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# Morphological and molecular characterization of *Pratylenchus lentis* n. sp. (Nematoda: Pratylenchidae) from Sicily

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*Abstract: Pratylenchus lentis* n. sp. parasitizing roots of lentil in Sicily, Italy, is described and illustrated. The new species is characterized by a relatively high lip region with three annuli, mean stylet length of 16 μm, with anteriorly flattened knobs, cylindrical body with a relatively anterior vulva, large and ovoid spermatheca full of sperm, plump tail with truncate, irregularly annulated terminus, and by the presence of males. Molecular ITS-RFLP and sequencing analyses of the new species showed clear differences from other most morphologically similar species, such as *P. thornei* and *P. mediterraneus*. Preliminary host range tests revealed that chickpea, pea, faba bean and durum wheat are good hosts of *P. lentis* n. sp., whereas common bean, alfalfa and barley are less robust hosts and tomato, bell pepper, eggplant, melon and sunflower are poor hosts for the nematode.

*Key words:* host-range, internal transcribed spacer, ITS1, ITS2, lentil, morphology, new species, *Pratylenchus*, root lesion nematode, sequencing, Sicily.

Lentil (*Lens culinaris* Medik.) is one of the most ancient and common leguminous crops in dry areas of the world. In Italy, its cultivation is restricted to hilly areas of the central (Umbria region) and southern (Apulia region, Sicily and neighboring smaller islands) parts of the country. In central Sicily, lentil cultivation is well adapted to semi-arid environments, where the Villalba ecotype evolved, is grown for its high quality (Sarno et al., 1988; Piergiovanni, 2000) and is, therefore, of local economic importance. A lentil field in Pitrusa locality at Villalba (province of Caltanissetta) in central Sicily contained large patches of chlorotic and stunted plants with roots marked by large necrotic lesions. The dissection of these lesions revealed large numbers of an amphimictic root-lesion nematode morphologically similar to *P. thornei* Sher & Allen, 1953 and to *P. mediterraneus* Corbett, 1983. We found that the morphological characters and DNA sequences of this population from lentil differ from those of *P. thornei* and *P. mediterraneus*, as well as from all other known *Pratylenchus* species. In this paper, we report these differences and describe the root-lesion nematode from lentil as a new species named *Pratylenchus lentis* n. sp. The specific epithet refers to the Latin name of the host plant genus. A list of the hosts of this new species is also included.

## Materials and Methods

*Morphological analysis:* Specimens used in this study were extracted from infested roots 24 to 48 hr after incubation (Young, 1954). They were then handpicked, killed and fixed with a solution of 4% formaldehyde + 1% propionic acid, heated to 80°C, then processed to pure glycerine for light microscope observations, according to Hooper (1970). Measurements were taken with the aid of a camera lucida and an ocular micrometer. Abbreviations used are defined by Siddiqi (2000). Means in the text are presented along with their standard deviations and ranges in parentheses.

*DNA extraction, PCR amplification and RFLP*: Twenty individual nematodes of *P. lentis* n. sp., *P. thornei* from Apulia (South Italy) and *P. mediterraneus* from Israel were handpicked, and each one was placed on a glass-slide in 3 μl of lysis buffer (10 mM Tris-HCl, pH 8.8, 50 mM KCl, 15 mM $MgCl_2$, 0.1% Triton X100, 0.01% gelatine with 90 μg/ml proteinase K) and then cut into small pieces by using a sterilized syringe needle under a dissecting microscope. The suspension was recovered and transferred to a cold 0.5 ml microcentrifuge tube. Each sample was overlaid with a drop of mineral oil and incubated at 60°C for 1 hr and then at 95°C for 10 min to deactivate the proteinase K. The crude DNA extracted from each individual nematode was directly amplified. The ITS-containing region spanning from the 3' end of the 18S rDNA to the 5' end of the 26S rDNA was amplified using forward primer 18S-Int (5' CGTAACAAGGTAGCTGTAGG 3') and 26S-Int (5' TCCTCCGCTAAATGATATGC 3'). PCR amplification was carried out in 100 μl containing 0.2 mM of each dNTP, 20 pmol of each primer (10 μM) and 2 units of Taq DNA polymerase (Roche, Germany). PCR cycles consisted of an initial denaturation step at 94°C for 2 min, followed by 35 cycles of 50 sec at 94°C (denaturation), 50 sec at 56°C (annealing), 50 sec at 72°C (elongation) and a final step at 72°C for 7 min. The size of amplification products was determined by comparison with the molecular weight marker Ladder 100 bp (Fermentas, St. Leon-Rot, Germany) following electrophoresis of 10 μl on a 1% agarose gel.

