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Research Article

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

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Abstract: Three species of *Paratylenchus* were collected and identified from vineyards and apple orchards in Kermanshah province, western Iran. Descriptions, morphometric data, line drawings and microscopic photographs are provided for these three species. *Paratylenchus audriellus* and *Paratylenchus straeleni* are the two very closely related species, with a stylet longer than 40 µm, four lateral lines and distinct vulval flaps. The third species, *Paratylenchus leptos*, has a shorter stylet, three lateral lines and distinct vulval flaps. *Paratylenchus leptos* is a new record for Iranian nematode fauna, and male of *P. audriellus* is reported from Iran for the first time.

Keywords: Description, *Gracilacus*, morphology, new record, *Paratylenchus*, pin nematode

Introduction

The pin nematodes of the genus *Paratylenchus* Micoletzky, 1922 now include 117 nominal species (Ghaderi *et al.*, 2014) with wide ranges of stylet length from 10 to 120 µm and finely annulated cuticle which is rarely ornamented with rows of tubercles. However, some authors (Raski, 1962, 1991; Andrássy, 2007; Ganguly and Khan, 1990) assign species bearing longer than 40 µm stylet to the genus *Gracilacus* Raski, 1962 and the species having cuticular tubercles to the genus *Gracilpaurus* Ganguly & Khan, 1990. To date, 26 species of this genus have been reported from different plants and localities in Iran; several of them are illustrated with morphometrics and descriptions (Barooti, 1981; Karegar *et al.*, 1995; Chitamber *et al.*, 2001; Nouri *et al.*, 2006; Jahanshahi Afshar *et al.*, 2006; Gharakhani *et al.*, 2007; Kashi *et al.*, 2009; Van den Berg *et al.*, 2011; Baadl *et al.*, 2012; Ghaderi and Karegar, 2013; Ghaderi *et al.*, 2014; Bahmani *et al.*, 2013, but others with no morphological data. In the present paper, we provided descriptions, morphometric data, line drawings and microscopic photographs for three studied species of the genus.

Materials and Methods

Soil and root samples were collected from the rhizosphere of different crops in Kermanshah province, western Iran. The samples were extracted using the tray method (Whitehead and Hemming, 1965). Specimens were killed by adding boiling 4% formaldehyde solution and processed to anhydrous glycerin according to De Grisse (1969). Measurements and drawings were performed using a drawing tube attached to an Olympus BH-2 light microscope. Species were identified using available identification keys (*e.g.* Raski, 1991; Ghaderi *et al.*, 2014).

Results

In present paper, two species with long stylet namely *P. audriellus* Brown, 1959 and *P.*

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straeleni (De Coninck, 1931) Oostenbrink, 1960 and a species with shorter stylet in comparison with the two aforementioned species namely *P. leptos* Raski, 1975 were collected and identified. The latter species is a new record for Iran's nematode fauna and males of *P. audriellus* are reported from Iran for the first time.

Iranian population of *Paratylenchus audriellus* Brown, 1959
(Figs 1 and 2; Table 1)

Female. Body slightly ventrally curved to open C-shaped after heat relaxation; anterior half almost straight. Cuticle marked by distinct transverse striae about 0.8-1 µm wide near mid-body. Gravid females appear slightly swollen in pre-vulvar region, the maximum width being at spermatheca level. Lateral fields narrow, about one-seventh of the body width, not areolated, with four incisures, inner ones faintly marked. Lip region truncated; submedian lobes indistinct in lateral view. Stylet well-developed, almost straight, with laterally directed knobs; the conus about three fourth of the total stylet. The orifice of dorsal gland opens at 4-5 µm distance posterior to the stylet knobs. Pharynx criconematoid, with relatively short isthmus and pyriform basal bulb. The excretory pore at level with the posterior half of isthmus to anterior end of pharyngeal basal bulb. Pharyngo-intestinal valve indistinct. Vulva a broad transverse slit with conspicuous, large lateral flaps. Spermatheca rounded, with relatively large sperm. No visible post-vaginal tissue. Post-vulval region tapering gradually, anus distinct or vestigial, tail always ending to a characteristic claw-like process with sharply pointed tip.

Male. Similar to female in general appearance. Body ventrally arcuate. Pharynx completely degenerated with no trace of stylet. Spicules slightly arcuate ventrally, with small rounded head and pointed tip. Gubernaculum minute, linear, fixed. Bursa absent. The conoid-arcuate tail ending to a claw-like process similar to that of the female.

This species has originally been described from the rhizosphere of white birch (*Betula papyrifera* Marsh.) from Ontario, Canada (Brown, 1959). Morphological and morphometric characters of Iranian population completely fit with those given in the original description, except the males from Canada have a longer (307-360 *vs* 200-273 µm) and thinner (a = 25.1-30 *vs* 15.4-21.0) body. These differences may be related to males having more functional roles in some populations than others. The species has previously been reported from Iran, recovered from the rhizosphere of hazelnut trees in Gilan province, northern Iran (Barooti *et al.*, 2000), with no morphological data and the authors only noted that stylet length is 56-58 µm (eight females). In the present study, a bisexual population of *P. audriellus* was recovered from the soil samples collected around roots of grapevine in Ghasr-e-Shirin.

Iranian population of *Paratylenchus straeleni* (De Coninck, 1931) Oostenbrink, 1960
(Figs 3 and 4; Table 2)

Paratylenchus straeleni was described based on a population from moss and soil from Baraque Michel, Belgium (De Coninck, 1931). This species has already been reported and described from oak trees in Saravan forest at Gilan province, Iran (Ghaderi and Karegar, 2013). Presently recovered population of the species was collected from the rhizosphere of apple trees in Gilan-e-Gharb and its morphometrics fit well with the Saravan population, except for lacking of post-vaginal tissue (post-vaginal tissue observed in some individuals of Gilan population).

