

Research Article

Three nematode species of the infraorder Tylenchomorpha (Nematoda: Tylenchina) from Iran

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Abstract: During a nematode survey and identification of plant-parasitic nematodes in Iran, several species belonging to the infraorder Tylenchomorpha were collected and identified from different localities of West Azerbaijan, Kermanshah and Golestan provinces. Among which three species, namely *Aphelenchoides xui* (Aphelenchoididae), *Paratylenchus recisus* (Tylenchulidae) and *Tylenchus skarduensis* (Tylenchidae) are new records for Iran nematode fauna. Description, measurements, line drawings and microscopic photographs are provided for these new records.

Keywords: morphology, morphometric, new record, nematode

Introduction

The genus *Aphelenchoides* Fischer, 1984 is the type genus of the family Aphelenchoididae Skarbilovich 1947 and includes more than 153 nominal species (Hunt, 2008; Kanzaki and Giblin-Davis, 2012). Although most species of the genus are fungivores (Kanzaki and Giblin-Davis, 2012), thirteen species have been reported as plant-parasitic in a wide variety of plants (Sánchez-Monge *et al.*, 2015). Up to now, thirty-seven *Aphelenchoides* species have been reported from Iran including seven new species (Adeldoost *et al.*, 2017; Esmaili *et al.*, 2016 a; b, 2017 a; b; c; Ghaderi *et al.*, 2012; Golhasan *et al.*, 2016 a, 2017; Miraeiz *et al.*, 2017).

The pin nematodes of the genus *Paratylenchus* Micoletzky 1922, belonging to the family Tylenchulidae Skarbilovich 1947 includes 118 nominal species (Ghaderi *et al.*, 2014; Wang *et al.*, 2016). Raski (1962) proposed the genus *Gracilacus* to include species of

Paratylenchus, with a stylet longer than 48 μ m. However, some nematologists have documented all species under *Paratylenchus* (Brzeski, 1998; Nguyen *et al.*, 2004; Decraemer and Hunt, 2006; Ghaderi *et al.*, 2014). Up to now, seventeen species of this genus have been reported from Iran (Gharakhani *et al.*, 2007; Ghaderi *et al.*, 2014; Esmaili *et al.*, 2015; Esmaili *et al.*, 2016c; Esmaili and Heydari, 2017).

Bastian (1865) proposed the genus *Tylenchus* with *T. davainei* as the type species. Till now, eight species of this genus have been reported from different regions of Iran (Mirbabaei Karani *et al.*, 2015; Golhasan *et al.*, 2016b).

In the present paper we report the presence of three species of mentioned genera in Iran, with detail morphologic and morphometric data, for the first time.

Materials and Methods

Eighty three bark, soil and root samples suspected of nematode infestation were collected from the different locations in West Azerbaijan, Kermanshah and Golestan provinces during 2014-2017. The samples were extracted using the tray method (Whitehead and Hemming, 1965). The

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specimens were killed by adding boiling 4% formaldehyde solution, and then processed to anhydrous glycerin (De Grisse, 1969). Permanent slides were prepared and studied by a light microscope (Nikon E200). Drawings were made using a drawing tube attached to this microscope. Photographs were taken by a digital camera attached to the microscope. Species identifications were done using the updated keys (Brzeski, 1998; Ghaderi *et al.*, 2014; Shahina 1996; Geraert, 2008) and original descriptions.

Results

About 30 known nematode species belonging to the infraorder Tylenchomorpha were identified. Among them three species, namely *Aphelenchoides xui* Wang, Wang, Gu, Wang, and Li 2013, *Paratylenchus recisus* Siddiqi 1996 and *Tylenchus skarduensis* Maqbool and Shahina 1987 are new records for Iran fauna.

Aphelenchoides xui Wang, Wang, Gu, Wang *et al.* 2013

(Figures 1 and 2; Table 1)

Female. Body slightly ventrally arcuate when heat-relaxed. Cuticle weakly annulated. Lateral field occupying three to four μm width at mid-body, with four incisures at vulval region (*i.e.*, three ridges), not areolated. Lip region rounded, offset, *ca* 2.1 μm high and 4.3 μm broad. Stylet slender with small basal swellings, conus occupying *ca* 41-46% of its total length. Procorpus cylindrical. Metacarpus rounded to oval with conspicuous valve plates situated centrally. Dorsal pharyngeal gland orifice opening into lumen of metacarpus midway between anterior end of metacarpal valve and anterior end of metacarpus. Nerve ring is situated at *ca* half stylet length posterior to metacarpus. Excretory pore located at 10-16 μm posterior to the base of metacarpus. Hemizonid invisible. Pharyngo-intestinal junction immediately posterior to metacarpus. Pharyngeal gland lobe is slender, *ca* three to four body diam. long, overlapping intestine dorsally. Reproductive tract is monodelphic-prodelphic. Oocytes are arranged in a single row even in the germinal zone; Spermatheca present, 2.5-3 body diam, filled with disc-like or

oblong sperm cells in a single row. Vagina oblique, wall not sclerotized. Vulva a simple slit in ventral view, without any vulval flap apparatus in lateral view. Post-vulval uterine sac (PUS) well developed *ca* 3.4 (2.6-3.8) vulva body diam. long, extending for *ca* 42 (30-51)% of vulva-anus distance, often containing sperm cells. Rectum and anus clearly visible. Tail is conical, terminating in a complicated step-like projection, usually with many tiny nodular protuberances.

Male: Body slender, cylindrical, and J-shaped when heat relaxed. Anterior region and cuticle are similar to female. Testis is single, anteriorly outstretched, locating left of intestine, spermatocytes in a single column. The Spicule is typically aphelenchoid, arcuate, apex and rostrum rounded, well developed, end of dorsal limb clearly curved ventrally. Bursa and gubernaculum were absent. Tail conical, bearing a short sharp mucro *ca* 2 μm long. Three pairs of sub-ventral caudal papillae present: first pair located just posterior to cloacal aperture, second pair in mid-tail region and third pair just anterior to tail end.

