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Short Paper

Some belonolaim species (Nematoda, Dolichodoridae) from Sabalan region, northwest of Iran

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Abstract: Six belonolaim species were collected from grasslands of Sabalan region. The found species belong to genera *Amplimerlinius*, *Merlinius*, *Nagelus*, *Neodolichorhynchus*, *Paramerlinius* and *Tylenchorhynchus*. Among the found species, *Neodolichorhynchus judithae* is reported for the first time from Iran and is characterized by having offset head, cuticule with 16 longitudinal ridges, delicate stylet 21-23 µm long, presence of post intestinal sac and tail with annulated terminus.

Keywords: Ardebil province, grasslands, new record, morphological characters, Tylenchina.

Introduction

Based on morphological characters (amphids, phasmids, deirids, lateral field and head sensory organs), Ryss (1993) suggested the combination of *Pratylenchoides* Winslow, 1958 and Merliniinae *sensu* Siddiqi 2000 members (*Nagelus* Thorne & Malek, 1968, *Merlinius* Siddiqi, 1970, *Amplimerlinius* Siddiqi, 1976, *Geocenamus* Thorne & Malek, 1968 and *Scutylenchus* Jairajpuri, 1971) into a single family and raised subfamily Merliniinae to the family rank (Merliniidae Siddiqi, 1971). Siddiqi (2000) placed the family Telotylenchidae Siddiqi, 1960 in the superfamily Dolichodoroidea Chitwood & Chitwood, 1950 and the genera of the subfamily Merliniinae in the family Telotylenchidae, mainly characterized by having six incisures in lateral field and having deirids. Geraert (2011) provided an excellent overview on the genera and species of the family Dolichodoridae Chitwood & Chitwood, 1950. The most recent review on taxonomy of this group of nematodes is made by Sturhan (2012). In this review, he considered the results of the molecular phylogenetic studies and presence of dierids in subfamily Merliniinae and the genus *Pratylenchoides* and placed them in family Merliniidae with two subfamilies, Merliniinae and Pratylenchoidinae. He placed *Pratylenchoides* under the subfamily Pratylenchoidinae and the remaining genera (*Merlinius*, *Nagelus*, *Amplimerlinius*, *Geocenamus*, *Paramerlinius* and *Macrotylenchus*) under Merliniinae, and also considered *Scutylenchus* as a synonym of *Geocenamus*. He proposed a new genus, *Paramerlinius* Sturhan, 2012, with *P. hexagrammus* (Sturhan, 1966) Sturhan, 2012 as the type species and 11 other species that previously were placed under the other genera. Finally, he established *Macrotylenchus* Sturhan, 2012 as a new genus with *M. hylophilus* Sturhan, 2012 as its species and two other species that were previously placed in *Geocenamus*.

Till date some species belonging to this group of nematodes have been reported from Iran. Kheri (1972) reported *Merlinius camelliae* n. sp. Mojtahedi *et al*., (1983) have reported 31

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species belonging to Belonolimidae of which 25 species were first records for Iran. Niknam *et al*., (2008), have reported nine species of Belonolimidae from lucerne farms in East Azarbaijan province, of which four species were new records for Iran. Pourjam *et al*., (2011) have reported seven species belonging to three genera, of which three species were new for nematode fauna of Iran.

Our surveys on plant parasitic nematode fauna occurring in grasslands of Sabalan region yielded several species belonging to Belonolaimidae. The morphological and morphometric study of the recovered species is the aim of the present article.

Materials and Methods

Several soil samples were collected from grasslands of Sabalan region during 2011. Nematodes were extracted by using tray method (Whitehead and Hemming, 1965). The extracted specimens were killed in hot 4% formaldehyde solution, transferred to anhydrous glycerin according to De Grisse (1969), and mounted on permanent slides. Morphological and morphometric characters were studied by Nikon E600 light microscope equipped with a drawing tube.

Results and Discussion

***Amplimerlinius macrurus* (Goodey, 1932) Siddiqi, 1976**

Measurements: See Table 1.

Morphological and morphometric characters of the studied population of this species are in agreement with those of original description. It is characterized by having long body, cylindrical tail with hemispherical and striated terminus. During our study, this species was collected from the rhizosphere of milk vetch (*Astragalus* sp.) from grasslands of Ghotour Soyi, Sabalan, Ardebil province, northwest of Iran.

***Merlinius brevidens* (Allen, 1955) Siddiqi, 1970**

Measurements: See Table 1.