Ten microliters of each PCR product was directly digested with the following restriction enzymes: Alu I, Ava II, Bam HI, Dde I, Hinf I and Rsa I (Roche, Germany), according to manufacturer's instructions, in a total volume of 20 μl. The digestions were conducted

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overnight or for 4 hr at 37°C. The resulting DNA fragments were separated by gel electrophoresis in a 2.5% agarose gel containing 0.003% ethidium bromide. The gels were then observed using UV light.

*DNA sequencing*: The amplified fragments from two individual nematodes were isolated from agarose gel using the High Pure PCR elution kit (Machinery-Nagel) and cloned into PCR 2.1-TOPO plasmid and TOP10 competent cells transformed using the TOPO-TA cloning kit (Invitrogen), according to the manufacturer's instructions. Twelve clones were sequenced using an ABI Prism 377 sequencer (Applied Biosystems, Foster City, CA). Both strands of each clone were sequenced using M13 forward and M13 reverse primers. Alignments were performed using ClustalW (Thompson et al., 1994). Formatting of alignments was performed with GeneDoc (Nicholas et al., 1997). The alignment of *P. lentis* sequences, not presented here, is available on request. The sequences have been submitted to GenBank with accession numbers AM933147-AM933158.

*Host test:* A preliminary experiment was conducted to study the host range and pathogenicity of the nematode on 13 plant species: alfalfa (*Medicago sativa* L.) 'Equipe', barley (*Hordeum vulgare* L.) 'Das 10', chickpea (*Cicer arietinum* L.) 'Ghab 1', common bean (*Phaseolus vulgaris* L.) 'Lingua di fuoco', durum wheat (*Triticum durum* Desf.) 'Simeto', eggplant (*Solanum melongena* L.) 'Violetta di Firenze', faba bean (*Vicia faba* L.) 'Aguadulce', lentil 'Villalba', melon (*Cucumis melo* L.) 'Napoletano giallo', pea (*Pisum sativum* L.) 'Progress 9', sweet pepper (*Capsicum annuum* L.) 'Yolo wonder', sunflower (*Heliantus annuus* L.) 'Isoleic' and tomato (*Solanum lycopersicum* L.) 'Rutgers'.

Three pre-germinated seeds/pot of each plant species were sown, and three seedlings of tomato, pepper and eggplant were transplanted in a clay pot containing 3 liters of steam-sterilized sandy soil. Two pots for each plant species were maintained in a glasshouse at 22 ± 2°C. Inocula (juveniles and adults) were obtained by rearing specimens of *P. lentis* n. sp. on chickpea in a glasshouse at 22 ± 2°C; nematodes were then collected by grinding in a blender and centrifuging chickpea roots. At plant emergence, a suspension of 15,000 juveniles and adults of the nematode per each pot was poured into six holes around seedling roots. Forty-five days after inoculation, the plants were uprooted, and the roots gently washed free of adhering soil and weighed. All stages of root-lesion nematodes were extracted from roots of plant species as described before and from soil of each pot by Coolen's method (Coolen, 1979) and counted. The reproduction rate of the nematode was calculated by dividing the total number of specimens found in the soil and in the roots by the number of nematodes inoculated at beginning of the experiment per each pot.

## Description

*Pratylenchus lentis* n. sp.
(Table 1, Figs. 1-3)

Morphometrics of the holotype female, and paratype females and males are reported in Table 1.

*Female:* Body cylindrical, slightly narrowing posterior to the vulva, with almost straight habitus after killing. Lip region relatively high, continuous, or barely offset in few specimens, 2.5 ± 0.3 (2-3) $\mu$m high and 8.3 ± 0.4 (8-9) $\mu$m wide, bearing three annules. Stylet slender, with anteriorly flattened basal knobs, about 5 $\mu$m across. Dorsal pharyngeal gland opening 2.3 ± 0.4 (2-3) $\mu$m posterior to the stylet knobs. Hemizonid just anterior to the excretory pore, two body annules long. Hemizonion not observed. Excretory pore located slightly anterior or, more often, posterior to the level of the pharyngeal-intestinal valve. Median pharyngeal bulb rounded, 14.7 ± 1.2 (13-16) $\mu$m high and 11.6 ± 0.6 (10.5-12.5) $\mu$m wide. Isthmus slender, 15.3 ± 1.3 (13-19) $\mu$m long, and encircled by the anterior half by the nerve ring. Pharyngeal glands sacciform, overlapping the intestine ventro-laterally for 42 ± 9.4 (29-60) $\mu$m. Lateral field composed of four lines, not areolate, becoming three in the distal end of tail, shortly posterior to the phasmid. Single genital tract well developed, exceptionally extending anteriorly to the level of the pharyngeal gland lobe. Spermatheca round to oval when full of sperm, but appearing also small and empty in a few specimens (Fig. 1G); its distal edge being 72 ± 18 (42-103) $\mu$m from the vagina. Vulva with two slightly prominent lips. Post-uterine sac long, 1.1 ± 0.2 (0.8-1.7) times the vulval body diameter, normally undifferentiated or with few distal cellular elements (Fig. 1G). Phasmids located at about middle tail, at 12.0 ± 2.4 (8-16) $\mu$m from the anus and 17.0 ± 3.1 (13-25) $\mu$m from tail end. Tail cylindrical, plump, bearing 20 ± 2.7 (17-26) annules on ventral side. Tail terminus coarsely annulated and truncate, often with a more or less pronounced indentation (Figs. 1L,M; 2G); a few specimens showed also slightly digitate or, more rarely, subhemispherical tail termini.