Iranian population of *Paratylenchus leptos* Raski, 1975
(Figs 5 and 6; Table 3)

Female. Body slightly arcuate to open C-shaped, anterior half almost straight after heat relaxation. Cuticle with distinct annuli, 1.5-1.8 µm in mid-body. Lateral field with three lines, 19-25% of corresponding body width. Lip region rounded, with truncate anterior end; small but distinct submedian lobes can be observed in lateral view. Stylet well-developed, with rounded basal knobs, slightly directed backward. The dorsal gland orifice, 4.5-6.5 µm posterior to stylet knobs. Pharynx criconematoid, with well-developed corpus, relatively slender isthmus and oval basal bulb, set off from intestine. Excretory pore at the

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level with anterior end of pharyngeal basal bulb. Ovary outstretched, spermatheca oval, containing spheroid sperm in all individuals. Vulva with distinct and relatively large lateral flaps. Post-vaginal tissue absent. Tail broadly conoid, usually ventrally curved, with rounded terminus, often bluntly digitate.

Male. Similar to female, but with smaller body size. Lateral fields with three incisures, about one-fifth of the body width. Lip region elevated and conoid, with truncate anterior end. Pharynx completely degenerated and stylet lacking. Spicules slightly arcuate ventrally, with small rounded head and pointed tip. Gubernaculum linear and simple. Bursa absent. Tail conoid, with finely to bluntly rounded-digitate terminus.

Raski (1975a) described *P. leptos* based on a population recovered from the rhizosphere of *Piper* sp. in Brazil. Presently studied population was collected from the rhizosphere of grapevine in Gilan-e-Gharb and is reported from Iran for the first time. Morphological and morphometric characters of the Iranian population fit well with those given in original description and the population from Ethiopia (Van den Berg *et al.*, 2004).

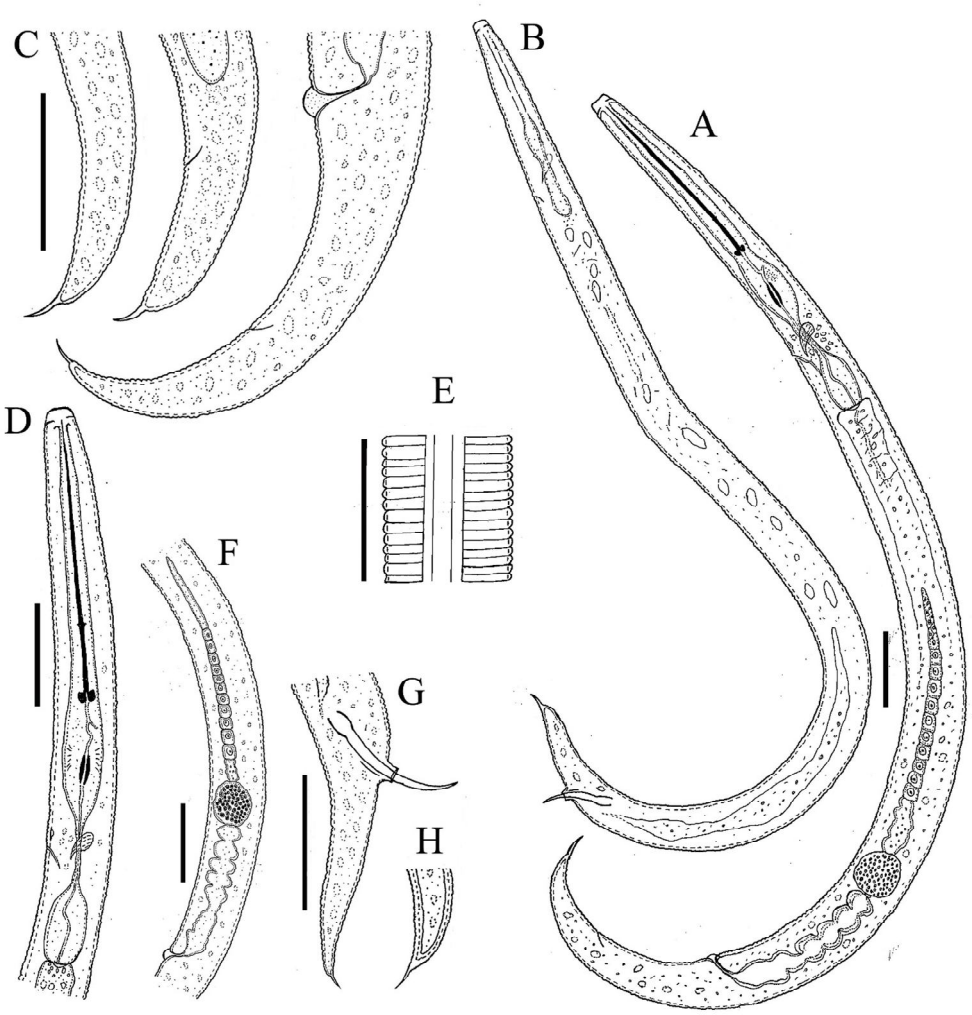

Figure 1 *Paratylenchus audriellus* from Kermanshah province, western Iran. A: Female entire body; B: Male entire body; C: Female posterior end; D: Female pharyngeal region; E: Lateral field; F: Total reproductive system of female; G: Male posterior end; H: Female tail terminus. (All scale-bars 20 µm).

