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Research Article

Some species of *Paratylenchus* Micoletzky, 1922 (Nematoda: Tylenchulidae) from vineyards in Kermanshah province, western Iran

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Abstract: Eight species of *Paratylenchus* were collected and identified from vineyards in Kermanshah province, western Iran. Description, measurements, line drawings and microscopic photographs are provided for two new records namely *P. humilis* and *P. prunii*. *Paratylenchus humilis* have a lip region truncate-conoid with distinct small submedian lobes in lateral view of female head, stylet shorter than 40 µm, three lateral lines and distinct vulval flaps. *Paratylenchus prunii* have a lip region rounded (slightly truncate in some specimens), without distinct submedian lobes in lateral view of female head, stylet shorter than 40 µm, four lateral lines and distinct vulval flaps. Male of *P. straeleni* is reported for the first time.

Keywords: Grapevine, identification, morphology, morphometric, new record, pin nematode

Introduction

Plant-parasitic nematodes have been reported in the vineyards of the different provinces in Iran such as: Hamadan (Karegar *et al.*, 1995), Markazi (Mohammad Deimi & Mitkowski, 2010) and Kurdistan (Ghaderi *et al.*, 2014) including several species of pin nematodes (*Paratylenchus* spp.) *P. peraticus* (Raski, 1962) Siddiqi & Goodey, 1964 and *P. arculatus* Luc & de Guiran, 1962 (Karegar *et al.*, 1995), *P. coronatus* Colbran, 1965 (Gharakhani *et al.*, 2007), *P. mexicanus* Raski, 1975, *P. projectus* Wu & Hawn, 1975 and *P. neoamblycephalus* Geraert, 1965 (Ashrafi *et al.*, 2012), *P. conicephalus* van den Berg, Eskandari, Teidt & Karegar, 2011 (Ghaderi *et al.*, 2014), and *P. audriellus* Brown, 1959 and *P. leptos* Raski, 1975 (Esmaeili *et al.*, 2015). *Paratylenchus* Micoletzky, 1922 now includes 118 nominal species (Ghaderi *et al.*, 2014; Wang *et al.*, 2016) with wide ranges of stylet length from 10 to 120 µm and finely annulated cuticle which is rarely ornamented with rows of tubercles. Based on our current knowledge, the occurrence of pin nematodes in Kermanshah province is not known so far. In order to study the diversity of *Paratylenchus* spp. in Kermanshah province, we conducted several samplings in vineyards of the province during the summer of 2015. As a result, eight known species of the genus *i. e. P. arculatus*, *P. audriellus*, *P. humilis* Raski, 1975, *P. leptos*, *P. nanus* Cobb, 1923, *P. neoamblycephalus*, *P. prunii* Sharma, Sharma & Khan, 1986 and *P. straeleni* (De Coninck, 1931) Oostenbrink, 1960 were identified. Two species *P. humilis*, *P. prunii* and male of *P. straeleni* as new records from Iran are described here.

Materials and Methods

Several soil samples were collected from the rhizosphere of grapevine in various localities of

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Kermanshah province, western Iran. The nematodes were recovered from the soil samples using the rapid centrifugal-flotation method (Jenkins, 1964) and tray method (Whitehead and Hemming, 1965). Specimens observed under light microscope (LM) were heat-killed by adding hot 4% formaldehyde solution and processed to pure glycerin using De Grisse's (1969) method. Permanent slides were prepared and studied using a light Olympus BH-2 microscope. Measurements and drawings were made using a drawing tube attached to the same microscope. Photomicrographs of live nematodes were taken by a digital camera attached to a Nikon E200 (Japan). Species identification was done using the available references (Raski, 1975; 1991; Brzeski, 1998; Ghaderi *et al.*, 2014).

Results and Discussion

Eight known species of the genus *Paratylenchus* were collected and identified: *P. arculatus* Luc & Guiran, 1962, *P. audriellus* Brown, 1959, *P. humilis* Raski, 1975a, *P. leptos* Raski, 1975, *P. nanus* Cobb, 1923, *P. neoamblycephalus* Geraert, 1965, *P. pruni* Sharma, Sharma & Khan, 1986 and *P. straeleni* (De Coninck, 1931) Oostenbrink, 1960. Among them, two species with short stylet namely *P. humilis* and *P. prunii* are new records for Iran nematode fauna. Furthermore, male of *P. straeleni* is reported and illustrated for the first time.

Paratylenchus humilis Raski, 1975 (Figs 1 and 2; Table 1)

Female. Heat-relaxed body posture slightly curved ventrad, an open letter C. Cuticle annuli about 1.3-1.5 µm wide at mid-body. Lateral field with three incisures. Lip region truncate-conoid, slightly off set; small submedian lobes distinct in lateral view. Stylet moderately slender, conus distinctly larger than the shaft; stylet knobs well developed, directed laterally to slightly posteriorly. Dorsal gland orifice opens at 3-4 µm posterior to stylet knobs. Pharynx criconematoid, with pyriform basal bulb. Excretory pore usually at level of the basal bulb. Ovary outstretched; spermatheca spherical, with rounded sperm cells. . Lips of vulva slightly protruded. Vulval flaps present. Anus obscure. Vulva-anus distance slightly longer than the tail length. Tail conoid, narrows gradually, almost tapering to an acute terminus, sometimes slightly digitate.

Male. Similar to the female in general characteristics. Lip region truncate-conoid or slightly rounded, not off set. Stylet absent, pharynx degenerate. Cloacal lips projected, forming a penial tube. Spicules slightly arcuate ventrally. Tail narrows abruptly posterior to cloaca, almost acute terminus.