*Male:* Less abundant than females (about 40% of specimens investigated). Body shape similar to female, except in posterior end of the body. Stylet slender, less robust than that of female; knobs ellipsoidal to cupshape with rounded margins. Pharynx slightly less developed than in females, with glands overlapping intestine 1 to 3 times the body width. Spicules curved, weakly cephalated; gubernaculum slightly curved. Tail conical, with crenate bursa enveloping tail and extending to the tail tip. Lateral field with four smooth incisures.

*Type host and locality:* Nematodes were extracted from root samples collected in May 2005 in a cultivated field of lentil (*Lens culinaris* Medik.) at Villalba (locality Pitrusa), province of Caltanissetta, in central Sicily

TABLE 1. Morphometric data of paratypes of *Pratylenchus lentis* n. sp. Measurements are in μm and expressed as means ± standard deviation (range).

| Characters | Female | | Male |
|---|---|---|---|
| | Holotype | Paratypes | Paratypes |
| n | - | 20 | 10 |
| L[a] | 689 | 638 ± 45.8 (556-717) | 553 ± 27.2 (512-611) |
| Stylet length | 16 | 16.3 ± 0.5 (15.5-17.0) | 15.5 ± 0.6 (14.5-16.5) |
| D.G.O. from stylet base | 2 | 2.5 ± 0.4 (2.0-3.0) | 2.3 ± 0.4 (1.5-3.0) |
| Anterior end to center of metacorpus | 62 | 60 ± 3.9 (50-66) | 56 ± 1.9 (53-59) |
| to cardia | 92 | 89 ± 6.2 (71-99) | 85 ± 3.1 (80-89) |
| end of pharyngeal gland lobe | 141 | 130 ± 12.1 (103-150) | 120 ± 3.4 (114-124) |
| secretory/excretory pore | 104 | 89 ± 8.4 (76-104) | 83 ± 4.6 (72-88) |
| Maximum body width | 21 | 22 ± 1.6 (20-26) | 19 ± 1.1 (16.5-20.0) |
| Anal body width | 15 | 15 ± 1.4 (12.5-18.5) | 11 ± 0.6 (10-12) |
| Vulva-anus distance | 118 | 108 ± 10.6 (85-125) | - |
| Tail length | 34 | 29 ± 3.7 (23-36) | 27.5 ± 3.3 (22.0-31.5) |
| Spicules | - | - | 17.5 ± 0.9 (16.5-19.0) |
| Gubernaculum | - | - | 5.0 ± 0.8 (3-6) |
| | Percentages | | |
| o | 12.5 | 13.9 ± 2.3 (11.8-18.2) | 14.8 ± 2.7 (9.7-18.8) |
| V or T | 77.5 | 78 ± 0.9 (76-80) | 39 ± 5.2 (31-47) |
| G | 42.0 | 39 ± 10.1 (25-59) | - |
| | Ratios | | |
| a | 32.8 | 29 ± 2.0 (26.0-32.8) | 29.5 ± 2.5 (26.9-33.9) |
| b | 7.5 | 7.2 ± 0.5 (6-8) | 6.5 ± 0.4 (6.0-7.5) |
| b' | 4.9 | 4.9 ± 0.4 (4.0-5.6) | 4.6 ± 0.2 (4.4-5.1) |
| c | 20.3 | 22.5 ± 2.4 (16.4-27.2) | 20.4 ± 2.8 (17.1-24.9) |
| c' | 2.3 | 1.9 ± 0.2 (1.6-2.3) | 2.5 ± 0.3 (1.8-2.8) |

[a]Abbreviations defined in Siddiqi (2000).

