Morphological variation and molecular study of the root lesion nematode *Pratylenchus thornei*

Nafiseh Divsalar[1], Ebrahim Shokoohi[2], Akbar Hoseinipour[1], Hendrika Fourie[3] and Esmat Mahdikhani Moqaddam[4]

[1]Department of Plant Protection, College of Agriculture, Shahid Bahonar University of Kerman, 7616-9133, Kerman, Iran
[2]Green Biotechnologies Research Centre of Excellence, University of Limpopo, Private Bag X1106, Sovenga 0727, South Africa
[3]Unit for Environmental Sciences and Management, North West University, 2520, Potchefstroom, South Africa
[4]Department of Plant Protection, College of Agriculture, Ferdowsi University of Mashhad, 91775-1163, Mashhad, Iran
e-mail: eshokoohi@gmail.com

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Summary. During a survey of plant-parasitic nematodes in the northern part of Iran, six populations of *Pratylenchus thornei* were studied by means of morphological and molecular characterisation. The results showed that body length has a highly significant correlation with all morphometric data indexed in the measurement table, except for the c‘ index and the dorsal pharyngeal gland opening. However, the highest correlation was detected between body length and ‘a’ index ($r = 0.805$). Furthermore, stylet length had a significant correlation with body length ($r = 0.511$), tail length ($r = 0.300$) and V ($r = 0.324$). On the study of ratios, the results revealed that b and a are more reliable to evaluate the species. A molecular study of the *P. thornei* populations using 28S rDNA showed close relationships of the Iranian and USA, Morocco, Moldova, Spain and United Kingdom populations extracted from NCBI Genbank. The results indicated monophyly of *P. thornei*. Measurement table, illustrations and phylogenetic analysis for this species are given.

Key words: Iran, morphometric, phylogeny, 28S rDNA.

Root-lesion nematodes belong to the genus *Pratylenchus* Filipjev, 1936 and, after root-knot and cyst nematodes, are listed as the third economically most important nematode pest genus that adversely affect crop production worldwide (Castillo & Vovlas, 2007; Jones *et al.*, 2013). These nematodes are distributed worldwide across different climatic zones and environments (Loof, 1991; De Goede & Bongers, 1998). This genus comprises 70 nominal species (De Luca *et al.*, 2011), with *Pratylenchus thornei* Sher & Allen, 1953 being one of the most frequently reported species (Castillo & Vovlas, 2007; Yan *et al.*, 2008; Smiley *et al.*, 2014). Members of the genus *Pratylenchus* migrate within the root tissues and feed on the cortical parenchyma (Castillo & Vovlas, 2007) and may multiply to a very large number (10000-35000 individuals (g root)$^{-1}$) (Loof, 1991). Thus, they cause severe damage and large necrotic lesions on roots, tubers or other below-ground parts of plants (Castillo & Vovlas, 2007; Jones *et al.*, 2013). In addition, the genus *Pratylenchus* has been shown to have morphological variation (Loof, 1991; Ryss, 2002; Fayazi *et al.*, 2012).

Accurate identification of *Pratylenchus* spp., is challenging because of limited diagnostic characters being available and the occurrence of intraspecific variability of morphological characteristics (Castillo & Vovlas, 2007). For example, lip region annuli of *P. hippeastri* was originally described with two incisures (Inserra *et al.*, 2007); however, later it was reported with two to three (De Luca *et al.*, 2010; Wang *et al.*, 2015). Hence, new tools for species identification recognition is needed. Furthermore, the occurrence of cryptic species and divergence between populations have been listed due to the discovery of new species using phylogenetic and molecular analysis (De Luca *et al.*, 2011). During the last decades, new methods such as molecular and phylogenetic studies have been provided for identification as well as for distinguishing between species of *Pratylenchus* (Subbotin *et al.*, 2008; De Luca *et al.*, 2011). Among the ribosomal DNA markers, the 28S rDNA gene has been mainly used

to separate species of *Pratylenchus* (Carta *et al.*, 2001; De Luca *et al.*, 2004, 2011; Subbotin *et al.*, 2005, 2008). *Pratylenchus thornei* can, for example, be easily distinguished from other closely related species using the partial D2-D3 segments of the 28S rDNA gene (Al-Banna *et al.*, 2004).

The main objectives of the present study were: *i*) to identify accurately six different populations of *P. thornei* using partial D2–D3 segments of the 28S rDNA gene; *ii*) to study the morphological variation that occurs among the different *P. thornei* populations; and *iii*) to study the phylogenetic relationships of the studied *P. thornei* populations and related species using 28S rDNA.

MATERIAL AND METHODS

Nematode materials. In October 2015, *Pratylenchus* specimens were extracted from rhizosphere soil samples of wheat, blackberry, wild and herbaceous plants from six localities (Table 1) within the Iranian provinces of Golestan, Guilan and Mazandaran using the Whitehead tray method (Whitehead & Hemming, 1965). Extracted nematode individuals were fixed with a hot 4% formaldehyde solution and transferred to anhydrous glycerin using the method of De Grisse (1969). Measurements of different structures and organs of the fixed nematode individuals were done with an Olympus CH-2 light microscope equipped with an ocular micro- and/or a curvimeter and drawing tube.

