J. Crop Prot. 2014, 3 (Supplementary): 691-709

Research article

Morphological and molecular characterization of three species of the genus *Pratylenchoides* Winslow, 1958 (Tylenchina, Merliniidae, Pratylenchoidinae) from Iran

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Abstract: Two species of *Pratylenchoides* recovered from the grasslands in Sabalan region and one species recovered from natural habitats of Tehran are illustrated based on morphological, morphometric and molecular characters. The first species, *P. crenicauda* is characterized mainly by its lip region with three-four annuli, lateral field with four incisures areolated throughout the length and having rod shaped sperm cells. It is further distinguished by the positions of the pharyngeal glands nuclei. *P. magnicauda* was found in Tehran and its morphological characters and phylogenetic relations with other species are discussed. The Iranian populations of *P. variabilis* are characterized by three lip annuli, stylet 20-22 µm long, four and six incisures in lateral field, rounded sperm and one of the pharyngeal glands nuclei located posterior to pharyngo-intestinal valve. The phylogenetic tree inferred from the partial sequences of D2-D3 segment of 28S rDNA revealed the three sequenced species are separate from each other and form a clade with high (1.00) Bayesian posterior probability (BPP) in Bayesian inference (BI) and 86% bootstrap support value (BS) in maximum likelihood (ML) analyses with other two sequenced species of the genus for this genomic region.

Keywords: Bayesian, maximum likelihood, Merliniidae, phylogeny, *Pratylenchoides crenicauda*, *P. magnicauda, P. variabilis*, Sabalan grasslands, Tehran, 28S rDNA

Introduction

The genus *Pratylenchoides* was erected by Winslow (1958), with *P. crenicauda* as type species. The list of the 24 valid species for this genus is given in Siddiqi, (2000). Since then, Ryss and Sturhan (2001) have described three species from Germany and Shao-Sheng and Su-Ling (2003) have described an additional species from China.

Based on the presence of deirids, the lateral field with six incisures and a distinctively thick cuticle in the tail terminus, Ryss (1993) transferred the genus *Pratylenchoides* to the family Merliniidae Siddiqi, 1971. Siddiqi (2000) placed the genus within the subfamily Radopholinae Allen & Sher, 1967, family Pratylenchidae Thorne, 1949. In a recent study, Sturhan (2012) placed the genus in a newly established subfamily Pratylenchoidinae Sturhan, 2012 within the family Merliniidae, a monotypic taxon. Phylogenetic analyses based on 18S rDNA, showed *Pratylenchoides* as forming monophyletic groups (clades) with members of Merliniinae Siddiqi, 1971 (Bert *et al*., 2008; Carta *et al.*, 2010;

Handling Editor: Vahe Minassian

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Received: 5 April 2014, Accepted: 25 July 2014
Published online: 1 August 2014

Holterman *et al*., 2009; van Megen *et al*., 2009). All previous phylogenetic studies focused on the genus *Pratylenchoides* were performed using the SSU rDNA. Recently, Majd Taheri *et al*. (2013) studying phylogenetic relationships among some pratylenchids from Iran, showed two species of *Pratylenchoides*, one species of *Amplimerlinius* Siddiqi, 1976 and one species of *Nagelus* Thorne & Malek, 1968 to form a fully supported clade. Till date, Pourjam *et al*. (2000) have reported *Pratylenchoides ritteri* Sher, 1970 and Majd Taheri *et al*. (2013) have reported *P. alkani* Yüksel, 1977 from Iran. One population of the genus, similar to *P. variabilis* is reported from West Azarbaijan (Ghaderi *et al*., 2014). The species *P. crenicauda* Winslow, 1958 is also reported in the latter study (Ghaderi *et al.*, 2014). There are two other reports in Persian (Farsi) by Ghahremani Nejad *et al*. (2012) and Hassanzadeh *et al*. (2005) reporting *P. magnicauda* (Thorne, 1935) Baldwin, Luc & Bell, 1983 and *P. leiocauda* Sher, 1970, respectively, from Iran.

The aims of the present research were to study the morphological and morphometric characters as well as the phylogenetic position of the three recovered species as inferred by the analyses of the D2-D3 domain of 28S rDNA.

Materials and Methods

Soil samples were collected from natural habitats near north western cities of Iran and Tehran province. The nematodes were extracted from soil using the tray method (Whitehead and Hemming, 1965) and then handpicked under a Nikon SMZ1000 dissecting microscope. The nematode specimens were heat killed by adding boiling 4% formalin solution and transferred to anhydrous glycerin according to De Grisse (1969). Measurements and drawings were performed using a drawing tube attached to a Nikon E600 light microscope. Photographs were taken using an Olympus DP72 digital camera attached to an Olympus BX51 microscope with differential interference contrast (DIC). Cross sections were prepared according to Atighi *et al*. (2013). The ratios and the morphometric symbols used in morphometric tables are according to Siddiqi (2000).

For the molecular study, a single nematode specimen (two isolates of the population with six lateral lines and two isolates of the population with four lateral lines in lateral fields of *P. variabilis*, one individual for *P. crenicauda* and one individual of *P. magnicauda*) was selected, observed in a drop of clean water (a temporary slide was made for each individual), transferred to a small drop of AE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0, QIAGEN Inc., Valencia CA, USA) on a clean slide and squashed using a clean slide cover glass. The suspension (DNA sample) for each individual was retrieved by adding 30 µl AE buffer and stored at -20 °C until later processed as PCR templates. Primers used for the amplification of D2-D3 domain were D2a (5'ACAAGTACCGTGAGGGAAAGT 3') and D3b (5'TGCGAAGGAACCAGCTACTA3') (Nunn, 1992). The 30 µl PCR mixture contained: 16.5 µl distilled water, 3 µl 10 × PCR buffer, 0.6 µl dNTP mixture, 1.2 µl 50 mM $MgCl_2$, 1.5 µl of each primer (10 pmoles/µl), 0.75 µl of Taq DNA polymerase (CinaGen, Tehran, Iran, 5 u/µl) and 5 µl of DNA template. The thermal cycling program was as follows: an initial denaturation at 95 ºC for 5 min, followed by 35 cycles of denaturation at 94 ºC for 30 s, annealing at 55 ºC for 30 s, and extension at 72 ºC for 1 min. A final extension was performed at 72 ºC for 10 min. The PCR products were sequenced in both directions using the same PCR primers using an ABI 3730XL sequencer (Bioneer Corporation, South Korea). Sequences produced in the present study can be consulted on GenBank database with accession numbers as follow: *P. crenicauda*: KC843487, *P. magnicauda*: KF026289, *P. variabilis* isolate 1 of the population with six lateral lines: KC843484, isolate 2: KC843483, *P. variabilis* isolate 1 of the population with four lateral lines: KC843486 and isolate 2: KC843485.

Additional DNA sequences of related taxa available in GenBank were selected using the BLAST homology search program and aligned with recently obtained sequences using Clustal X2 (http://www.clustal.org/). The model of base substitution (GTR + G + I) was selected using

MrModeltest 2 (Nylander, 2004) according to the Akaike criterion. Bayesian analysis was performed to confirm the tree topology using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) running the chain for one million generations. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analyses. The Markov Chain Monte Carlo (MCMC) method within a Bayesian framework was used to estimate the BPP of the phylogenetic trees (Larget and Simon, 1999) using the 50% majority rule. For maximum likelihood analysis, the same dataset as for the Bayesian tree was used and it was analyzed using raxmlGUI version 1.1 (Silvestro and Michalak, 2011) using the same model of nucleotide substitution (GTR + G + I) as in the previous analysis. For both BI and ML methods, *Aphelenchus avenae* (JQ348400) was used as outgroup.

***Pratylenchoides crenicauda* Winslow, 1958**

(Figs 1 & 2)

MEASUREMENTS

See Table 1.