The found population of this species is characterized by broadly rounded head with 5-6 fine annuli, 14-16 µm stylet length, absence of post intestinal sac, sub-cylindrical tail with smooth rounded to flattened tip bearing 34-56 fine annuli. This species was recovered from the rhizosphere of nettle (*Urtica* sp.) naturally growing in Alvandlou, Sabalan region, Ardebil province, northwest of Iran.

***Nagelus obscurus* (Allen, 1955) Powers, Baldwin & Bell, 1983**

Measurements: See Table 2.

It is characterized by having long body, head continuous with the rest of body, stylet robust with large knobs, short basal bulb and conical tail with striated terminus. The morphological and morphometric characters of recovered population of this species from grasslands of Sabalan region are in congruence with those of the population found by Ali-ramaji *et al.*, (2013) and the populations reported by Brzeski, 1997 (Table 2). Brzeski (1997) compared the species *Nagelus leptus* (Allen, 1955) Siddiqi, 1979 with *N. obscurus* and pointed out that these two species differ in their body length, stylet length, and absence of male in *N. leptus*. The morphometric characters of presently studied population of *N. obscurus* are close to the ranges of morphometric data of *N. leptus* and by having males in the population, this population is identified as *N. obscurus* (Table 2).

***Paramerlinius hexagrammus* (Sturhan, 1966) Sturhan, 2012**

Measurements: See Table 4.

The collected population is characterized by its 1092-1361 µm body length, head continuous with body contour, strong and very refractive cephalic framework, robust stylet, lateral field with six lines, areolated at neck region, deirids prominent, located in four line zone of lateral field, tail sub-cylindrical with smooth terminus. During our survey, it was recovered from grasslands of Arkhalti, Sabalan region, Ardebil province, northwest of Iran.

Table 1 Morphometric characters of *Amplimerlinius macrurus* & *Merlinius Brevidens* adults collected from pastures of Sabalan region, northwest of Iran (All measurements in µm and in the form: mean ± sd. (Min-Max)).

Origin	*Amplimerlinius macrurus*		*Merlinius brevidens*	
Character	**Female**	**Male**	**Female**	**Male**
n	**10**	**5**	**10**	
L	972 ± 52.1 (913-1046)	987 ± 65.4 (895-1067)	618 ± 44.1 (611-747)	659 ± 33.4 (615-695)
a	27.2 ± 2.5 (22.8-30.5)	31.1 ± 3.6 (24.8-33.5)	30.9 ± 2.3 (26.7-33.9)	34.5 ± 3.1 (30.8-38.2)
b	4.9 ± 0.2 (4.6-5.1)	5.0 ± 0.2 (4.7-5.4)	4.8 ± 0.3 (4.4-5.1)	4.5 ± 0.3 (4.2-4.8)
c	14.2 ± 0.7 (12.7-14.8)	11.2 ± 0.4 (10.7-11.8)	14.0 ± 1.8 (11.4-17.6)	12.2 ± 1.3 (11.0-14.2)
c'	2.7 ± 0.3 (2.1-3.1)	3.6 ± 0.3 (3.2-4.0)	3.1 ± 0.4 (2.4-3.6)	3.7 ± 0.1 (3.5-3.8)
V or T	56.8 ± 1.3 (55.1-58.3)	36.4 ± 8.6 (22.6-43.9)	56.7 ± 1.6 (54.4-59.8)	43.6 ± 4.1 (36.6-46.8)
Stylet length	27.5 ± 1.0 (26.0-29.0)	27.6 ± 2.5 (25.0-30.0)	15.5 ± 0.6 (15.0-16.5)	15.1 ± 0.5 (14.5-16.0)
MB	52.0 ± 1.3 (50.0-54.3)	52.1 ± 1.0 (51.1-53.4)	44.5 ± 1.7 (41.4-46.2)	57.0 ± 3.8 (50.4-59.8)
S. E. pore	141 ± 8.7 (131-158)	150 ± 10.5 (140-165)	112 ± 6.2 (103-124)	110 ± 6.1 (102-119)
Oesophagus	199 ± 9.2 (186-217)	196 ± 17.2 (180-225)	142 ± 3.5 (137-148)	147 ± 3.6 (144-153)
Tail length	68.6 ± 5.5 (62.0-79.0)	88.2 ± 8.1 (76-96)	49.5 ± 7.8 (38-63)	54.2 ± 4.1 (49.0-60.0)
Tail Annules	46.6 ± 4.7 (38-53)	–	44.3 ± 7.0 (34-53)	48.6 ± 4.6 (43-55)
Spicule	–	34.8 ± 3.1 (31-38)	–	23.4 ± 1.1 (22.0-25.0)
Gubernaculum	–	13.0 ± 1.9 (11-15)	–	8.6 ± 1.3 (7.0-10.0)