DNA extraction, PCR and phylogenetic analysis. DNA extraction was done using the Chelex method (Holovachov *et al.*, 2013). Twelve *Pratylenchus* specimens from each of the six localities sampled were hand-picked with a fine tip needle and transferred to a 1.5 ml tube containing 20 µl double distilled water. The nematodes in the tube were crushed with the tip of a fine needle and vortexed. Twenty microliters of 5% Chelex® 100 and 2 µl of proteinase K were added to each of the microcentrifuge tubes that contained the nematode specimens of the different localities, and mixed well with the crushed nematode material. These separate microcentrifuge tubes with the nematode solutions were incubated at 56°C for 2 h. Then, the solutions were incubated at 95°C for 10 min to deactivate the proteinase K (Holovachov *et al.*, 2013). The supernatant was then extracted from each of the tubes and stored at –20°C. Following this step, the forward and reverse primers, D2A (5’-ACAAGTACCGTGAGGGAAAGTTG-3’) and D3B (5’-TCGGAAGGAACCAGCTACTA-3’), respectively (Subbotin *et al.*, 2006), were used in the PCR reactions for partial amplification of the 28S rDNA region. PCR was conducted with 8 µl of the DNA-extract from the nematode specimens of each tube added to 12.5 µl of 2× PCR Master Mix Red (Pishgam Company), 1 µl of each primers (10 pmol μl^{-1}) and ddH_2O to comprise a final volume of 25 µl. The amplification was processed using an Eppendorf master cycler gradient (Eppendorf, Hamburg, Germany), with the following programme: initial denaturation for 3 min at 94°C, 37 cycles of denaturation for 45 s at 94°C, extension for 45 s at 56°C and annealing for 1 min at 72°C, and finally an extension step of 6 min at 72°C followed by a temperature on hold at 4°C. After DNA amplification, 4 µl of products from each tube were loaded on a 1% agarose gel in TBE buffer (40 mM Tris, 40 mM boric acid, and 1 mM EDTA) for evaluation of the DNA bands. The bands were stained with 50 mM ethidium bromide and visualised and photographed on a UV transilluminator. The amplicons of each population was next stored at –20°C. Finally, the PCR products were purified for sequencing with the primers used for amplification by the Pishgam Corporation. Available sequences for other *Pratylenchus* spp. were obtained from NCBI GenBank for comparison to the sampled species obtained during this study. Also, an outgroup, *Zygotylenchus guevarai* (Tobar Jiménez, 1963) Braun & Loof, 1966 (JQ917439) based on a study by Shokoohi (2013), was obtained for comparison. The ribosomal LSU sequences were analysed and aligned using BioEdit (Hall, 1999). The length of the alignment was 837 bp and the base substitution model was evaluated using jModeltest 0.1.1 (Posada, 2008). Phylogenetic trees were elaborated using the Bayesian inference method as implemented in the program Mr Bayes 3.1.2 (Ronquist & Huelsenbeck, 2003). The analysis HKY+G model was selected using jModeltest 0.1.1 (Posada, 2008). The selected model was initiated with a random starting tree and run with the Markov chain Monte Carlo (MCMC) for 10^6 generations. The Bayesian tree was ultimately visualised using the TreeView program (Page, 1996). Genetic pairwise distance was estimated using options implemented in Mega V.7.0.14 (Kumar *et al.*, 2016). The partial D2-D3 segment of 28S rDNA sequences of *P. thornei* obtained from the sampled populations as a result of this study were deposited in GenBank under accession numbers: KX258735 (province of Golestan, Gorgan region), KX258736 (province of Golestan, Ziarat region), KX258737 (province of Guilan, Roodsar region), KX258738 (province of Guilan, Langerood region), KX258739 (province of Mazandaran, Nowshahr region) and

KX258740 (province of Mazandaran, Sari region) (Table 2).

Statistical analysis. Correlation of morphometric data was done using the two-tailed Pearson correlation. All analyses were done using