Remarks

According to grouping of *Aphelenchoides* species *sensu* Shahina (1996), *Aphelenchoides xui* is within Group 2, in this group “tail with one or sometimes two mucronate structures”. Dimensions and morphological characters of the present specimens conform to the descriptions of those given by Wang *et al.* (2013) from packaging wood of *Pinus* sp. in South Africa. Iranian population of *Aphelenchoides xui* is closest to *A. arcticus* Sanwal, 1965, *A. haguei* Maslen, 1979 and *A. fuchsi* Esmaeili *et al.* (2016b). It differs from *A. arcticus* by the offset vs non-offset lip region, female tail terminus (ending of a step-like projection usually with many tiny nodular protuberances vs a shallow constriction narrowed sharply with a very fine mucro at the tip) and male spicule shape (dorsal limb with a hook-like tip vs smoothly rounded tip). It differs from *A. haguei* by the vagina angled obliquely forward vs at 90° to the body axis, longer spicule (dorsal limb = 30-32 μm vs 16-25 μm) and shape of tail terminus. It also differs from *A. fuchsi* by having longer stylet (12-13 μm vs 8-10 μm) and the male

spicule length in dorsal limb (30-32 vs 15-16 μ m). The present specimens were recovered from bark and wood samples of a pine tree (*Pinus eldarica*) in Naqadeh, West Azerbaijan province,

northwestern Iran. The present study expands the morphological and morphometric characters of the species, and is the second report of *A. xui* after Wang Gu Wang *et al.* (2013).

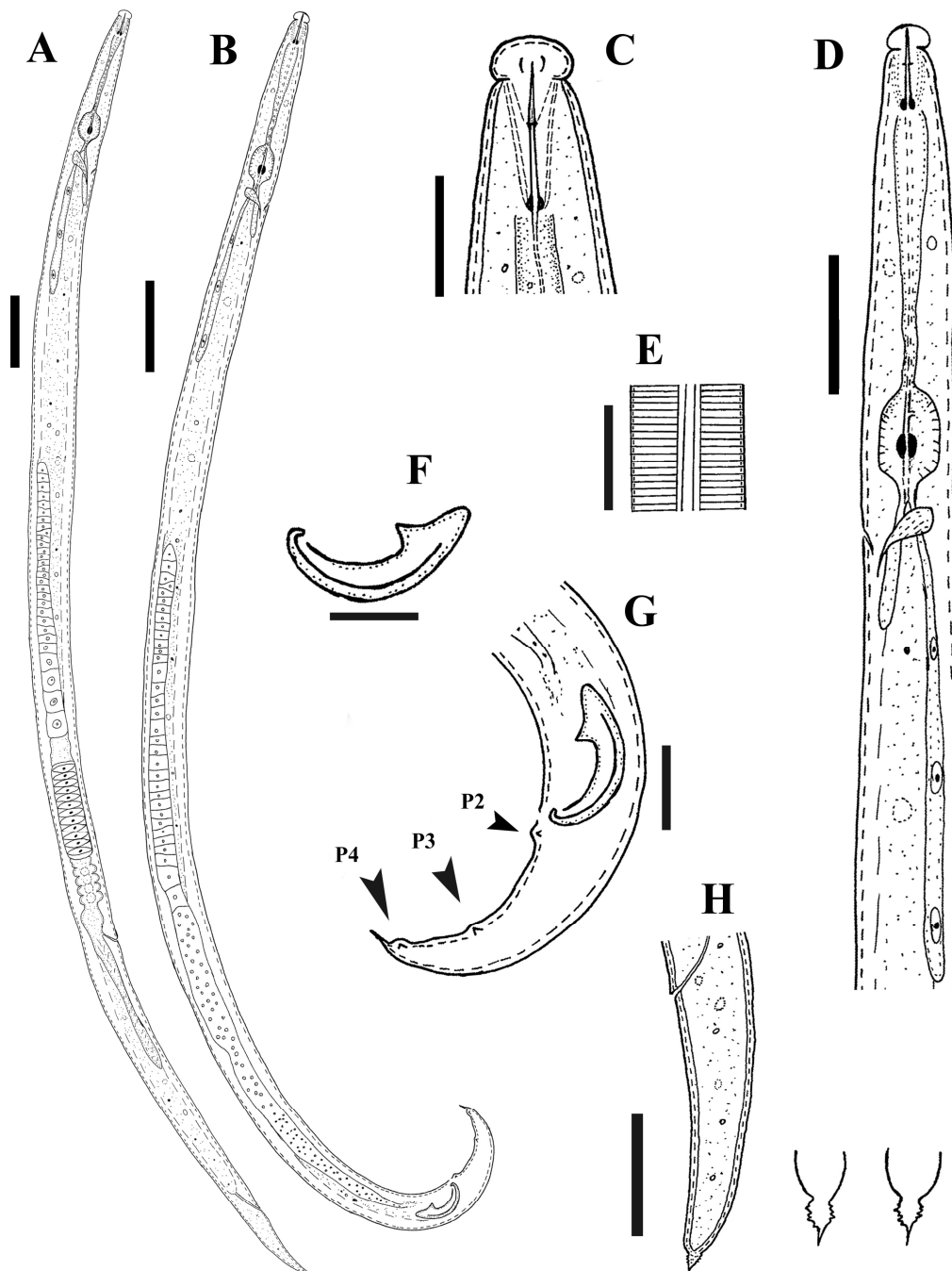


Figure 1 *Aphelenchoides xui*. A, B: Female and male entire body; C: Female head in detail; D: Female pharynx region; E: Lateral field; F: Spicule in detail; G: Male posterior body showing genital papillae (arrowheads, ventral view); H: Female tail. (Scale bars: A, B = 40 μ m; D, E, H = 20 μ m; C, F, G = 10 μ m).