Table 2 Morphometric characters of *Nagelus obscurus* adults recovered from pastures of Sabalan region compared with other populations (All measurements in µm and in the form: mean ± sd. (Min-Max))

Origin	Present study population		Brzeski, 1997		Ali-ramaji *et al*., 2013 (Iran)	
Character	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
n	**13**	**1**	**60**	**60**	**19**	**13**
L	934 ± 69.4 (844-1075)	1010	740 ± 100 (520-970)	714 ± 104 (530-910)	784 ± 77.1 (677-907)	754 ± 131 (542-915)
a	34.3 ± 1.3 (32.3-36.8)	40.4	23.1 ± 1.0 (20-27)	28 (23-33)	30 ± 2.7 (24.5-34.5)	32.8 ± 1.8 (29.8-35.2)
b	5.4 ± 0.4 (5.0-6.3)	5.8	5.3 ± 0.3 (4.1-6.3)	5.2 ± 0.3 (4.3-5.8)	5.1 ± 0.2 (4.7-5.5)	5.1 ± 0.5 (4.0-6.0)
c	12.9 ± 1.2 (11.0-14.8)	12.6	133 ± 0.5 (10.7-14.9)	13.8 ± 1.4 (10.3-13.4)	13.7 ± 0.8 (12.3-14.7)	11.6 ± 0.7 (10.4-12.9)
c'	4.2 ± 0.6 (3.2-5.5)	3.3	3.1 ± 0.2 (2.3-3.6)	3 ± 0.2 (2.5-3.6)	3.3 ± 0.2 (2.9-3.7)	3.4 ± 0.4 (2.7-3.9)
V	54.2 ± 1.2 (51.7-56.5)	34.5	54.4 ± 1.2 (50-59)	–	55.4 ± 1.1 (53.3-57.2)	–
Stylet length	27.2 ± 1.1 (25.0-29.0)	24.0	23 ± 0.8 (20.5-25)	22 ± 1.4 (19.5-25)	24 ± 2.7 (20-27)	23.0 ± 3.2 (19.0-27.0)
MB	52.1 ± 1.1 (50.3-53.6)	51.4	53.4 ± 1.1 (49-58)	53.9 ± 1.8 (50-59)	51.5 ± 1.6 (49.6-54.3)	53 ± 1.2 (50-54.5)
S. E. Pore	140 ± 5.7 (130-149)	151	116 ± 6.9 (95-141)	115 ± 9.9 (92-138)	124 ± 11.1 (107-144)	119 ± 15 (98-141)
Oesophagus	174 ± 6.5 (163-190)	175	142 ± 11.1 (113-169)	136 ± 14.9 (118-168)	154 ± 12.7 (130-183)	148 ± 13.7 (132-175)
Body Width	27.5 ± 2.3 (24-32)	25	–	–	–	–
Tail length	73.2 ± 4.5 (67-80)	80	58 ± 8.3 (36-85)	60 ± 10.2 (43-81)	57 ± 7.1 (47-71)	65.5 ± 12.3 (46.0-81.0)
Tail Annules	60.1 ± 4.8 (54-69)	–	64 ± 11.4 (38-97)	–	56 ± 7.8 (46-70)	–
Spicule	–	31	–	28 ± 2.7 (23-32)	–	28.0 ± 3.9 (21.0-33.0)
Gubernaculum	–	11	–	10 ± 0.9 (8-12)	–	11.0 ± 1.8 (8.5-14.0)

Table 3 Morphometric characters of *Neodolichorhynchus judithae* adults recovered from pastures of Sabalan region-compared with other populations (All measurements in µm and in the form: mean ± sd. (Min-Max).