DESCRIPTION

Female

Body slender, slightly tapering towards both ends, slightly curved ventrally when heat relaxed. Cuticle *ca.* 2 µm thick at vulva, clearly annulated, annuli 2.0-2.5 µm wide at mid-body. Lateral field with four incisures, areolated throughout the body length (Fig. 2, K-M). Lip region continuous with body contour, flattened anteriorly and bearing three-four distinct annuli. Stylet robust with rounded and posteriorly sloping knobs. Procorpus wide, *ca.* 3.5 times stylet length, median bulb large with refractive valves, isthmus slender, 30-37 µm long, encircled by nerve ring at level ranging from the anterior third to middle of isthmus, pharyngeal glands with three nuclei; glands overlapping intestine dorsally for 9-15 annuli or 0.7-1.5 times body width. The nuclei of dorsal and one subventral glands located anterior to pharyngo-intestinal valve, the other subventral gland nucleus located posterior to the intestinal valve. Deirid position at the level of hemizonid or slightly posterior. Reproductive system didelphic, amphidelphic with both genital branches equally developed, anterior branch 173-283 µm long, posterior one 136-314 µm long, vulva slightly posterior to mid-body, vagina with internal walls slightly sclerotised, 13-16 µm long, ovaries straight, spermatheca functional, rounded, filled with bacilliform sperm (the rounded shape of sperm is due to vertical position of sperm cells and observing the transverse section of them, see Fig. 2D). Phasmid 13-17 annuli (30-35 µm) posterior to the anus. Tail subcylindrical, bearing 25-37 annuli, its terminus annulated.

Male

Common, almost as abundant as female. General morphology similar to that of female except for slightly shorter body and reproductive system. Testis single, anteriorly outstretched with bacilliform sperm in proximal zone. Spicules tylenchoid, *ca.* 7.5 times longer than wide, and ventrally curved. Gubernaculum distinct. Bursa 65-90 µm long with crenate edge, enveloping tail. Phasmid distinct and occurring on the tail at the mid-region or posterior half.

REMARKS

This population was recovered from soil samples collected about the rhizosphere of grasses (not identified) in Sabalan grasslands, Meshkinshar, Ardebil province, northwest Iran. In one examined female, four pharyngeal nuclei were observed (see Fig. 2, I & J). Iranian population of *P. crenicauda* has a slightly longer body compared to the range for body length of the original data (768-1001 *vs* 570-910 µm), range given by Sher, 1970 (768-1001 *vs* 530-860 µm) and to the body length of syntypes given by Siddiqi, 1974 (768-1001 *vs* 530-630 µm). By having a longer body, the two indexes a and b have also greater ranges (Table 1). The most remarkable difference between the data of Iranian population and the data in other reports corresponds to the range of the index c′. Iranian population has a greater range for this index compared to the range given by Siddiqi, 1974 and to the syntypes (2.6-4.3 *vs* 2.0-2.3). On the other hand, Castillo and Gomez Barcina, 1988 reported the c′ value equal to 2.8 ± 0.3 for the studied population of *P. crenicauda* from Spain. Unfortunately, in their work, the maximum value of c′ is not correct (2.6-2.4), a typing error, but the

minimum value (2.6) shows that the range of this index for both Iranian and Spanish populations are similar. Both populations have a close range for tail length (46-62 µm in Iranian population and 39-50 µm in Spanish population). Present study shows the variability of index c′ for this species.

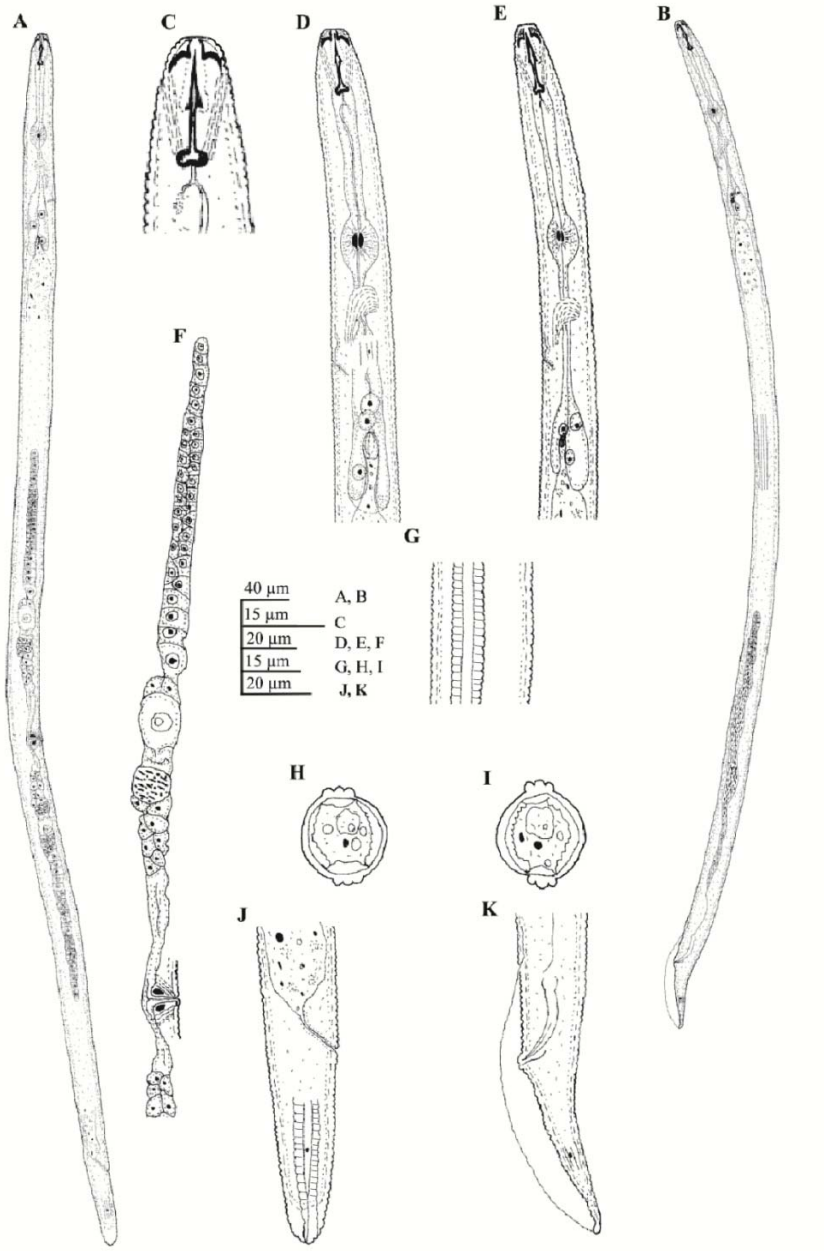

Figure 1 *Pratylenchoides crenicauda*. A: Female entire body, B: Male entire body, C: Female anterior end in detail, D & E: pharyngeal region showing the position of the pharyngeal glands nuclei, F: Part of female reproductive system, G: Lateral field of female, H & I: Cross sections, J & K: Female and male posterior end, respectively.