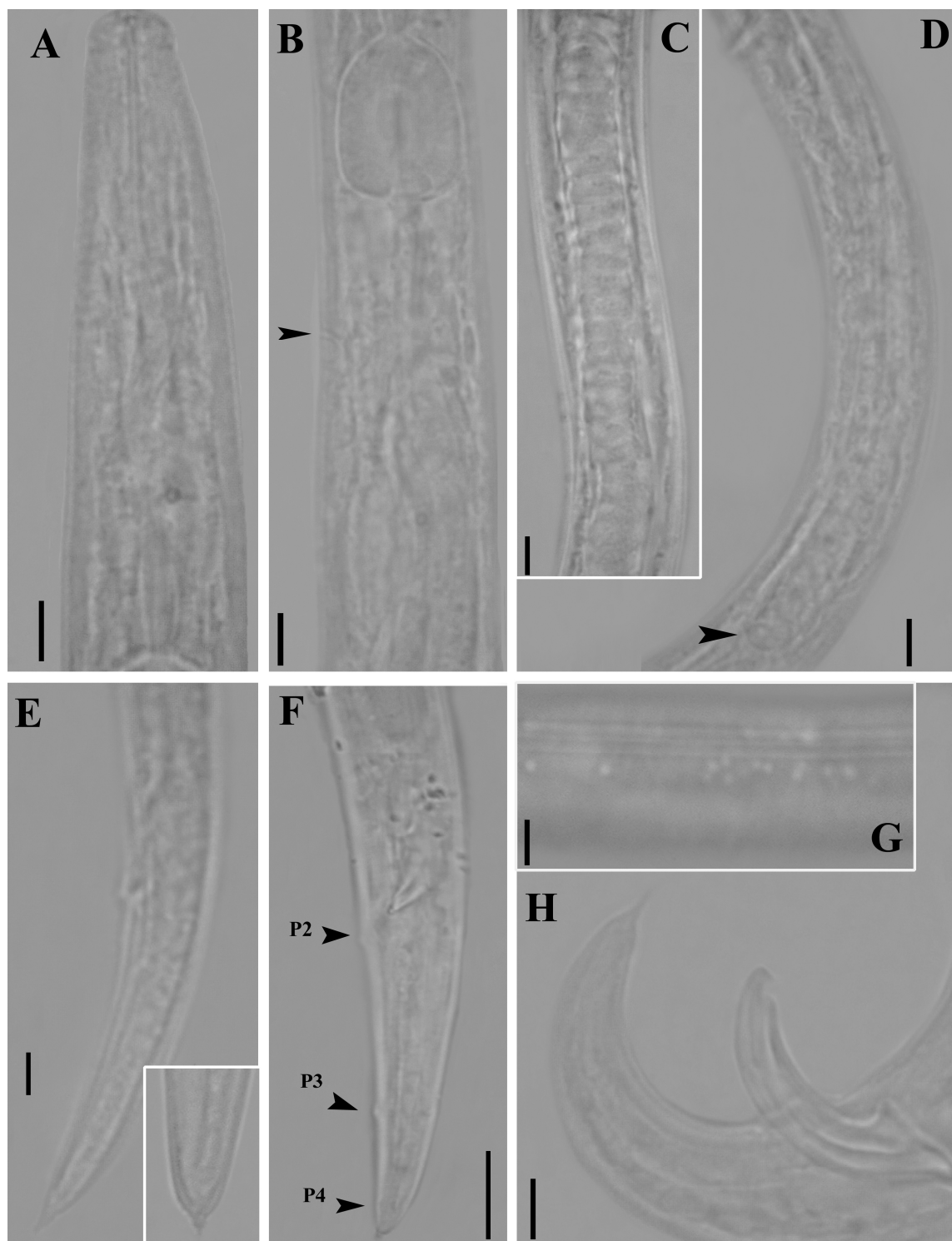


Figure 2 *Aphelenchoides xui*. A: Female anterior body; B: Metacarpus region showing excretory pore (arrowhead); C: Female genital tract; D: Vulval region showing post uterine sac (arrowhead); E: Female tail; F: Male posterior body showing genital papillae (arrowheads, ventral view); G: Lateral field; H: Male posterior body showing spicules. (All scale bars = 5 μm; except in F = 10 μm).

Table 1 Morphometric data of *Aphelenchoides xui* from West Azarbayjan province, northwestern Iran and its comparison with original description.