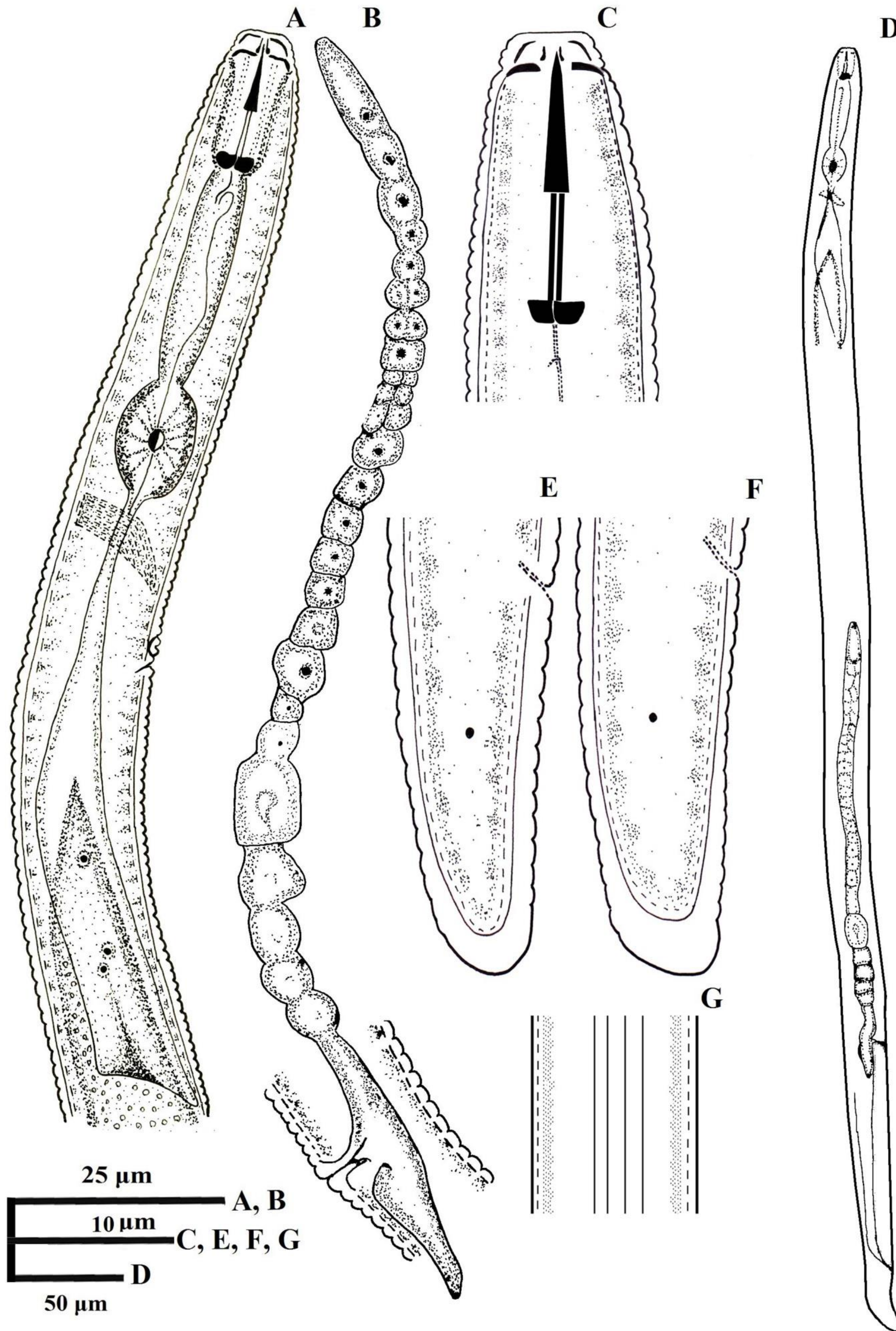

Fig. 1. *Pratylenchus thornei* Sher and Allen, 1953. A: Neck. B: Female reproductive system. C: Anterior end. D: Entire female. E, F: Female posterior end. G: Lateral field. (population code: IR5, Mazandaran, Nowshahr region, collected from wheat rhizosphere).

the SPSS 13 (SPSS Inc., 2005) statistical package. Hierarchical Clustering analysis was done by using morphometric data and Rstudio, pvclust package (Suzuki & Shimodaira, 2015). According to the analyses, 19 specimens obtained as a result of this study belonged to *P. thornei*. The populations used for comparative purposes, hierarchical clustering as well as their morphometric data are available in the databases from USA (Sher & Allen, 1953), Russia (Ryss, 1988), Canada (Yu, 1997), Germany (Loof, 1960), Italy (D'Errico, 1970) and India (Khan & Singh, 1975). In addition, ten specimens from Iran and those previously reported (Pourjam *et al.*, 1999; Mirzaipour *et al.*, 2016) were analysed. The eleven morphometric traits used for identification purposes and hierarchical clustering analysis included: de Man's indices (a, b, c, c' and V), body length, pharynx length (distance from anterior to pharynx-intestinal junction), stylet length, post vulva sac length, tail length and position of the secretory excretory pore from the anterior end (de Man, 1888). The data for these characters were obtained from the fixed specimens. Data on the morphometric measurements of the populations were analysed using the bootstrap method.

RESULTS

Pratylenchus thornei Sher & Allen, 1953 (Figs 1-3)

Material examined. Eight females from Nowshahr, in good state of preservation.

Measurements. See Table 1.

Females. Body 420-680 µm long, slightly curved after fixation, cuticle finely annulated with 1.0-1.5 µm wide at midbody. Maximum body diameter 15-20 µm. Lip region round to flat, with slightly depression, bearing three annuli, continuous with the body. Lateral field with four lines, two outer lines crenated, occupying about 30% of midbody diameter. Stylet length 14.0-17.6 µm; basal knobs usually rounded and flatted. Dorsal pharyngeal gland opening (DGO) at 2-3 µm posterior to stylet base. Median bulb oval to rounded, nerve ring located just after bulb, at 48-57% of the neck. Secretory excretory pore at 70-100 µm from anterior body, at 68-76% of the neck. Hemizonid one annuli anterior to secretory excretory pore. Pharyngeal glands overlapped with intestine about 30-57 µm. Pharynx 61-99 µm long, body length about 5.5-9.9 times pharynx length. Ovary not reached to pharyngeal glands. Oocytes in one or two rows. Vulva occupied 71-82% of body length. Spermatheca not visible. Post-vulval uterine sac 20-31 µm long, vulva with a transverse slit. Tail terminus shows variation, round, truncate, smooth and in two populations slightly spatulate. Tail 19-32 µm long, about 2.0-2.8 times anal body diameter. Phasmid located at posterior half of the tail, 45-57% of tail length.

Male. Not found.

Other material examined. The specimens studied were similar with those fixed and measured from the provinces of Gorgan, Guilan and Mazandaran. However, they differ in body length (427-688 *vs* 440-790 µm) and 'a' index (5.3-8.9 *vs* 6-9.9). Also, the stylet length of the specimens from the Gorgan population was smaller (14-15 µm) than those from the other populations (Table1).

Remarks. This species is distributed worldwide and reported by many scientists (Sher & Allen, 1953; Loof, 1960; D'Errico, 1970; Khan & Singh, 1975; Ryss, 1988; Yu, 1997; Pourjam *et al.*, 1999; Castillo & Vovlas, 2007; Fayazi *et al.*, 2012; Mirzaipour *et al.*, 2016). All the populations studied fit well with those studied by Sher & Allen (1953); however, they differ in stylet length (14-17 *vs* 17-19 µm). Compared to the Dutch population studied by Loof (1960), a shorter 'c' index (16-25 *vs* 18-29) was recorded. In comparison with the Italian population studied by D'Errico (1970), specimens of the Iranian populations studied differed in 'b' value (5-9 *vs* 4.8-7.8). Compared with the specimens studied by Khan & Singh (1975), the studied populations had a shorter 'C' value (11-21 *vs* 18-29). Ryss (1988) studied a population of this species having a longer stylet compared to the studied Iranian populations (14-17 *vs* 16-18 µm) and compared to specimens studied by Yu (1997), these Iranian populations differed in tail shape (truncate and slightly spatulate *vs* round, conical round, truncate). In comparison with the material studied by Castillo & Vovlas (2007), the Iranian populations studied differed in post-vulva uterine sac length (20-31 *vs* 33-46 µm) and 'b' index (5.5-9.9 *vs* 4.7-8.7). Compared with Fayazi *et al.* (2012) there is no significant differences. They have indicated morphological variation in *P. thornei* as found in the present study.