Origin	Present Study population		Andrassy, 2007		Sturhan, 1966	
Character	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
n	**9**	**5**	**–**	**–**	**3**	**1**
L	994 ± 68.3 (903-1100)	872 ± 41.5 (838-929)	700-970	720-820	750 (690-790)	720
a	34.0 ± 1.5 (30.9-35.8)	33.7 ± 2.8 (29.2-36.2)	30-35	33-35	31 (30-31)	33
b	6.0 ± 0.3 (5.6-6.5)	5.5 ± 0.2 (5.3-5.7)	5.4-7.2	5.2-6.3	5.8 (5.3-6.3)	5.2
c	19.6 ± 0.9 (18.2-21.5)	17.2 ± 1.3 (15.7-19.2)	15-23	17-18	19	17
c'	2.3 ± 0.1 (2.1-2.5)	2.7 ± 0.2 (2.5-3.0)	2.2-2.8	–	–	–
V or T	54.0 ± 0.9 (52.7-55.5)	50.1 ± 6.6 (40.6-55.8)	52-58	–	57 (53-60)	53
Stylet length	22.2 ± 0.7 (21-23)	21.4 ± 1.1 (20.5-23.0)	20-24	–	22	22
MB	52.9 ± 1.4 (50.3-54.4)	52.4 ± 1.3 (50.6-53.9)	–	–	–	–
S. E. Pore	137 ± 7.5 (122-148)	134 ± 6.3 (129-142)	–	–	–	–
Oesophagus	164 ± 8.0 (157-180)	159 ± 6.6 (152-170)	–	–	–	–
Tail lenght	50.9 ± 4.5 (42-55)	51.0 ± 3.4 (47-55)	–	–	–	–
Tail Annules	41.6 ± 4.3 (32-47)	–	–	–	–	–
Spicule	–	26.4 ± 1.1 (25-28)	–	24	–	24
Gubernaculum	–	14.6 ± 1.3 (14-17)	–	13-14	–	14

Table 4 Morphometric Characters of *Paramerlinius hexagrammus*, *Tylenchorhynchus dubius and T. maximus* adults recovered from pastures of Sabalan (All measurements in µm and in the form: mean ± sd. (Min-Max)).

Origin	*Paramerlinius hexagrammus*		*Tylenchorhynchus dubius*		*T. maximus*
Character	**Female**	**Male**	**Female**	**Male**	**Female**
n	**10**	**5**	**10**	**5**	**10**
L	1253 ± 83 (1092-1361)	1125 ± 52 (1060-1174)	750 ± 66.(660-881)	738 ± 64 (639-802)	1329 ± 75 (1120-1348)
a	32.6 ± 1.9 (29.6-35.8)	35.2 ± 3.0 (31.7-39.1)	33.3 ± 2.7 (30.3-40.1)	35.0 ± 1.8 (33.23-37.6)	41.6 ± 3.2 (36.2-46.5)
b	5.8 ± 0.3 (5.4-6.4)	5.4 ± 0.4 (4.9-5.9)	5.4 ± 0.4 (5.1-6.4)	5.2 ± 0.3 (4.8-5.6)	6.7 ± 0.4 (6.0-7.3)
c	15.7 ± 1.0 (13.8-17.4)	12.0 ± 0.9 (11.2-13.3)	16.5 ± 1.3 (14.8-19.6)	16.0 ± 1.6 (14.1-18.3)	20.4 ± 1.5 (18.7-23.5)
c'	3.1 ± 0.2 (2.8-3.4)	4.2 ± 0.4 (3.7-4.6)	2.7 ± 0.2 (2.5-3)	2.7 ± 0.3 (2.5-3.2)	2.9 ± 0.5 (2.3-4.0)
V	52.9 ± 0.9 (51.8-54.6)	51.5 ± 3.7 (47.3-56.1)	56.7 ± 0.9 (55.5-58.6)	59.5 ± 7.4 (52.5-71.7)	52.4 ± 1.2 (50.4-53.7)
Stylet length	31.2 ± 1.5 (29-33)	30.4 ± 0.5 (30-31)	19.3 ± 1.0 (18-21)	19.1 ± 0.9 (18-20)	22.4 ± 0.9 (21.5-24)
MB	51.7 ± 1.7 (49.5-54.2)	54.2 ± 1.9 (51.5-56.1)	49.1 ± 1.9 (46.9-53.7)	49.0 ± 1.5 (47.1-50.7)	54.2 ± 1.1 (52.6-56.6)
S. E. pore	172 ± 9.7 (160-195)	164 ± 2.8 (161-168)	112 ± 6 (106-123)	107 ± 6 (101-116)	143 ± 5 (134-151)
Oesophagus	217 ± 7 (201-227)	203 ± 10 (190-217)	139 ± 10 (123-160)	141 ± 9 (132-157)	186 ± 3 (181-190)
Tail length	80.1 ± 6.6 (70-89)	93.8 ± 7.1 (85-101)	45.4 ± 3.3 (40-50)	46.6 ± 7.1 (40-57)	61.0 ± 5.4 (53-70)
Tail Annules	47.4 ± 4.5 (42-55)	54.7 ± 3.8 (52-59)	34.4 ± 2.4 (31-38)	–	40.9 ± 2.3 (39-47)
Spicule	–	34.4 ± 1.5 (32-36)	–	27.0 ± 3.2 (24-31)	–
Gubernaculum	–	11.6 ± 0.9 (10-12)	–	11.6 ± 1.1 (10-13)	–