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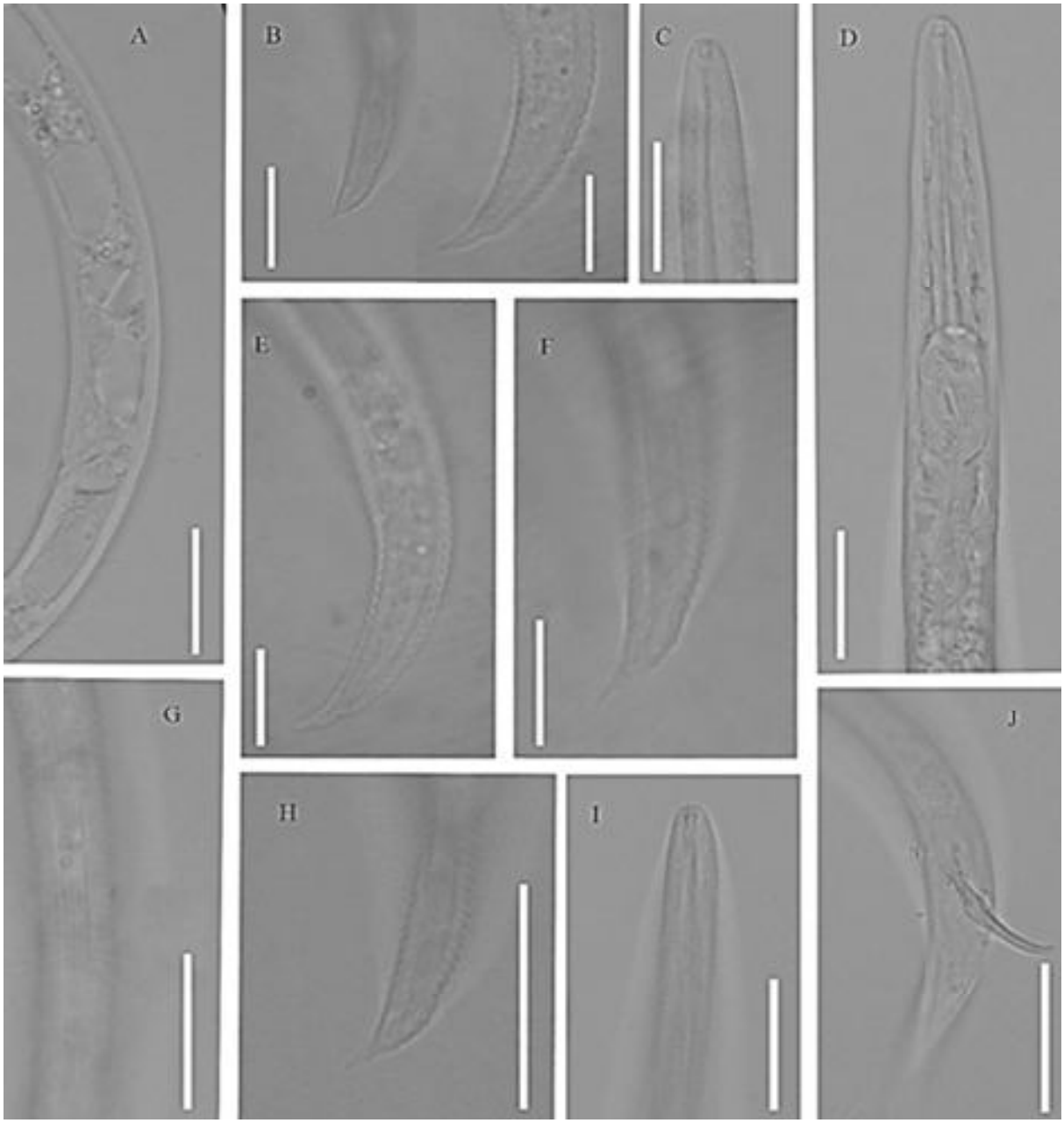

Figure 2 *Paratylenchus audriellus* from Kermanshah province, western Iran.A. Part of female reproductive system; B, H: Female tail terminus; C: Female head; D; Female pharyngeal region; E & F: Female posterior end; G: Lateral field; I: Male head; J: Male posterior end showing spicule. (All scale-bars 20 µm, except E & F which are 10 µm).

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Table 1 Morphometric data of *Paratylenchus audriellus* from Kermanshah province, western Iran and its comparison with original description (measurements are in µm).

Characters\Origin	Present study		Brown, 1959	
	Female	Male	Female	Male
n	12	3	15	15
L	323 ± 24.3 (278 - 363)	239 ± 36.7 (200 - 273)	304 - 381	307 - 360
a	21.9 ± 0.9 (20.3 - 23.2)	17.5 ± 3.0 (15.4 - 21.0)	17.6 - 22.9	25.1 - 30.0
b	3.0 ± 0.1 (2.8 - 3.3)	-	3.1 - 4.4	-
c	10.8 ± 1.2 (9.3 - 12.8)	11.8 ± 3.0 (8.3 - 13.8)	11.0 - 18.9	10.0 - 12.8
c'	4.1 ± 0.4 (3.3 - 4.7)	27.4 ± 2.7 (25.0 - 30.3)	-	-
V	81.4 ± 1.3 (80.0 - 84.2)	-	79.4 - 83.0	-
Stylet	55.2 ± 4.2 (51 - 61)	-	48.0 – 55.0	-
Conus	41.5 ± 2.7 (37 - 45)	-	-	-
m%	75.3 ± 2.5 (72.1 - 79.6)	-	-	-
Median bulb	70.4 ± 4.7 (62 - 77)	-	-	-
MB	66.3 ± 4.4 (57.5 - 75.5)	-	-	-
Excretory pore	86.2 ± 5.2 (72 - 93)	77.7 ± 2.5 (75 - 80)	-	-
Nerve ring	75.4 ± 4.6 (70 - 79)	72.3 ± 2.5 (70 - 75)	-	-
Pharynx	106 ± 8.1 (95 - 120)	-	-	-
Head - Vulva	261 ± 20.1 (239 - 299)	-	-	-
Body width	14.6 ± 0.7 (14 - 16)	13.7 ± 1.2 (13 - 15)	-	-
Vulval body width	12.8 ± 0.8 (12 - 14)	-	-	-
Vulva - Anus	29.8 ± 6.5 (18 - 40)	-	-	-
Anal body width	7.4 ± 0.5 (7 - 8)	8.7 ± 0.6 (8 - 9)	-	-
Tail	29.8 ± 1.9 (26 - 33)	20.7 ± 3.1 (18 - 24)	-	-
St / L%	17.3 ± 1.4 (15.2 - 17.9)	-	-	-
EP / L%	26.9 ± 1.0 (24.8 - 28.4)	33.0 ± 4.2 (29.3 - 37.5)	-	-
Spicules	-	19.7 ± 0.6 (19 - 20)	-	17.5 - 22.5
Gubernaculum	-	4.3 ± 0.6 (4 - 5)	-	-