According to the key presented by Castillo & Vovals (2007), *P. thornei* is similar to *P. brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941 and *P. cruciferus* Bajaj & Bhatti, 1984. The species is distinguished from *P. brachyurus* by the number of lip annuli (three annuli *vs* two annuli) and from *P. cruciferus* by labial shape (flat and slightly round *vs* flat) and hemizonid position (one

annuli anterior to secretory excretory pore *vs* 2-8 annuli anterior to secretory excretory pore).

Table 1. Morphometrics of *Pratylenchus thornei* Sher & Allen, 1953 populations studied form different localities in Iran. All measurements are in µm.

Character	Populations					
Province	Golestan		Mazandaran		Guilan	
Locality of sampling	Gorgan	Ziarat	Nowshahr	Sari	Roodsar	Langrood
n	6♀♀	6♀♀	8♀♀	9♀♀	7♀♀	8♀♀
L	507±56.5 (465-567)	571±42.4 (534-641)	540±52.7 (486-623)	548±88.6 (427-688)	627±45.4 (548-683)	598±59.4 (520-669)
a	31.2±3.8 (26.8-34.0)	29.8±2.2 (27.1-33.2)	31.5±3.7 (26.8-36.4)	30.1±3.4 (26.4-34.9)	33.1±1.8 (31.7-36.7)	33.3±3.4 (29.0-37.0)
b	6.2±0.8 (5.3-6.7)	7.8±0.7 (7.2-8.9)	6.3±0.5 (5.5-6.9)	6.7±0.9 (5.5-8.0)	7.2±1.0 (6.0-9.2)	7.3±1.3 (6.2-9.9)
c	22.5±0.9 (21.4-23.2)	21.0±1.9 (18.1-23.1)	26.0±2.4 (21.6-28.3)	22.9±2.3 (18.9-26.4)	28.3±3.0 (22.4-29)	24.5±3.2 (21.2-29.3)
c’	2.4±0.3 (2.0-2.6)	2.4±0.2 (2.0-2.6)	2.3±0.3 (2.0-2.8)	2.3±0.3 (1.9-2.7)	2.0±0.1 (1.8-2.3)	2.3±0.1 (1.9-2.5)
V	76.5±3.1 (74-80)	76.9±5.6 (71-81)	76.2±6.0 (70-82)	77±2.5 (71-78)	77±3.1 (73-81)	78±2.5 (74-81)
Lip height	7.3±0.2 (7-7.5)	7.9±0.7 (7.2-8.6)	7.8±0.5 (7.2-8.1)	7.7±0.4 (7.2-8.1)	7.9 ± 0.3 (7.5-8.2)	7.9±0.1 (7.8-8.1)
Lip region diameter	2.8±0.2 (2.7-3.1)	2.8±0.2 (2.7-3.2)	2.9±0.2 (2.7-3.2)	2.7±0.2 (2.5-3.1)	2.7 ± 0.4 (2.3-3.2)	2.6±0.4 (2.2-3.1)
Stylet length	14.8±0.4 (14-15)	15.5±1.3 (14-17)	15.5±1.2 (14-17)	15±1.1 (14-16)	15.8±1.1 (14-17)	15.8±1.3 (14-17)
Conus length	7.9±0.9 (7-9)	8.4±1.1 (7-10)	8±1.5 (7-10)	8±0.6 (7-9)	8±0.6 (7-9)	8.1±1.0 (6.4-9.0)
Pharynx length	86±2.1 (83-87)	73±6.6 (61-78)	84±4.2 (79-90)	81±8.5 (72-99)	87±10.2 (68-95)	83.1±8.8 (67-93)
Pharyngeal glands	125.7±6.3 (121-134)	111.8±6.8 (103-119)	118.7±7.3 (109-129)	123.1±11.9 (99-134)	133.4±122.8 (110-145)	129.8±10.3 (119-146)
Secretory excretory pore	80.6±2.2 (78-82)	81±4.4 (75-87)	84.5±12.9 (70-109)	83.8±13 (69-97)	82.9±11.5 (61-93)	81.2±4.6 (74.7-86.5)
MB	41.5±0.8 (41-42)	42.8±2.6 (38-44)	42.4±1.9 (39-44)	41.7±2.3 (38-46)	40.3±4.3 (34-46)	40.3±3.0 (36-43.7)
PVS	21.5±2.5 (22-26)	29.1±3.6 (24-32)	22.6±1.1 (21-24)	22±1.9 (20-24)	22.7±2.3 (20-26)	23.3±1.3 (22-25)
Max. body diameter	17.7±1.1 (16-19)	19.1±0.7 (18-20)	17.2±0.9 (16-18)	18.1±1.5 (16-20)	18.9±0.9 (17-20)	18.0±1.5 (17-21)
Lateral field width	5.3±0.7 (5-6)	7±0.5 (6-7)	5.5± 0.9 (5-6)	5.7±0.2 (5-6)	6.1± 0.6 (5-7)	5.9±0.3 (5-6)
Tail length	22±3.3 (20-26)	27±2.7 (24-32)	20.8±1.7 (19-23)	24.7±3.5 (20-29)	22.2±1.6 (20-25)	24.6±2.1 (22-28)
Anal body diameter	9.1±0.2 (9-10)	11.4±0.4 (11-12)	8.7±0.9 (7-10)	10±0.8 (9-11)	10.6±0.4 (10-11)	10.5±1.2 (9-12)

DNA characterisation. The 28S rRNA gene fragment (about 658 to 712 bp) of the six *P. thornei* populations studied during this research was amplified by the two primers D2A and D3B. Nblast (https://blast.ncbi.nlm.nih.gov/Blast.cgi) results indicated that the populations studied (Gorgan, Roodsar and Langerood; KX258735, KX258737, KX258738) are similar to a population from the USA (EU130880; 100% identity). The *P. thornei* population from Sari, province of Mazandaran (KX258740) shows only one nucleotide difference with the population from the USA (EU130880; 99% identity). Compared to other populations from the USA (EU130880, 98% identity), the Iranian populations studied (Nowshahr and Ziarat; KX258739, KX258736) differed by 15 base pairs.