(latitude 37°39′21″ N; longitude 13°50′37″ E), on clay soil, 600 m above sea level.

*Type designations:* Holotype female and female and male paratypes (collection numbers T-663t, T-5798p-T-5800p) in the USDA Nematode Collection, Beltsville, MD, and in the Istituto per la Protezione delle Piante of Bari, CNR, Via G. Amendola, 122/D, 70126 Bari, Italy (collection numbers IPP-H0735 to -37, H0739 to -44, H0776, -78, -80, -83, -85). Additional paratypes deposited at the University of California-Riverside Nematode Collection (collection number IPP-H0777), University of California Davis Nematode Collection (collection number IPP-H0784) and Nematode Collection of Wageningen, Wageningen University and Research Center, Laboratory of Nematology, Wageningen, The Netherlands (collection number IPP-H0782).

*Diagnosis and relationships: Pratylenchus lentis* n. sp. is a bisexual species characterized by a relatively high, mostly continuous lip region, composed of three annuli; stylet 16 μm in mean length, with anteriorly flattened knobs; body cylindrical, with a relatively anterior vulva; spermatheca large and full; tail plump, truncate and irregularly annulated at terminus.

*Pratylenchus lentis* n. sp. most closely resembles *P. thornei* in head shape and the range of main morphometric diagnostic values, but differs by having a tail terminus mostly annulated or coarsely crenate vs. smooth in *P. thornei*. It further differs by males being common vs. rare in *P. thornei* (Fortuner, 1977), by females not being contracted posterior to the vulva as in *P. thornei* and by shorter male spicules (15.0-17.5 vs. 21). Based on the original description (o. d.), it also has a shorter stylet (15.5-17.0 vs. 17-19). Because of the relatively high lip region, truncate tail and common males, *P. lentis* n. sp. is somewhat morphologically similar to *P. mediterraneus*, but differs by having an annulated tail terminus unlike the smooth tail tip of *P. mediterraneus*, a longer body (556-717 vs. 428-577 o. d.), a slightly longer stylet in mean value (16.3 vs. 15) and a more posterior excretory pore (76-104 vs. 65-84). The female of *P. mediterraneus* narrows posterior to the vulva, unlike in *P. lentis* n. sp., and the outer bands of the lateral fields are crenate with the middle one variously ornamented (oblique striae becoming double lines near vulva) in mid-body vs. lateral fields that lack areolation or ornamentation in *P. lentis* n. sp.

The few other *Pratylenchus* species having three lip annules, common males and female tails with more or less blunt, coarsely annulated termini are *P. convallariae* Seinhorst, 1959, *P. fallax* Seinhorst, 1968, and *P. pratensis* (De Man, 1880) Filipjev, 1936. *Pratylenchus convallariae* has a less slender body (a = 23–27) compared to *P. lentis* n. sp. (a = 26–33), a narrow vs. plump tail in *P. lentis* n. sp., with a fine striation at tip unlike the coarser, irregular striations at the tail tip of *P. lentis* n. sp. *Pratylenchus fallax* has flattened vs. high cephalic region in *P. lentis* n. sp., shorter body length, shorter pharyngeal overlap, conical tail with rounded or irregular terminus

