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Morphological and molecular characterization of *Pratylenchus thornei* Sher & Allen, 1953 (Rhabditida: Pratylenchidae) from Iraq

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Abstract

During an investigation on the biodiversity of plant-parasitic nematodes in Misan province (southeast of Iraq), two populations of *Pratylenchus thornei* were isolated from the rhizosphere of faba bean. The morphological and morphometric data were provided for the recovered populations. Both populations were similar to each other, but they were somewhat different in terms of the tail shape and body length. The tail in the Alkahla population was subcylindrical, tail terminus truncated and sometimes with a small projection. The tail in the Ali-Algharbi population was conical with an almost round to broadly rounded terminus. The morphological and morphometric characters of both populations agree with the type population of the species and some other populations reported from different areas. Molecular phylogenetic analysis of the Iraqi populations of *P. thornei* using the large subunit ribosomal RNA gene (LSU rDNA D2-D3) and internal transcribed spacer (ITS rDNA) sequences using Bayesian inference (BI), showed that they form maximally supported clades with other sequences of the species. The present study is the first report of *P. thornei* from Iraq based on morphological and molecular data.

Keywords: D2-D3 LSU, ITS rDNA, Misan province, phylogeny, root-lesion nematodes

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شناسایی ریخت‌شناختی و مولکولی *Pratylenchus thornei* Sher & Allen, 1953 (Rhabditida: Pratylenchidae) از عراق

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چکیده:

طی بررسی تنوع نماتدهای انگل گیاهی در استان میسان (جنوب‌شرقی عراق)، دو جمعیت از گونه *Pratylenchus thornei* از ریزوسفر باقلا جداسازی گردید. ویژگی‌های ریخت‌شناختی و ریخت‌سنجی برای جمعیت‌های یافت شده تهیه گردید. هر دو جمعیت با یکدیگر شباهت داشتند ولی از نظر شکل دم و طول بدن تا اندازه‌ای از هم متفاوت بودند. دم در جمعیت به‌دست آمده از شهر الکحلا نیمه‌استوانه‌ای با انتهای تخت و گاهی همراه با زایده‌ای کوچک بود. دم در جمعیت به‌دست آمده از شهر علی‌الغربی مخروطی با انتهای تقریبا گرد تا گرد پهن بود. ویژگی‌های ریخت‌شناختی و ریخت‌سنجی هر دو جمعیت، با جمعیت تیپ و برخی از جمعیت‌های گزارش شده این گونه از نقاط مختلف مطابقت داشت. واکاوی تبارزایی جمعیت‌های عراقی *P. thornei* با استفاده از توالی‌های ناحیه D2-D3 از ژن زیرواحد بزرگ RNA ریبوزومی و توالی‌های ناحیه بین ژنی ITS rRNA با استفاده از روش بیس (Bayesian inference) نشان داد که آن‌ها با توالی‌های دیگر این گونه با احتمال در حد بالا در یک کلاد قرار گرفتند. در تحقیق حاضر، ویژگی‌های ریخت‌شناختی و مولکولی جمعیت‌های عراقی این گونه برای اولین بار ارائه می‌گردد.

واژه‌های کلیدی: استان میسان، تبارزایی، نماتدهای مولد زخم، D2-D3 LSU و ITS rDNA

Introduction

The faba bean (*Vicia faba* L.) is one of the important and economic crops in Iraq, which is cultivated on large areas because its significance for human consumption. It is placed in crop rotation with cereals and other legumes (Ansari *et al*. 1980). Faba bean is considered as one of the imperative crops in Misan province, and its cultivation is centered in the Ali-Algharbi and the Alkahla cities, with large areas and different cultivars (Anonymus 2021).

Root-lesion nematodes are considered worldwide as one of the major restrictions of crop cultivation from economic importance aspect. *Pratylenchus* Filipjev, 1936 species are ranked after root-knot and cyst nematodes as having the highest economic significance on crops worldwide. They are migratory endoparasites that cause root damage on a wide range of crops. *Pratylenchus thornei* Sher & Allen, 1953 in cereals and legumes is one of the most common species of the genus (Castillo & Vovlas 2007).