Pairwise Maximum Composite Likelihood distance among the 28S rDNA region of *P. thornei* ranges from 0.000 to 0.073. The highest genetic variation was observed for a population from Australia (EU130872) and one from Iran (JX261963). Among the Iranian populations

identified during this study, the genetic distance ranges from 0.000 to 0.071. The highest variation was observed for *P. thornei* specimens of the Golestan (present study; KX258736) and Lorestan (JX261963) populations.

Table 2. Nematode species and GenBank accession numbers used for the phylogenetic study.

Species	GenBank accession number	Reference	Origin	Sample codes
P. thornei	KX258735	Present study	Iran (Golestan, Gorgan)	IR1
P. thornei	KX258736	Present study	Iran (Golestan, Ziarat)	IR2
P. thornei	KX258737	Present study	Iran (Guilan, Roodsar)	IR3
P. thornei	KX258738	Present study	Iran (Guilan, Langerood)	IR4
P. thornei	KX258739	Present study	Iran (Mazandaran, Nowshahr)	IR5
P. thornei	KX258740	Present study	Iran (Mazandaran, Sari)	IR6

Table 3. Correlation analysis for populations of *P. thornei* Sher & Allen, 1953.

Character	L	a	b	c	c’	Tail	V	Pharynx	Stylet	Secretory excretory pore	PVS
L	1										
a	0.830(**)	1									
b	0.713(**)	0.526(**)	1								
c	0.424(**)	0.415(**)	0.283	1							
c’	0.000	0.043	–0.146	–0.558(**)	1						
Tail	0.442(**)	0.309	0.375(*)	–0.599(**)	0.483(**)	1					
V	–0.072	–0.300	0.019	–0.098	–0.022	0.042	1				
Pharynx	0.362(*)	0.306	–0.227	0.173	–0.013	0.091	–0.129	1			
Stylet	0.293	0.037	0.153	0.119	0.131	0.112	0.109	0.196	1		
Secretory excretory pore	0.476(**)	0.364(*)	0.002	–0.017	0.356(*)	0.409 (*)	–0.282	0.404(*)	0.375 (*)	1	
PVS	0.353(*)	0.013	0.531(**)	0.256	0.515(**)	0.080	0.139	0.139	0.158	0.425(**)	1

* – correlation is significant at the 0.05 level (2-tailed).
** – correlation is significant at the 0.01 level (2-tailed).

Morphometric analysis on the *P. thornei*. The results based on two-tailed Pearson correlation within the six *P. thornei* populations showed that some morphometric data, obtained from 46 females, showed significant correlations with others. Some important morphometric data, such as body length and length of the post vulval sac, need to be considered to understand the correlation with each other. The results indicate that body length has significant correlation with other morphometric data (a, b and c index, tail, pharynx, secretory excretory pore and post vulval sac) in females (Table 3). On the other hand, body length had no correlation with some important morphometric characters such as c‘ and V indices in females. Interestingly, c index had a significant correlation ($r = -0.599$, $P \leq 0.01$) with tail length. Stylet length only showed correlation with the positionof the secretory excretory pore ($r = 0.375$, $P \leq 0.01$). Post vulval sac length had a significant correlation with the indices b ($r = 0.531$), c’ ($r = 0.515$), body length ($r = 0.353$) and secretory excretory pore ($r = 0.425$).

Hierarchical clustering. The dendrograms (Fig. 4) show the average linkage method of hierarchical clustering with *P* values of 14 populations of *P. thornei* from different localities in the world (Sher & Allen, 1953; Loof, 1960; D’Errico, 1970; Khan & Singh, 1975; Ryss, 1988; Yu, 1997; Pourjam *et al.*, 1999; Mirzaipour *et al.*, 2016). The hierarchical analysis based on important morphometric characters clustered the *P. thornei* populations

studied into two groups. One group consisted of populations from Germany, Iran and Italy and with 99 and 100 AU (approximately unbiased) values. The other group consisted of populations from