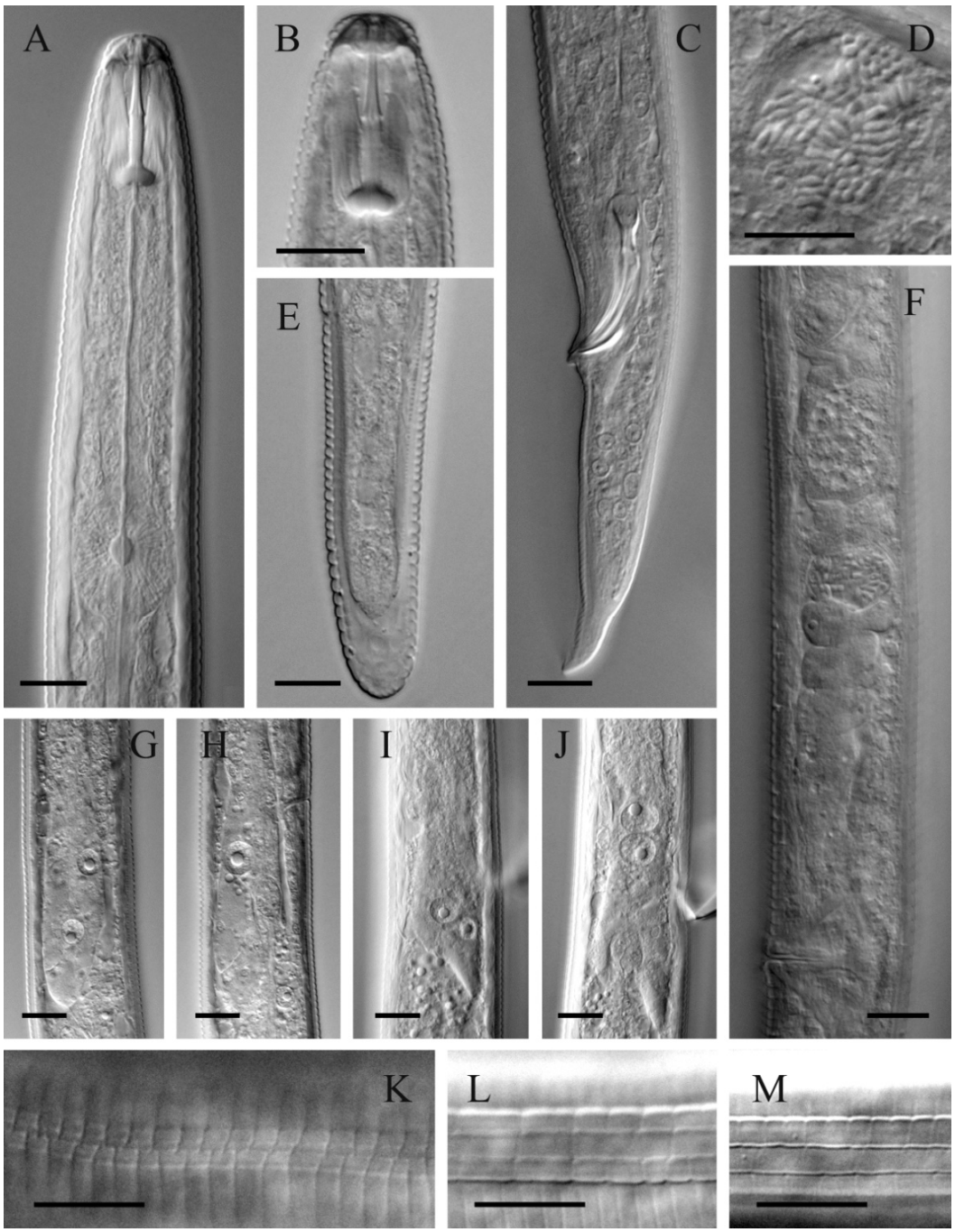

Figure 2 *Pratylenchoides crenicauda*. A: Female anterior part, B: Anterior end in detail, C: Male posterior end, D: Bacilliform sperm in female spermatheca, E: Female tail, F: Part of female reproductive system, G & H: Common pharyngeal glands nuclei position, I & J: Exceptional number of pharyngeal nuclei (4) observed in one female only, K-M: Areolated lateral line in anterior end (K), mid-body (L) and tail (M) regions. All scale bars = 10 µm.

Table 1 Morphometrics of *Pratylenchoides crenicauda* from Iran and the data from other report.

Characters	Iran, Sabalan grasslands		Ghaderi, 2014	Winslow, 1958		Sher, 1970		Siddiqi, 1974 (syntypes)	Castillo and Gomez Barsina, 1988	
	Female	Male	Female	Female	Male	Female	Male	Female	Female	Male
n	15	9	9	24	6	15	4	5	9	4
L	898 ± 74 (768 - 1001)	764.5 ± 55.4 (675 - 841)	604 - 787	570 - 910	640 - 740	640 (530 - 860)	660 (610 - 720)	530 - 630	668.0 ± 77.6 (574 - 797)	516.0 ± 68.2 (431 - 595)
a	35.9 ± 2.8 (32.2 - 43.4)	35.8 ± 1.4 (33.5 - 38.0)	21 - 31	19 - 32	26 - 33	25 (21 - 29)	–	24 - 28	28.5 ± 1.5 (26.4 - 31.3)	30.3 ± 3.2 (26.9 - 34.3)
b	5.6 ± 0.3 (5.1 - 6.0)	5.6 ± 0.4 (5.1 - 6.1)	4.8 - 5.8	3.3 - 6.4	4.5 - 6.2	4.6 (4.1 - 5.2)	–	4.4 - 5.2	4.2 ± 0.6 (3.6 - 5.6)	4.4 ± 0.6 (3.7 - 5.0)
b′	4.6 ± 0.3 (3.9 - 5.3)	4.9 ± 0.4 (4.6 - 5.7)		–	–	4.2 (3.5 - 5.2)	5.5 (5.2 - 5.7)	4.0 - 4.3	4.3 ± 0.06 (4.2 - 4.3)	-
c	16.9 ± 1.4 (14.9 - 20.3)	14.7 ± 1.1 (12.7 - 16.1)	14 - 19	12.9 - 17.6	11.7 - 15	15 (13 - 18)	–	16 - 18	14.3 ± 1.1 (12.9 - 16.5)	11.5 ± 1.2 (10.0 - 12.8)
c′	3.0 ± 0.4 (2.6 - 4.3)	3.2 ± 0.3 (2.8 - 4.0)	2.0 - 3.4	–	–	–	–	2.0 - 2.3	2.8 ± 0.3 (2.6 - ?)	3.4 ± 0.3 (3.1 - 3.8)
V or T	56.7 ± 0.9 (54.6 - 57.9)	36.6 ± 4.0 (30 - 43)	55 - 59	54 - 61	–	58 (56 - 62)	–	56 - 60	57.0 ± 1.6 (54 - 59)	35.0 ± 4.2 (31 - 40)
Stylet	21.1 ± 0.8 (20 - 22)	19.9 ± 0.8 (18 - 21)	19 - 22	–	–	22 (20 - 23)	22 (20 - 24)	19.5 - 21.0	20.0 ± 0.5 (19 - 21)	17.0 ± 0.7 (16.6 - 18.0)
MB	52.8 ± 2.1 (49.1 - 56.9)	52.9 ± 3.9 (49.3 - 62.2)		–	–	–	–	46 - 49	52.0 ± 0.8 (51 - 53)	56.0 ± 0.7 (56 - 57)
Head to ex. pore[1]	136.0 ± 8.9 (124 - 153)	118.0 - 9.6 (102 - 133)		–	–	–	–	–	103.0 ± 19.8 (83 - 125)	95.0 ± 3.2 (91 - 98)
Pharynx[2]	160.0 ± 10.3 (137 - 172)	138.0 ± 8.6 (119 - 146)		–	–	–	–	–	157.0 ± 9.4 (142 - 170)	117.0 ± 3.7 (114 - 122)
Pharyn. overlapping[3]	33.6 ± 7.7 (20 - 46)	18.3 ± 6.1 (9 - 26)		–	–	–	–	–	-	-
Head to vulva	509.0 ± 42.3 (437 - 573)	–		–	–	–	–	–	-	-
Max. body width	25.2 ± 3.1 (18 - 28)	21.3 ± 1.2 (19 - 23)		–	–	–	–	–	23.5 ± 3.1 (19 - 29)	17.0 ± 0.7 (16 - 17)
Vulva – anus	338.0 ± 33.3 (280 - 389)	–		–	–	–	–	–	-	-
Tail	53.4 ± 5.3 (46 - 62)	52.3 ± 5.2 (45 - 64)		–	–	–	–	–	47.0 ± 6.7 (36 - 57)	45 ± 5 (39 - 50)
Tail annules	30.0 ± 3.7 (25 - 37)	–	23 - 37	–	–	28 - 36	–	–	29.0 ± 2.2 (27 - 32)	-
Spicule	–	24.6 ± 0.9 (23 - 26)		–	–	–	22 (20 - 24)	–	-	22.0 ± 1.4 (20 - 23)
Gubernaculum	–	7.7 ± 0.5 (7 - 8)		–	–	–	6 (4 - 7)	–	-	5.7 ± 0.4 (5.5 - 6.2)

All measurements are in µm and in the form: mean ± s.d. (range), [1]Head to excretory pore, [2]Anterior end to Pharyngo - intestinal valve, [3]Pharyngeal overlapping.

***Pratylenchoides magnicauda* (Thorne, 1935) Baldwin, Luc & Bell, 1983**

(Fig. 3)

MEASUREMENTS

See Table 2.