Remarks. *Paratylenchus humilis* has been documented as a synonym of *P. aquaticus* Merny, 1966 in some literature (Geraert & Ali, 1978; Raski & Luc, 1987; Ebsary, 1991; Siddiqi, 2000), but Raski (1975, 1991), Esser (1992), Brzeski (1995, 1998), van den Berg *et al*. (2014) and Ghaderi *et al*. (2014) have regarded it as a valid taxon. Brzeski (1995) stated that the c value of the male needs more study, moreover the male tail of *P. humilis* is shorter than that of *P. aquaticus* (c' = 1 *vs* 2.6-3.4). Also, bursa is present in the population of *P. aquaticus* collected from Mexico (Brzeski, 1995), but was absent in the illustration of *P. humilis* by Raski (1975). The male spicule length of *P. aquaticus* is given as 21-22 µm and 16-18 µm in the populations from Côte d' Ivoire (Merny, 1966) and Mexico (Brzeski, 1995), respectively, but this is 12-14 μm for *P. humilis* from Brazil (Raski, 1975). Considering stable differences in body length and position of excretory pore in females, as well as length of tail and spicules in males in different populations of *P. aquaticus* and *P. humilis*, Brzeski (1995) and van den Berg *et al.* (2014) regarded *P. humilis* as a valid species. Females of our population are completely fit with those of *P. humilis* (Raski, 1975). However, females in our population are smaller than *P. aquaticus* (183-225 *vs* 256-409 µm) and the distance of the excretory pore from the anterior end is much shorter (44-59 *vs* 58-80 µm). Also, males of the Iranian population have shorter

spicules (13-14 *vs* 16-22 µm) and longer tail (c' = 2.2 *vs* 1) than those of the reported populations of *P. aquaticus* (Merny, 1966; Brzeski, 1995). *Paratylenchus humilis* can be easily distinguished from the other closely related species, *P. leptos*, *P. perminimus* Siddiqi, 1996 and *P. thysanolus* Pramodini & Mohilal, 2009 by having female tail with acute terminus *vs* bluntly rounded terminus. This new record for Iran was collected from vineyards in Ghasr-e Shirin county, Kermanshah province, western Iran.

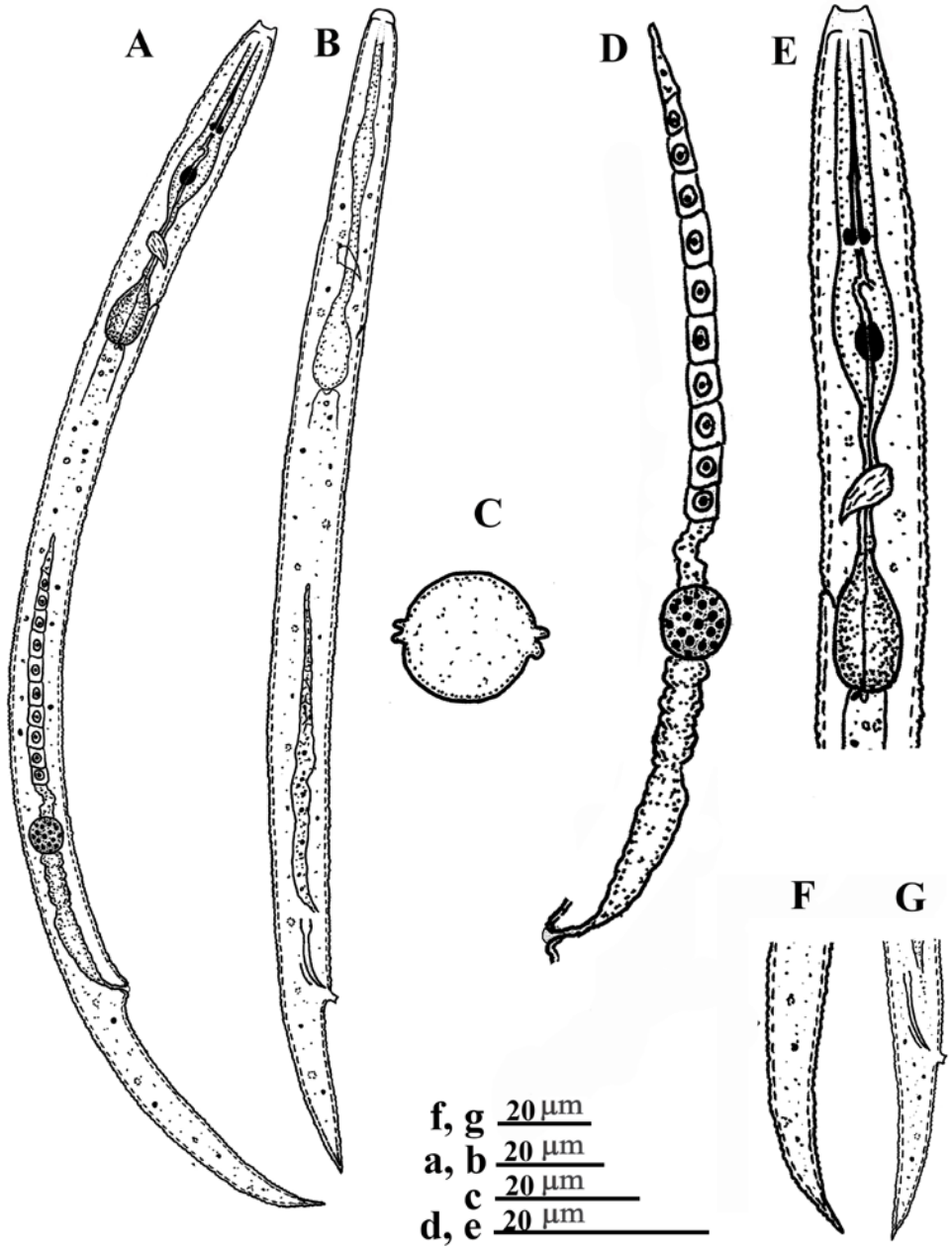

Figure 1 *Paratylenchus humilis*. A: Female entire body; B: Male entire body; C: Lateral field; D: Female reproductive system; E: Female pharyngeal region; F: Female posterior end; G: Male posterior end.