The morphological identification of *Pratylenchus* species is exceedingly difficult due to the large number of species and small number of diagnostic features available. The other point is remarkable intraspecific variabilities of some morphological characteristics (Subbotin *et al*. 2008).

Based on available data, 43 species of plant-parasitic nematodes including *P. thornei* have been reported from vineyards in Iraq (Stephan *et al*. 1985). Morphological, morphometric and molecular data were however not provided for any of the reported species in the study. During the present study, two populations of *P. thornei* were isolated from soil samples collected from the Misan province in southeast of Iraq. The present study aimed to characterize the Iraqi populations of the species based on morphological and morphometric characteristics. Additionally, molecular data obtained from D2-D3 segment of rDNA gene and ITS rDNA region were used to reconstruct the phylogenetic relationships of the recovered populations.

Materials and methods

Nematode extraction and morphological observations

Several soil samples were collected from the rhizosphere of the faba bean in Misan province, Iraq. The centrifugal flotation technique (Jenkins 1964) and the tray method (Whitehead & Hemming 1965) were used to extract the nematodes from soil samples. The collected nematodes were killed by adding 4% hot formaldehyde solution and transferred to anhydrous glycerin according to De Grisse (1969). Observations and measurements were conducted using a Leitz SM-LUX light microscope equipped with a drawing tube. Some of the specimens were photographed using an Olympus digital camera attached to an Olympus BX51 light microscope.

DNA extraction, PCR and sequencing

For molecular analyses, single female specimens were picked out, examined in a drop of distilled water on a temporary slide under the light microscope, and transferred to 5 µl of TE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0) on a clean slide, and then crushed using a cover slip. Each suspension was collected by adding 15 µl TE buffer. The DNA samples were stored at –20°C until used as a PCR template. Primers for LSU rDNA D2-D3 amplification were forward primer D2A (5′-ACAAGTACCGTGAGGGAAAGT-3′) and reverse primer D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Nunn 1992). Primers for amplification of ITS rDNA were forward primer rDNA1 (5′-TTGATTACGTCCCTGCCCTTT-3′) and reverse primer rDNA1.58S (5′-ACGAGCCGAGTGATCCACCG-3′) (Subbotin *et al*. 2000). To amplify the above-mentioned segments of DNA, the polymerase chain reactions (PCRs) were performed as described previously (Jumaah & Azimi 2022). The obtained PCR products with successful amplification as observed after staining with Green Viewer™ and observation under UL light were subjected to sequencing using Applied Biosystems 3500 (ABI) sequencer, Pishgam corporation, Tehran, Iran. The newly obtained sequences were deposited into the GenBank database (accession numbers OQ683864, OQ683865, for LSU D2-D3 and OQ683868, OQ683869 for ITS rDNA).

Phylogenetic analyses

The newly obtained sequences of the D2-D3 fragments of LSU rDNA and ITS rDNA and additional sequences of relevant species selected after the nucleotide basic local alignment search tool (BLASTn) were aligned by Clustal X version 2 using the default parameters (Larkin *et al*. 2007). Both performed alignments were edited manually in the MEGA7 program (Kumar *et al*. 2016). Based on the Akaike information criterion, the base substitution model was selected using MrModeltest2 (Nylander 2004). A general time reversible model, including among-site rate heterogeneity and estimates of invariant sites (GTR + G + I), was used in the both phylogenies. The Bayesian analysis was performed to infer the phylogenetic trees using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003), running the chains for four 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 determine equilibrium distribution and help estimate the posterior probabilities of the phylogenetic trees (Larget & Simon 1999) using the 50% majority rule. Bayesian posterior probability (BPP) values higher than 0.50 are given on appropriate clades. The output files of the phylogenetic program were visualized using Dendroscope v3.2.8 (Huson & Scornavacca 2012) and were digitally drawn in CorelDRAW software version 17.