DESCRIPTION

Female

Body slender, slightly tapering towards both ends, straight to slightly ventrally curved when heat relaxed. Cuticle *ca.* 1.5-2.0 µm thick at vulva, clearly annulated, annuli 1.5-2.0 µm wide at mid-body. Lateral field with six lines, areolated throughout the length (Fig. 3, D & G). Lip region continuous with body contour, bluntly conoid in end and bearing five distinct annuli. Stylet robust with rounded knobs, slightly sloping posteriorly. Procorpus *ca.* 2.6-3.0 times

stylet length, median bulb large with refractive valves, isthmus slender, 35-50 µm long, encircled by nerve ring, pharyngeal glands with three nuclei; glands overlapping intestine dorsally for 5-10 annuli or 0.2-0.5 times body width. The nuclei of dorsal and two subventral glands located anterior to pharyngo-intestinal valve. Deirid position at the level of hemizonid or slightly posterior. Reproductive system didelphic, amphidelphic with both genital branches equally developed, anterior branch 166-287 µm, posterior one 169-243 µm long, vulva slightly posterior to mid-body, vagina with slightly sclerotised internal walls, 10-14 µm long, ovaries straight, spermatheca small, round to oval, devoid of sperm. Phasmid 12-20 annuli (23-30 µm) posterior to anus. Tail subcylindrical, bearing 27-36 annuli, its terminus annulated.

Male

Not found.

REMARKS

Thorne (1935) and Allen (1955) reported four lines in lateral field of *P. magnicauda*. Siddiqi (1976) reexamined Thorne's material and reported six lines in lateral field, reducing to four posteriorly. Loof (1971) observed four lines too. Baldwin *et al*. (1983) examined the holotype and found that only four of the six lines in lateral field were visible, due to sublateral position of the nematode in the slide. The population of the present study has six distinct lines in lateral field, areolated throughout the length in accordance with the observation of Baldwin *et al*. (1983) on a population from Utah (USA). Present population was recovered from muddy soil samples collected in the rhizosphere of grasses (not identified) in village of Ahar, Tehran province, Iran.

***Pratylenchoides variabilis* Sher, 1970**

(Figs 4-7)

MEASUREMENTS

See Tables 3.

DESCRIPTION

Female

Body slender, slightly ventrally curved after heat relaxation. Cuticle 1.5-2.0 µm thick at midbody, distinctly annulated, annuli 1.8-2.0 µm wide at midbody. Lateral field with four or six (in two separate populations) incisures, outer lines crenated, becoming irregularly areolated in tail. In transverse dissections, the lateral lines protrude in the population with four lines (Figs 4F, 5G), but are smooth in the population with six lines (Figs 6G, 7F). Lip region continuous with body contour, flattened anteriorly with three annuli. Stylet robust with large, rounded knobs, sloping slightly posteriorly, stylet conus about as long as the shaft. The dorsal gland orifice (DGO) 2-4 µm posterior to stylet knobs. Procorpus muscular, 1.5-1.8 times longer than stylet, median bulb large with refractive valves, isthmus narrow and slender, pharyngeal glands overlapping intestine dorsally about 0.5-1.5 times corresponding body diameter, two glands nuclei anterior and one gland nucleus posterior to the pharyngo-intestinal valve. In examined specimens, some individuals had three nuclei located anterior to the valve. Hemizonid usually slightly posterior to nerve ring and excretory pore 0-2 annuli posterior to hemizoind. Deirid often at the level of the hemizoid. Reproductive system didelphic, amphidelphic with both genital branches equally developed; anterior branch 119-220 µm long, posterior branch 122-199 µm long, ovaries straight, spermatheca rounded, axial, filled with rounded sperm, vulva posterior to midbody, vagina with slightly sclerotised lining, 12-16 µm long. Phasmid at almost mid level of the tail. Tail varying in shape from cylindrical to subcylindrical bearing 20-30 annuli and with rounded to truncate terminus.

Male

Not found.

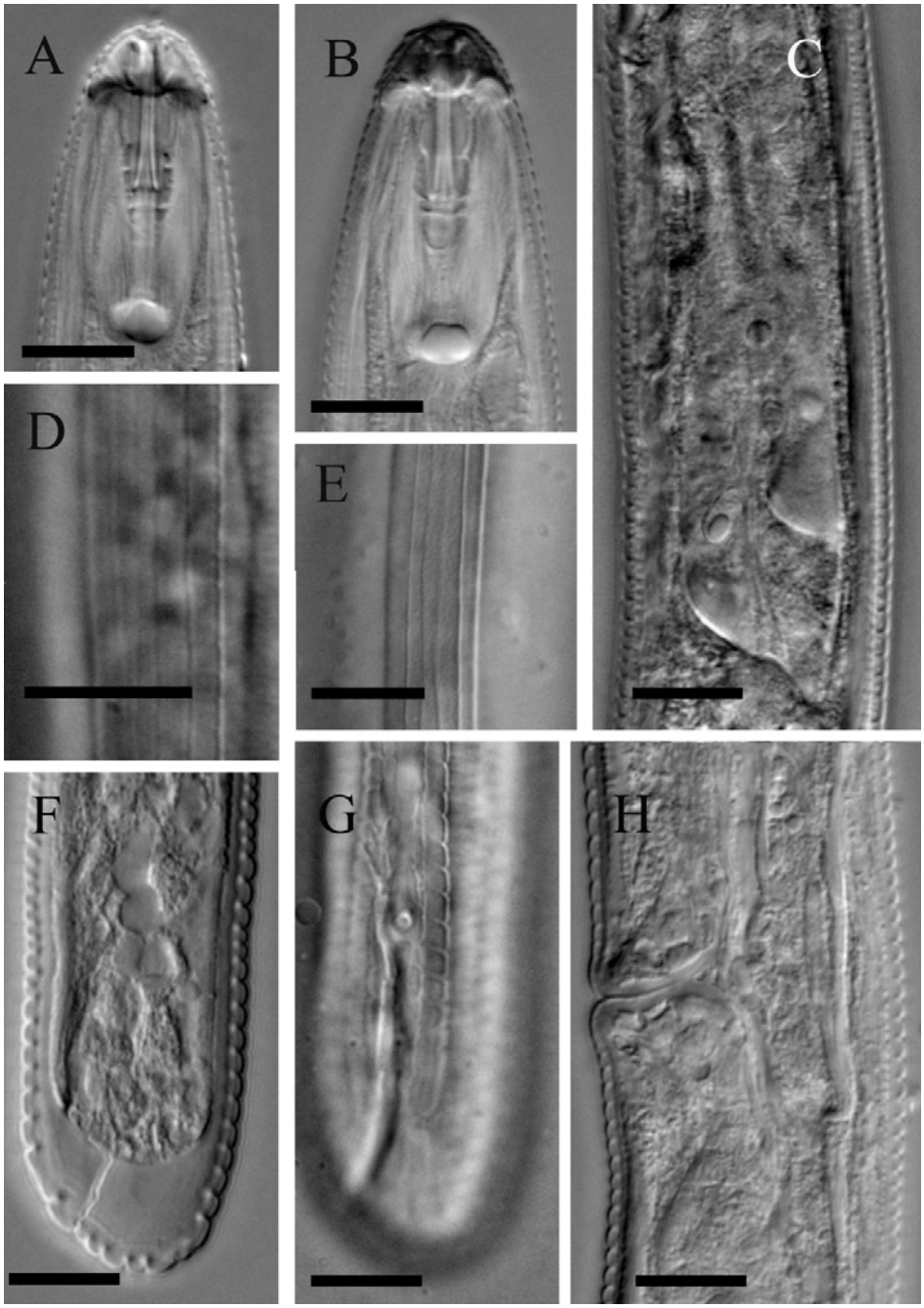

Figure 3 *Pratylenchoides magnicauda*. A & B: Anterior end in detail, C: Short overlapping of pharyngeal glands and cardia, D: Areolation in mid-body, E: Lateral lines, F: Tail, G: Areolation in tail and phasmid, H: Vagina. All scale bars = 10 µm.

Table 2 Morphometrics of *Pratylenchoides magnicauda* female, from Iran and the data from other report.