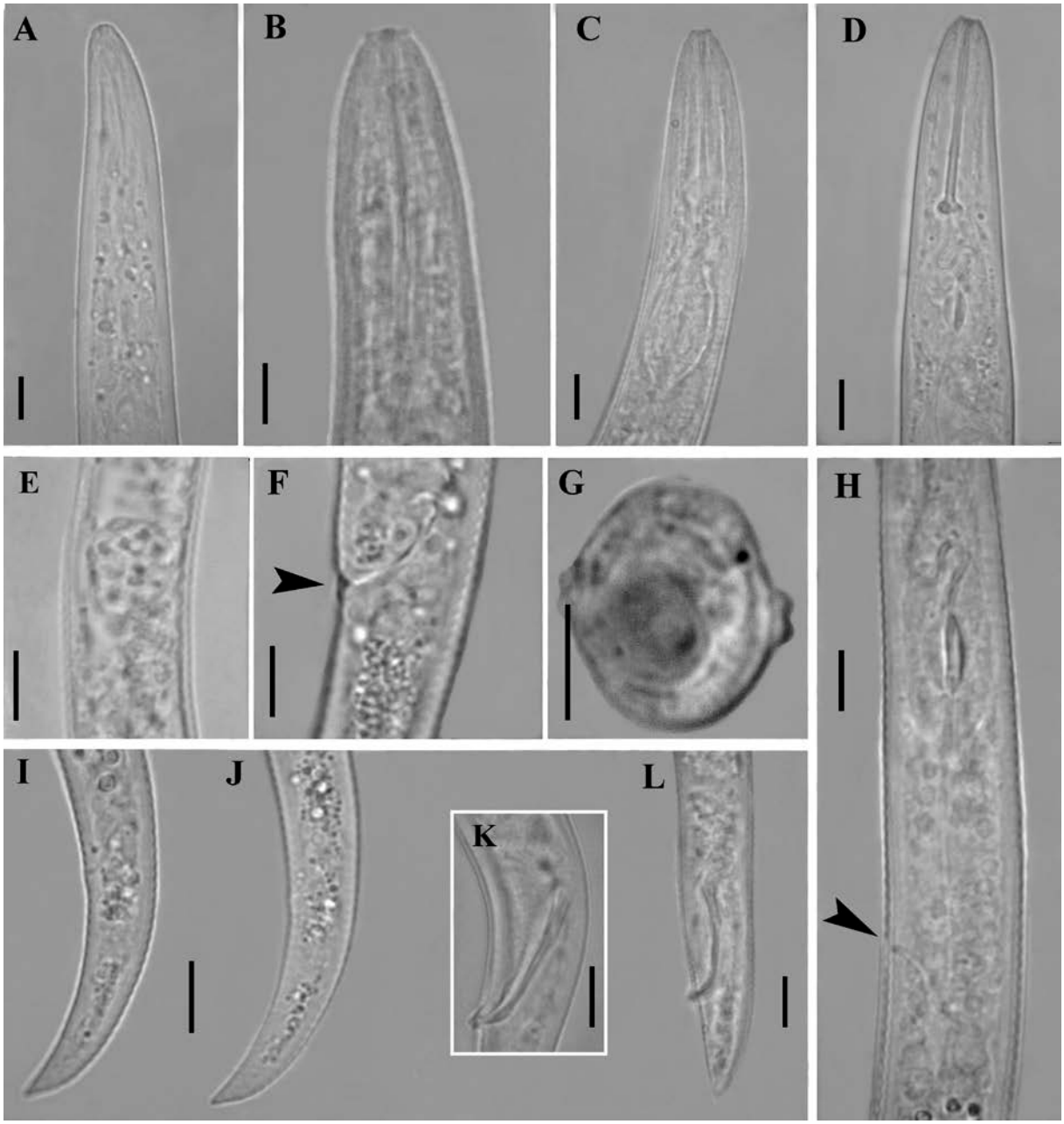

Figure 2 *Paratylenchus humilis*. A: Male pharyngeal region; B-D: Female anterior end; E: Spermatheca in detail; F: Vulva region showing vulval flap; G: Cross section of female; H: Female pharyngeal region and showing excretory pore; I, J: Female posterior end; K; Spicules in detail; L: Male posterior end. (All scale-bars 5 µm).

Table 1 Morphometric characters of *Paratylenchus humilis* collected from vineyards and its comparison with original description.

Character	Present study		Raski, 1975	
	10 ♀	2 ♂	6 ♀	14 ♂
L	204 ± 15.3 (183-225) 7.5	190,200	180 (170-190)	200 (180-210)
a	16.5 ± 1.5 (15.0-18.8) 9.0	15.4,15.8	18 (16-20)	24 (21-28)
b	3.1 ± 0.2 (3.0-3.3) 4.9	-	3.4 (3.2-3.5)	-
c	14.7 ± 1.8 (13.1-18.8) 12.2	10.6,13.3	17 (15-18)	26 (20-30)
c'	1.9 ± 0.2 (1.6-2.3) 11.1	2.0,2.5	-	-
V	81.9 ± 1.5 (80.0-85.0) 1.9	-	83 (81-84)	-
Stylet	17.4 ± 0.8 (16.0-19.0) 4.8	-	18 (16-19)	-
Conus	11.5 ± 1.0 (10.0-13.0) 8.5	-	-	-
m (conus/stylet %)	66.2 ± 5.7 (58.8-76.5) 8.7	-	-	-
Pharynx	65.9 ± 5.0 (60.0-75.0) 7.7	-	-	-
Excretory pore	49.5 ± 5.6 (44.0-59.0) 11.2	40,43	47 (43-50)	48 (41-45)
Head - Vulva	167.3 ± 11.3 (152-184) 6.7	-	-	-
Head - anus	190.3 ± 15.1 (169-213) 7.9	172,185	-	-
Tail length	14.0 ± 1.4 (12.0-17.0) 10.1	15,18	-	-
Body width	12.4 ± 0.8 (11.0-14.0) 6.8	12,13	-	-
S.E. / L (%)	24.2 ± 2.0 (20.5-26.2) 8.1	-	-	-
St / L (%)	8.6 ± 0.7 (8.0-10.4) 8.6	-	-	-
Spicules	-	13,14	-	13 (12-14)

Measurements are in µm.