Results and discussion

Iraqi population of *Pratylenchus thornei* Sher & Allen, 1953

(Figs 1, 2)

Measurements

See Table 1.

Description

Female

Body ventrally arcuate upon fixation. Cuticle with fine striation, 0.7-1.2 µm wide at mid-body. The lateral fields marked by four smooth incisures, sometimes additional line in the central band may occur, becoming four on the tail, occupying about 37-40% of the body diameter at mid-body. Lip region not offset from the body, bearing three annuli, 2.4-3.3 µm high and 5.8-7.4 µm wide, outer margins of cephalic framework extending back about two body annuli. Stylet with rounded basal knobs. Median bulb oval, 13.5-16.0 µm long and 8.0-11.0 µm wide (in Alkahla population) and 13.8-15.7 µm long and 8.2-11.9 µm wide (in Ali-Algharbi population), pharyngeal glands overlapping intestine ventrally. Hemizonid is about two body annuli long, two to three annuli anterior to the secretory-excretory pore. Reproductive system monodelphic-prodelphic, ovary with oocytes arranged in one or two rows, spermatheca small, rounded to slightly oval, empty, hardly visible in some specimens, vulva a transverse slit, vagina 7-9 µm long, PUS one to one and a half times body width in vulva region. Tail subcylindrical with truncate tip with a small projection (in the Alkahla population) to conical with round to broadly rounded terminus (in the Ali-Algharbi population). Phasmids nearly at mid-tail, 9-15 annuli anterior to the tail terminus.

Male

Not found.

Remarks

The recovered populations of the species in the present study were similar to each other, but they were somewhat different in terms of the tail shape. Both populations agreed with the type of population (Sher & Allen 1953), and some other populations reported from different areas. However, the stylet length is slightly shorter compared to that in type population (14.0-17.2 *vs* 17-19 µm). This variation in the stylet length has already reported (Castillo & Vovlas 2007; Geraert 2013; Movahedifar & Azimi 2020). No significant difference was observed compared to the data given by Geraert (2013).

The presently studied populations of the species were recovered from the rhizosphere of faba bean in Misan province, southeast Iraq. The GPS coordinates for Alkahla and Ali-Algharbi populations are as follows: 31°41'02.46"N, 47°16'50.05"E and 32°28'57.13"N, 46°37'48.78"E, respectively. Morphological and morphometric data of *P. thornei* from Iraq are presented herein for the first time.

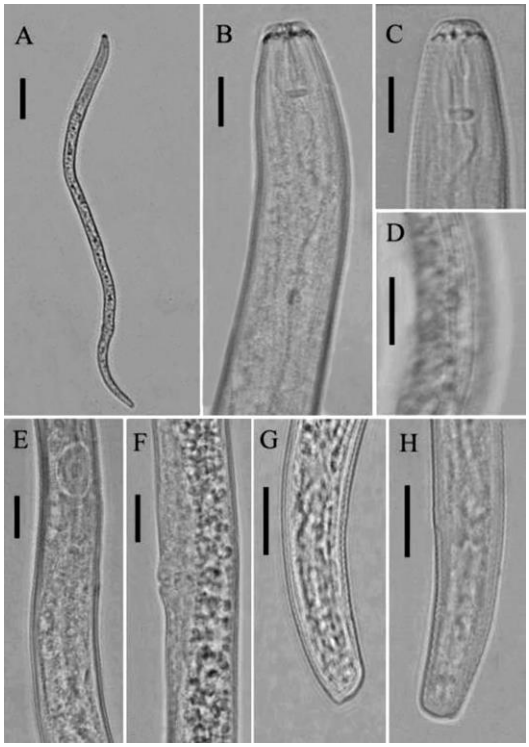

Fig. 1. Light microphotographs of *Pratylenchus thornei* Sher & Allen, 1953 female from Iraq (Alkahla population). A: Entire body; B, C: Anterior body region; D: Lateral field at mid-body; E: Part of pharynx; F: Vulval region; G, H: Posterior body region. (Scale bars: A = 50 µm; B-H: 10 µm).