Characters	Iran, Tehran	Ghahremani Nejad, 2012	Allen, 1955	Loof, 1971	Baldwin *et al*., 1983	Brzeski, 1998
n	10	4	10	9	20	–
L	859 ± 70 (769-984)	804 ± 99 (697-903)	790-1000	680-920	890 ± 40 (750-1070)	560-1100
a	31.7 ± 2.9 (27.8-35.8)	34.2 ± 2.0 (32-36.5)	23-32	25-30	30.7 ± 1.2 (26.0-36.9)	24-37
b	4.5 ± 0.3 (4.1-5.0)	4.2 ± 0.4 (4-4.5)	4.4-6.0	4.0-4.7	–	3.1-5.3
b´	4.3 ± 0.3 (3.9-4.7)	–	–	–	4.7 ± 0.2 (3.7-5.1)	–
c	15.9 ± 1.4 (14.3-18.6)	16.0 ± 3.2 (13.5-20.5)	13-19	15-18	16.6 ± 0.8 (14.6-20.5)	13-23
c´	2.8 ± 0.2 (2.5-3.1)	3.1 ± 0.5 (2.4-3.5)	2.5	2.1-2.8	2.4 ± 0.2 (1.8-3.1)	1.7-3.4
V	59.2 ± 2.5 (54.4-62.7)	60.6 ± 1 (59-62)	56-62	57-61	61.0 ± 0.8 (58-64)	54-66
Stylet	27.2 ± 0.6 (26-28)	25.0 ± 0.3 (24.5-25.5)	26.7-29.7	25-28	32 ± 0.6 (29.5-34.0)	25-34
MB	48.0 ± 1.7 (46.3-52.1)	–	–	–	49.5 ± 1.9 (35-53)	–
Head-ex. pore[1]	136 ± 8 (128-155)	–	–	–	–	–
Pharynx[2]	190 ± 13 (169-205)	189 ± 7 (180-197)	–	–	–	152-212
Pharyn. overlapping[3]	9.6 ± 3.0 (5-13)	–	–	–	–	–
Head-vulva	508 ± 27 (469-562)	–	–	–	–	–
Max. body width	27.3 ± 2.9 (22-30)	23.5 ± 2 (21-25.5)	–	–	–	–
Vulva–anus	291 ± 36 (245-353)	–	–	–	–	–
Tail	54.0 ± 3.1 (49-58)	50.8 ± 6.2 (42.5-57)	–	–	54.0 ± 3.6 (40.5-71.0)	35-71
Tail annules	31.6 ± 3.0 (27-36)	33	–	34-42	–	–

All measurements are in µm in the form: mean ± s.d. (ranges), [1]Head to excretory pore, [2]Anterior end to pharyngo-intestinal valve, [3]Pharyngeal overlapping.

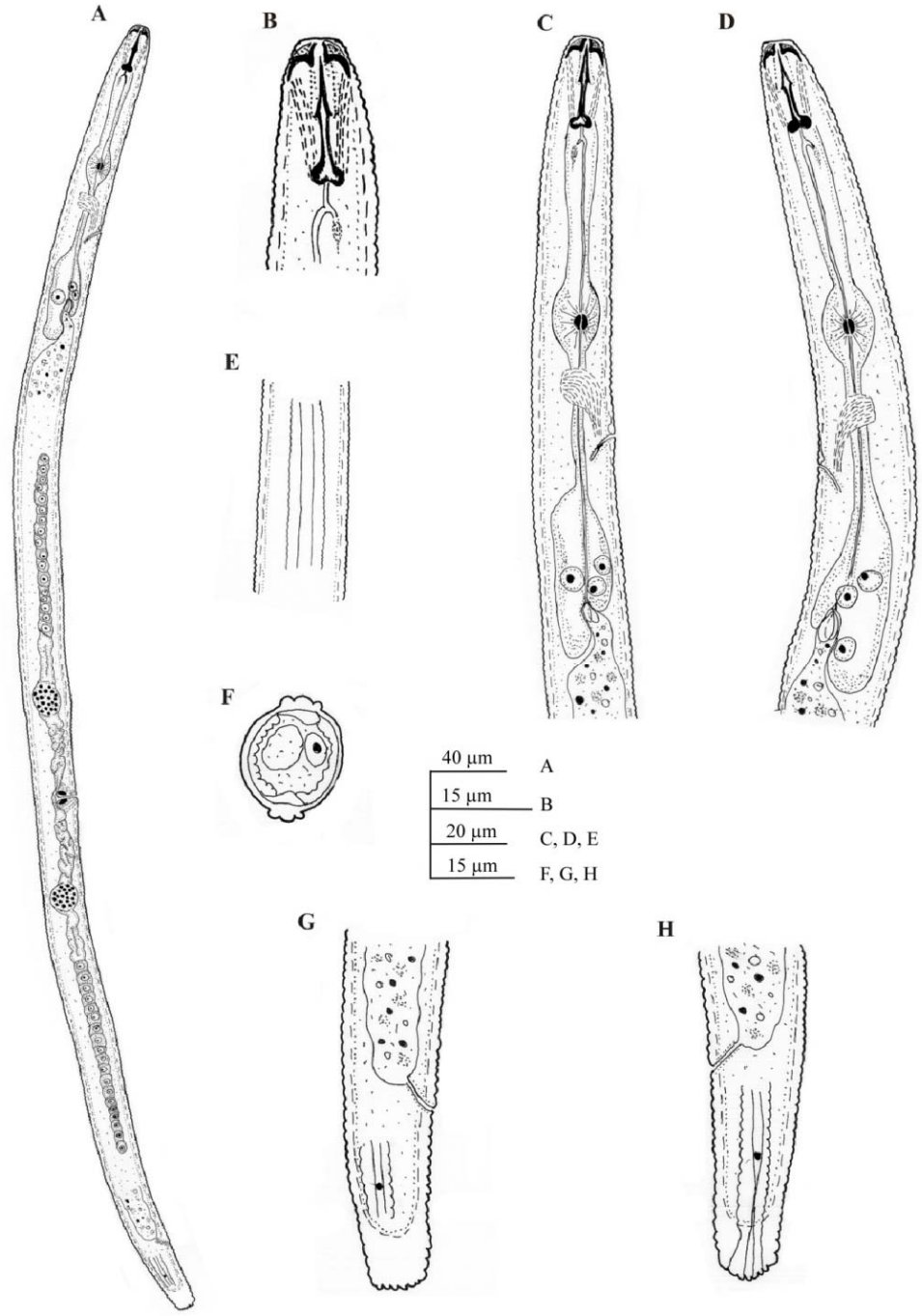

Figure 4 *Pratylenchoides variabilis* (the population with four lateral lines, line drawings). A: Entire body, B: Anterior end, C & D: pharyngeal region showing the variation in position of the pharyngeal glands nuclei, E: Lateral lines, F: Cross section, G & H: Tail.

Table 3 Morphometrics of *Pratylenchoides variabilis* from Iran and the data from other reports.

Characters	Iran, Sabalan (the population with four lateral lines)	Iran, Sabalan (the population with six lateral lines)	Ghaderi, 2014	Bernard, 1984	Sher, 1970
	Female	Female	Female	Female	Female
n	11	17	5	16	20
L	633.5 ± 43.1 (552-688)	572.4 ± 48.1 (481-651)	573-665	554 (475-671)	580 (500-660)
a	26.7 ± 1.6 (24.1-30.0)	25.4 ± 2.0 (19.2-27.7)	26-29	30.4 (26-34)	30 (26-32)
b	4.3 ± 0.3 (3.9-4.8)	4.2 ± 0.3 (3.8-4.9)	4.5-5.0	4.4 (3.9-5.1)	3.9 (3.5-4.4)
b´	3.8 ± 0.3 (3.3-4.3)	3.7 ± 0.3 (3.3-4.3)	–	4.0 (3.6-4.9)	3.4 (3.1-4.4)
c	17.6 ± 2.3 (13.5-20.6)	17.7 ± 1.4 (14.4-19.7)	16-20	15.1 (14-17)	16 (13-19)
c´	2.2 ± 0.2 (1.9-2.5)	2.1 ± 0.3 (1.7-2.7)	2.2-2.5	2.9 (2.4-3.2)	–
V	60.9 ± 1.4 (59.1-63.8)	59.7 ± 2.5 (55.4-64.6)	58-61	58 (52-61)	58 (56-61)
Stylet	20.6 ± 0.7 (20-22)	19.9 ± 1.2 (17-22)	19-21	21 (20-22)	22 (21-24)
MB	49.7 ± 2.6 (46.2-55.3)	50.5 ± 3.3 (42.4-54.6)	–	–	–
Head-ex. pore[1]	108.0 ± 6.1 (99-118)	101 ± 7 (95-126)	–	–	–
Pharynx[2]	146.5 ± 12.2 (114-160)	136.0 ± 9.7 (119-161)	–	–	–
Pharyn. overlapping[3]	18.5 ± 5.9 (10-29)	17.9 ± 3.5 (10-24)	–	–	–
Head-vulva	386 ± 31 (329-439)	341.5 ± 27.3 (295-382)	–	–	–
Max. body width	23.8 ± 1.9 (21-27)	22.6 ± 1.6 (20-25)	–	–	–
Vulva - anus	210.0 ± 14.1 (185-227)	195.8 ± 27.9 (150-242)	–	–	–
Tail	36.5 ± 5.0 (27-44)	32.5 ± 3.8 (25-42)	–	–	–
Tail annuli	25.0 ± 2.4 (20-30)	21.3 ± 3.1 (20-26)	22-29	–	24 (21-27)

All measurements are in µm in the form: mean ± s.d. (ranges), [1]Head to excretory pore, [2] Anterior end to pharyngo-intestinal valve, [3]Pharyngeal overlapping.