***Paratylenchus prunii* Sharma, Sharma & Khan, 1986**

(Figs 3 and 4; Table 2)

Female. Heat-relaxed body posture ranging from slightly curved ventrad, an open letter C to a figure "6". Cuticle annuli about 1.0-1.2 µm wide at mid-body. Lateral field with four incisures. Lip region rounded (slightly truncate in some specimens), continuous with body contour, submedian lobes indistinct in lateral view. Stylet moderately slender, conus distinctly larger than the shaft; stylet knobs well developed, directed laterally to slightly posteriorly. Dorsal gland orifice opens at 3.0-4.5 µm posterior to stylet knobs. Pharynx criconematoid, with pyriform basal bulb. Excretory pore located in the region of basal pharyngeal bulb. Hemizonid present, situated in the region of excretory pore. Ovary outstretched; spermatheca rounded, with rounded sperm cells. Posterior uterus branch reduced, obscure. Lips of vulva not protruding. Vulval flaps present. Anus obscure. Vulva-anus distance slightly longer than the tail length. Tail ventrally arcuate with distinct striations ending with a bluntly rounded terminus.

Male. Similar to the female in general characteristics. Lip region truncate-conoid or slightly rounded, not off set. Stylet completely degenerate, pharynx barely distinguishable. Cloacal lips projected, forming a distinct penial tube. Spicules slightly arcuate ventrally. Tail conoid with rounded terminus.

Juvenile. One fourth-stage juvenile was found in its population. Similar to female. Stylet apparently absent. Tail tip more broadly rounded.

Remarks. This species originally has been described from soil around roots of peach trees (*Prunus persica* Batsch.) from Solan, India (Sharma *et al.*, 1986). All morphological and morphometric characters of our population conform closely to those given by Sharma *et al*. (1986). *Paratylenchus prunii* resembles *P. arculatus* Luc & de Guiran, 1962, *P. dianthus* Jenkins & Taylor, 1956 and *P. mexicanus* Raski, 1975. It is distinguished from *P. arculatus* by having lip region without submedian lobes *vs*

with prominent submedian lobes and also in absence of stylet in juveniles (J4) *vs* presence. It can be distinguished from *P. dianthus* by having a spherical *vs* oval spermatheca, as well as having shorter spicules (14-15 *vs* 18-29 µm). The other species, *P. mexicanus*, has an oval spermatheca and larger spicules (21 µm). This species was collected from vineyards in Gilan-e Gharb county, Kermanshah province, and is reported for the first time from Iran.