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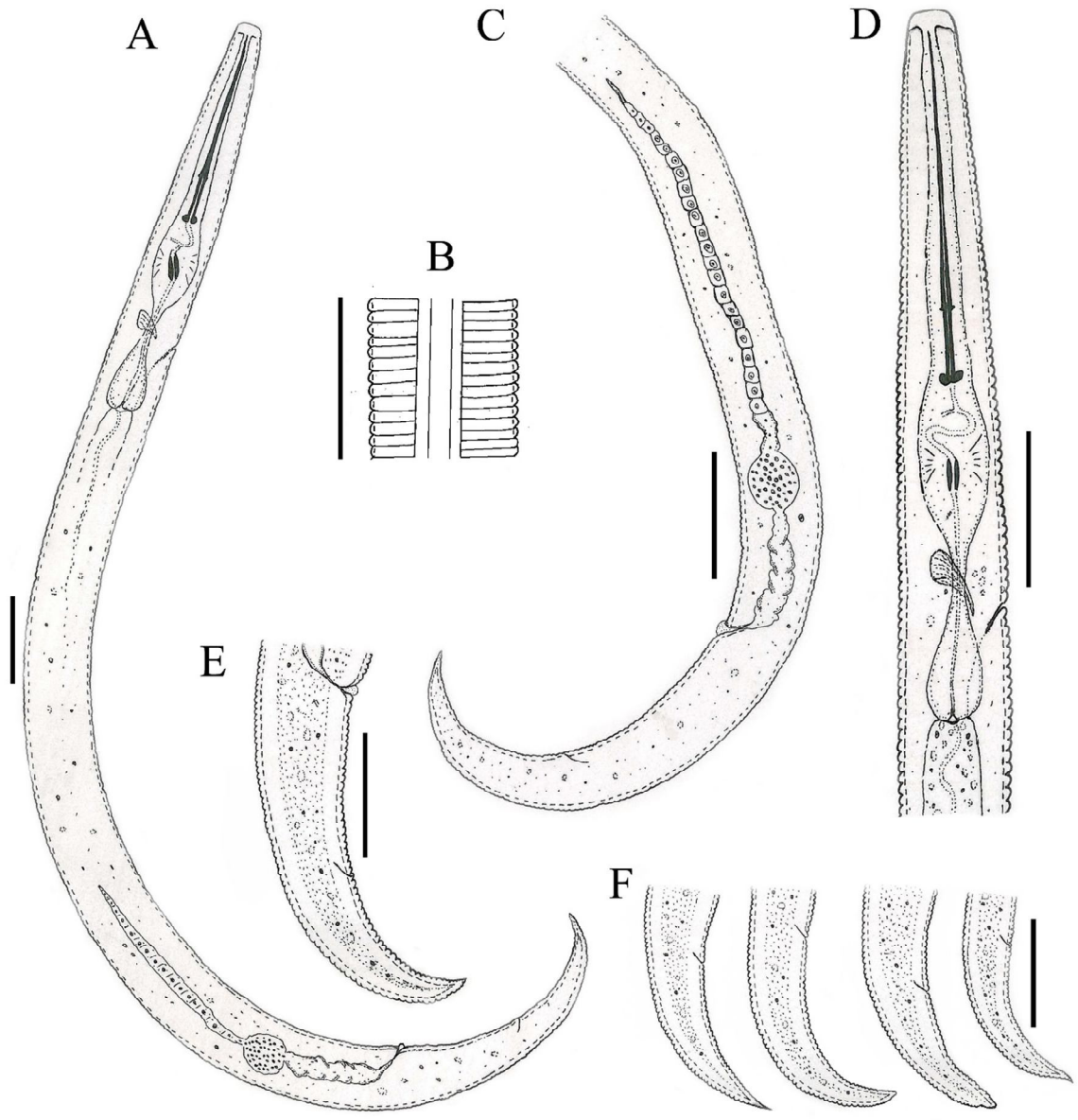

Figure 3 *Paratylenchus straeleni* from Kermanshah province, western Iran. A: Female entire body; B: Lateral field; C: Total reproductive system; D: Female anterior end; E: Vulva region and posterior end; F; Female tail showing variation in tail terminus. (All scale - bars 20 µm).

