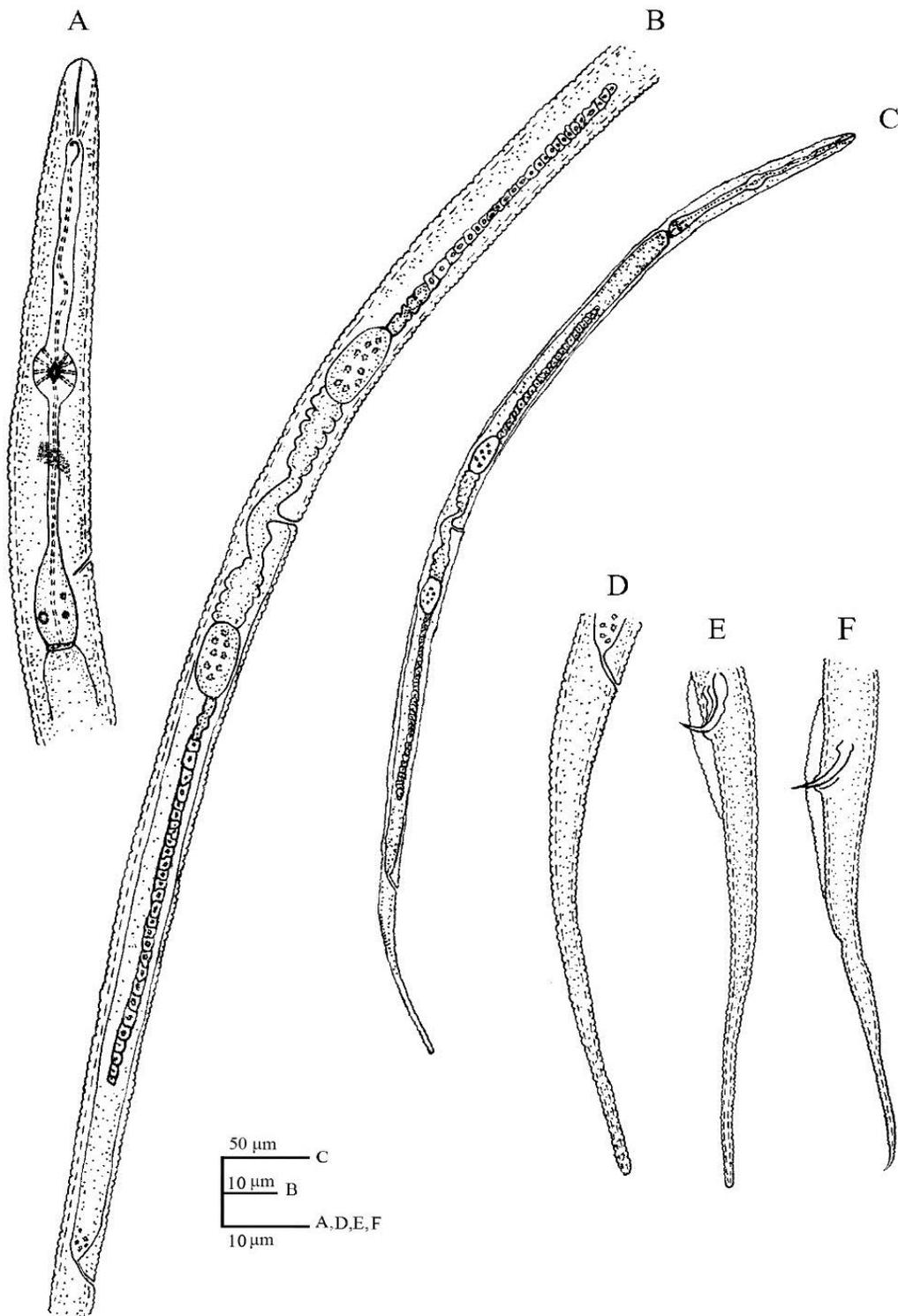
**Remarks:** Specimens of *P. pratensis* were collected from pistachio gardens of Khorasan Razavi province as a new record of Iran. The measurements of the specimens are within the range of *P. pratensis* Doucet, 1996.



**Fig. 1.** *Psilenchus bahiablancae* (A-E). A. Oesophageal region; B. Female tail; C. Male tail with cloacal region; D. Reproductive system; E. Whole body of female.