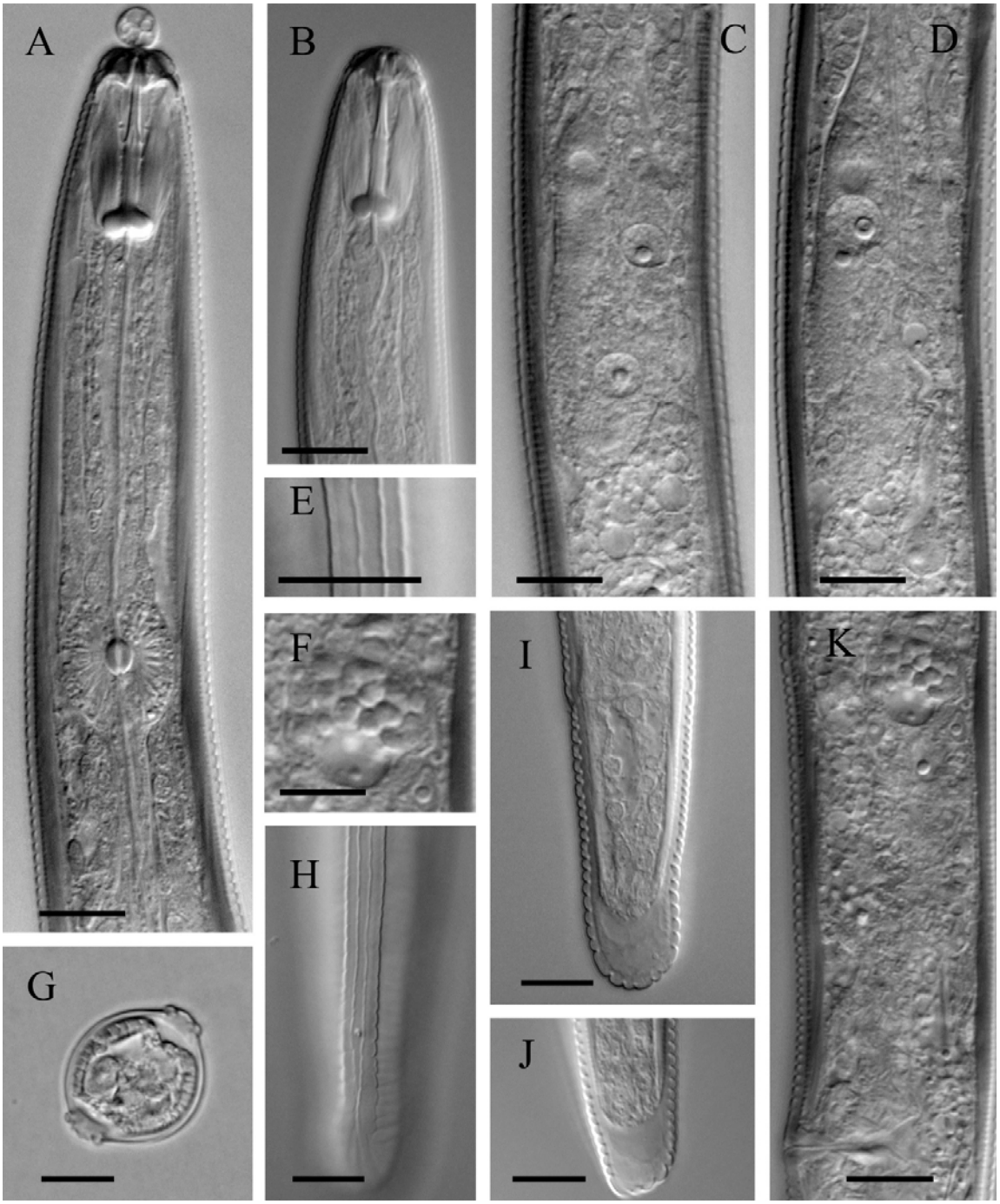

Figure 5 *Pratylenchoides variabilis* (the population with four lateral lines, LMs). A: Anterior part, B: Anterior end, C & D: Position of the pharyngeal glands nuclei, E: Lateral lines, F: Rounded sperm in female spermatheca, G: Cross section, H: Lateral lines and phasmid, I & J: Tail and variation in the shape of its end, K: Part of female reproductive system. All scale bars = 10 µm.

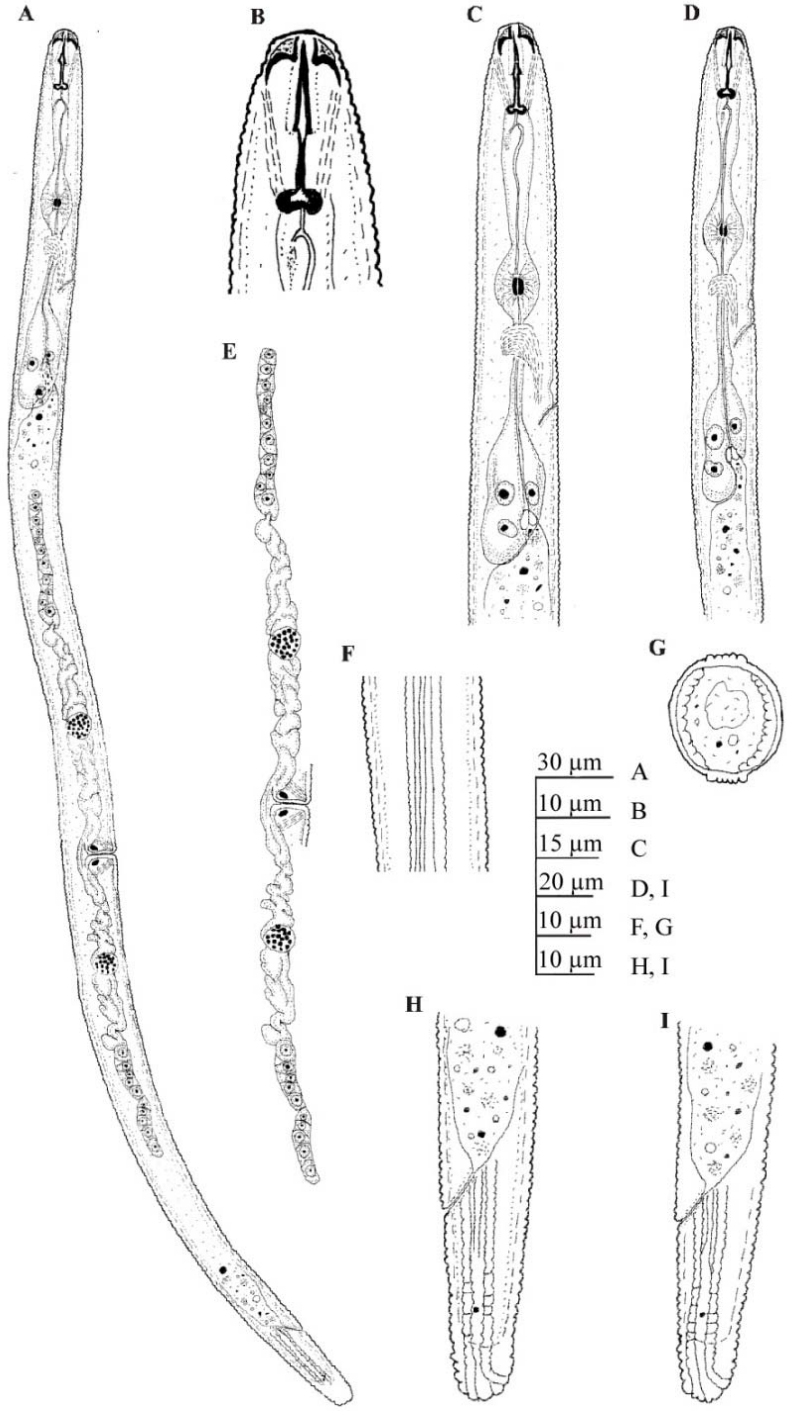

Figure 6 *Pratylenchides variabilis* (the population with six lateral lines, line drawings). A: Female entire body, B: Anterior end, C & D: pharyngeal region, E: Reproductive system, F: Lateral lines, G: Cross section, H & I: Tail.