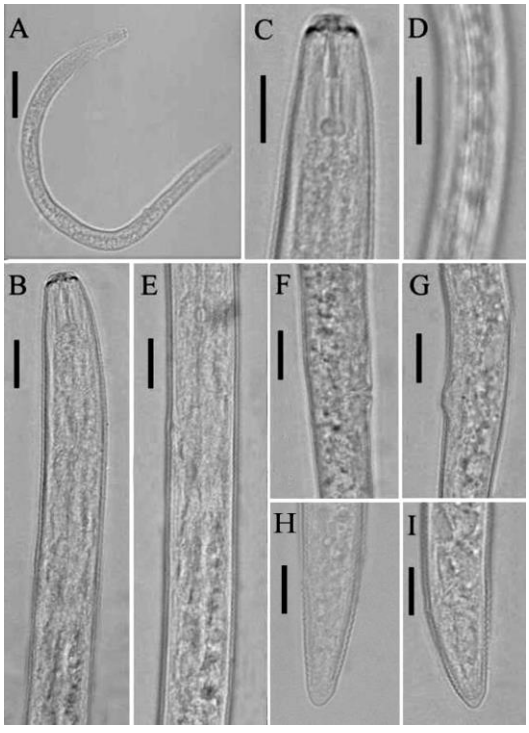

Fig. 2. Light microphotographs of *Pratylenchus thornei* Sher & Allen, 1953 female from Iraq (Ali-Algharbi population). A: Entire body; B, C: Anterior body region; D: Lateral field at mid-body; E: Part of pharynx; F, G: Vulval region; H, I: Posterior body region. (Scale bars: A = 50 µm; B-I: 10 µm).

Table 1. Morphometrics of *Pratylenchus thornei* Sher & Allen, 1953 from Alkahla and Ali-Algharbi regions in Misan province, Iraq. All measurements are in µm in form: mean ± SD (range).

Characters	*Pratylenchus thornei*	
	Alkahla	Ali-Algharbi
n	11	9
L	555±26.4 (586-592)	450±23.5 (423-489)
a	31.6±0.7 (28.5-35.4)	29.8±1.2 (27.7-33.2)
b	6.2±0.2 (5.8-7.5)	5.3±0.3 (4.8-5.9)
b′	4.4±0.3 (3.9-4.8)	4.1±0.2 (3.7-4.5)
c	23.2±1.7 (21.2-30.4)	21.4±2.7(16.2-24.4)
c′	2.3±0.2 (1.9-2.5)	2.4±0.2 (2.0-2.7)
V	74.2±2.8 (71.6-77.0)	73.1±3.7 (71.4-78.1)
DGO	3.1±0.1 (2.4-3.3)	2.8±0.1 (2.2-3.3)
Stylet length	15.4±0.7 (14.0-16.5)	15.6±1.4 (14.7-17.2)
Stylet shaft length	6.3±0.3 (5.8-6.6)	5.8±0.4 (5.4-6.9)
Stylet knob diameter	3.1±0.1 (2.4-3.3)	2.9±0.2 (2.2-3.2)
Stylet knob height	1.5±0.1 (1.4-1.6)	1.5±0.2 (1.3-1.6)
Pharynx length	99.7±9.7 (95.5-113.8)	93.2±12.4 (85.7-108.7)
Anterior end to excretory pore	73.0±4.7 (66.4-80.4)	74.0±6.4 (67.4-83.2)
MB	45.4±3.3 (43.5-51.2)	47.5±1.8 (44.1-50.2)
Pharyngeal overlap	28.1±2.7 (24.5-31.5)	28.2±2.9 (27.2-31.2)
Maximum body width	16.4±0.7 (15.0-17.4)	15.5±0.7 (14.6-17.0)
Anal body width	9.6±0.9 (8.3-11.6)	8.7±0.5 (8.4-9.1)
Vulva body width	15.8±4.1(13.2-16.9)	13.7±0.6 (13.2-14.6)
V-A	110.0±6.2 (96.3-119.3)	98.7±9.5 (88.4-112.1)
PUS	18.8±4.1 (15.7-22.4)	15.7±1.2 (14.8-16.5)
Tail length	24.4±2.0 (22.4-26.5)	23.7±1.5 (21.4-24.1)
Tail annuli	20±4.1 (18-24)	19±1.2 (17-22)