***Neodolichorhynchus judithae* (Andrássy, 1962) Jairajpuri & Hunt, 1984 (Figs. 1 & 2)**

Measurements: See Table 3.

Female: Body ventrally curved, assuming an open C after fixation. Cuticule with fine annulation, annuli 1.0-1.5 µm wide at mid-body. Lateral field with four lines, two border lines crenated. Cuticule bears 10 longitudinal ridges (except lateral field), five ventral and five dorsal. Head distinctly offset, rounded and bearing 5-6 annuli. Cephalic framework weakly developed. Stylet delicate, with rounded to slightly

posteriorly sloping knobs. DGO opens at 2-3 µm distance posterior to stylet knobs. Median bulb rounded to oval with refractive valves. Isthmus long, basal bulb small, pyriform. Deirids absent. Excretory pore located at posterior part of isthmus, hemizoid not distinct. Vulva a simple slit, without epiptygma. Spermatheca axial, oval to rounded and filled with small and rounded sperm. Uterus showing a quadricolumella arrangement. Post intestinal sac long, reaching about half the tail. Phasmids distinct, located at proximal half of the tail. Tail slightly ventrally curved, conical with annulated terminus.

Male: General morphology similar to that of female, but smaller. Spicule tylenchoid. Gubernaculum arcuate. Bursa with crenate border and enveloping the tail. Tail conical.

Discussion

Morphological and morphometric characters of the Iranian population of *N. judithae* are in agreement with those given in the original description and those given by Sturhan (1966). In the original description of this species, 14 longitudinal ridges (1 mid-dorsal, 1 mid-ventral, 6 ventral and 6 dorsal) are reported. Andrássy, 2007 has given 12-14 ridges. However, in Iranian population, the cuticle bears 16 longitudinal ridges (1 mid-dorsal, 1 mid-ventral, 7 ventral and 7 dorsal) (Fig. 1 G). This species is characterized by having offset head, lateral field with four lines, delicate stylet, presence of post intestinal sac and tail with annulated terminus (Fig. 1 I).