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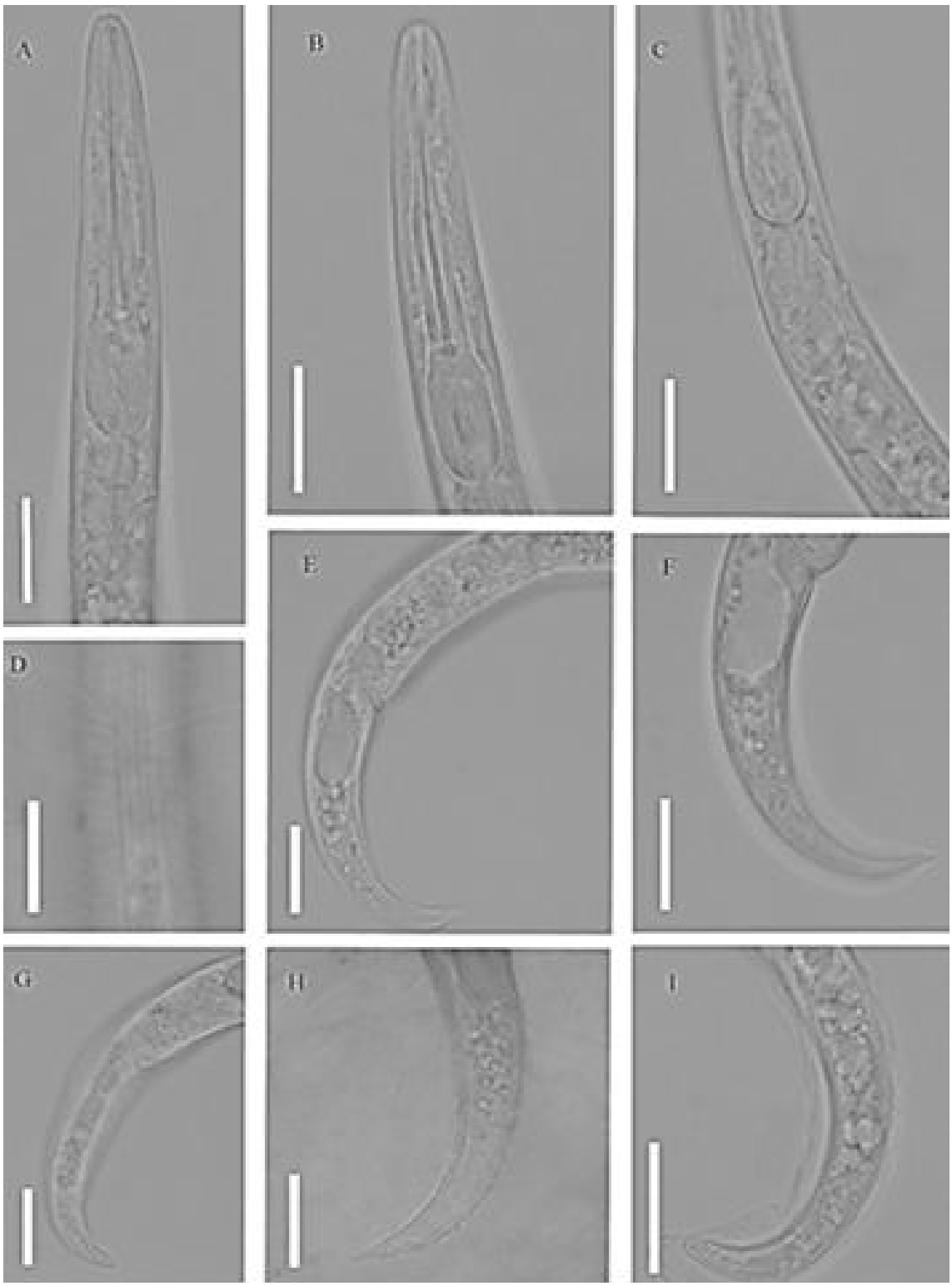

Figure 4 *Paratylenchus straeleni* from Kermanshah province, western Iran. A, B: Female anterior end; C: Female pharyngeal region; D: Lateral field; E; Vulva region; F - I: Female posterior end (All scale - bars 20 µm).

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Table 2 Morphometric data of females of *Paratylenchus straeleni* from Kermanshah province, western Iran and its comparison with other populations (measurements are in µm**).**

Characters\Origin	Present study	Ghaderi & Karegar, 2013	Brzeski & Hanel, 1999
n	12	15	56
L	325 ± 23.4 (280 - 365)	340 ± 18.6 (312 - 387)	339 (284 - 386)
a	22.5 ± 1.7 (20.0 - 25.1)	24.7 ± 1.1 (22.8 - 27.4)	24 (20 - 27)
b	3.0 ± 0.2 (2.7 - 3.3)	3.2 ± 0.2 (2.9 - 3.7)	3.4 (3.0 - 3.8)
c	11.0 ± 1.5 (8.7 - 14.0)	13.0 ± 0.8 (10.8 - 13.9)	13.1 (11 - 15)
c'	3.9 ± 0.3 (3.3 - 4.3)	3.3 ± 0.2 (3.1 - 3.5)	3.0 (2.5 - 3.5)
V	81.6 ± 2.5 (78.3 - 85.7)	81.9 ± 0.6 (80.6 - 82.9)	82 (80 - 84)
Stylet	54.8 ± 3.7 (50.0 - 60.0)	53.3 ± 2.2 (47.6 - 56.5)	54 (48 - 58)
Conus	41.4 ± 2.6 (37.0 - 45.0)	40.8 ± 1.9 (34.5 - 43.4)	42 (38 - 47)
m%	75.7 ± 2.1 (72.7 - 80.0)	76.4 ± 2.0 (72.5 - 79.8)	78 (76 - 81)
Median bulb	68.8 ± 3.8 (60.0 - 75.0)	-	-
MB	63.7 ± 4.3 (57.8 - 75.0)	-	-
Excretory pore	84.9 ± 3.6 (80.0 - 90.0)	86.8 ± 4.5 (78.0 - 93.8)	79 (69 - 92)
Nerve ring	77.3 ± 5.1 (70.0 - 84.0)	81.4 ± 2.8 (76.0 - 86.9)	-
Pharynx	108 ± 6.8 (98 - 118)	106 ± 3.2 (102 - 114)	101 (92 - 111)
Head - Vulva	265 ± 20.2 (240 - 289)	279 ± 14.8 (255 - 317)	-
Body width	14.4 ± 0.6 (14.0 - 15.5)	13.8 ± 1.0 (12.5 - 17.0)	-
Vulval body width	13.0 ± 0.7 (12.0 - 14.0)	-	-
Vulva - Anus	33.9 ± 3.7 (24.8 - 43.0)	35.1 ± 3.3 (28.0 - 40.7)	-
Anal body width	7.7 ± 0.4 (7.0 - 8.0)	-	-
Tail	29.8 ± 2.5 (26.0 - 34.0)	26.3 ± 2.6 (24.0 - 32.0)	26 (20 - 31)
St / L%	16.9 ± 1.4 (14.8 - 19.6)	15.7 ± 0.8 (13.6 - 16.9)	-
EP / L%	26.2 ± 1.7 (24.2 - 29.3)	25.5 ± 1.1 (23.9 - 27.3)	23 (21 - 27)