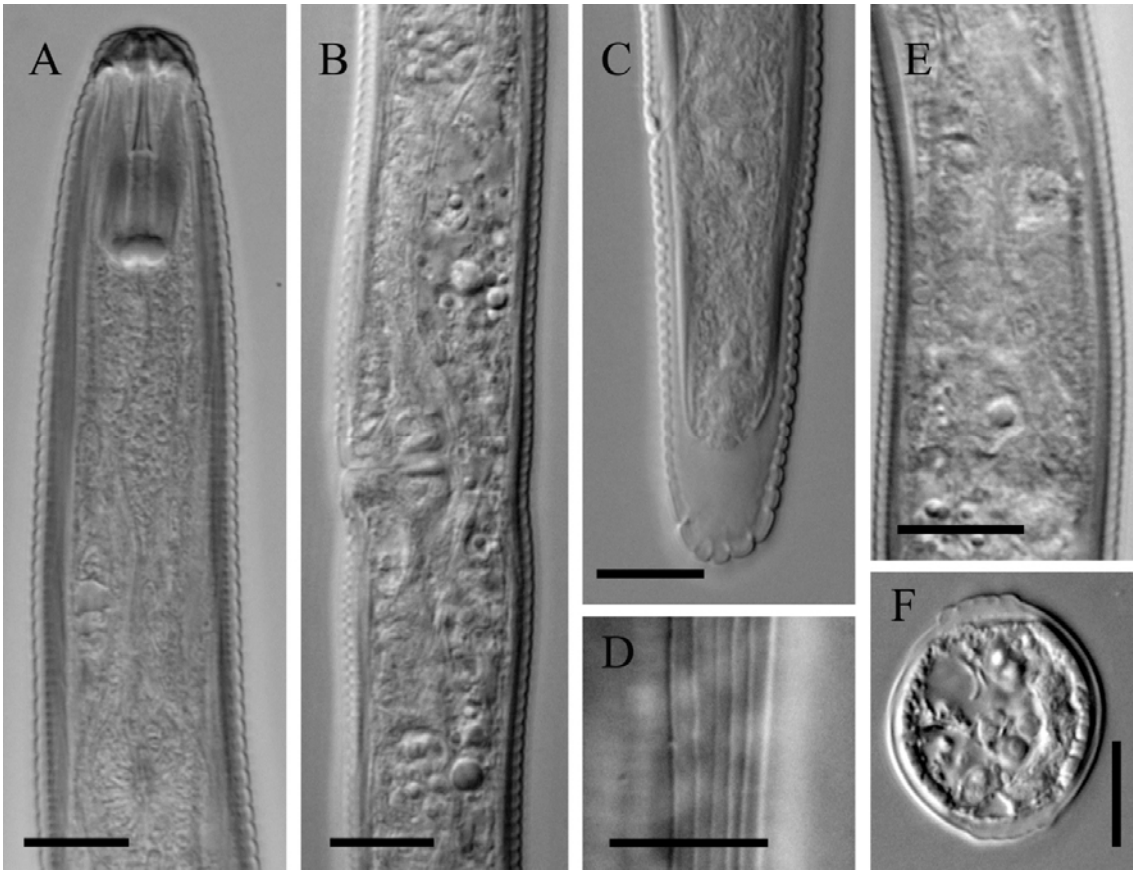

Figure 7 *Pratylenchoides variabilis* (the population with six lateral lines, LMs). A: Anterior part, B: Part of female reproductive system, C: Tail, D: Lateral lines, E: pharyngeal glands nuclei, F: Cross section showing six lateral lines. All scale bars = 10 µm.

REMARKS

During this study, two populations of *P. variabilis* were found. The first population collected from Meshkinshahr had four incisures in lateral field that were seen and confirmed in cross sections (see material and methods) as delimiting bands (alae) that protrude from the body contour (see Figs 4F, 5G). The tail terminus of this population is rounded to truncate and the tail lateral field lacks areolation. In a second population collected from Meshkinsahr, the lateral field had six incisures, that were seen in cross section as delimited bands that do not protrude from the body contour (see Figs 6G, 7F). The tail terminus of this population is rounded and the tail lateral field is irregularly areolated. With respect to morphometrics, these two populations were fully congruent with each other. In the original description of *P. variabilis*, it was noted that lateral field has four or six incisures; however, no observations from cross sections were reported. In a Canadian population of this species, reported by Bernard (1984), a drawing of the cross section from a four-lined population shows protruding bands. Our morphological study of two populations clearly demonstrates this species is surprisingly variable with respect to some morphological features (*i.e.* number of lateral lines and position of pharyngeal glands nuclei). The two Iranian populations of *P. variabilis* are morphologically and morphometrically congruent with the original description and the characters of the Canadian population (Bernard, 1984).

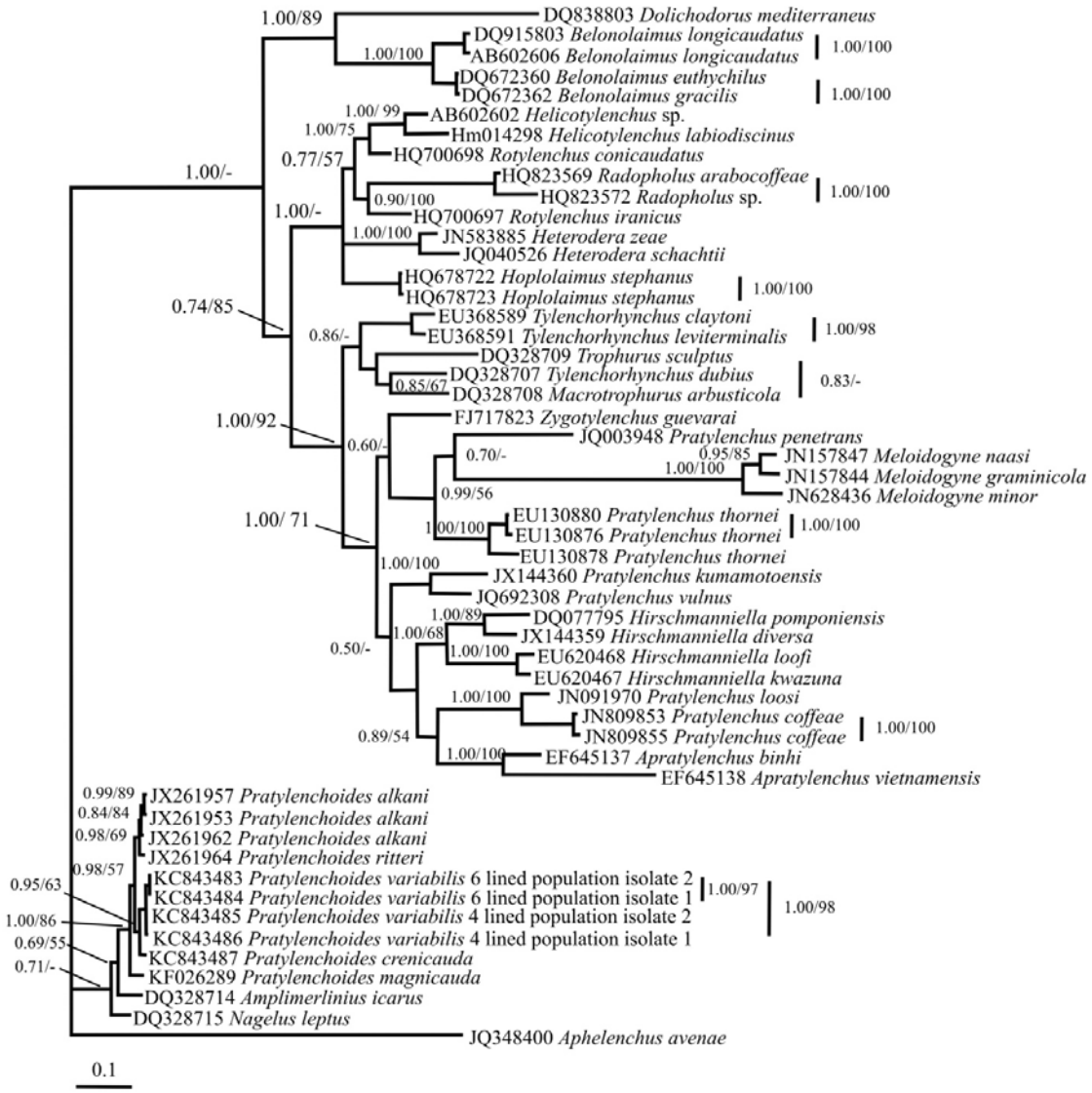

Figure 8 Bayesian 50% majority rule consensus tree inferred from 49 sequences of the D2-D3 domains of the 28S rDNA under the GTR + I + G model. BPP and ML BS values are given for each appropriate clade in the shape BPP/ML BS. The newly sequenced taxa/isolates are in bold.