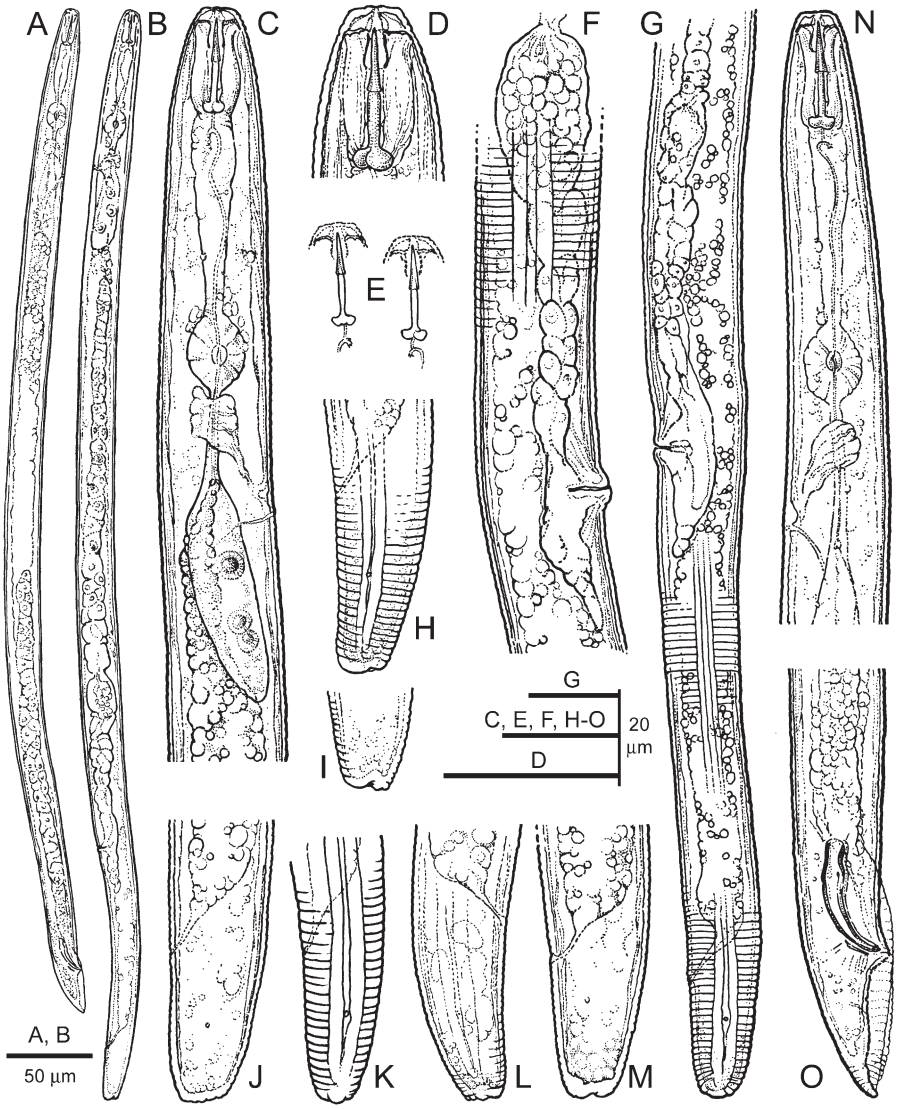


FIG. 1. *Pratylenchus lentis* n. sp. females (B-G) and males (A, N,O) drawn from fixed specimens. A, B: Entire body; C: Pharyngeal region; D: Anterior region; E: Stylets; F: Vulval region with spermatheca full of sperm; G: Vulval and posterior region; H-M: Variation in tail shape; N: Anterior region; O: Tail.

vs. cylindrical, bluntly rounded to truncate tail in *P. lentis* n. sp. and lateral fields with the inner band furrowed by oblique incisures in midbody, unlike in the lateral fields of *P. lentis* n. sp. *Pratylenchus pratensis* (Loof, 1974) shares with *P. lentis* n. sp. the annulated tail, although in the former it is variably shaped, but never truncate as in *P. lentis* n. sp.; *P. pratensis* also has a slightly shorter stylet, a more anterior vulva (V = 75-78 vs. 77-80), a finer, inconspicuous cuticular annulation (0.9 vs. 1.3 at midbody) and a spermatheca almost rectangular in shape vs. roundish in *P. lentis* n. sp.

*Molecular characterization*: Morphology and morphometrics of *P. lentis* n. sp. are primarily similar to *P. thornei* and *P. mediterraneus*. However, despite the morphological similarity, the three species show substantial genetic differences both in the size and RFLP patterns of the ITS region. The amplification of the ITS-containing region produced a single fragment of approximately 700 bp for *P. lentis* n. sp., whereas *P. thornei* and *P. mediterraneus* showed amplified products of 828 and 756 bp, respectively (Fig. 3), suggesting that the amplicon size can readily distinguish the three *Pratylenchus* species. Congeneric species usually display identically sized ITS regions, but in *Pratylenchus* large differences in ITS size have been widely reported (Orui, 1996; Powers et al., 1997; Uehara et al., 1998a & b; Waeyenberge et al., 2000).

The ITS-RFLP analyses clearly identified and differentiated *P. lentis* n. sp. from *P. mediterraneus* and *P. thornei* (Fig. 3). The enzymes Alu I and Ava II produced extra bands in all individual nematodes of *P. lentis* n. sp. included in this study. These extra bands were present even after prolonged digestions, suggesting that some of the ITS regions are divergent in their sequences.