Characters	Present study		Wang <i>et al.</i> (2013)	
	Female	Male	Female	Male
n	8	5	15	15
L	743 ± 44 (678-805)	629 ± 57 (564-710)	770 (548-882)	729 (564-819)
a	33.0 ± 2.2 (30.5-37.0)	32.5 ± 1.1 (31.0-34.0)	30.5 (26.2-44.3)	31.3 (28.6-35.5)
b	9.6 ± 0.4 (8.8-10.0)	8.0 ± 0.5 (7.6-8.7)	8.3 (6.5-9.4)	7.7 (6.1-8.6)
b'	4.5 ± 0.4 (3.8-4.8)	4.1 ± 0.3 (3.8-4.6)	4.4 (3.5-5.7)	4.3 (3.7-5.3)
c	17.4 ± 1.5 (15.7-20.1)	14.0 ± 1.1 (13.0-16.0)	15.8 (13.9-20.8)	16.6 (13.9-18.6)
c'	3.6 ± 0.3 (3.0-4.0)	3.6 ± 0.4 (3.1-4.1)	3.8 (3.4-4.0)	3.1 (2.8-3.6)
V or T	71.0 ± 1.6 (69.0-73.5)	53.8 ± 3.4 (50.0-59.0)	69.0 (66.7-76.7)	53.3 (25.4-67.1)
Stylet	12.5 ± 0.5 (12.0-13.0)	12.4 ± 0.5 (12.0-13.0)	12.3 (11.1-13.2)	11.7 (11.0-12.5)
Overlapping	90.0 ± 14 (72.0-110.0)	75.2 ± 8.7 (67.0-90.0)	-	-
Median bulb	66.0 ± 3.3 (60.0-70.0)	75.2 ± 8.7 (65.0-75.0)	-	-
Pharynx	77.1 ± 4.9 (70.0-85.0)	77.2 ± 3.4 (73.0-82.0)	-	-
MB	85.7 ± 3.3 (81.3-91.9)	89.4 ± 1.5 (87.2-91.5)	-	-
Nerve ring	84.1 ± 7.0 (77.0-95.0)	85.8 ± 6.2 (80.0-94.0)	-	-
Excretory pore	86.8 ± 6.0 (80.0-97.0)	90.8 ± 6.5 (84.0-98.0)	87.0 (75.0-100)	90.0 (81.0-99.0)
Body width	22.3 ± 1.2 (20.0-24.0)	19.4 ± 1.3 (18.0-21.0)	26.5 (18.4-33.4)	23.4 (16.7-27.7)
Post-uterine sac	71.5 ± 13.2 (50.0-90)	-	93.0 (68.0-132)	-
PUS/BW	3.2 ± 0.6 (2.3-4.1)	-	-	-
PUS/ vulva to anus %	42.1 ± 7.7 (29.4-51.7)	-	50.0 (39.0-65.0)	-
Vulva-Anus	170 ± 4.4 (163-175)	-	192 (132-217)	-
Anal /cloacal body diam	11.9 ± 1.5 (10.0-14.0)	12.6 ± 1.1 (11.0-14.0)	-	-
Spicule (curved median line)	-	24.4 ± 0.5 (24.0-25.0)	-	22.5 (18.1-25.2)
Spicule (dorsal limb)	-	31.0 ± 1.0 (30.0-32.0)	-	29.2 (21.7-33.4)
Spicule (ventral limb)	-	18.8 ± 0.8 (18.0-20.0)	-	16.8 (12.8-19.4)

Measurements are in μm .***Paratylenchus recisus* Siddiqi, 1996****(Figures 3 and 4; Table 2)**

Female. Heat-relaxed body posture ranging from slightly curved ventrally, an open letter C to a figure 6. Cuticle annuli about 1.0-1.3 μm wide at mid-body. Lateral field with four incisures. Lip region truncate, 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 3.5-5.5 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 to longitudinally oval, axial, with small 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 to a small rounded, sometimes conoid terminus.

Male. Not found.

Remarks

This species was originally described from soil around roots of *Stylosanthes guianensis* at Carinagua, Llanos Oriental, Colombi (Siddiqi, 1996). Morphometrics of females of Iranian population completely fit those of *P. recisus* (Siddiqi, 1996). *Paratylenchus recisus* comes close to *P. pernoxius* Siddiqi, Baujard and Mounport, 1993 and *P. rostrocaudatus* Huang and Raski, 1987. It is distinguished from the former by having a differently shaped cephalic region lacking distinct submedian lobes, a slightly arcuate female tail and from the latter by having four incisures in the lateral field vs. three,

and a conoid-rounded female tail tip vs. beak-shaped. We collected this species for the first

time from Iran in soil samples of a vineyard in Ghasr-e Shirin county, Kermanshah province.