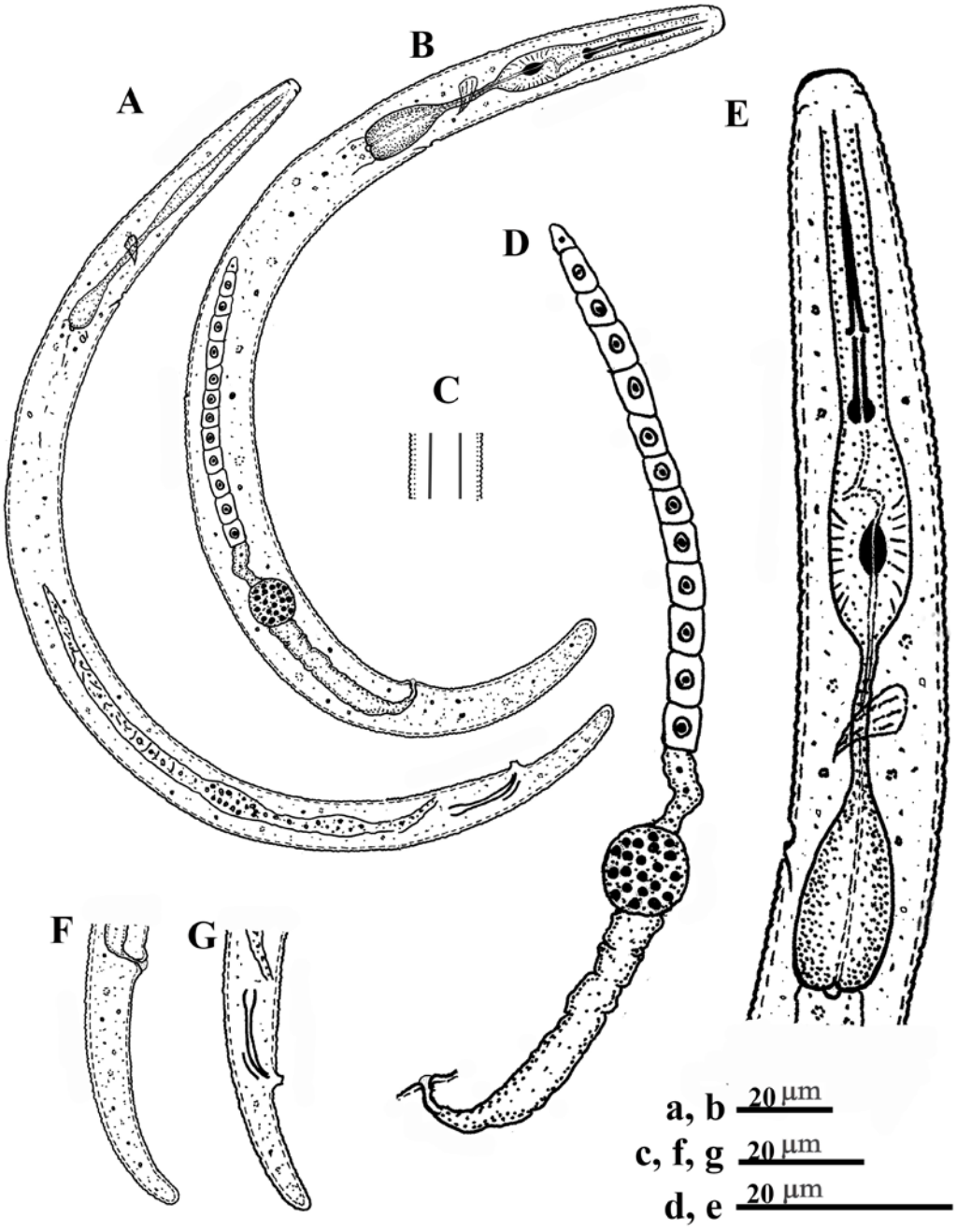

Figure 3 *Paratylenchus prunii*. A: Male entire body; B: Female entire body; C: Lateral field; D: Female reproductive system; E: Female pharyngeal region; F: Female posterior end; G: Male posterior end.

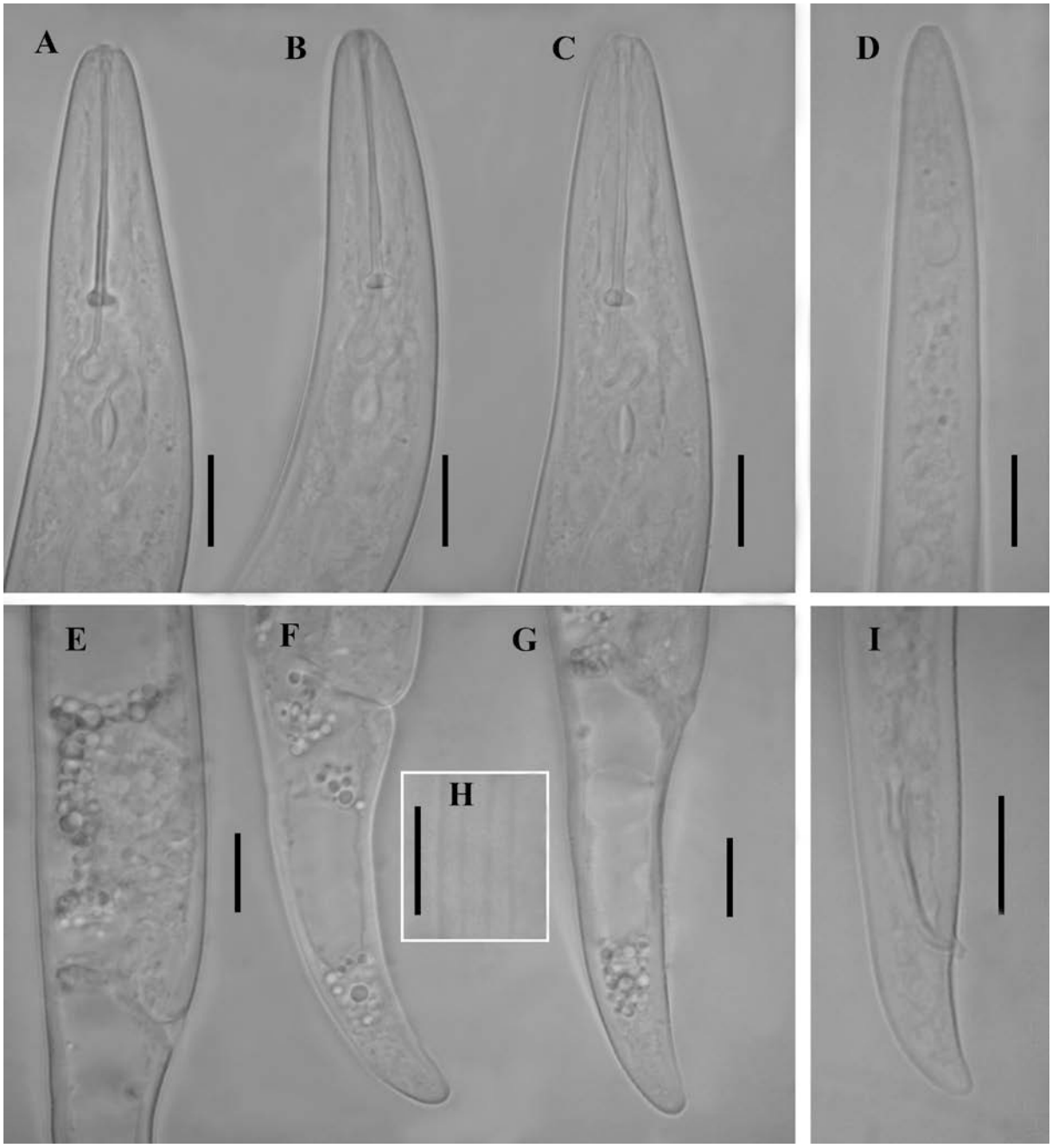

Figure 4 *Paratylenchus prunii*. A-C: Female anterior end; D: Male pharyngeal region; E; Vulva region; F, G: Female posterior end; H: Spicule in detail; I: Male posterior end. (All scale-bars 10 µm).

Table 2 Morphometric characters of *Paratylenchus prunii* collected from vineyards and comparison with other populations.