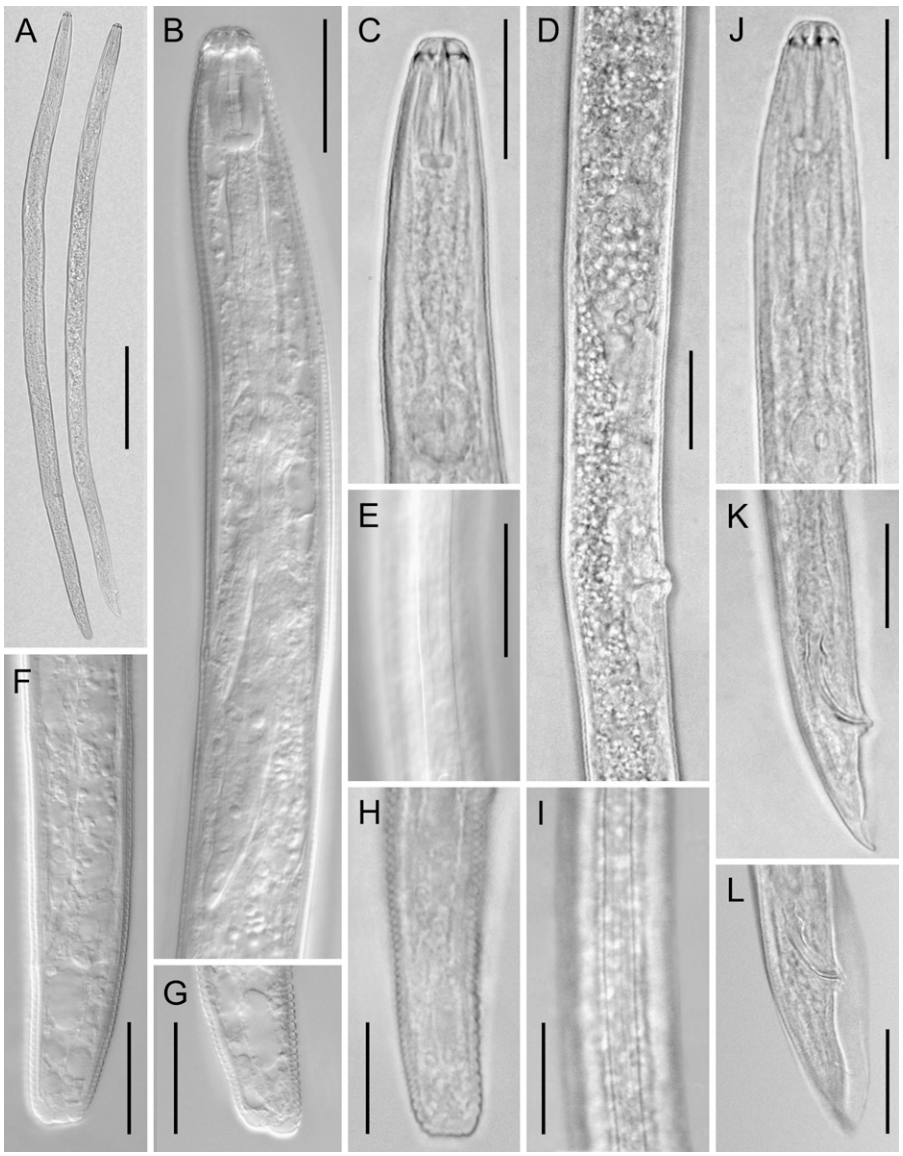


FIG. 2. Photomicrographs of females (A-I) and males (A, J-L) of *Pratylenchus lentis* n. sp. A: Female (left) and male (right) entire body; B: Pharyngeal region; C: Anterior end; D: Vulval region; E, I: Lateral fields at mid-body; F-H: Variation in tail shape; J: Anterior end; K, L: Tail at different foci. (Scale bars: A = 100 $\mu$m; B-L = 20 $\mu$m)

The sequence analysis of 12 cloned ITS amplicons of *P. lentis* n. sp. confirmed variability in length and in sequence in the ITS regions (ITS1 and ITS2) since the 5.8S rDNA and the 3' and 5' ends of the 18S and 26S, respectively, were constant in length. The ITS1 and ITS2 sizes ranged between 260 to 267 bp and between 171 to 177 bp, respectively. The alignment of the sequences also revealed the presence of many nucleotide variations and few insertions/deletions (indels) inside the population under study. Those indels of 1 to 3 bp clearly contributed to the length differences observed in both ITS1 and ITS2 among the cistrons (Fig. 4). The pairwise comparison between these sequences showed a percentage of dissimilarity varying from 0 to 5%. The level of ITS sequence variation observed among individuals of the same species is about the same as that observed among ITS repeats within individuals, typically $\leq 1\%$ (Heise et al., 1999; Nadler et al., 2000). This finding suggests that *P. lentis* n. sp. is characterized by substantial intra-specific variation in the ITS region. Sequence microheterogeneity, which is the presence in individual nematodes of more than one ITS or D2-D3 pattern in their genome, has been widely reported in nematodes and other invertebrates; the main cause of the heterogeneity is the presence of microsatellites and nucleotide differences (Orui, 1996; Zarlenga et al., 1996; Powers et al., 1997; Duncan et al., 1999; Hugall et al., 1999; Marrelli et al., 1999; van Herwerden et al., 1999; Harris and Crandall, 2000; van Herwerden et al., 2000; Carta et al., 2001; Conole et al., 2001; Handoo et al., 2001; von der Schulenburg et al., 2001; De Luca et al., 2004a, 2004b; Vovlas et al., 2007; Subbotin et al., 2008).