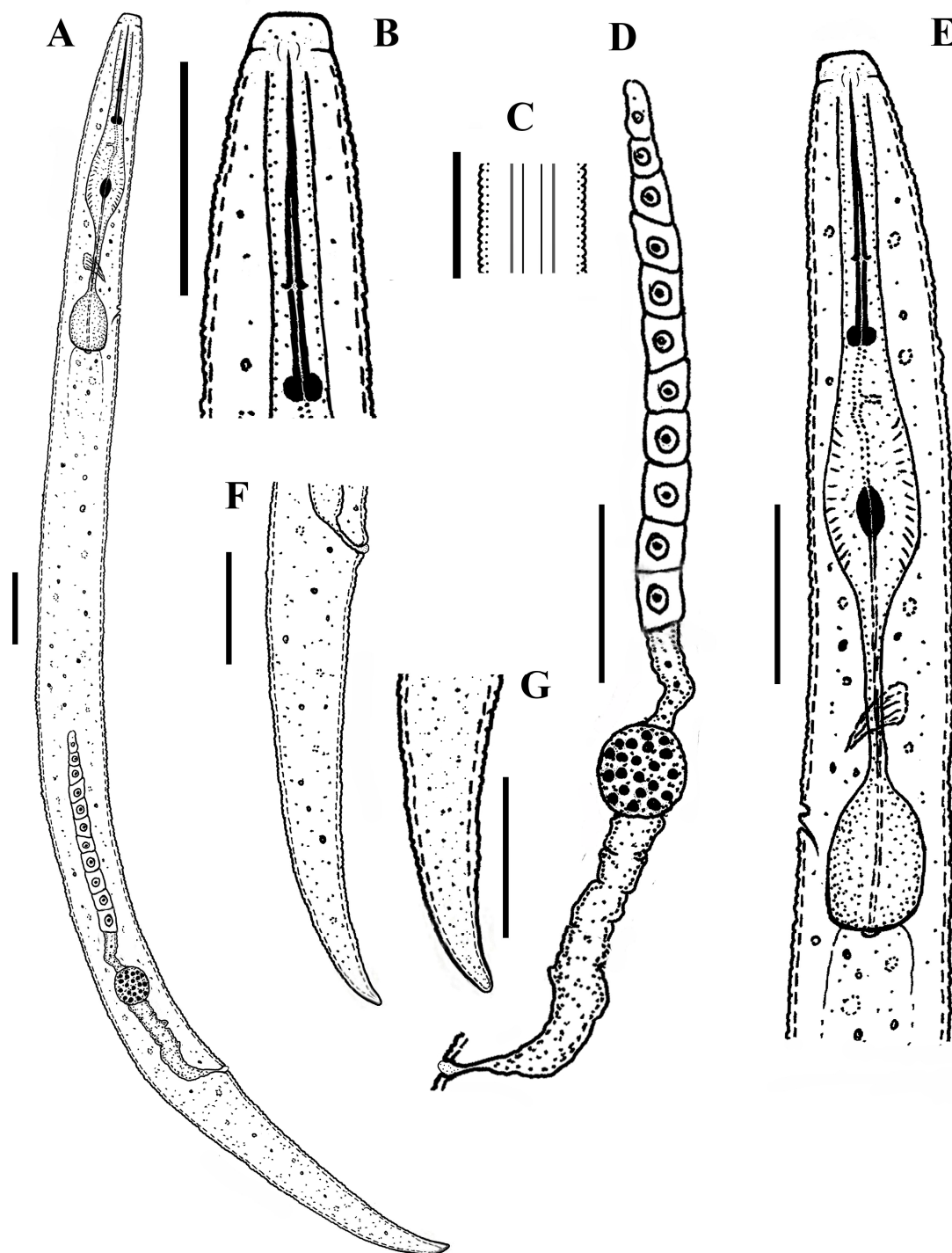


Figure 3 *Paratylenchus recius*. A: Female entire body; B: Female head in detail; C: Lateral field; D: Reproductive system; E: Pharyngeal region; F, G: Posterior end. (Scale bars: A, B, D, E = 10µm; C, F, G = 20µm).

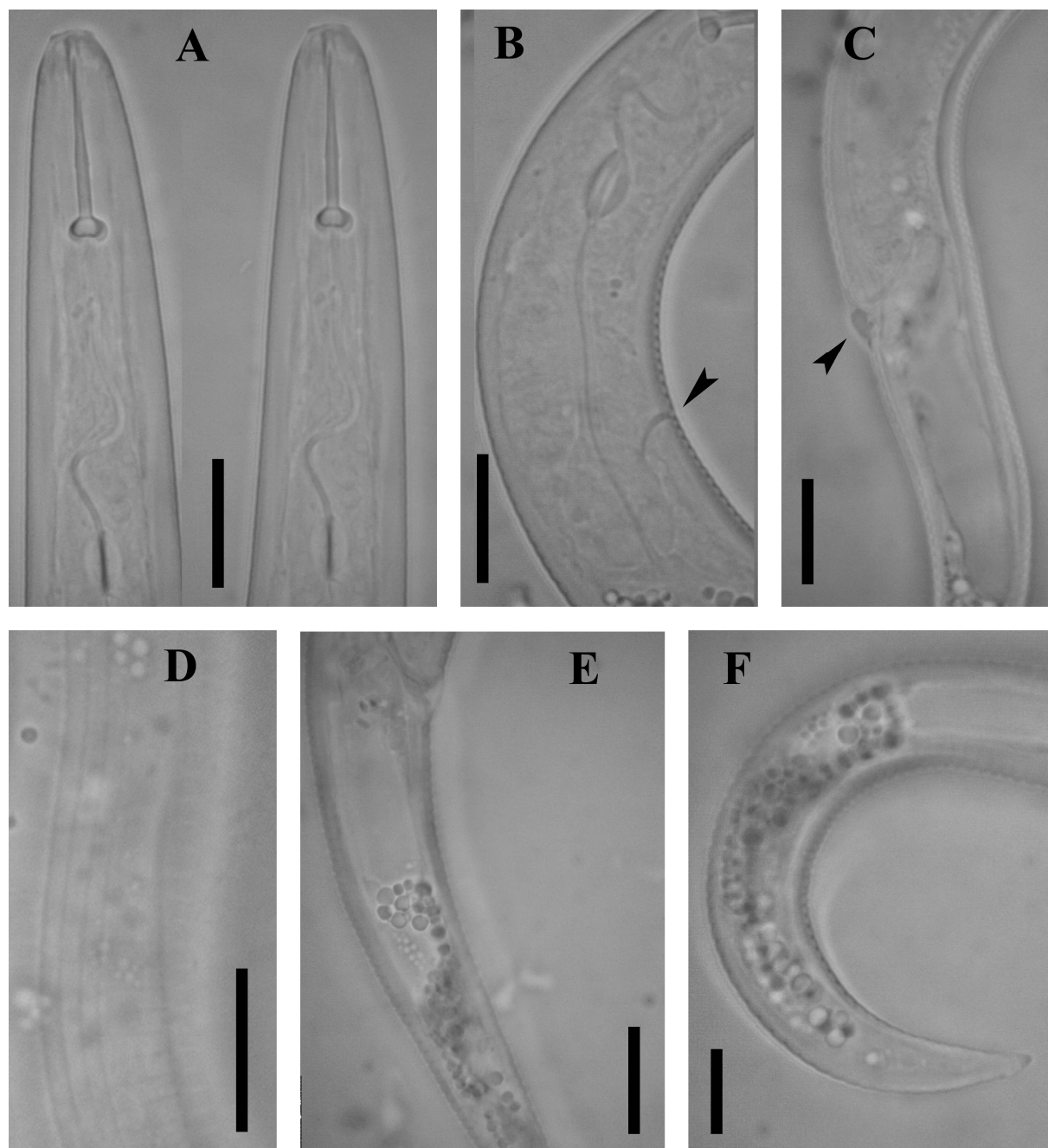


Figure 4 *Paratylenchus recius*. A: Anterior ends; B: Pharyngeal region and showing excretory pore; C: Vulva region and showing vulval flap; D: Lateral fields; E: Vulva region; F: Posterior end. (All scale-bars 10 µm).