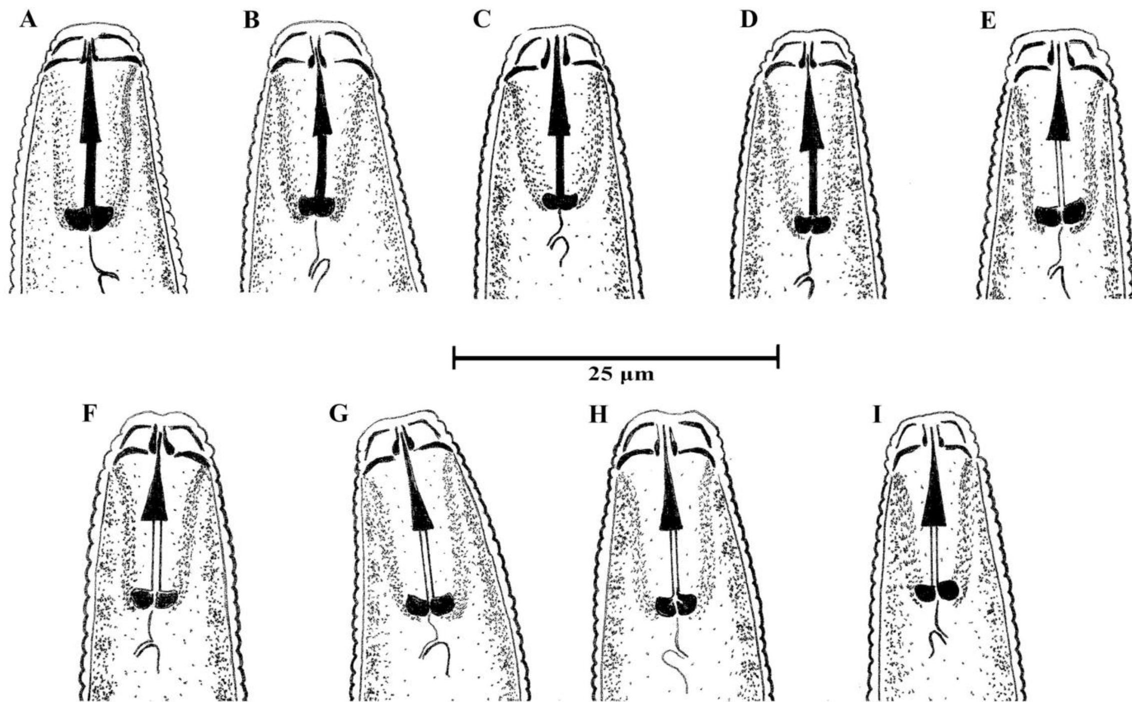

Fig. 2. *Pratylenchus thornei* Sher and Allen, 1953. A-I: Anterior region. Sampled from three provinces in the north of Iran (Golestan, Guilan and Mazandaran) A: (IR1, Golestan, Gorgan region); B: (IR5, Mazandaran, Nowshahr region) C: (IR4, Guilan, Langerood region); D, E: (IR2, Golestan, Ziarat region); F, G: (IR3, Guilan, Roodsar region); H, I: (IR6, Mazandaran, Sari region).

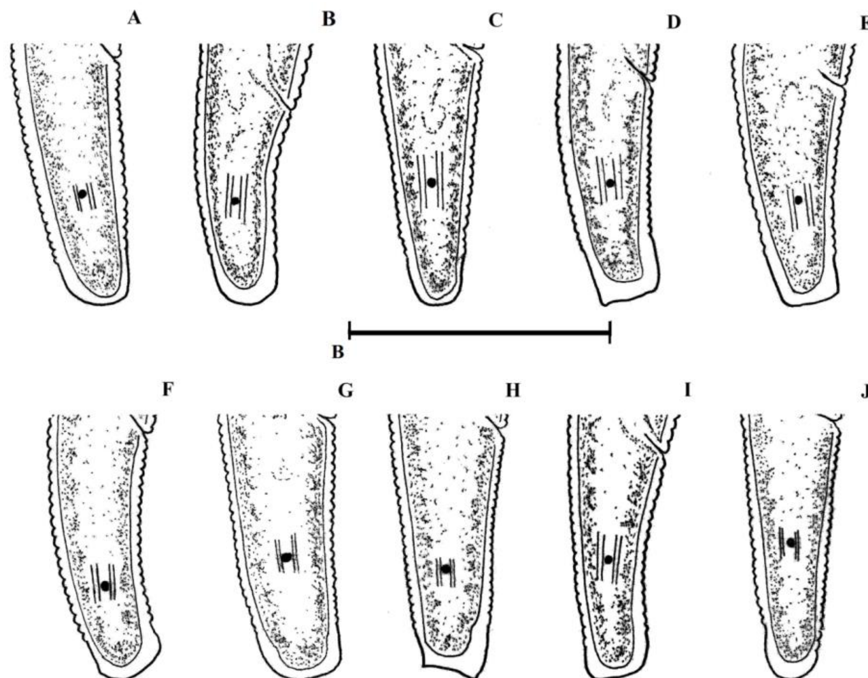

Fig. 3. *Pratylenchus thornei* Sher and Allen, 1953. A-J: Posterior end of females. Sampled from three provinces in the north of Iran (Golestan, Guilan and Mazandaran). A, B: (IR6, Mazandaran, Sari); C: (IR5, Mazandaran, Nowshahr);

D, E: (IR4, Guilan, Langerood); F, G: (IR2, Golestan, Ziarat); H, I: (IR3, Guilan, Roodsar); J: (IR1, Golestan, Gorgan) [scale bar 25 µm].

Canada, USA and Russia with 100 AU (approximately unbiased) values (Fig. 4). This analysis demonstrated that the Iranian populations are very closely related to Italian populations, representing a sister group. Moreover, the geographic pattern comprises of three continents with two of them (Asia and America) plotting completely separate from the European populations of *P. thornei*. On the other hand, the Russian population of *P. thornei* is placed in a group separate from the European populations.

DISCUSSION

The usefulness of morphometric analyses as a tool to identify *Pratylenchus* spp., due to significant correlations existing between some morphometric characteristics of females of the six *P. thornei* populations examined, has been demonstrated as a result of this study. In addition, morphometric cluster analysis is also suggested as a useful tool to characterise *Pratylenchus* spp. However, use of a