**Fig. 2.** *Psilenchus bilineatus* (A-E). A. Oesophageal region; B. Male tail with cloacal region; C. Female tail; D. Reproductive system; E: Whole body of female.



**Fig. 3.** *Psilenchus pratensis* (A-F). A. Oesophageal region; B. Reproductive system; C. Whole body of female; D. Female tail; E & F: Male tail with cloacal region.

**Table 3. Morphometric characters of the Iranian population of *Psilenchus bahiablanceae*, *P. bilineatus*, *P. pratensis* and comparison with original descriptions (Measurements are in  $\mu\text{m}$ ).**

Characters	<i>Psilenchus bahiablanceae</i>	Doucet, 1996	<i>Psilenchus bilineatus</i>	Mizukubo & Nakasono, 1987	<i>Psilenchus pratensis</i>	Doucet, 1996
<b>L</b>	1280±119/2 (1150-1450)	1.1-1.5	898.8±77.4 (783-1020)	0.76-0.98	895±126 (770-1080)	0.97-1.24
<b>Body width</b>	19.1±1.5 (18-22)	-	19±1.8 (17-22)	-	19.2±2.6 (17-23)	-
<b>a</b>	47.9±3.4 (43.3-50.6)	46 - 70	47.4±2.4 (44.5-50)	36-50	46.7±2 (44.4-50)	45-59
<b>b</b>	6.6±0.6 (6.1-7.5)	-	6.3±0.6 (5.6-7.3)	-	6.3±0.7 (5.5-7.1)	-
<b>c</b>	7.2±0.7 (6.4-8.1)	7.4-11.5	7.2±0.5 (6.6-7.9)	6.3-8.5	7.9±1.2 (6.8-9.9)	7.2- 9.8
<b>c'</b>	10 ±0.8 (9.4-11.1)	6.7-11.1	10.7±0.7 (9.9-11.8)	7-12	9.5±1.2 (7.3-10.7)	8.3-11
<b>V</b>	49±1.6 (47.3-50.6)	43-50%	49±1.1 (47.1-50)	45-50 %	50.2±0.8 (49.1-51.1)	46-55%
<b>V'</b>	54.8± 7 (54.2-59.3)	48.4-56%	57±1.8 (53.9-58.6)	-	57.6±1.8 (54.6 - 59.1)	-
<b>G<sub>1</sub></b>	28.2 ±1.7 (25.9-30)	-	12.7±3.7 (9.6-19)	-	12.8 ±5.2 (9.1 - 21.9)	-
<b>G<sub>2</sub></b>	23 ±0.9 (21.7-23.7)	-	13.2± 5.2 (7.9-23.2)	-	13.2±4.3 (10.4-20.9)	-
<b>Stylet</b>	14.8 ±0.5 (14-15)	13-17	14.3±0.5 (14-15)	12-15	15±0.9 (14-16)	12-15
<b>Pharynx length</b>	136±8.9 (123-142)	122-148	143.7±4 (139-150)	117-139	142.5±5.2 (138 -152)	130-166
<b>Excretory pore</b>	107.2 ±13 (89-123)	94-132	105.2±2.6 (102-110)	-	117.8±21.9 (97-160)	106-118
<b>MB%</b>	56±4.5 (52.8-62.7)	51-58	55.3±1.4 (53.6-57.6)	55-60	53.5±1.7 (51.4 -55.9)	53.6-58 %
<b>DGO</b>	7.4±0.5 (6-8)	7-8	3.5±0.2 (3-5)	3-5.5	9.3±0.8 (9-10)	9-10
<b>Tail/Vulva-anus</b>	0.4±0.1 (0.3-0.5)	-	0.4±0.0 (0.3-0.4)	0.3-0.5	0.4±0.1 (0.2-0.4)	0.4
<b>Tail length</b>	124±5.9 (117-131)	114-192	124.7±4.7 (118-130)	107-146	114.2 ±5.6 (106-121)	110-144
<b>Male characters</b>						
<b>Spicule length</b>	36.5±2.5 (32-37)	30-38	19.2±1.2 (18-21)	18-21	26.8±1.2 (24-30.5)	25-32
<b>Gubernaculum</b>	12±0.9 (11-13.5)	10-15	7.5±0.6 (6-8.5)	6-9	10.5±0.7 (8.5-12)	8-12

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