Table 2 Morphometric data of *Paratylenchus recisus* from Kermanshah province, western Iran and its comparison with original description.

Character	Present study	Siddiqi (1996)
	Female	Female
n	10	20
L	324 ± 16.1 (298-343)	330 (275-390)
a	20.6 ± 0.8 (20.1-23.2)	21.4 (16.5-26.6)
b	3.2 ± 0.1 (3.0-3.5)	4.18 (3.8-5.0)
c	12.3 ± 1.2 (11.1-14.1)	14.8 (12.9-16.2)
V	80.6 ± 1.2 (78.5-82.5)	80.3 (78.5-83.2)
Stylet	15.3 ± 1.2 (14.0-17.0)	15.4 (15.0-16.8)
Conus	9.8 ± 0.8 (9.0-11.0)	-
m	63.9 ± 5.1 (56.3-71.4)	-
Pharynx	98.9 ± 5.1 (92.0-108)	-
Excretory pore	81.2 ± 3.4 (78.0-88.0)	-
Head-Vulva	250 ± 13.1 (235-267)	-
Head-anus	287 ± 16.1 (272-314)	-
Tail length	25.6 ± 1.9 (22.0-27.0)	-
Body width	14.3 ± 0.5 (14.0-15.0)	-
S.E./L%	26 ± 1.4 (23.0-27.9)	-
St/L%	4.9 ± 0.5 (4.1-5.7)	-

Measurements are in µm

Tylenchus skarduensis* Maqbool and Shahina, 1987*(Figures 5 and 6, Table 3)**

Female: Body ventrally curved upon fixation and appearing C-shaped. Cuticle coarsely annulated, annuli 1.4-2.0µm wide at mid-body. Lateral field occupying 5-6µm diameter at mid-body, with four incisures at vulval region, outer incisures strongly areolated on entire body (*i.e.* three ridges). Cephalic region continuous with body contour, anteriorly truncate and bearing four to five distinct annuli. Stylet slender with well-developed rounded basal knobs, 13.2-14.5µm long; conus occupying *ca* 46-50% of its total length. The dorsal gland orifice just posterior to stylet knobs. Median bulb oval to almost round in outline, located *ca* 48-58µm from the anterior end. Nerve ring encircling middle of isthmus. Excretory pore variable in position, mostly opposite to basal bulb of pharynx and immediately posterior to hemizonid, 94-112µm from the anterior end. Basal bulb offset; isthmus slender, ending to a

pyriform basal pharyngeal bulb. Deirids at center of lateral field, at level of excretory pore. Female reproductive tract monodelphic-prodelphic 175-305 µm long, ovary outstretched with oocytes arranged in single row, crustaformeria quadricolumellate, following a rounded spermatheca, usually empty and hardly visible in some specimens. Vulva a transverse slit, vulval lips not protruding, vagina perpendicular to body axis, reaching near halfway across body. Post-uterine sac 11-18 µm, 0.4-0.7 vulval body diameter. Tail 7.5-10 times anal body daim., strongly curved ventrally, in some specimens forming a hook, regularly tapering towards end to a bluntly rounded stout terminus with conspicuous annulation.

Male: Similar to females in the general characteristics, except in sexual organs. Testis outstretched, 257-327µm long, spermatocytes in one single column. Spicules tylenchoid, 19-22µm long; gubernaculum simple, 6-7µm long. Bursa crenate, adanal, 38-44µm long in arc line.