Molecular characterization and phylogenetic relationships

D2-D3 fragments of 28S rDNA phylogeny

Two 724 (Alkahla population) and 711 (Ali-Algharbi population) nt long D2-D3 expansion segments of LSU rDNA (OQ683864 and OQ683865) were obtained for the Iraqi populations of *Pratylenchus thornei*. The sequence variation between these two sequences showed four mismatches and no gap. The BLAST search using these sequences revealed that they have 99.86% and 99.72% identity with another sequence of the same species (MT856382). Sequence variation between Iraqi populations and this sequence was one mismatch and one to two gaps.

A total of 67 sequences of *Pratylenchus* spp. and two sequences of *Meloidogyne enterolobii* Yang & Eisenback, 1983 and *M. ichinohei* Araki, 1992 as outgroup taxa (EF029862 and KX823403), were selected for the LSU phylogeny. The outgroup taxa were chosen according to the previous studies (Nguyen *et al*. 2019; Movahedifar & Azimi 2020; Abdolkhani & Azimi 2021).

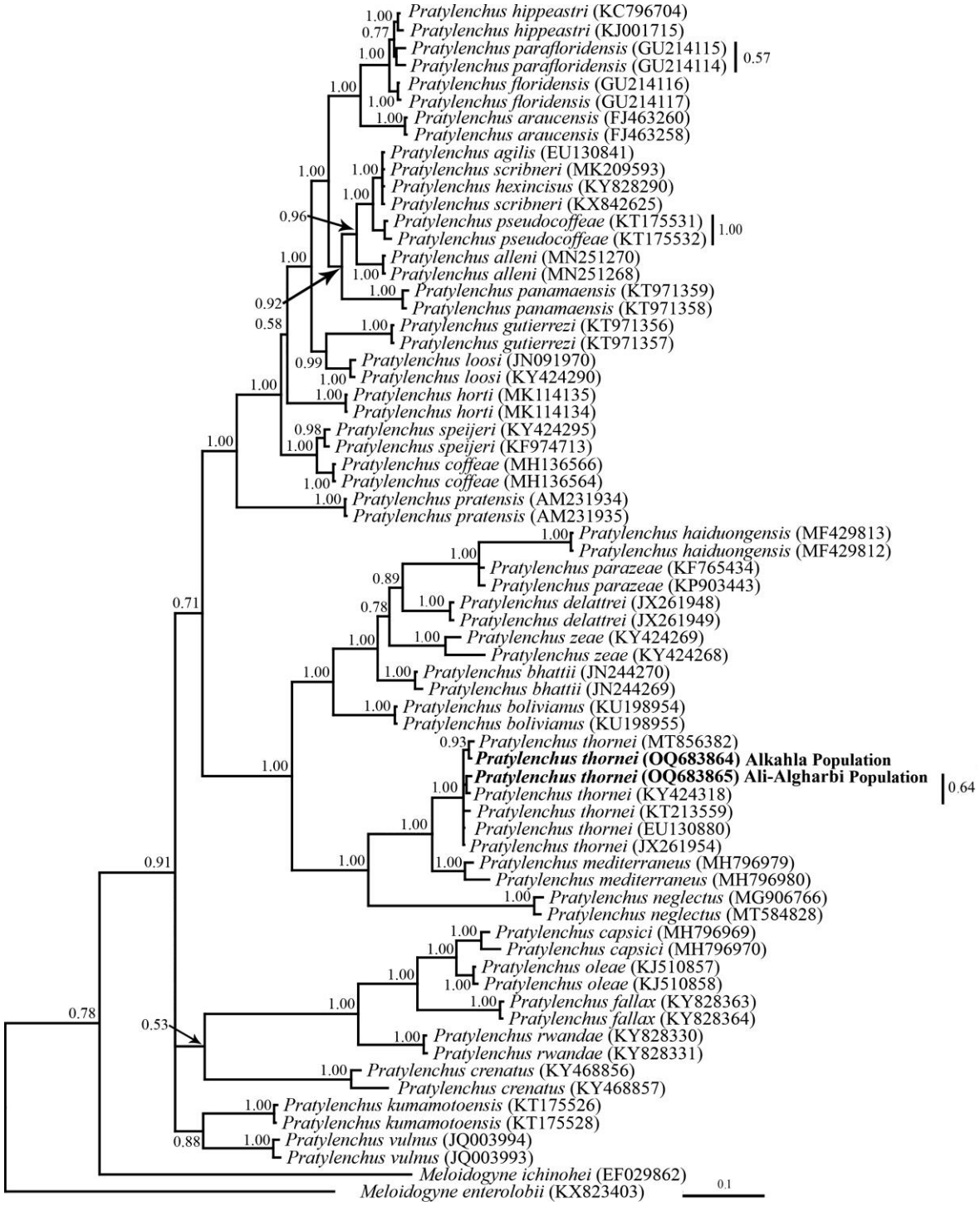

Fig. 3. Bayesian 50% majority rule consensus tree inferred from analysis of the D2-D3 domains of the LSU rDNA sequences of Iraqi populations of *Pratylenchus thornei* Sher & Allen, 1953 under the GTR + G + I model. Bayesian posterior probability values of more than 0.50 are given for appropriate clades. New sequences are indicated in bold.