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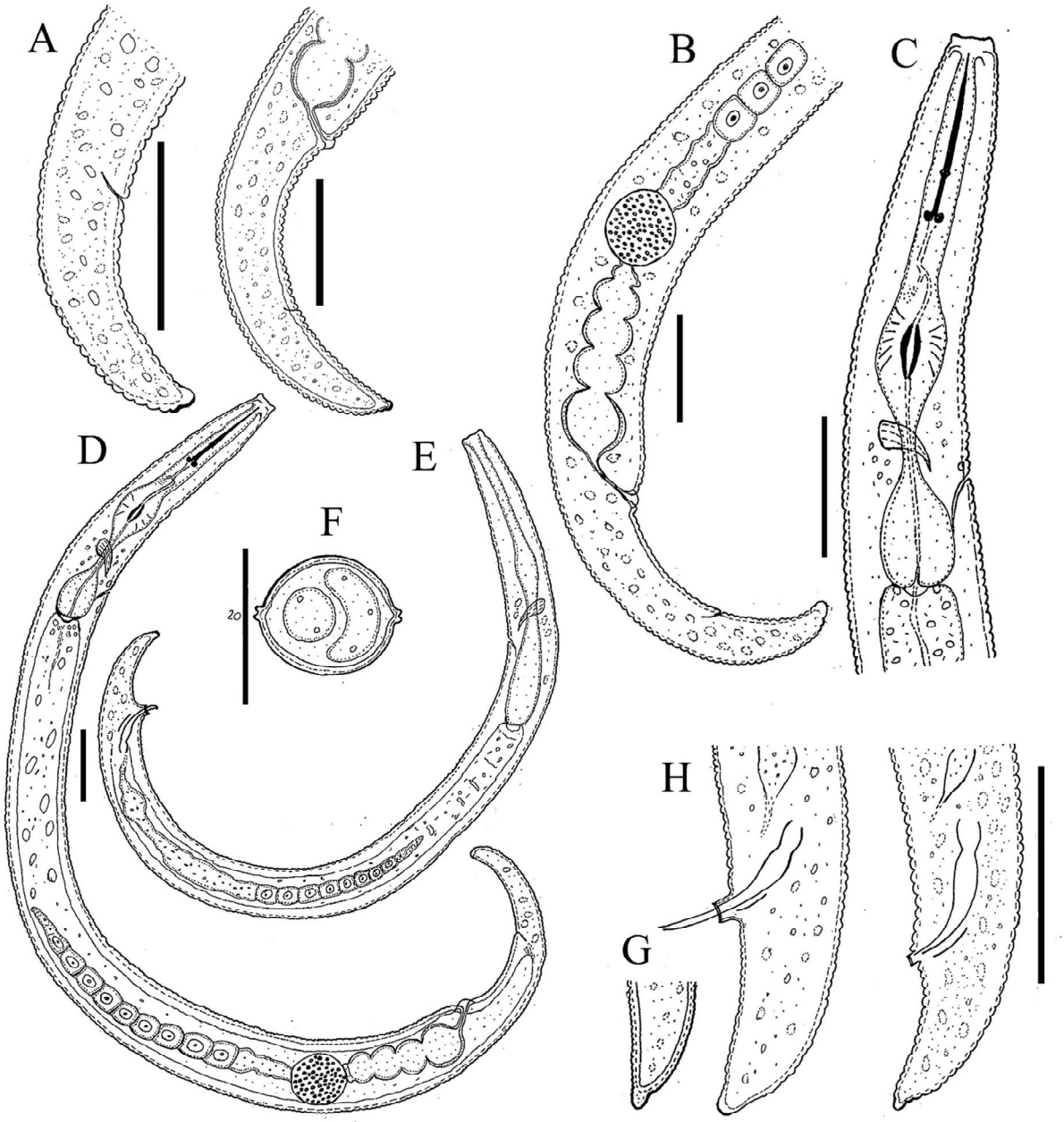

Figure 5 *Paratylenchus leptos* from Kermanshah province, western Iran. A: Female posterior end; B: Total reproductive system of female; C: Female pharyngeal region: D: Female entire body; E: Male entire body; F: Lateral field; G: Female tail terminus in detail; H: Male posterior end. (All scale - bars 20 µm).