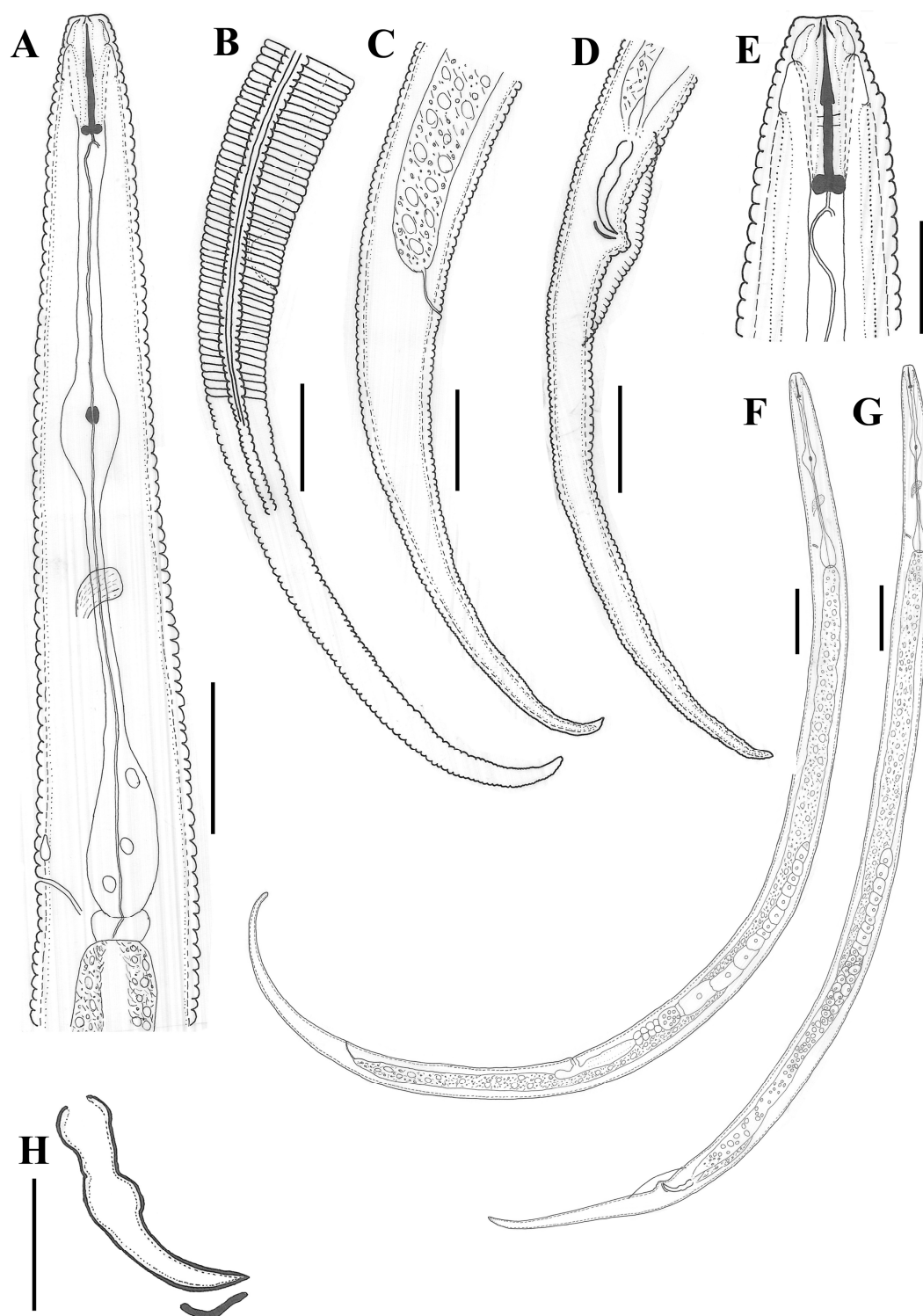


Figure 5 *Tylenchus skarduensis*. A: Female pharyngeal region; B: Female posterior end showing lateral field; C: Female tail; D: Male posterior end showing spicule; E: Anterior end; F: Female entire body; G: Male entire body; H: Spicule in detail. (Scale bars: A, G, H = 20µm; B- F = 10µm).

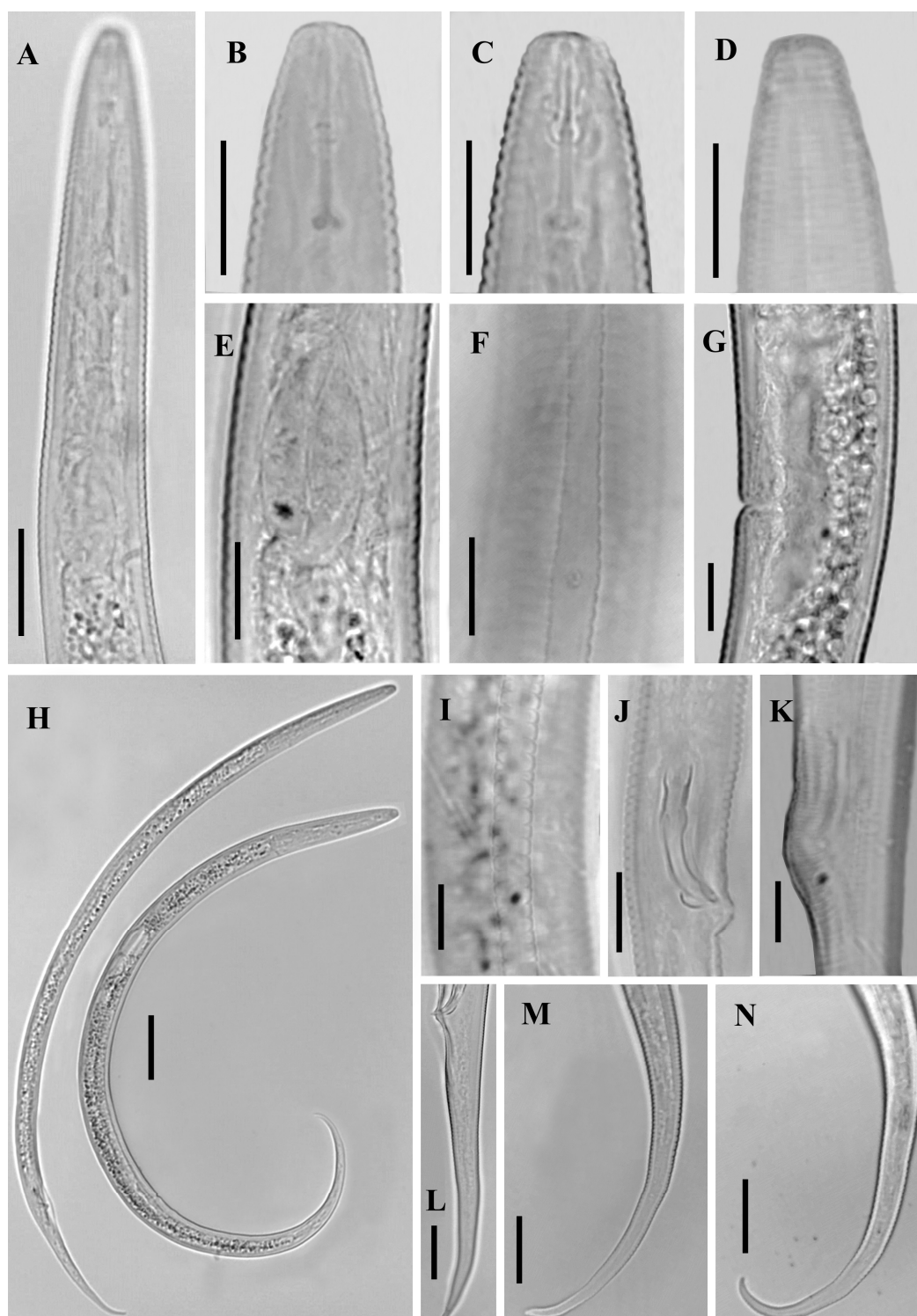


Figure 6 *Tylenchus skarduensis*. A: Female pharyngeal region; B-D: Anterior end; E: Terminal region and showing excretory pore; F: Dieris; G: Vulval region showing post uterine sac; H: Female and male entire body; I: Lateral field at mid-body; J: Male posterior end showing spicule; K: Male caudal alae; L: Male tail; M and N: Female tail. (Scale bars: A, L-N = 20µm; H = 50µm; B-G, I-K = 10µm).