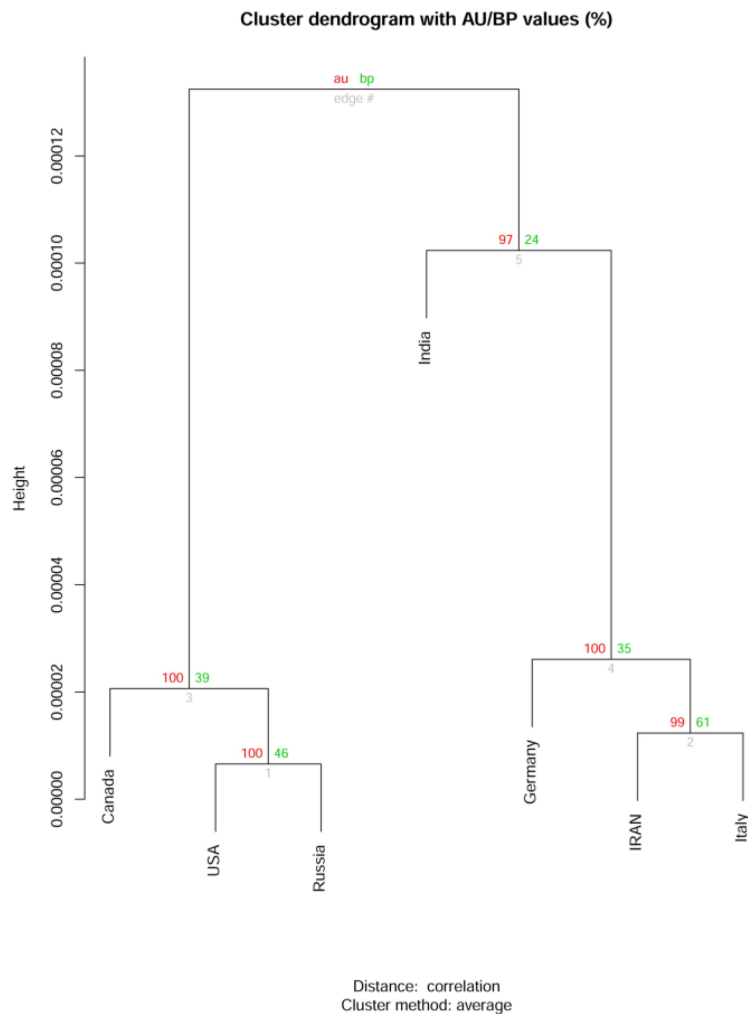

Fig. 4. Cluster dendrogram for different populations of *Pratylenchus thornei* using morphometric data. Red values on the left branch represent AU (Approximated Unbiased) values, while green values on the right branch indicate BP (Bootstrap Probability) values in percentage, and cluster labels (bottom).

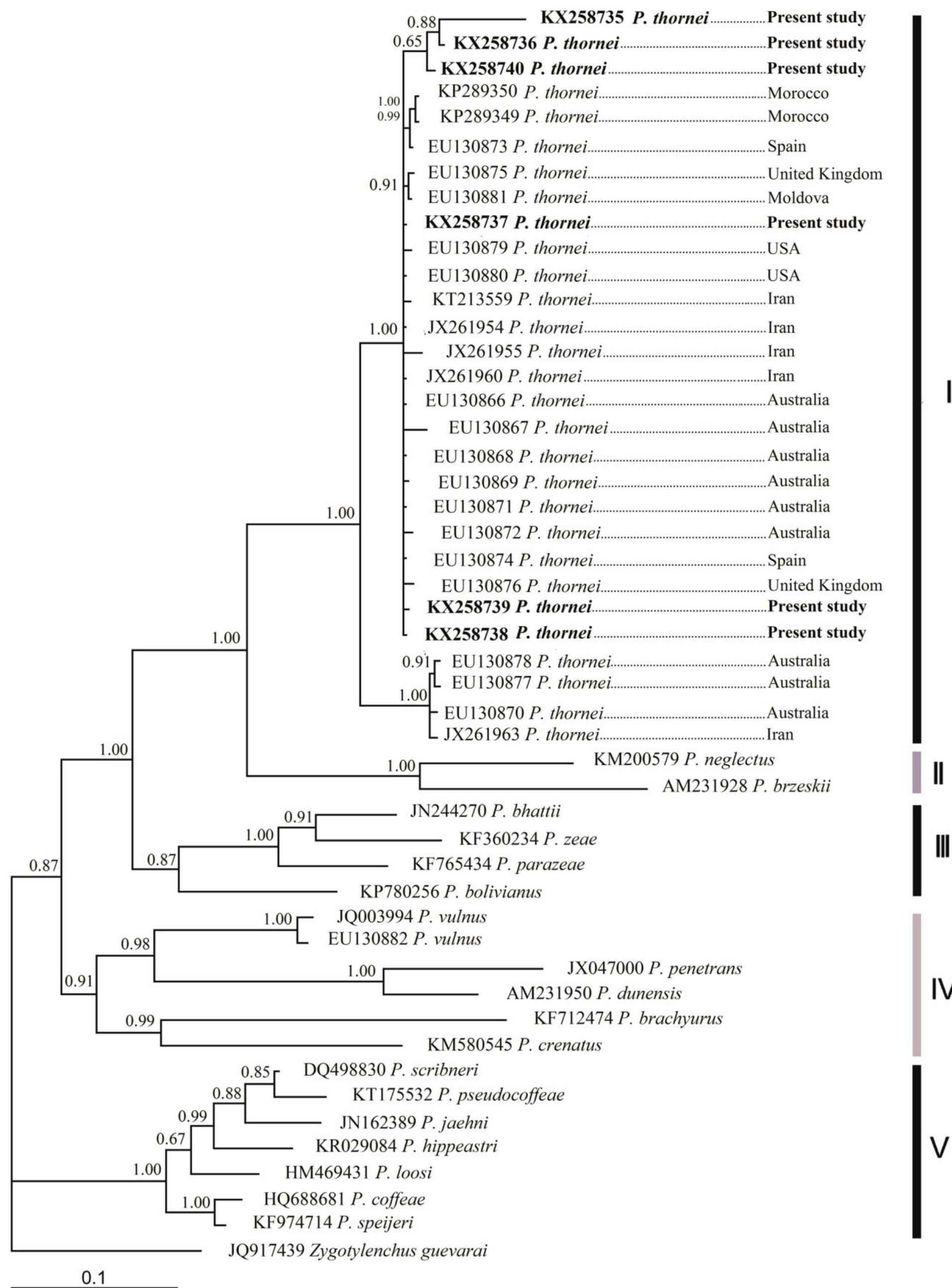

Fig. 5. The Bayesian tree inferred of *Pratylenchus thornei* Sher and Allen, 1953 newly sequences from Iran and the other sequences of the Gene Bank, based on sequences of the 28S rDNA region.

third method, namely molecular analysis of the six *P. thornei* populations according to the 28S rDNA gene, is suggested to be more accurate than morphometric analysis to identify *Pratylenchus* spp., as has also been shown by Subbotin *et al.* (2008).