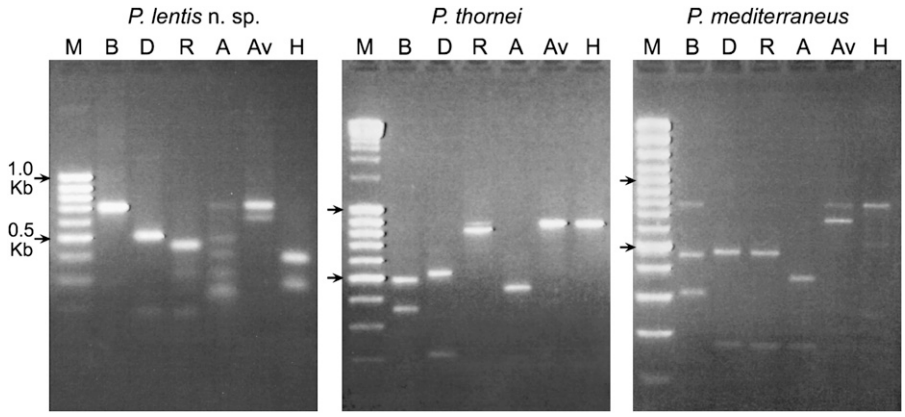


FIG. 3. Restriction fragments of amplified ITS region of *Pratylenchus lentis* n. sp., compared with those of *P. thornei* and *P. mediterraneus*. A: Alu I; Av: Ava II; B: Bam HI; D: Dde I; H: Hinf I; R: Rsa I and M: 100 bp ladder.

Therefore, in *P. lentis* n. sp., there are very few and short microsatellites, usually stretches of (A)n, (T)n, (G)n and (C)n. All together, these observations confirm that in *Pratylenchus* species, the ITS region is one of the most variable loci so far identified (Uehara et al., 1998a; Waeyenberge et al., 2000).

Pairwise comparisons of the ITS sequence of *P. lentis* n. sp. were carried out with the only two ITS sequences available in the database, belonging to *P. coffeae* Goodey, 1951 (Ay561436), and with the ITS sequence of *P. penetrans* (Cobb, 1917) Filipjev & Schuurmans-Stekhoven, 1941, not released in the database, reported by Uehara et al. (1998b). These comparisons revealed a high nucleotide dissimilarity (44% and 45%, respectively) and considerable variation in length, with longest gaps present in the ITS2 of *P. lentis* n. sp. These findings suggest that the high level of intra-population variability observed in the ITS containing region of *P. lentis* n. sp., also found in other *Pratylenchus* species, may reflect different dynamics of homogenization in the rDNA repeat families or can be due to the sequencing of pseudogenes (Marquez et al., 2003).

*Host-range characterization:* The nematode is common in the area of Villalba, where it causes severe damage to lentils. The crop showed patches with stunted and yellowish symptoms, and the roots were full of necrotic lesions of different sizes.

Greenhouse investigations revealed that chickpea, pea, faba bean and durum wheat are also good hosts for the nematode. In other hosts, such as common bean, alfalfa and barley, the nematode reproduction rate ranged from 0.5 to 0.8, while in vegetable crops (tomato, pepper, eggplant, melon and sunflower), the nematode reproduction rate was very low (0.1 – 0.2).