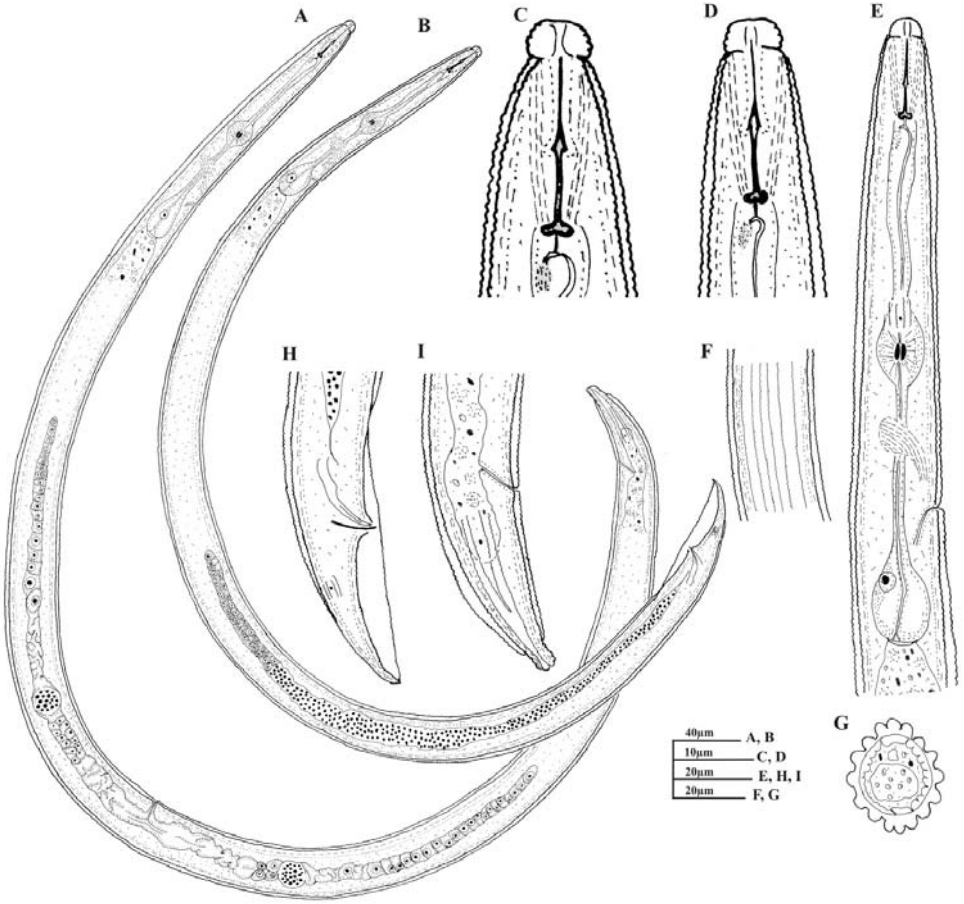

Figure 1 Iranian population of *Neodolichorhynchus judthae*. A: Entire female; B: Entire male; C, D: Female, head region; E: Female, anterior region; F: Female, cuticle surface; G: Female, mid-body cross section; H: Male, tail; I: Female, tail.