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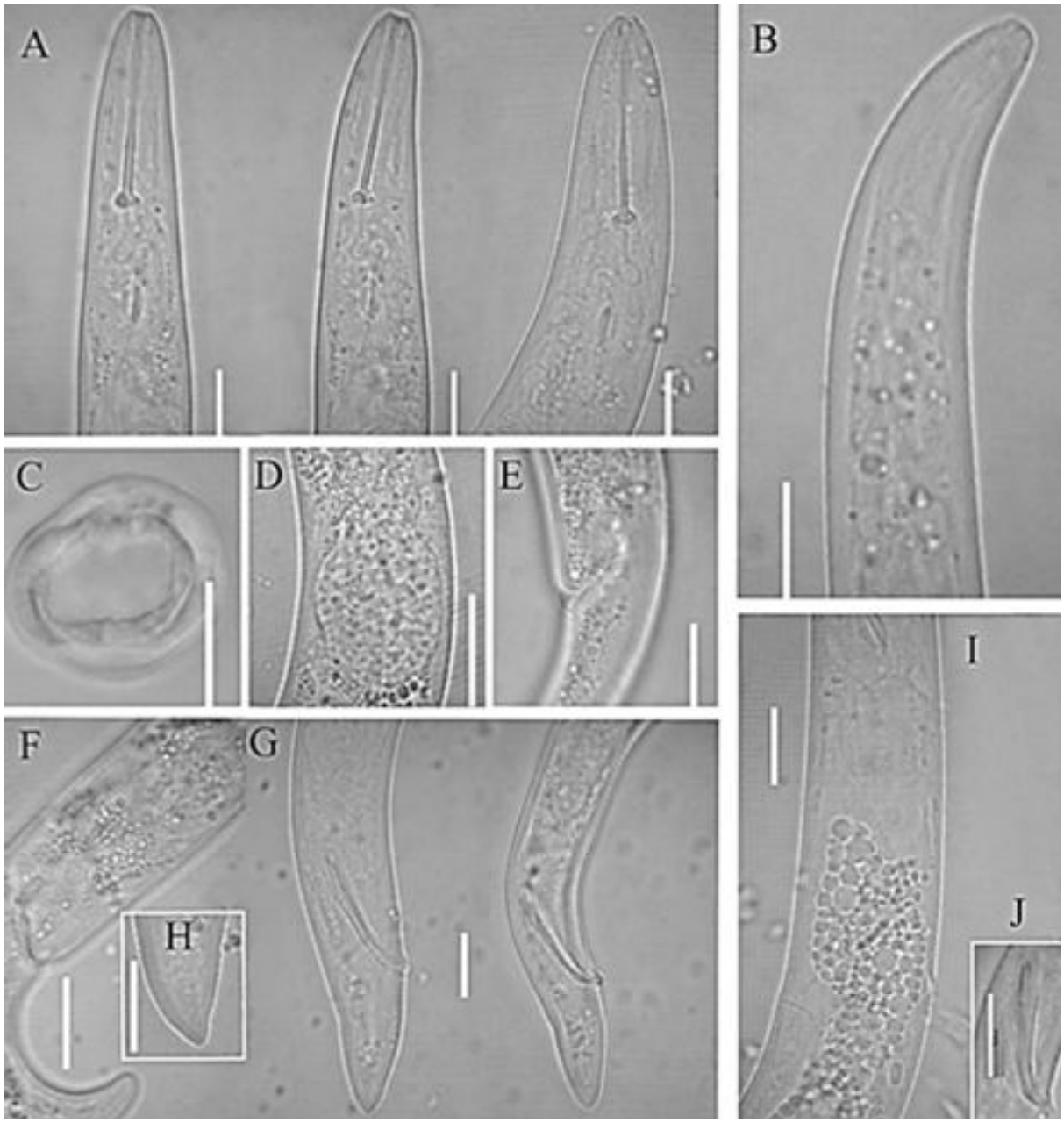

Figure 6 *Paratylenchus leptos* from Kermanshah province, western Iran. A: Female anterior end; B: Male pharyngeal region: C: Cross section of female; D: Spermatheca in detail; E: Vulva region; F: Female posterior end; H: Tail terminus in detail; G: Male posterior end; I: Female pharyngeal region; J: Spicule in detail. (All scale - bars 10 μm).

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Table 3 Morphometric data of *Paratylenchus leptos* from Kermanshah province, western Iran and its comparison with other populations (measurements are in µm).