Molecular characterization and phylogenetic relationships

The partial sequencing of the 28S rDNA D2-D3 segment for *Pratylenchoides crenicauda* yielded 701 bp nucleotides. It was 670 bp for *P. magnicauda*. The partial sequencing of the same segment of isolates 1 and 2 of the population of *P. variabilis* with six lateral lines yielded 713 bp nucleotides for both and no differences between the sequences of the two isolates were observed. Sequencing of the same segment of isolates 1 and 2 of the population of *P. variabilis* with four lateral lines yielded 592 bp nucleotides, after several amplifying and sequencing repeats. Again, no differences were observed between the sequences of the two

isolates. Alignment of the sequences of four isolates of *P. variabilis* yielded 592 characters (after manually editing of the alignment) with only two different nucleotides (0.33%) between the two populations varying in line number. In position 142, the population with four lateral lines had G (*vs* A in the population with six lines) and in position 190, the population with four lines had C (*vs* T in the population with six lines).

A phylogenetic tree was inferred from Bayesian analysis of a multiple alignment with 700 total characters in which 208 characters were conserved, 481 characters were variable and 392 characters were parsimony informative (Fig. 8). The BPP values are given on the clades together with the BS in ML analysis in the shape: BPP /ML BS. Values less than 50% are not indicated. The nucleotide composition of this dataset is as follow: T: 23.6%, C: 22.9%, A: 20.1%, G: 33.4%. Using *Aphelenchus avenae* (JQ348400) as outgroup, the four Iranian populations (*P. crenicauda*, *P. magnicauda*, four- and six-lateral lined populations of *P. variabilis*) form a well-supported clade with 1.00 BPP and 86% ML BS with two other sequenced species of the genus (*P. ritteri* and *P. alkani*), indicating the monophyly of the genus (based on the current information and the level of species sampled).

Discussion

From the known species of *Pratylenchoides*, three species namely *P. megalobatus* Bernard, 1984, *P. bacilisemenus* Sher, 1970 and *P. arenarius* Ryss & Sturhan, 2001 are known by having bacilliform sperm. Also, most reports of *P. crenicauda* have not discussed the morphology of sperm in detail. Sher (1970) and Geraert (2013) pointed out that the species has usually an inconspicuous or not seen spermatheca, or has irregularly rounded sperm. On the other hand, Baldwin *et al*., 1983 stated: "sperm in *P. crenicauda* are elongate and spindle shaped". Our observation is in agreement with the observation of Baldwin *et al*., 1983 and confirms that the species has rod shaped (bacilliform) sperm. The other observed variation in morphological/morphometric characters, corresponds to the index c′ that has a greater range and is a new record for the species (Table 1). Finally, the nuclei of dorsal and one subventral glands of this species are located anterior to pharyngeal-intestinal valve and the other subventral gland nucleus is located posterior to the intestinal valve. In one examined female, two nuclei were observed posterior to the intestinal valve and it seems that the nucleus of one of subventral glands is doubled, again a new observation for the species.

Finding of two populations of a nematode species with variation in a morphological character, like the number of lateral lines, as observed for *P. variabilis*, at first causes to make a hypothesis of occurring a mixed population. According to the original description, *P. variabilis* is known by having individuals with four and six lines in lateral field. In our studied populations, some individuals had three nuclei located anterior to the pharyngo-intestinal valve. Beside morphological similarities and the same morphometric data ranges, two populations with four and six lateral lines had almost identical sequences of 28S rDNA D2-D3 (with only two nucleotide differences). The third species, *P. magnicauda* was also studied considering its morphological and molecular characters. The phylogenetic tree inferred from the partial sequences of D2-D3 segment of 28S rDNA, revealed the three sequenced species are separate from each other and form a clade with high (1.00) BPP in BI and 86% BS in ML analyses with other two sequenced species of the genus for this genomic region. The two genera *Nagelus* and *Amplimerlinius* form a moderately supported (0.71 BPP) clade in BI with species of the genus *Pratylenchoides*, a moderate support to the recently proposed subfamily (Pratylenchoidinae) for the genus under the family Merliniidae.

In our tree, the position of the genus *Pratylenchoides*, relative to the other genera of the family Pratylenchidae (*sensu* Siddiqi, 2000)

suggests the non-monophyly of the family. It furthermore shows a close relationship of *Pratylenchoides* with genera *Amplimerlinius* and *Nagelus*. Such a position is consistent with the placement of *Pratylenchoides* under Merliniidae by Ryss (1993). On the other hand, our phylogenetic study using the 28S rDNA D2-D3 partial sequences gave the same result reached by analyzing of the 18S rDNA by Bert *et al*. (2008), Holterman *et al.* (2009), Van Megen *et al*. (2009) and Carta *et al.* (2010), supporting the recently proposed monotypic subfamily Pratylenchoidinae by Sturhan, 2012 under the family Merliniidae.

Acknowledgements

The authors appreciate the kind help of James G. Baldwin (Department of Nematology, University of California, Riverside) who read critically and improved the manuscript. They also acknowledge the Iranian National Science Foundation (INSF) and Tarbiat Modares University (Iran) for financial supports. The kind help of Milka Elshishka for providing some old references and valuable comments of Tiago José Pereira (Ph. D. student, Department of Nematology, University of California, Riverside) are appreciated.

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مطالعه مولکولی و ریخت‌شناسی سه گونه از جنس *Pratylenchoides* Winslow, 1958 (Tylenchina, Pratylenchoidinae) از ایران

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چکیده: دو گونه از جنس *Pratylenchoides* از مراتع سبلان و یک گونه دیگر از اطراف تهران از نظر شاخص‌های ریخت‌سنجی، ریخت‌شناسی و مولکولی مورد بررسی قرار گرفتند. اولین گونه، *P. crenicauda* به‌واسطه داشتن سر متمایز از بدن با سه تا چهار شیار عرضی، سطوح جانبی با چهار شیار کاملاً مضرس، اسپرم دوکی شکل و موقعیت هسته‌های غدد مری از سایر گونه‌ها متمایز می‌گردد. گونه *P. magnicaud* از اطراف تهران جمع‌آوری شد و مشخصات ریخت‌شناسی و رابطه فیلوژنی آن با سایر گونه‌ها مورد بررسی قرار گرفت. جمعیت ایرانی گونه *P. variabilis* با داشتن سه شیار عرضی در سر، استایلت به طول ۲۲-۲۰ میکرومتر، سطوح جانبی با چهار یا شش شیار عرضی، اسپرم گرد و قرار گرفتن یکی از هسته‌های غدد مری بعد از محل اتصال مری-روده تفکیک می‌شود. درخت تبارشناسی با استفاده از توالی ناحیه D2-D3 ژن رمزگردان RNA زیرواحد بزرگ ریبوزومی ترسیم شد و مشخص شد این سه گونه از همدیگر جدا بوده و یک گروه تک‌نیا را در روش آنالیز بیس (Bayes) با احتمال پسین بالا (۱/۰۰) و بوت‌استرپ ۰/۸۶ درصد در روش maximum likelihood با دو گونه توالی‌یابی شده دیگر از این جنس تشکیل می‌دهند.

واژگان کلیدی: تبارشناسی، تهران، خانواده Merliniidae، سبلان، روش آنالیز فیلوژنی بیس، روش آنالیز فیلوژنی maximum likelihood