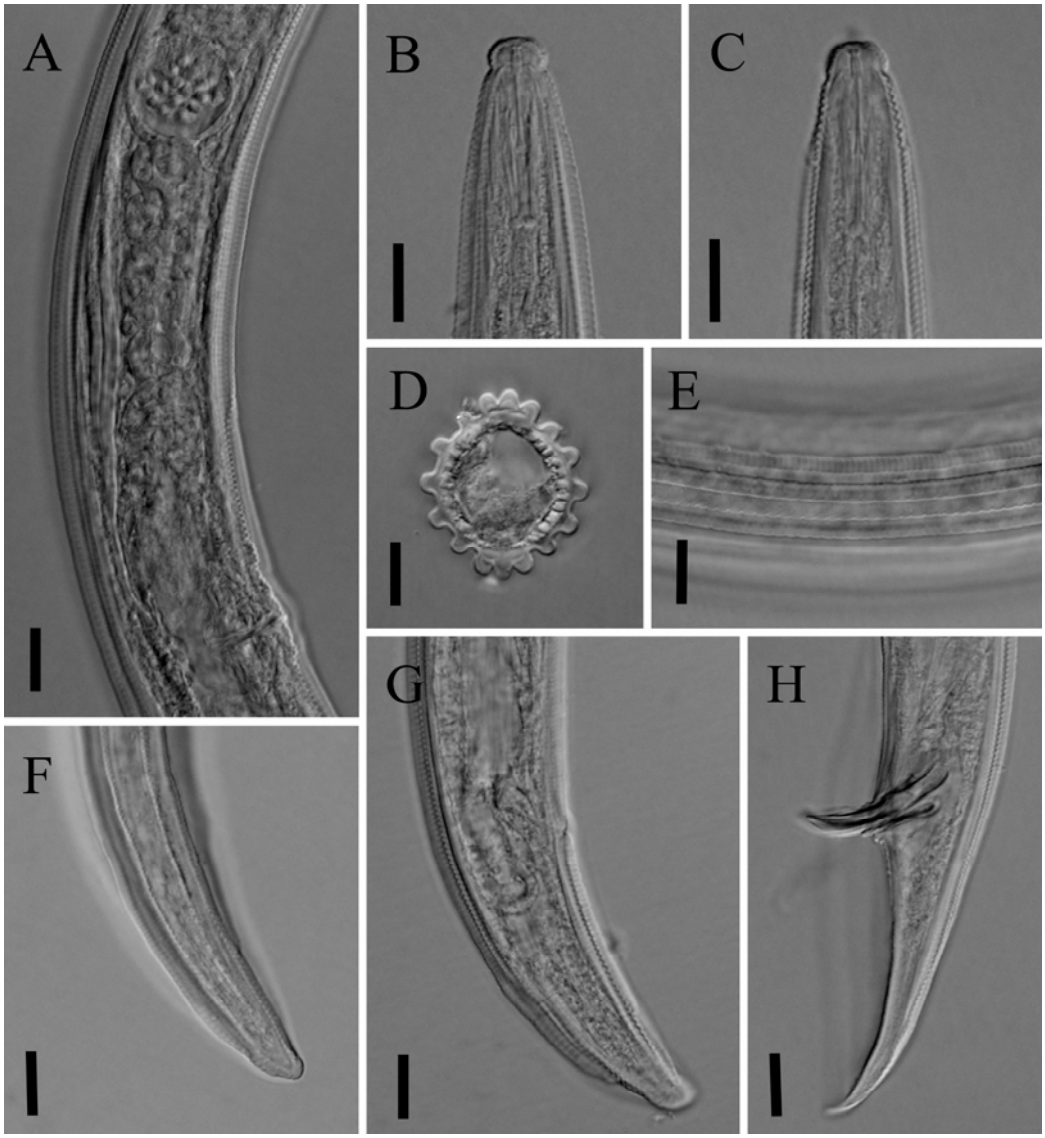

Figure 2 Iranian population of *Neodolichorhynchus judthae*. A: Part of female reproductive system; B & C: Female, anterior end; D: Female, mid body cross section; E: Female, longitudinal ridges; F & G: Female, tail; H: Male, cloacal region. All scale bars 10 µm.

This species (the Iranian population) resembles *N.microphasmis* (Loof, 1960) Jairajpuri & Hunt, 1984 and *N. Sulcatus* (de Guiran, 1967) Jairajpuri & Hunt 1984. This species differs (the data of the Iranian population is used for the comparisons with close species) from *N. microphasmis* by having shorter stylet (21-23 vs 24-27 µm), less number of longitudinal ridges (16 vs 12-20), tail with annulated terminus (vs smooth). Brzeski (1991) remarked that *N. judthae* is identical with *N. microphasmis* stating that these two species differ in stylet length and shape of knobs. It differs from *N. sulcatus* by having longer stylet (21-23 vs 19-22 µm), presence of post intestinal sac (vs absent) and tail with annulated terminus (vs smooth).

Locality and habitat

This population was collected from the rhizosphere of grass from grasslands of Korlar, Sabalan region, Ardebil province, northwest of Iran.

***Tylenchorhynchus dubius* (Bütschli, 1873) Filipjev, 1936**
(Measurements: See Table 4.
The found population of this species is characterized by having offset head with six fine annuli, 18-21 µm stylet length, lateral field irregularly areolated, presence of post intestinal sac, tail sub-cylindrical, its terminus hemispherical, annulated and bearing 31-38 fine annuli. During our survey, it was recovered from grasslands of Sardabeh, Sabalan region, Ardebil province, northwestern Iran.

***Tylenchorhynchus maximus* Allen, 1955**
Measurements: See Table 4.
The studied population of this species is characterized by having long body, 21-24 µm long stylet, spear knobs sloping posteriorly, presence of post intestinal sac, vulva without epiprygma, tail cylindrical to sub-cylindrical, occasionally clavate with broadly rounded and striated terminus bearing 39-47 fine annuli. During this study, it was recovered from grasslands of Kakalar, Sabalan region, Ardebil province, northwestern Iran.

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گزارش چندین گونه نماتد belonolaim (Nematoda, Dolichodoridae) از مراتع منطقه سبلان، شمال غربی ایران

یوسف پناهنده و ابراهیم پورجم*

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چکیده: طی بررسی نماتدهای انگل گیاهی مراتع سبلان شش گونه متعلق به جنس‌های *Amplimerlinius*، *Merlinius*، *Nagelus*، *Neodolichorhynchus* و *Paramerlinius* از خانواده Dolichodoridae شناسایی گردید. از میان گونه‌های شناسایی شده، گونه *Neodolichorhynchus judithae* برای اولین بار از ایران گزارش می‌شود. این‌گونه به‌واسطه داشتن سر متمایز از بدن، کوتیکول با ۱۶ شیار طولی، استایلت ظریف به‌طول ۲۱-۲۳ میکرومتر، امتداد روده پس از مخرج و دم مخروطی با انتهای شیاردار متمایز می‌گردد.

واژگان کلیدی: استان اردبیل، مرتع، گزارش جدید، مشخصات ریخت‌شناسی، Tylenchina