Regarding morphometric analysis, the authors could not find reports that demonstrated that correlations of such measurements or indices have been used to aid in identifying *Pratylenchus* spp. In our study, the indices a, b and c generally correlated with the body length of the *P. thornei* female specimens studied from the six Iranian populations.

Hence, it is suggested to be more useful than other indices accurately to identify the species. By contrast, some characteristics, *e.g.* stylet with body length; *r* = 293, showed no or insignificant correlations, which may be an indication that the genes responsible for these characteristics are located at different loci or far apart (high genetic distances). Correlation between morphological characteristics of nematode specimens has been suggested as a useful method to identify species of nematodes. For example, Fortuner (1984) noted that the indices a, c and c' are often useful for accurate identification of species of *Helicotylenchus* Steiner, 1945. Amirzadi *et al.* (2013) also showed that b and c are more useful in identifying *Acrobeles* spp. Fortuner (1990) also revealed that the features related to body size (length and indices of a and b) showed high correlation to each other for *Hirschmanniella belli*. Subbotin *et al.* (1999) also stated that morphometric character analysis is suitable for separating populations within the *H. avenae* group. As a result of this study, cluster analysis according to morphometric characteristics grouped the six Iranian populations of *P. thornei* close to, but in a separate clade from, the European populations previously reported (Ryss, 1988).

The phylogenetic position of the *P. thornei* populations was studied by the extensive use of 42 large subunit rDNA sequences of nematodes belonging to the genus *Pratylenchus* from GenBank. The consensus tree based on LSU, showed that the *P. thornei* species is represented by a monophyletic group. The phylogenetic analysis grouped *Pratylenchus* spp. into five clades: I) *P. thornei*; II) *P. neglectus* and *P. brzeskii*; III) *P. bhatii*, *P. zeae*, *P. parazeae* and *P. bolivianus*; IV) *P. vulnus*, *P. penetrans*, *P. dunensis*, *P. brachyurus* and *P. crenatus*; and V) *P. scribneri*, *P. pseudocoffeae*, *P. coffeae*, *P. jaehni*, *P. hippeastri*, *P. loosi* and *P. speijeri*. *Pratylenchus brezeski* and *P. neglectus* grouped as a sister group and close to the *P. thornei* populations, as indicated by Subbotin *et al*. (2008). The six *P. thornei* populations from Iran studied grouped together with the same species from USA, Australia, Spain, Iran and others countries (Subbotin *et al.*, 2008; Majd Taheri *et al.*, 2013). Although the *P. thornei* populations grouped close to *P. brezeski* and *P. neglectus* phylogenetically, *P. thornei* specimens are distinguished from *P. brezeski* by having three lip annuli and indistinct spermatheca, but both of them have a smooth tail terminus. *Pratylenchus neglectus* differs by having two lip annuli and also due to conoid-round tail shape. The spermatheca of both species is reduced. These three species also placed closely together in the study presented by Subbotin *et al.* (2008), while according to ITS rDNA *P. thornei* was placed in a group separated from *P. neglectus* (De Luca *et al.*, 2011). The latter authors indicated that *P. thornei* is more closely related to *P. mediterraneus* Corbett, 1983. These two species are similar in lip annuli, tail shape and tail with smooth tip. However, they differ in lip shape, spermatheca and the presence of sperm. According to morphological characters, these species hence do not group closely together. Phylogenetic analysis based on morphological characters, as reported by Ryss (2002), showed that *P. thornei* places close to *P. pinguicaudatus* and *P. coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941 and *P. dasi* Fortuner, 1985. It is similar to *P. pinguicaudatus* in lip annuli, stylet length, and smooth tip tail but different in lip shape. It resembles *P. coffeae* due to its smooth tip tail but differs in lip annuli (two annuli), shape and spermatheca. Consequently, the phylogenetic position of these species based on morphological characters are not acceptable because they differ substantially in terms of morphological traits. Therefore, use of molecular analyses, especially with data that has been published on the 28S rDNA sequences as presented, is a more accurate way to identify these species.

In addition, more studies on DNA sequence data will improve our knowledge of the phylogenetic position of *Pratylenchus* spp.

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Divsalar, N., E. Shokoohi, A. Hoseinipour, H. Fourie and E. M. Moqaddam. Морфологическая изменчивость и молекулярное изучение нематод *Pratylenchus thornei*.

Резюме. При проведении исследований по фитопаразитическим нематодам на севере Ирана были выявлены 6 популяций *Pratylenchus thornei*, исследованные затем морфологическими и молекулярными методами. Было покзано, что длина тела этих нематод четко коррелирует с другими морфометрическими показателями, за исключением показателя c' и положения поры дорсальной железы пищевода. Наиболее выраженная корелляция была отмечена между длиной тела и индексом 'a' (r = 0.805). Также, значительная корелляция была отмечена между длиной стилета и длиной тела (r = 0.511), длиной хвостового отдела (r = 0.300) и индексом V (r = 0.324). Среди индексов Де-Мана, значения 'b' и 'a' наиболее надежны для разграничения видов. Молекулярно-филогенетический анализ последовательностей 28S rDNA *P. thornei* показал значительное сходство иранских популяций с формами этого вида из США, Марокко, Молдавии, Испании и Великобритании. Показана монофилия *P. thornei*. Представлены карта распределения и филогенетическое древо этого вида.