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                    *        20         *        40         *        60         *        80
P.lentis21      TGTTTGCGTATTTA---CAT-GAGCTTATTAA-T-ATAACTCGTTGTGAAAATGTACACTTGGTTCACAAATGCGGATAATTGAAATTA
P.lentis24      TGTTTGCGTAATTATTACAT-GAGCTTATTAA-TTATAACTCGTTGTGAAAATGTTCACTTGGTTCACAAATGCGGATAATTGAAATTA
P.lentis18      CATTTGCGTAA---TTACAT-GAGCTTATTAA-GTATATCTCGTTGTGGAAATGTACACTTGGTTCACAAATGCGGATAATTGAAATTA
P.lentis20      CAATTGCGTAAAT-TTACAT-GAGCTTATTAA-G-ATAACTCGTTGTGAAAATGTACACTTGGTTCACAAATGCGGATAATTGAAATTA
P.lentis2       CAATTGCGTAATT-TTACAT-GAGCTTATTTA-C-ATAACTCGTTGTGAAAATGCACACTTGGTTCACAAATGCGGATAATTGAAATTG
P.lentis25      CATTTGCGTAA---TTACAT-GAGCTTATTAA-T-ATAACTCGTTGTGAAAATGTACACTTAGTTCACAAATGCGGATAATTGAAATTA
P.lentis1       TGTTTGCGTATCTTTAATG--GAGTTTATTTTTATATAACTCGTTGTGAAAATGTACACTTGGTTCACAAATGCGGATAAATGAAATTA
P.lentis19      TGTTTGCGTATCTTTAATG--GAGTTTATTTTTATATAACTCGTTGTGAAAATGTACACTTGGTTCACAAATGCGGATAAATGAAATTA
P.lentis17      TGTTTGCGTATCTTTAATG--GAGTTTATTTTTATATAACTCGTTGTGAAAATGTACACTTGGTTCACAAATGCGGATAAATGAAATTA
P.lentis11      TGTTTGCGTAT-TGTAATGAAGAGTTTATTTT-ATATAACTCGTTGTGAAAATGTACACTTGGTTCACAAATGCGGATAATTGAAATTA
P.lentis15      TGTTTGCGTAT-TGTAATGAAGAGTTTATTTT-ATATAACTCGTTGTGAAAATGTACACTTGGTTCACAAATGCGGATAATTGAAATTA
P.lentis23      TGTTTGCGTAT-TGCAGTGAAGAGTTTATTTT-ATATAGCTCGTAGTGAAAATGTACACTTGATTCACAAATGCGGATAATTGAAATTA

                *       100         *       120         *       140         *       160         *
P.lentis21      CGACATTATATTTCTAATTATTTTGCTTTATGCATTTTGATGGACCAAATGTCAAGTCTGTGTGGCAAATATCATTTTCGA
P.lentis24      TGACATTACATTTC-GCTTATCTTCCTTTATGCATTTTGCTGAACCAAATGTCAAGCCTGTGTGGTAAATATCATTTTCGA
P.lentis18      TGGCAGTATATTTCTCATTATCTTGCTTTATGCATTTTGCTGAATCAAATGTTAAAGCC-TGTGGCAAATCTCATTTTCGA
P.lentis20      TGACATTATATTTCA-ATTGTCTTGCTTTATGCATTTTGCTGAATCAAATGTCAA-GCC-TGTGGCAAATATCATTTTCGA
P.lentis2       TGACATTATATTTCA-ATTGTCTTGTTTTATGCATTGTGATGGACTAAATGTCAA-GCC-TATGGCAAATATCATTTTCGA
P.lentis25      TGACATTATATTTCT-CTTATCTTGCTTTATGCATTCTGATGGACCAAATGTCAAGCCTGTGTGGCAAATATCATTTTCGA
P.lentis1       TGACAGTATATTTCTCATTATCTTGCTTTATGCATTTTGATGGACCAAATGTCGAGCCTGTGTGGCAAATATCATTTTCGA
P.lentis19      TGACAGTATATTTCTCATTATCTTGCTTTATGCATTTTGATGGACCAAATGTCGAGCCTGTGTGGCAAATATCATTTTCGA
P.lentis17      TGACAGTATATTTCTCATTATCTTGCTTTATGCATTTTGATGGACCAAATGTCGAGCCTGTGTGGCAAATATCATTTTCGA
P.lentis11      TGGCAGTATGTTTCTCATTATCTTGCTTTATGCATTTTGCTGGACCAAATGTCAAGCCTGTGTGGCAAATATCATTTTCGA
P.lentis15      TGGCAGTATGTTTCTCATTATCTTGCTTTATGCATTTTGCTGGACCAAATGTCAAGCCTGTGTGGCAAATATCATTTTCGA
P.lentis23      TGTCAGTATGTTTCA-ATTGTCTTGCTTTATGCATTTTGCTGAACTAAATGTCAAGCCTGTTTGGCAAATATCATTTTCGA
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FIG. 4. Portion of the alignment of different cloned ITS amplicons of *P. lentis*. The alignment corresponds to a region located at the 5' end of the ITS2.

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