Character	Present study		Sharma, Sharma & Khan, 1986	
	10 ♀	2 ♂	50 ♀	5 ♂
L	324 ± 18 (290-343) 5.6	275,290	28 (230-320)	270 (250-310)
a	21.8 ± 0.9 (20.3-23.2) 4.2	19.3,21.2	20.3 (18.0-22.6)	26.9 (25.4-28.4)
b	3.1 ± 0.1 (3.0-3.5) 4.4	-	3.8 (3.5-4.4)	3.4 (2.9-4.1)
c	12.8 ± 2.0 (10.7-17.5) 15.8	12.6,13.8	12.3 (10.2-16.7)	13.5 (12.7-14.6)
V	81.0 ± 1.1 (78.7-82.5) 1.4	-	82.5 (79.2-84.1)	-
Stylet	26.1 ± 1.4 (24.0-28.0) 5.3	-	24.8 (22-28)	-
Conus	17.4 ± 1.3 (16.0-20.0) 7.8	-	-	-
m (conus / stylet %)	66.6 ± 2.6 (64.0-71.4) 3.9	-	-	-
Pharynx	99.7 ± 5.5 (93.0-110.0) 5.5	-	-	-
Excretory pore	84.8 ± 4.5 (79.0-90.0) 5.4	78,83	-	-
Head - Vulva	253 ± 13.2 (239-270) 5.2	-	-	-
Head - anus	288 ± 19 (263-316) 6.6	255,267	-	-
Tail length	24.9 ± 2.7 (19.0-27.0) 11	20,23	-	-
Body width	14.4 ± 0.5 (14.0-15.0) 3.6	13,15	-	-
S.E. / L (%)	27.1 ± 0.7 (25.8-28.4) 2.7	-	-	-
St / L (%)	8.3 ± 0.5 (8.0-9.5) 5.8	-	-	-
Spicules	-	14,15	-	15.2 (14-16)

Measurements are in µm.

***Paratylenchus straeleni* (De Coninck, 1931) Oostenbrink, 1960**
(Figs 5 and 6; Table 3)

Paratylenchus straeleni was described based on a population from the soil of moss of the sewage gutter in Belgium (De Coninck, 1931). Females of this species have already been reported and described from oak trees in Saravan forest at Gilan province, Iran (Ghaderi and Karegar, 2013), and from the rhizosphere of apple trees in Gilan-e Gharb, Kermanshah province (Esmaeili *et al*., 2015). Present population of this species was collected from the rhizosphere of grapevine in Gilan-e Gharb and fits well with other populations, but this population is bisexual. Males are similar to the females in general characteristics. Lip region truncate-conoid or slightly rounded, not off set. Stylet absent, pharynx more or less degenerated. Cloacal lips forming a distinct penial tube. Spicules slightly arcuate ventrally. Tail conoid, narrows abruptly posterior to cloaca, almost with acute terminus.

Remarks. *Paratylenchus straeleni* is close to *P. audriellus*. In 1965, Geraert discussed that *P. straeleni* is not so different from *P. audriellus* and noted that *P. audriellus* has a more sharply pointed tail and shorter stylet. Raski (1976) inspecting the paratypes of *P. audriellus*, stated that they are similar to several collections of *P. straeleni* and therefore, synonymized these two species. He also noted that the paratypes of *P. audriellus* have conoid, sharply-pointed tails in some females, but typical claw-like process on others. Most authors accepted and followed this synonymy (*e. g.* Brzeski, 1998; Brzeski & Háněl, 1999; Siddiqi, 2000; Andrássy, 2007; Ghaderi *et al*., 2014). In our previous work (Esmaeili *et al*., 2016), the Iranian population of *P. audriellus* was separated morphologically

and morphometrically from populations of *P. straeleni* (Ghaderi & Karegar, 2013; Van den Berg *et al*., 2014) in having a distinct claw-like tail process in all individuals *vs* tail tip not claw-shaped, and large *vs* short vulval flaps. Furthermore, the molecular characterisation of these two very similar nematode species, using the D2-D3 of 28S rRNA and the partial 18S rRNA gene sequences, revealed that *P. straeleni* species is clearly separated from *P. audriellus* and has been considered as a valid taxon (Esmaeili *et al*., 2016).