Characters\Origin	Present study		Raski, 1975	Van den Berg *et al.*, 2004	
	Female	Male	Female	Female	Male
n	10	3	8	15	2
L	204 ± 15.8 (180 - 225)	206 ± 14..9 (195 - 223)	220 (200 - 260)	223 (204 - 260)	197, 207
a	14.8 ± 1.4 (12.8 - 16.9)	14.8 ± 2.1 (13.0 - 17.2)	17.0 (15 - 22)	20.0 (15 - 24)	24, 25
b	2.6 ± 0.3 (2.2 - 3.1)	-	3.2 (3.0 - 3.5)	3.5 (3.3 - 4.3)	4.3
c	15.6 ± 2.2 (12.5 - 18.8)	11.8 ± 1.4 (10.8 - 13.3)	-	17.5 (15 - 19)	16, 15
c'	1.8 ± 0.3 (1.5 - 2.3)	1.9 ± 0.3 (1.7 - 2.2)	-	2.1 (1.8 - 2.6)	1.7, 1.9
V	77.7 ± 2.0 (76.0 - 82.5)	-	82 (77 - 84)	82 (80 - 85)	-
Stylet	20.4 ± 1.8 (18 - 23)	-	22 (20 - 23)	22.5 (20 - 26.5)	-
Conus	13.6 ± 1.6 (12 - 17)	-	-	15 (13 - 18)	-
m%	66.6 ± 2.8 (65.0 - 73.9)	-	-	-	-
Median bulb	51.4 ± 4.5 (45 - 57)	-	-	-	-
MB	65.5 ± 6.2 (54.9 - 76.0)	-	-	-	-
Excretory pore	61.4 ± 2.6 (58 - 65)	61.0 ± 3.6 (58 - 65)	55 (51 - 57)	54 (51 - 61)	46, 50
Nerve ring	55.7 ± 3.8 (53 - 60)	72.3 ± 2.5 (70 - 75)	-	-	-
Pharynx	78.5 ± 2.3 (75 - 82)	-	-	64 (60 - 68)	46, 48
Head - Vulva	158 ± 11.8 (141 - 176)	-	-	-	-
Body width	13.9 ± 0.8 (13 - 15)	14.0 ± 1.0 (13 - 15)	-	12 (9 - 14)	8
Vulval body width	11.8 ± 0.6 (11 - 13)	-	-	-	-
Vulva - Anus	32.2 ± 6.0 (22 - 41)	-	-	-	-
Anal body width	7.4 ± 0.5 (7 - 8)	9.3 ± 0.6 (9 - 10)	-	-	-
Tail	13.3 ± 2.1 (11 - 17)	17.7 ± 2.5 (15 - 20)	-	13 (11 - 16)	12,14
St / L%	10.0 ± 1.1 (8.0 - 11.5)	-	-	-	-
EP / L%	30.3 ± 3.0 (26.2 - 36.1)	29.6 ± 2.8 (26.9 - 32.5)	-	24 (21 - 26)	23
Spicules	-	17.0 ± 1.0 (16 - 18)	-	-	14
Gubernaculum	-	5.0 ±.0 1 (4 - 6)	-	-	3

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Discussion

If we accept validity of the genus *Gracilacus* as regarded by Raski (1962, 1991) and Andrássy (2007), two of our recovered species with stylet longer than 40 µm, i.e. *P. audriellus* and *P. straeleni*, fall into the genus *Gracilacus*, but *P. leptos* still remains under the genus *Paratylenchus*. Comparison of recovered populations with other populations of the above mentioned species are presented in Tables 1 - 3.

Brown (1959) pointed out that presence of a distinct claw - like process on tail tip of both male and female distinguishes *P. audriellus* from other species of *Paratylenchus*. Geraert (1965) believed 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 and Hanel, 1999; Siddiqi, 2000; Andrássy, 2007; Ghaderi *et al.*, 2014). In present study, two populations tentatively assigned to two species *P. audriellus* and *P. straeleni* recovered from from Ghasr-e-Shirin and Gilan-e-Gharb were compared. The assigned population to *P. audriellus* was different from populations of *P. straeleni* from Iran (Ghaderi and Karegar, 2013; present study) and USA (Van den Berg *et al.*, 2014) in having a distinct claw-like process in all individuals, as well as in having larger vulval flaps. However, the importance and usefulness of these traits as diagnostic characters for species delimitation must be determined with more detailed morphological and/or molecular studies using several populations of both species from different geographic locations. Therefore, present study fails to make a final decision on the validity or synonymy of the *P. audriellus*, but morphological data and drawings are presented separately for the two presently recovered populations, allowing readers to make a more accurate comparison. Two species *Paratylenchus goodeyi* Oostenbrink, 1953 and *Paratylenchus ivorensis* Luc & de Guiran, 1962 are most closely related species to *P. straeleni sensu lato*, but could be distinguished from it by having rounded lip region and more anterior position of vulva, respectively.

The latter species, *P. leptos* is the first representative of the genus from Iran having a stylet shorter than 40 µm in length and three incisures in lateral field. It can be distinguished from four closely related species namely *Paratylenchus perminimus* Siddiqi, 1996, *Paratylenchus thysanolus* Pramodini & Mohilal, 2009, *Paratylenchus humilis* Raski, 1975b and *Paratylenchus aquaticus* Merny, 1966 by having a more bluntly rounded and digitate tail (*vs* non-digitate). It also differed from *P. perminimus* by having longer spicules in males (16-18 *vs* 12 µm), and from *P. thysanolus* by having longer stylet in females (18-23 *vs* 17-19 µm) and different shape of tail terminus in males (bluntly rounded *vs* acute).

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معرفی سه گونه از جنس *Paratylenchus* (Nematoda: Tylenchulidae) در استان کرمانشاه، غرب ایران

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چکیده: سه گونه از جنس *Paratylenchus* از تاکستان‌ها و باغ‌های سیب استان کرمانشاه در غرب ایران جمع‌آوری و مورد شناسایی قرار گرفت. توصیف کامل، داده‌های ریخت‌سنجی، ترسیم‌ها و عکس‌های میکرسکوپ نوری برای این سه گونه ارائه شده است. گونه‌های *P. audriellus* و *P. straeleni* دو گونه بسیار نزدیک به هم بوده و دارای استایلت بلندتر از ۴۰ میکرومتر، چهار شیار جانبی و پرده کوتیکولی مشخص اطراف فرج هستند. گونه دیگر، *P. leptos*، دارای استایلت کوتاه‌تر، سه شیار جانبی و پرده کوتیکولی اطراف فرج است. گونه *P. leptos* و جنس نر گونه *P. audriellus* برای اولین بار از ایران گزارش می‌شود.

واژگان کلیدی: توصیف کامل، *Gracilacus*، ریخت‌شناسی، گزارش جدید، *Paratylenchus*، نماتد سنجاقی

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