Table 3 Morphometric data of *Tylenchus skarduensis* from Golestan province, northern Iran and its comparison with original description.

Characters	Present study		Maqbool and Shahina (1987)	
	Female	Male	Female	Male
n	12	6	10	3
L	707 ± 46.0 (642-791)	655 ± 56 (632-723)	800 (640-960)	670 (640-710)
a	29 ± 1.6 (26.0-31.0)	33.0 ± 1.7 (28.0-35.0)	30.3 (26.0-35.0)	38.0 (34.0-39.0)
b	6.2 ± 0.4 (5.7-6.8)	5.6 ± 0.7 (4.6-6.6)	6.4 (6.0-7.0)	5.6 (5.5-6.3)
c	6.2 ± 0.5 (5.6-7.4)	5.9 ± 0.4 (5.2-6.4)	7.7 (7.1-8.3)	6.4 (6.2-6.8)
c'	8.3 ± 1.0 (7.4-10.0)	7.1 ± 0.8 (6.6-8.3)	6.3 (6-8.2)	6.8 (6.0-7.0)
V or T	65.0 ± 2.2 (63.0-69.0)	-	68.0 (67.0-69.0)	-
Stylet	13.8 ± 0.4 (13.2-14.5)	13.5 ± 0.4 (13.0-14.0)	16.0 (15.0-16.0)	14.4 (14.0-15.0)
Conus	6.6 ± 0.4 (6.0-7.0)	6.3 ± 0.4 (6.0-7.0)	-	-
MB	46.0 ± 1.1 (44.5-48.2)	46.0 ± 2.0 (43.0-49.0)	44.0	-
Excretory Pore	103 ± 5.6 (94.0-112)	95.0 ± 3.0 (90.0-99.0)	114 (94.0-122)	-
Pharynx	113 ± 5.6 (104-123)	113 ± 3.6 (109-118)	-	-
Nerve ring	82.0 ± 3.4 (78.0-86.0)	80.0 ± 5.0 (76.0 – 86.0)	-	-
Head-Vulva	459 ± 28.0 (418-493)	-	-	-
Body width	25.0 ± 2.2 (22.0-27.5)	20.0 ± 1.0 (19.0-21.0)	-	-
Post uterine sac length	21.0 ± 1.4 (19.0 – 23.0)	-	15.0 (12.0-16.0)	-
Vulva-Anus	130 ± 11.0 (113-149)	-	184 (140-190)	-
Anal/cloacal body diam.	14.0 ± 1.4 (12.0-16.0)	16.0 ± 1.8 (14.0-19.0)	-	-
Tail	115 ± 12.0 (90.0-136)	112 ± 6.7 (105-124)	(88.0-112)	104 (100-114)
Spicule	-	20.5 ± 1 (19.0-22.0)	-	18.5 (18.0-20.0)
Gubernaculum	-	6.3 ± 0.5 (6.0-7.0)	-	6.4 (6.0-7.0)

Measurements are in μm .

Remarks

Dimensions and morphological characters of the present specimens conform to the descriptions of *T. skarduensis* given by Maqbool and Shahina (1987) reported from the rhizosphere of mulberry (*Morus rubra* L.) except for shape of female body after fixation. As far as we know there is no report of this rare species after original description, so this is the second report of occurrence of the species. Because of strong areolation in two outer lines in lateral field on entire body, relatively robust stylet 13.2-14.5 μm long, spicule length 19-22 μm the Iranian population of *T. skarduensis* is distinguishable from all other known species of the genus. However, the population is mostly close to *T. safroni* (Fotadar and Handoo, 1979) Siddiqi, 1986 and *T. stachys* Brzeski, 1996, but Iranian population differs from *T. safroni* by having shorter spicule and gubernaculum (19-22 vs 27-30 μm and 6-7 vs 7-10 μm , respectively),

different cephalic region shape (truncate vs rounded) and simple excretory pore duct vs cuticularized. The population is distinguishable from the latter by having more posterior position of vulva (V = 63-69 vs 59-63), shorter tail length (90-136 vs 133-174 μm), shorter spicule length (19-22 vs 24-28 μm). The present specimens was recovered from the rhizosphere of pomegranate tree from Takhshi Mahalleh, a village located in west of Gorgan, Golestan province, northern Iran. The present study expands the morphometric and morphological characters of the species and reports, describes and illustrates *T. skarduensis* for second time after Maqbool and Shahina (1987).

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معرفی سه گونه از نماتدهای فوق بالاخانواده Tylenchomorpha (Nematoda: Tylenchina) از ایران

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چکیده: طی بررسی که به منظور شناسایی نماتدهای انگل گیاهی در برخی نقاط ایران انجام شد، چندین گونه متعلق به فوق بالاخانواده Tylenchomorpha از مناطق مختلف استان‌های آذربایجان غربی، کرمانشاه و گلستان جمع‌آوری و شناسایی شدند. از بین آن‌ها سه گونه *Aphelenchoides xui* از خانواده Tylenchulidae و گونه *Tylenchus skarduensis* از خانواده Tylenchidae، *Paratylenchus recisus* از خانواده Tylenchulidae و گونه *Tylenchus skarduensis* از خانواده Tylenchidae، گزارش‌های جدیدی برای فون نماتدهای ایران می‌باشند. توصیف کامل، داده‌های ریخت‌سنجی، ترسیم‌ها و عکس‌های میکروسکوپ نوری برای این سه گونه ارائه شده است.

واژگان کلیدی: ریخت‌شناسی، ریخت‌سنجی، گزارش جدید، نماتد