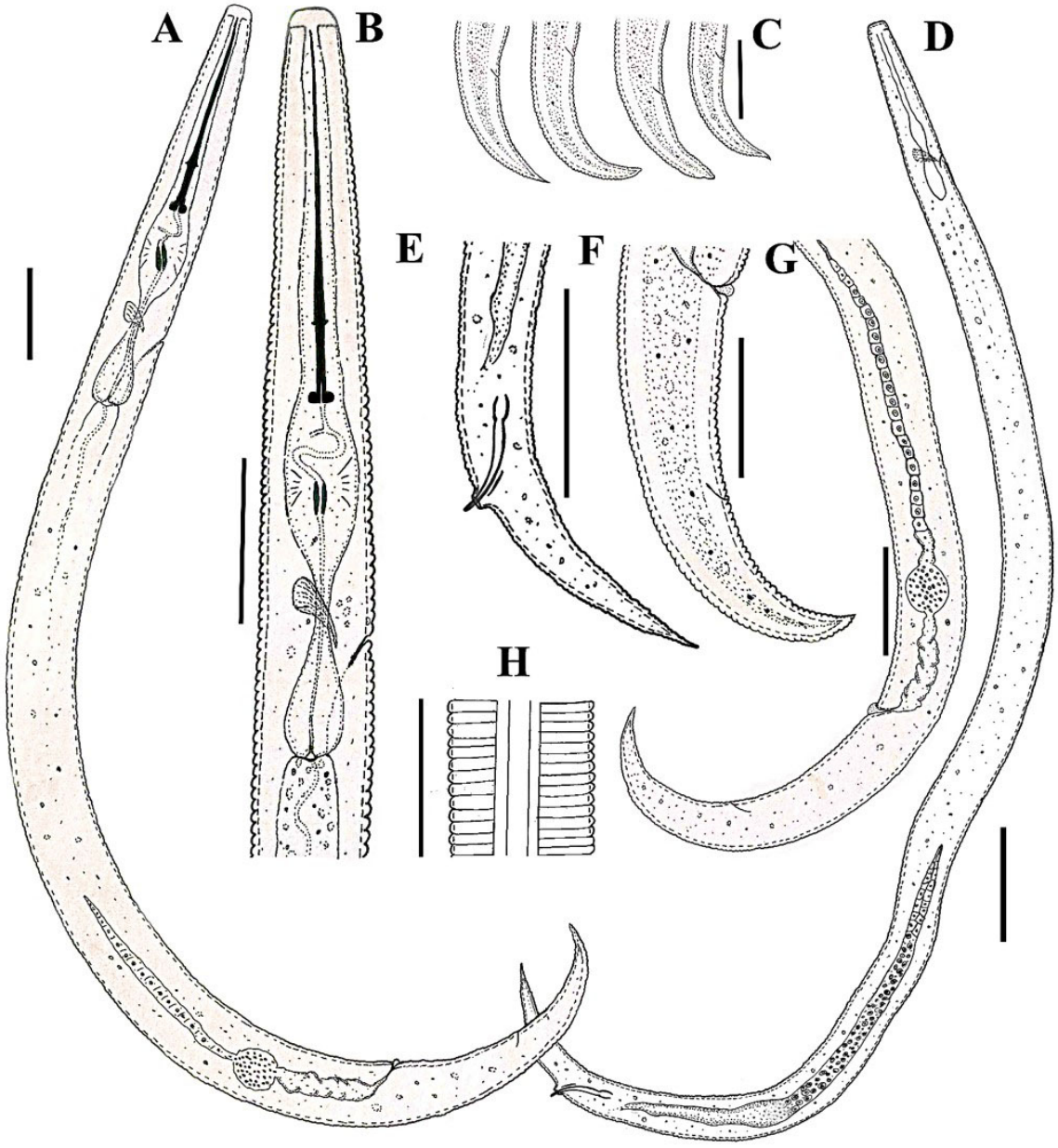

Figure 5 *Paratylenchus straeleni*. A: Female entire body; B: Female pharyngeal region; C: Female posterior end; D: Male entire body; E: Male posterior end; F: Female posterior end; G: Female reproductive system; H: Lateral field. (All scale-bars 25 µm, except D & E which are 30 µm).

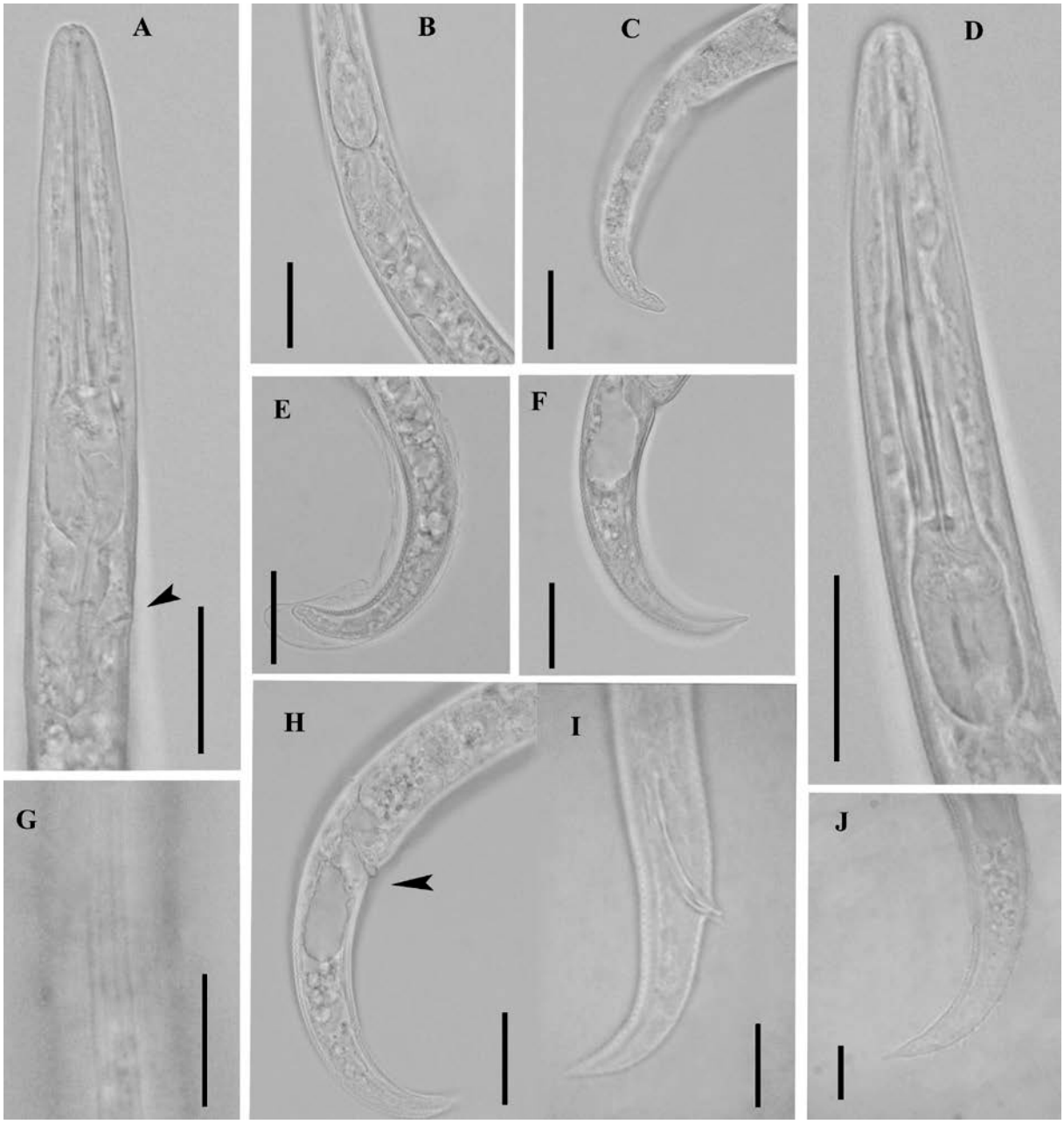

Figure 6 *Paratylenchus straeleni*. A: Female pharyngeal region and showing excretory pore; B: Female posterior part of pharyngeal region; C: Female posterior end; D: Female anterior end; E, F, H, J; Female posterior end; G: Lateral field; H; Vulva region and showing vulval flaps; I: Male posterior end. (All scale-bars 20 µm).