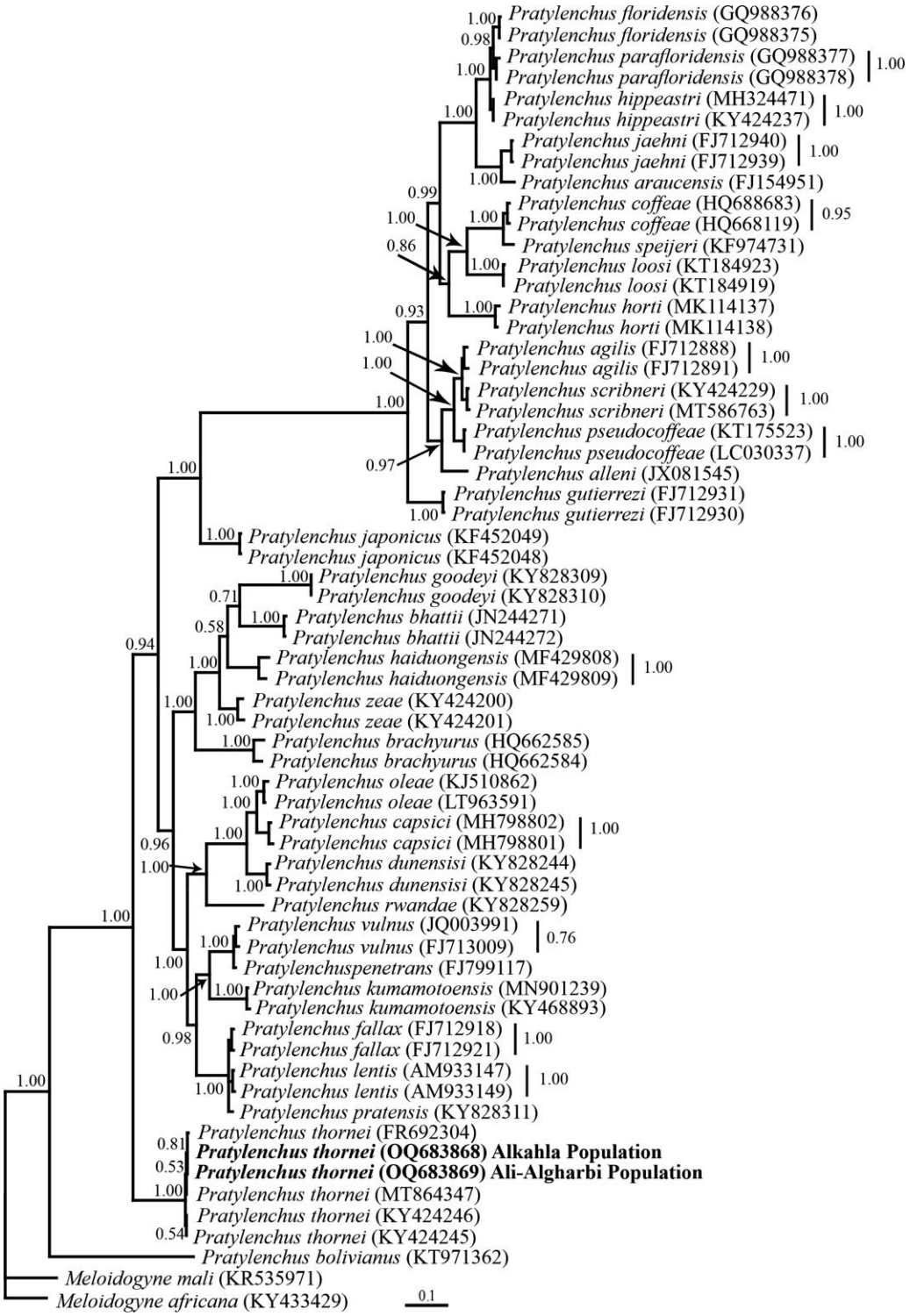

Fig. 4. Bayesian 50% majority rule consensus tree inferred from analysis of the ITS rDNA of Iraqi populations of *Pratylenchus thornei* Sher & Allen, 1953 under the GTR + G + I model. Bayesian posterior probability values of more than 0.50 are given for appropriate clades. New sequences are indicated in bold.

This dataset comprised 834 total characters. The phylogenetic tree inferred using this dataset is presented in Figure 3. The newly generated sequences of the Iraqi populations of *P. thornei* have formed a maximally

supported clade with other sequences of the species in this tree.

Partial ITS rDNA phylogeny

The sequencing of the ITS rDNA of the Iraqi populations of *Pratylenchus thornei* yielded two fragments with 584 (Alkahla population) and 561 (Ali-Algharbi population) nt long (OQ683868 and OQ683869). The sequence variation between these two sequences showed one mismatch and no gap. The BLAST search using these sequences revealed they have 99.49% and 99.64% identity (two to three mismatches and no gap) with another ITS sequence of the species (MT864347).

A total of 61 sequences of *Pratylenchus* spp. and two sequences of *Meloidogyne mali* Itoh, Ohshima & Ichinoche, 1969 and *M. africana* Whitehead, 1960 as outgroup taxa (KR535971 and KY433429), were selected for ITS phylogeny. The outgroup taxa were chosen according to the previous studies (Nguyen *et al*. 2019; Movahedifar & Azimi 2020). This dataset comprised 1293 total characters. The phylogenetic tree inferred by this dataset is presented in Figure 4. The sequences of the Iraqi populations of *P. thornei* formed a maximally supported clade with other sequences of the species in this tree.

Pratylenchus thornei populations from all over the world were split into two highly supported clades. One of these clades includes most populations, and another containes Australian populations of *P. thornei* with *P. mediterraneus* Corbett, 1983 (Subbotin *et al*. 2008). In the studies that a large number of sequences of the species, including some Australian sequences of the species or sequences that are placed in a separate clade were used, similar results have been obtained in one or more gene regions (Majd Taheri *et al*. 2013; Janssen *et al*. 2017a; Divsalar *et al*. 2018; Movahedifar & Azimi 2020; Abdolkhani & Azimi 2021). On the other hand, in the studies that a smaller number of sequences of the species were used in the analysis and some sequences that are placed in a separate clade were not included, all sequences were placed in one clade (Janssen *et al*. 2017b; Qing *et al*. 2019). The present study is also of the second type.

Conflict of interest

All the authors certify that they do not have any conflict of interest.

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