Table 3 Morphometric characters of *Paratylenchus straeleni* collected from vineyards and comparison with other populations.

Characters	Present study		Brzeski & Háněl, 1999		Ghaderi & Karegar, 2013	Esmaeili *et al*., 2015
	10 ♀	3 ♂	56 ♀	14 ♂	15 ♀	12 ♀
L	324 ± 32.1 (276-360) 9.9	253 ± 316.8 (243-273)	339 (284-386)	336 (307-371)	340 ± 18.6 (312-387)	325 ± 23.4 (280-365)
a	22.5 ± 1.7 (19.7-25.1) 7.5	18.7 ± 2.4 (16.2-21.0)	24 (20-27)	29.3 (27-32)	24.7 ± 1.1 (22.8-27.4)	22.5 ± 1.7 (20.0-25.1)
b	3.0 ± 0.2 (2.7-3.3) 6.2	-	3.4 (3.0-3.8)	3.7 (3.2-4.1)	3.2 ± 0.2 (2.9-3.7)	3.0 ± 0.2 (2.7-3.3)
c	11.1 ± 1.7 (8.7-14.4) 15	12.5 ± 1.9 (10.2-13.7)	13.1 (11-15)	12.2 (11-13)	13.0 ± 0.8 (10.8-13.9)	11.0 ± 1.5 (8.7-14.0)
V	82.1 ± 2.9 (78.3-88.0) 3.6	-	82 (80-84)	-	81.9 ± 0.6 (80.6-82.9)	81.6 ± 2.5 (78.3-85.7)
Stylet	55.2 ± 3.2 (52.0-60.0) 5.9	-	54 (48-58)	-	53.3 ± 2.2 (47.6-56.5)	54.8 ± 3.7 (50.0-60.0)
Conus	41.4 ± 2.9 (36.0-46.0) 6.9	-	42 (38-47)	-	40.8 ± 1.9 (34.5-43.4)	41.4 ± 2.6 (37.0-45.0)
m (%)	75.1 ± 2.6 (69.2-78.2) 3.4	-	78 (76-81)	-	76.4 ± 2.0 (72.5-79.8)	75.7 ± 2.1 (72.7-80.0)
Excretory pore	85.1 ± 3.2 (81.0-89.0) 3.7	7.7 ± 2.5 (75-80)	79 (69-92)	74 (64-81)	86.8 ± 4.5 (78.0-93.8)	84.9 ± 3.6 (80.0-90.0)
Pharynx	108 ± 6.3 (100-117) 5.8	-	101 (92-111)	-	106 ± 3.2 (102-114)	108 ± 6.8 (98-118)
Head-Vulva	265.5 ± 19.1 (243-288) 7.2	-	-	-	279 ± 14.8 (255-317)	265 ± 20.2 (240-289)
Body width	14.4 ± 0.6 (14.0-15.5) 3.9	13.7 ± 1.2 (13-15)	-	-	13.8 ± 1.0 (12.5-17.0)	14.4 ± 0.6 (14.0-15.5)
Vulva-Anus	34.2 ± 9.5 (20-46) 27.8	-	-	-	35.1 ± 3.3 (28.0-40.7)	33.9 ± 3.7 (24.8-43.0)
Tail	29.4 ± 2.7 (25.0-34.0) 9.2	20.7 ± 3.1 (18-24)	26 (20-31)	28 (24-30)	26.3 ± 2.6 (24.0-32.0)	29.8 ± 2.5 (26.0-34.0)
St/L (%)	17.1 ± 1.5 (14.8-19.9) 8.5	-	-	-	15.7 ± 0.8 (13.6-16.9)	16.9 ± 1.4 (14.8-19.6)
EP/L (%)	26.4 ± 1.8 (24.4-29.7) 6.9	29.6 ± 2.8 (26.9-32.5)	23 (21-27)	-	25.5 ± 1.1 (23.9-27.3)	26.2 ± 1.7 (24.2-29.3)
Spicules	-	19 ± 1.0 (18-20)	-	20 (19-21)	-	-

Measurements are in µm.

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چند گونه از جنس *Paratylenchus* Micoletzky, 1922 (Nematoda: Tylenchulidae) در تاکستان‌های استان کرمانشاه، غرب ایران

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چکیده: هشت گونه متعلق به جنس *Paratylenchus* از تاکستان‌های استان کرمانشاه، غرب ایران جمع‌آوری و شناسایی شد. توصیف کامل، داده‌های ریخت‌سنجی، ترسیم‌ها و عکس‌های میکروسکوپ نوری برای دو گونه *P. humilis* و *P. prunii* به‌عنوان گزارش جدید برای فون نماتدهای ایران ارایه شده است. گونه *P. humilis* داری سر هم‌طراز با بدن، اغلب مخروطی در جلو تخت و دارای برجستگی‌های کنار میانی ریز، استایلت کوتاه‌تر از ۴۰ میکرومتر، سه شیار طولی در سطوح جانبی بدن و پرده کوتیکولی مشخص اطراف فرج است. گونه *P. prunii* دارای سر هم‌طراز با بدن، اغلب گرد و فاقد برجستگی‌های کنار میانی ریز، استایلت کوتاه‌تر از ۴۰ میکرومتر، چهار شیار طولی در سطوح جانبی بدن و پرده کوتیکولی مشخص اطراف فرج است. نر نماتد *P. straeleni* نیز برای اولین بار از ایران گزارش می‌شود.

واژگان کلیدی: انگور، شناسایی، شکل‌شناسی، ریخت‌سنجی، اولین گزارش، نماتد سنجاقی